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Inflammatory Reproductive White Adipose Tissue Characterizes The Obese Preeclamptic-like BPH/5 Mouse prior to Pregnancy

By

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INTRODUCTION

Preeclampsia (PE) is a serious disorder of pregnancy characterized by late gestational hypertension (systolic ≥140 mmHg and diastolic ≥90 mmHg) combined with at least one other accompanying sign/symptom, such as proteinuria, liver or renal dysfunction, and neurologic deficits among others[1]. PE has been shown to impact up to 8% of pregnancies worldwide, though it is difficult to diagnose due to its heterogeneous presentation with multiple systems affected during pregnancy[1]. Additionally, PE/eclampsia is responsible for 10-15% of maternal deaths, 12% of small-for-gestational age infants, and 20% of infants born preterm[2]. Even when consequences are not immediately evident, PE can have lasting effects on the lives of both mother and child. Women with PE have been shown to have increased incidence of cardiovascular diseases, a leading cause of mortality, in later life. Children of PE pregnancies have also been observed to have increased hypertension and incidence of stroke as they become older[3]. Though the disorder has a devastating impact on the lives of mothers and babies around the world, the etiology of PE remains unknown and the only way to relieve life threatening maternal symptoms of PE is delivery of both the baby and placenta[4]. Therefore, the placenta has been identified as a causal organ in the pathogenesis of PE.

Because PE manifests in the second half of pregnancy, it has been historically difficult to diagnose early, before symptoms are observed. However, it is widely accepted that PE is caused by placental injury occurring in early pregnancy resulting in reduced blood flow to the fetus[4]. Optimal placental function is essential to a healthy pregnancy. This temporary organ is responsible for supplying oxygen and essential nutrients to the fetus while removing waste like carbon dioxide.

However, these roles are all dependent on a functional placenta, the formation of which depends on a number of factors[5]. Once implantation has occurred, trophoblast cells can proliferate and differentiate into villous and extravillous cell types. Extravillous cytotrophoblasts are then responsible for invading the maternal decidua and remodeling blood vessels to become high capacitance, low resistance vessels that promote blood flow to the placenta[5]. Once maternal vessels have been remodeled, vasculogenesis takes over to form a vascular network in both embryo and placenta. Soon after, angiogenesis begins to predominate to contend with the increasing size of the placenta and metabolic needs of the fetus[5]. Angiogenesis is essential to a healthy pregnancy and is controlled by pro and anti-angiogenic factors. It has been hypothesized that an imbalance in pro- and anti-angiogenic factors may contribute significantly to the etiology of PE. Decreased serum levels of pro-angiogenic factors, vascular endothelial growth factor (VEGF) and placental growth factor (PLGF), combined with increased levels of the anti-angiogenic soluble fms-like tyrosine kinase (s-Flt) are associated with the development of PE[4]. This is just one example of how tightly regulated different responses and processes must be during pregnancy. Because the mother's body must tolerate a semiallogenic fetus, immune system regulation is paramount to a successful pregnancy. However, the immune system, particularly the complement system has been identified as another area where imbalance can negatively impact pregnancy outcomes[6]. Ultimately, PE is thought to relate to inadequate trophoblast invasion and remodeling of spiral arteries brought on by dysregulation of angiogenic, immune, and inflammatory factors at the maternal-fetal interface[4]. To understand the source of the dysregulation, scientists have focused on risk factors for PE to identify what might be the stimulus of this disorder.

PE has many risk factors including chronic hypertension, pre-gestational diabetes, and obesity. In fact, women with a body mass index (BMI) ≥ 35 kg/m^2 have a 30% increased risk of

developing PE. This is extremely concerning considering the fact that obesity itself affects 30% of reproductive-age women. Obesity is associated with expansion of adipose tissue as well as a state of low-grade inflammation throughout the body, because adipose tissue is a source of various adipokines, cytokines, and even complement proteins[6,7]. Though the exact relationship between obesity and PE has yet to be elucidated, the complement system has been implicated.

The complement system is an arm of the immune system responsible for defense against pathogens, but also clearing of apoptotic cells and cell debris. This system can be activated systemically, i.e. in serum, or locally, i.e. in adipose tissue[6]. The complement system is comprised of over 50 proteins and can be activated through 3 different pathways: the classical, lectin, or alternative pathway. All 3 converge at complement factor 3 (C3), which continues through the cascade eventually forming the membrane attack complex[8]. The alternative complement pathway has been isolated as a particular area of interest in the study of PE[9]. Lynch and colleagues looked at the relationship between obesity, PE, and the level of complement activation fragments Bb and C3a in the serum of pregnant women. Bb is specifically involved in the alternative pathway, while C3a is a direct product of the cleavage of C3[10]. This study measured both BMI and complement fragments in serum during early pregnancy and found that women who were obese and had increased levels of complement activation fragments in serum were up to 10 times more likely to develop PE compared to nonobese women who did not have increased complement activation fragments in serum[10]. Adipsin (complement factor D) has also been identified as a part of the alternative complement system thought to contribute to PE. It has even been suggested as a biochemical marker of PE to be measured via quantification of its concentration in maternal urine[11]. We sought to examine the relationship between this increase in complement activation and increase in adipose tissue that predisposes women to PE using an animal model.

It has been shown that visceral adipose tissue localized to the central compartment (intra-abdominal depot) may contribute to adipose tissue accumulation at the maternal-fetal interface. This could potentially be the source of the increased complement activation observed in PE pregnancy[6]. To investigate this phenomenon, we utilized the preeclamptic-like BPH/5 mouse model. These mice develop gestational hypertension and proteinuria during late pregnancy that is resolved after delivery, as well as decreased litter size and low birth weight pups[12]. The similarities to PE make this an ideal mouse model in which to study early pregnancy events that may contribute to the etiology of PE[13]. Interestingly, it has previously been shown in the BPH/5 mouse model that in early pregnancy at embryonic day (e) 7.5, complement factor B (CfB) and C3 mRNA expression is significantly upregulated in the implantation sites before placentation takes place[14]. This came after observation of an angiogenic imbalance at e5.5 in the BPH/5 implantation sites[14]. Furthermore, complement inhibition rescued BPH/5 pregnancies and was shown to improve spiral artery remodeling[15]. This mouse model has also been shown to be hyperphagic compared to C57BL/6 controls and subsequently these mice have increased white adipose tissue (WAT) deposition compared to C57BL/6 controls before pregnancy[16]. Furthermore, the reproductive WAT (reproWAT) of non-pregnant BPH/5 female mice exhibited an increase in inflammatory markers, interleukin (IL)-6 and tumor necrosis factor (TNF) α[16].

The first aim of this study was to understand whether adipose tissue could be a source of complement factors contributing to dysregulated inflammation observed in PE pregnancies. The second aim was to understand the effect of weight loss via calorie restriction on the presumed dysregulation. Therefore, we hypothesized that pre-pregnancy WAT is a source of complement factors that contribute to the inflammatory milieu at the maternal-fetal interface in BPH/5 mice, and that calorie restriction via pair feeding will attenuate this imbalance.

MATERIALS AND METHODS

Animals

Virgin 8-12 week C57 and BPH/5 mice were used from colonies maintained at the LSU School of Veterinary Medicine for all experiments. Mice were exposed to a standard 12 hour light/dark cycle. Throughout all experiments, mice were fed standard 5001 chow. In order to collect tissue, mice were euthanized via CO_2 inhalation. All animal procedures were reviewed and approved by Louisiana State University Animal Care and Use Committee. Care of the mice met the standards set forth by the NIH guidelines on the care and use of animals, USDA regulations, and the American Veterinary Medical Association (AVMA) Panel on Euthanasia. Tissue was collected, immediately snap frozen, and stored in a -80°C freezer for downstream analyses.

Study Design

Experiment 1: 8-12 week old multi housed C57BL/6 (C57) and BPH/5 females were fed ad libitum over 14 days. We then measured *CfB, C3,* and *adipsin* mRNA expression in the reproWAT of these mice (Figure 1 top). We have previously shown that BPH/5 females eat an average of 4 grams of 5001 chow per day, while C57 mice eat an average of 3 grams of 5001 chow per day[16]. Based on this data, we pair fed 8-12 week old BPH/5 females 3 grams of 5001 chow per day over 14 days as a method of calorie restriction. This translated to a 25% calorie reduction. Calorie restriction via pair feeding has previously been shown to reduce reproWAT weight in non-pregnant BPH/5 females[16]. Therefore, after

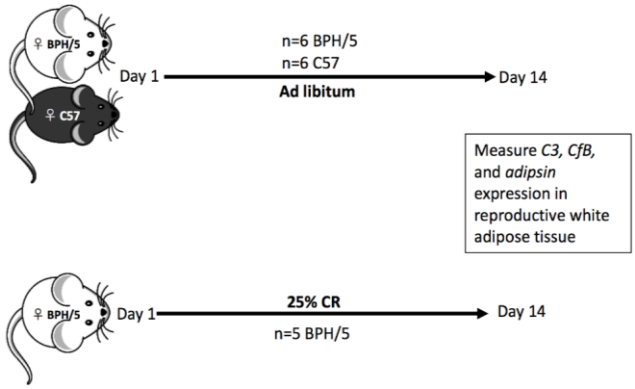

Figure 1. Experiment 1: Nonpregnant BPH/5 and C57 females were fed ad libitum and monitored over 14 days (n=6) prior to humane euthanasia. A cohort of non-pregnant BPH/5 females were then calorie restricted (CR) via pair feeding (n=5) for 14 days prior to humane euthanasia. Repro WAT was collected from all mice and gene expression was measured.

calorie restriction, we measured expression of *CfB, C3,* and *adipsin* mRNA in the reproWAT to determine whether reduction in adiposity would affect complement expression (Figure 1 bottom).

Experiment 2: Next, we investigated complement gene expression in reproWAT and implantation sites of BPH/5 and C57 mice during pregnancy. To test our hypothesis, a cohort of 8-12 week old BPH/5 and C57 females were mated with strain matched males. Day of plug detection was designated as e0.5. Females were separated from males at e0.5 and single housed for the remainder of the experiments. These mice were fed ad libitum from e0.5 through e7.5. At e7.5, body and reproWAT weight were recorded, reproWAT and implantation sites were collected for gene expression (Figure 2 top). To determine effect of calorie restriction during pregnancy on the complement mileu of the BPH/5 reproWAT and implantation sites in early pregnancy, another cohort of 8-12 week old BPH/5 females were mated with strain matched males. BPH/5 females were separated from males at e0.5 and single housed for the remainder of the experiment. Previous unpublished data from the Sones lab has shown that C57 females continue to eat an average of 3 grams of food per day throughout the first 7 days of gestation. Therefore, we employed the same method of calorie restriction via pair feeding as used in the non-pregnant BPH/5 females. This cohort of BPH/5 females was calorie restricted beginning at e0.5 through e7.5. At e7.5, body and reproWAT weight was recorded, and reproWAT and implantation sites were collected for gene expression (Figure 2 bottom).

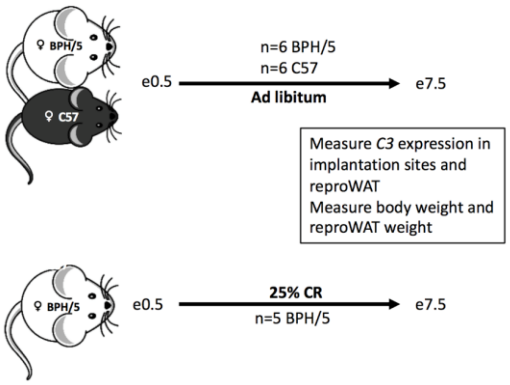

Figure 2. Experiment 2: BPH/5 and C57 females were mated with age and strain matched males. At plug detection (e0.5), females were separated from males and fed ad libitum until e7.5 (n=6) prior to humane euthanasia. Body weight and reproWAT weight was measured, and tissue was collected for gene expression. Another cohort of BPH/5 females were calorie restricted (CR) via pair feeding (n=5). After 14 days of calorie restriction mice were humanely euthanized, tissue was collected, and gene expression was measured in implantation sites and reproWAT.

Gene expression analysis

Quantitative real-time PCR (qRT-PCR) was used to measure mRNA expression levels of complement factors in reproWAT and e7.5 implantation sites collected from BPH/5 and C57 mice. Tissue was homogenized in Trizol and phase separated with chloroform prior to RNA isolation with a Qiagen RNeasy kit (Qiagen). cDNA was then synthesized using a Quanta kit following manufacturers instructions. qRT-PCR was used to determine the amount of complement factor 3 (C3), complement factor B (CfB), and complement factor D (adipsin) mRNA (Table 1). qPCR was performed using SYBR green. Each qRT-PCR was performed in duplicate using 25ng of cDNA. Relative expression was quantified and expressed as a fold change relative to 18s mRNA levels using the 2^-deltadelta Ct method. These procedures were performed as previously described[17].

Gene	Forward Sequence	Reverse Sequence	Reference
C3	CACCGCCAAGAATCGCTAC	GATCAGGTGTTTCAGCCGC	Rupprecht et al. *J Immunol. 2007*. [18]
CfB	GAAACCCTGTCACTGTCATTC	CCCCAAACACATACACATCC	Zou et al. *J Immunol. 2016.*[19]
Adipsin	GCTATCCCAGAATGCCTCGTT	CCACTTCTTTGTCCTCGATTGC	Searfoss et al. *J Biol Chem, 2003*. [20]

Table 1: Forward and reverse primer sequences for complement factor 3 (C3), complement factor B (CfB), and Complement factor D (adipsin) were obtained from published literature referenced above. These primer sequences were then used in qRT-PCR to measure expression of the respective genes.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism, Version 6.0f. Normality was tested and either a 2-tail t test or one-way analysis of variance (ANOVA) was used to analyze data. Post-hoc Tukey comparisons were performed with least square means for the effect. Data are presented as mean ± standard error of the mean (SEM). Statistical significance was defined as $p<0.05$.

RESULTS

Body Weight and Adiposity of e7.5 Pregnant BPH/5 and C57 Females

Non-pregnant BPH/5 females have previously been described as overweight compared to C57 females with increased presence of reproductive WAT[16]. Furthermore, when calorie restricted via pair feeding, both BPH/5 reproductive WAT and body weights were significantly decreased[16]. We then measured these parameters at e7.5 to understand whether this trend continued throughout pregnancy. It was observed that BPH/5 ad libitum fed female body weights and reproductive WAT weights were increased compared to C57 ad libitum fed females at e7.5 (25.26 ± 0.7502 g vs. 21.17 ± 0.1375 g and 932.6 ± 282.7 mg vs. 243.6 ± 44.35 mg respectively; $p<0.05$ and $n=6$ for all groups). When e7.5 BPH/5 mice were calorie restricted via pair feeding their body weight and reproductive WAT weight was decreased compared to ad libitum fed e7.5 BPH/5 females (22.65 ± 0.5795 g vs 25.65 ± 0.7502 g and 548.0 ± 298.8 mg vs. 932.6 ± 282.7 mg respectively; $p<0.05$ and $n=6$ for all groups) (Figure 3).

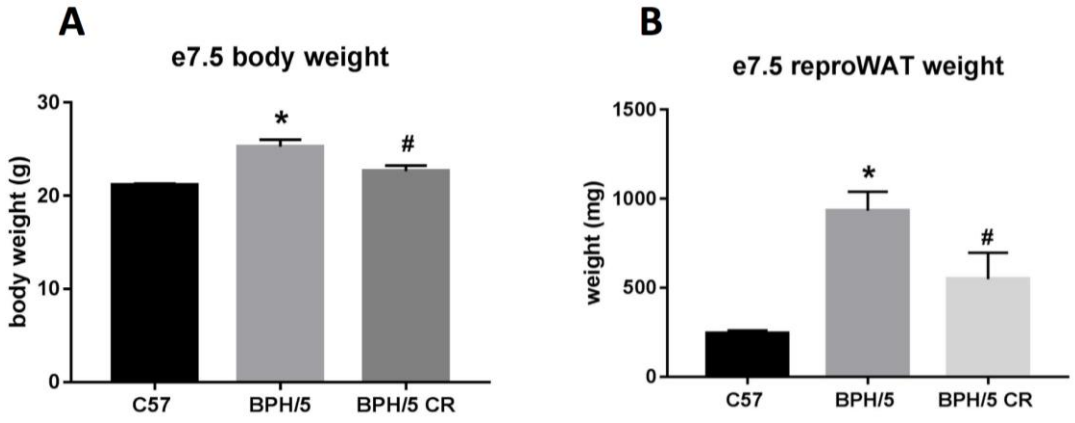

Figure 3: Measurement of body weight and reproductive WAT weight during early pregnancy. (A) Body weight of ad libitum fed BPH/5 and C57 and calorie restricted (CR) BPH/5 mice was measured at e7.5 of pregnancy. n=6; *p<0.05 compared to C57. #p<0.05 compared to BPH/5. **(B)** Reproductive white adipose tissue (reproWAT) was measured at e7.5 in ad libitum fed BPH/5 and C57 and calorie restricted BPH/5 mice n=6; *p<0.05 compared to C57 ad lib. #p<0.05 compared to BPH/5 ad lib. Data are expressed as mean ± SEM.

CfB*,* Adipsin*, and* C3 *mRNA in Reproductive WAT of Non-pregnant BPH/5 and C57 Females*

To determine whether reproWAT could be a source of alternative complement components contributing to an altered inflammatory profile during pregnancy, we measured *CfB*, *adipsin*, and *C3* mRNA expression in reproWAT of ad libitum fed non-pregnant C57 and BPH/5 females. *CfB* mRNA levels were comparable in the reproWAT of ad libitum fed non-pregnant C57 and BPH/5 females (1.0331 ± 0.1118 vs. 1.899 ± 0.05839; $p>0.05$, n=6). *Adipsin* was also not differentially expressed in reproWAT of non-pregnant C57 and BPH/5 females (1.259 ± 0.3871 vs. 1.67 ± 0.3937; $p>0.05$, n=6). However, *C3* mRNA was increased in the reproWAT of ad libitum fed nonpregnant BPH/5 females compared to C57 females (2.78 ± 0.7432 vs. 1.097 ± 0.206; $p<0.05$, n=6). Following this observation, we wanted to determine whether the increase in *C3* expression could be attenuated through calorie restriction to reduce reproWAT. When non-pregnant BPH/5 females were calorie restricted, *C3* mRNA levels were reduced compared to the ad libitum fed BPH/ 5 females (0.3583 ± 0.09633 vs. 2.78 ± 0.7432; $p<0.05$, n=6) (Figure 4).

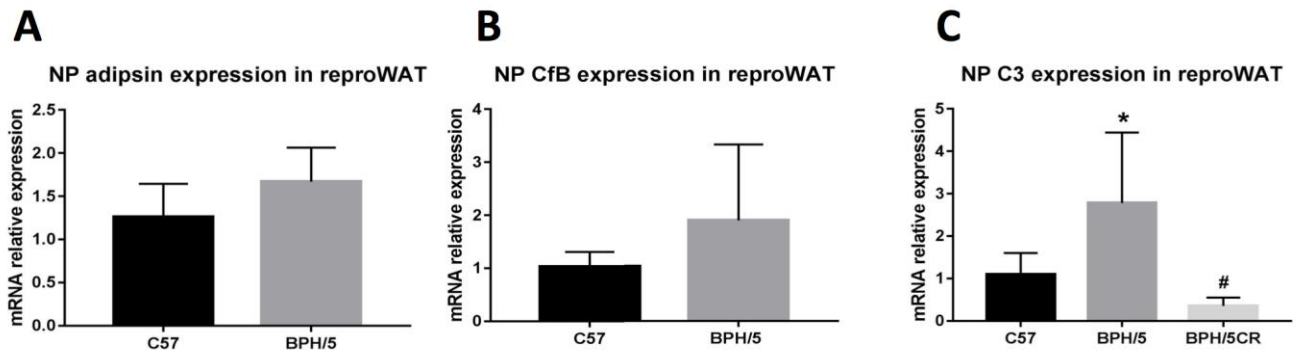

Figure 3: qRT-PCR expression of CfB, adipsin, and C3 in nonpregnant reproductive WAT. (A) mRNA expression of complement factor B (CfB) was measured in reproWAT of ad libitum fed non-pregnant C57 and BPH/5 mice (n=6, $p>0.05$) (B) mRNA expression of complement factor D (adipsin) was measured in reproWAT of ad libitum fed non-pregnant C57 and BPH/5 mice (n=6, $p>0.05$). (C) mRNA expression of complement factor C3 (C3) was measured in reproWAT of ad libitum fed non-pregnant BPH/5 and C57 mice as well as calorie restricted (pair fed) BPH/5 mice (n=6). *$p<0.05$ compared to C57. #$p<0.05$ compared to BPH/5. Data are expressed as mean ± SEM

Expression of C3 *in reproductive WAT and implantation sites of e7.5 BPH/5 and C57 females.*

To understand whether C3 dysregulation carried over into early pregnancy and occurred at the maternal-fetal interface, *C3* mRNA was measured in the reproWAT and implantation sites of e7.5 female mice. *C3* expression in the reproWAT did not differ between e7.5 C57 and BPH/5 e7.5 female mice (1.235 ± 0.3182 vs. 1.756 ± 0.3906; $p>0.05$, n=6). *C3* mRNA levels were increased in e7.5 BPH/5 ad libitum fed females compared to e7.5 C57 ad libitum fed females (5.16 ± 0.4028 vs. 1.101 ± 0.2149; $p<0.05$, n=6). We then observed that calorie restriction via pair feeding beginning at e0.5 could reduce *C3* mRNA expression in implantation sites of BPH/5 females at e7.5 when compared to gestation matched ad libitum fed BPH/5 females (3.456 ± 0.5793 vs. 5.16 ± 0.4028; $p<0.05$, n=5) (Figure 5).

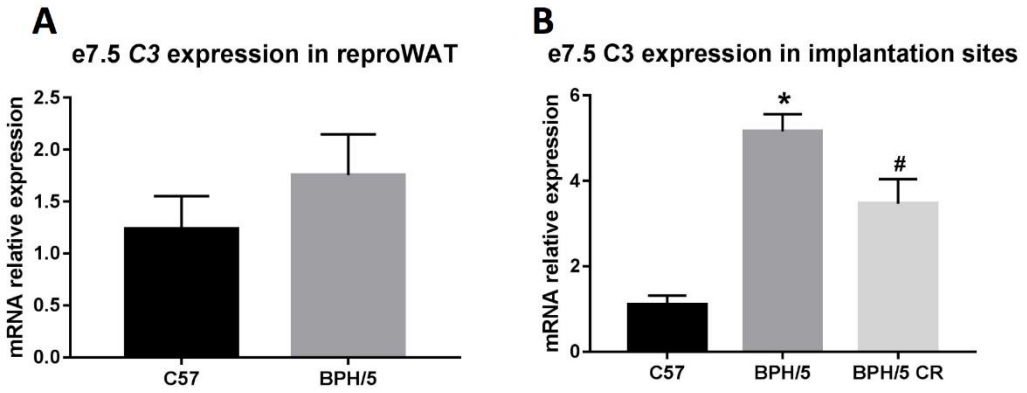

Figure 5. qRT-PCR expression of *C3* in e7.5 reproductive WAT and implantation sites (**A**) mRNA expression of C3 was measured in reproductive white adipose tissue (reproWAT) of ad libitum fed e7.5 C57 and BPH/5 mice (n=6; $p>0.05$). (**B**) mRNA expression of *C3* was measured in implantation sites of ad libitum fed e7.5 C57 and BPH/5 mice (n=6) and calorie restricted (CR) BPH/5 mice and compared to ad libitum fed e7.5 BPH/5 mice (n=5; *$p<0.05$ compared to C57, #$p<0.05$ compared to ad libitum fed BPH/5). Data are expressed as mean ± SEM.

DISCUSSION

Maternal obesity is a risk factor for several disorders in pregnancy including gestational diabetes, gestational hypertension, PE, and macrosomia. These disorders can increase risk for cesarean deliveries and perinatal mortality.[17] However, the link between obesity and these adverse pregnancy outcomes is not fully understood; therefore, further studies are warranted. We focus on PE, an adverse outcome of pregnancy that is a leading cause of maternal and fetal morbidity and mortality. Though the etiology is not fully understood, it is widely accepted that the placenta plays a causative role in the development of this disorder. A prominent risk factor for PE is obesity, which is characterized by increased adipose tissue and a state of low grade inflammation. Interestingly, PE has also been related to the dysregulation of inflammatory mediators, particularly complement proteins, at the maternal-fetal interface.[6] To understand whether WAT might be a source of local inflammation at the maternal-fetal interface, we specifically studied the visceral WAT localized around the reproductive tract of the female mouse (reproWAT). Therefore, these studies looked at the role of inflammation in both reproductive white adipose tissue and the maternal-fetal interface to characterize the relationship between maternal obesity and PE. We utilized an obese mouse model of PE to better understand this interaction. The BPH/5 model is ideal to study risk factors in early pregnancy as these mice develop a PE phenotype spontaneously during pregnancy mirroring the maternal syndrome[12]. Interestingly, it has previously been shown that the BPH/5 mouse model has an increase in reproWAT and inflammatory factors within reproWAT prior to pregnancy.[15] We hypothesized that reproWAT could be a source of inflammatory factors in early pregnancy contributing to the etiology of PE in this model. Furthermore, we sought to understand whether a reduction in maternal adiposity via pair feeding would have an effect on the expression of the inflammatory factors at the maternal-fetal interface.

This model has been previously characterized as obese compared to a C57 control prior to pregnancy. We discovered that this increase in body weight continues throughout pregnancy. The obesity epidemic in humans has been addressed in a number of ways, particularly reduction of adiposity by some form of calorie restriction. Dietary restriction has proven effective in reducing adiposity and some inflammatory factors in human adults[21]. Furthermore, dietary intervention aimed at limiting gestational weight gain during pregnancy has been shown to improve pregnancy outcomes[22]. Our data demonstrates a successful reduction in body weight and reproWAT during early pregnancy by pair feeding. Similar to the effects observed in humans, previous data from the Sones laboratory has shown that calorie restriction which starts before and continues throughout gestation can rescue BPH/5 pregnancies.

To understand whether reproWAT is the source of inflammatory dysregulation observed at the maternal-fetal interface during PE, we then examined the inflammatory profile of the reproWAT prior to pregnancy. In human studies, PE has been linked to a dysregulation of the alternative complement pathway,[9] however we did not see differential expression of *CfB* and *adipsin* mRNA in the reproWAT of BPH/5 mice prior to pregnancy. We did see increased expression of *C3* mRNA, which is common to all complement pathways. Reduction of adipose tissue via pair feeding was successful in reducing the expression of *C3* mRNA in reproWAT of BPH/5 mice close to C57 levels. These data suggest that reducing adiposity could be successful in correcting a dysregulated inflammatory profile that results from obesity.

Complement activity is thought to contribute to PE by contributing to an angiogenic imbalance localized at the maternal-fetal interface.[6] Based on this localization, we investigated whether complement dysregulation occurred at the maternal-fetal interface in early pregnancy and in the reproWAT surrounding it. There was a trend of *C3* increase in reproWAT at e7.5 in BPH/5

pregnancy, but it was not significantly increased compared to C57. As previously shown, *C3* mRNA was increased in e7.5 implantation sites of BPH/5 mice compared to C57 gestation matched counterparts. The early pregnancy time point, e7.5, represents peak decidualization in mice, an important event for successful pregnancy. Dysregulation during this important time could contribute to placental injury observed during PE. However, reducing reproWAT via pair feeding attenuated the upregulation of *C3* mRNA at the maternal- fetal interface. This indicates that a reduction in adiposity may have an anti-inflammatory effect on the environment at the maternal-fetal interface in this model and potentially obese women.

In summary, this data suggests that maternal obesity during early pregnancy may contribute to inflammatory dysregulation at the maternal fetal interface thereby playing a role in PE. Pair feeding was utilized to decrease adiposity which subsequently attenuated the observed complement dysregulation. These findings are significant because they describe a possible biochemical marker that could identify PE patients early in pregnancy. This study also demonstrates a potential intervention by reduction of adiposity to improve adverse pregnancy outcomes. This does come with limitations, because calorie restriction is a very general intervention and affects the entire body systemically, thus it is difficult to elucidate the exact effects. In addition, this study was done in a mouse model which gives us insight into the mechanisms behind PE. However, it is necessary to be extremely careful when extrapolating interventions from animal models to humans[23]. Further studies are needed to understand the effect of maternal adiposity reduction via calorie restriction on pregnancy disorders and adverse pregnancy outcomes. This data gives us an avenue of study to pursue in order to further elucidate the mechanism behind PE and potential interventions for the disorder.

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Appendix:

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PERSPECTIVES | *Genomics of Metabolic and Tumor/Cancer Traits*

Obesity "complements" preeclampsia

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Olson KN, Redman LM, Sones JL. Obesity "complements" preeclampsia. *Physiol Genomics* 51: 73–76, 2019. First published February 4, 2019; doi:10.1152/physiolgenomics.00102.2018.—Preeclampsia (PE) is a devastating adverse outcome of pregnancy. Characterized by maternal hypertension, PE, when left untreated, can result in death of both mother and baby. The cause of PE remains unknown, and there is no way to predict which women will develop PE during pregnancy. The only known treatment is delivery of both the fetus and placenta; therefore, an abnormal placenta is thought to play a causal role. Women with obesity before pregnancy have an increased chance of developing PE. Increased adiposity results in a heightened state of systemic inflammation that can influence placental development. Adipose tissue is a rich source of proinflammatory cytokines and complement proteins, which have been implicated in the pathogenesis of PE by promoting the expression of antiangiogenic factors in the mother. Because an aggravated inflammatory response, angiogenic imbalance, and abnormal placentation are observed in PE, we hypothesize that maternal obesity and complement proteins derived from adipose tissue play an important role in the development of PE.

complement; immune; obesity; preeclampsia; pregnancy

INTRODUCTION

Preeclampsia (PE) is a devastating disorder of pregnancy and leading cause of maternal and fetal morbidity and mortality, affecting 2–8% of pregnancies worldwide (15). It is characterized by late gestational hypertension (systolic $\geq$ 140 mmHg or diastolic $\geq$ 90 mmHg) combined with another sign/symptom such as proteinuria, renal insufficiency, or thrombocytopenia, among others (20). PE predisposes women to cardiovascular complications and exerts direct consequences on the offspring, including intrauterine growth restriction and cardiometabolic disease later in life (20). The etiology of PE remains unknown. However, because the only known cure is delivery of both the fetus and placenta, it is widely accepted that abnormal placentation plays a causal role in PE. A number of preexisting maternal conditions are risk factors for PE, including obesity. This review highlights one proposed mechanism whereby adipose tissue, rich in inflammatory mediators and complement proteins, contributes to altered placental angiogenesis and development in PE.

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PREGNANCY, PREECLAMPSIA, AND ANGIOGENESIS

Normal placental development is essential for pregnancy success. First, embryo implantation is followed by trophoblast cell proliferation and differentiation before invasion and remodeling of maternal spiral arteries (27). To support the developing placenta, the fetoplacental unit then produces angiogenic factors including vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) (19). Once the placenta is functional, soluble fms-like tyrosine kinase-1 (sFlt-1), an antiangiogenic factor and a VEGF antagonist, is produced to modulate VEGF activity (13, 21). A delicate balance of pro- and antiangiogenic factors is necessary in a healthy pregnancy. Furthermore, tight regulation of the maternal immune system, including complement system activation at the maternal-fetal interface, is needed to establish and maintain pregnancy (3). Inadequate trophoblast remodeling of spiral arteries, a key feature of PE, is believed to result from perturbations in the maternal immune response and placental angiogenesis (16). The exact mechanisms causing impaired placentation in PE have yet to be elucidated, but several risk factors such as chronic hypertension, gestational diabetes mellitus, and obesity have been described (1).

OBESITY AND PREECLAMPSIA

Increasing body mass index (BMI) is positively associated with an increased risk of PE (2, 35). Women with a prepregnancy BMI of 35 kg/m^2 or above have a 30% increased risk of developing PE (38). This relationship is confirmed by evidence illustrating that bariatric surgery done to decrease obesity can reduce the incidence of PE (8). The association between obesity and PE is concerning considering that obesity is rapidly rising among women throughout much of the world, especially in North America and Europe (37). Obesity is characterized by expansion of adipose tissue (fat) in the body. Furthermore, maternal obesity along with circulating factors, such as nonesterified fatty acids, may contribute to excess lipid accumulation in the placenta (14, 36). This can interfere with placental development, including trophoblast invasion and angiogenesis as well as nutrient transport between mother and fetus, resulting in increased oxidative stress and inflammation at the maternal-fetal interface (36). These placental injuries often characterize PE pregnancies. The localization of many proinflammatory factors, including tumor necrosis factor (TNF)-α and interleukin (IL)-6, to adipose tissue led to the understanding that obesity presents a state of low-grade systemic inflammation (7). The connection between maternal obesity and PE is hypothesized to involve immune cells within the mother's

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adipose tissue and in the placenta contributing to impaired placentation (11).

COMPLEMENT ACTIVATION AND OBESITY IN PREECLAMPSIA

The complement system has been implicated as a link between excess adipose tissue and PE (4, 32). Complement components increase with obesity and are positively correlated with BMI as well as subcutaneous, visceral, and total fat area, while decreasing with weight loss (30). The complement system consists of over 50 well-regulated proteins generated primarily by the liver, but also by other organs and tissues. The complement system can therefore be activated systemically in circulation or locally within specific tissue environments, i.e., adipose tissue (4, 22, 32). Previously, complement activity was thought to primarily exist in extracellular compartments. Current research, however, indicates that the complement system is also active intracellularly. This novel intracellular complement system has been termed the "complosome" (12, 41). It is hypothesized that complement proteins are responsible for protecting the body from pathogens. More recently, it has been shown that complement proteins are involved in many diverse physiological processes such as clearance of apoptotic cells and cellular debris, and regulating humoral and innate immune responses (39). The complement cascade is activated through three primary pathways described as alternative, lectin, and classical pathways. Although each pathway contains distinct proteins that respond to different stimuli and activate the complement cascade, all pathways eventually converge at complement component 3 (C3). C3 produces effector molecules including the membrane attack complex and anaphylatoxins C3a and complement fragment 5 (C5)a. These fragments act via G protein-coupled receptors C3a receptor (C3aR) and C5a receptor (C5aR), respectively (39).

A tightly regulated immune system is essential to placentation because the embryo presents with both maternal (self) and paternal (nonself) antigens (29). The complement system is no exception to this tight regulation; it must be downregulated to allow adequate placentation, yet simultaneously activated to protect the mother and fetus from foreign pathogens (10). Serum concentrations of C3 have been shown to be upregulated in pregnancies affected by PE, while C4 is reduced (17). Furthermore, single nuclear polymorphisms in the *C3* gene of women may be predictive for determining PE risk (23). The source of the complement dysregulation is under current investigation. Concentrations of complement components, specifically adipsin (Complement Factor D) in serum or urine detected early in pregnancy have been suggested to identify patients susceptible for developing PE (24, 25, 40). Adipsin is primarily produced by adipocytes (30). Women with increased serum concentrations of complement fragments Bb and C3a combined with obesity were most likely to develop PE compared with those with either only obesity or higher levels of circulating Bb and C3a, or neither (24, 25). This observation highlighted the potential interaction between adiposity and the complement system in the pathogenesis of PE with excess adipose tissue being a source of the increased complement proteins noted in pregnancies affected by PE. Interestingly, C3aR mRNA is highly expressed in adipose tissue of C57BL/6 mice, and this expression has been shown to significantly increase when mice are put on a high-fat diet (28). This illustrates a role for the complement system in adipose tissue signaling during diet induced obesity. Interestingly, Kestlerová et al. (17) reported 42% of the women with PE were obese with a BMI >30. While these findings provide strong evidence for obesity-mediated complement activation in PE, further studies are needed to elucidate the precise mechanism.

COMPLEMENT AND ANGIOGENESIS

Visceral white adipose tissue of the intra-abdominal depot (the central fat compartment) may contribute to fat accumulation at the placenta and could be the source of increased complement in PE pregnancies (5, 36). Complement dysregulation has been hypothesized to influence the development of PE via interactions with angiogenic factors localized at the maternal-fetal interface (11). An angiogenic imbalance both in the placenta and in maternal circulation is a hallmark of PE (6, 33). In healthy pregnancies, an increase in antiangiogenic factors is observed from ~33 wk onward (21). Whereas in PE, there is an increase in circulating sFlt-1 with a corresponding decrease in the proangiogenic factor PlGF beginning at 20 wk (21). sFlt-1 binds free VEGF and PlGF, rendering them inactive, thus producing an apparent systemic angiogenic imbalance in the PE mother early in pregnancy (21, 31). This phenomenon may be influenced by the complement system.

Compared with healthy pregnant women, increased localization of the complement component C5a was evident at the maternal-fetal interface in women with PE (26). Moreover, C5a increased the expression of antiangiogenic factors including TNF-α and sFlt-1 and, in tandem, decreased expression of

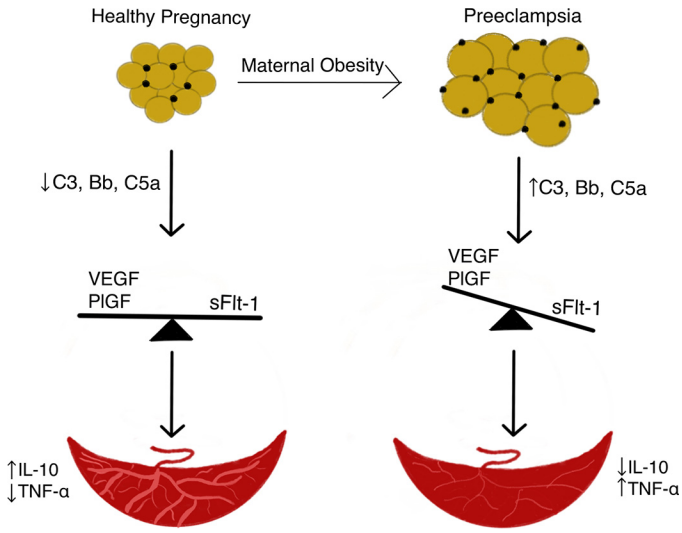

Fig. 1. Working hypothesis linking maternal obesity and preeclampsia. We hypothesize that excess maternal adipose tissue proximal to the reproductive tract is the source of increased complement components and fragments (C3, Bb, C5a) observed in preeclamptic pregnancies. These complement proteins, found in maternal circulation and placenta, may promote increased production of antiangiogenic factors including soluble fms-like tyrosine kinase (sFlt-1), which in turn, decreases proangiogenic factors vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), creating an angiogenic imbalance. This imbalance results in placental injury, leading to decreased blood flow to the placenta concomitant with alterations in placental cytokines, interleukin (IL)-10 and tumor necrosis factor (TNF)-α, before the presentation of preeclampsia. Yellow circles represent adipocytes. Black circles represent adipose tissue immune cells.

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proangiogenic PlGF and anti-inflammatory IL-10 in trophoblast cells, all of which have been implicated as key players in PE. In this study, C5a was predominantly localized in macrophages, while the C5aR was found on trophoblasts (26). These data suggest that macrophage infiltration promotes complement factor C5a binding to its receptor on trophoblast cells, thus restricting adequate trophoblast invasion and angiogenesis, thereby contributing to the etiology of PE. In support of this mechanism, in a murine model of hypoxia-induced retinal vascularization, it was shown that C5a will polarize macrophages to produce the antiangiogenic factors sFlt-1 and TNF-α (18). The relationship between C5a and angiogenic dysregulation was further confirmed by Girardi and colleagues (11), who demonstrated that C5a could induce monocytes to produce increased sFlt-1, thus decreasing the VEGF required for placental angiogenesis. Furthermore, inhibition of C3 activation early in pregnancy improved outcomes in a mouse model of spontaneous PE, including fetal loss and growth restriction, as well as increasing placental weight and restoration of spiral artery remodeling (9). The maternal hypertensive phenotype was not assessed with complement inhibition in this study; however, this outcome is important in understanding the relationship between complement inhibition and PE. The therapeutic potential of complement inhibition is being investigated in a number of animal models of adverse pregnancy outcomes and holds promise for treating women with PE (34).

Maternal obesity in pregnancy is characterized by increased complement activation, which is hypothesized to mediate an angiogenic imbalance at the maternal-fetal interface, accompanying an increased development of PE (Fig. 1). It is critical to understand the source of complement dysregulation as well as the mechanism by which increased complement fragments and components contribute to the placental injury characteristic of PE pregnancies. Future studies should investigate whether visceral adipose tissue proximal to the female reproductive tract could be a source of complement components infiltrating into the maternal-fetal interface, and whether decreasing the maternal adipose tissue before or during pregnancy could prevent PE in women with obesity.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.N.O. and J.L.S. performed experiments; K.N.O. and J.L.S. analyzed data; K.N.O., L.M.R., and J.L.S. interpreted results of experiments; K.N.O. prepared figures; K.N.O. drafted manuscript; K.N.O., L.M.R., and J.L.S. edited and revised manuscript; K.N.O., L.M.R., and J.L.S. approved final version of manuscript.

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