2021

Characterization of Spontaneous Preeclampsia in the African Green Monkey (*Chlorocebus aethiops sabaeus*)

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Digital Object Identifier: https://doi.org/10.13023/etd.2021.156

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CHARACTERIZATION OF SPONTANEOUS PREECLAMPSIA IN THE AFRICAN GREEN MONKEY (*CHLOROCEBUS AETHIOPS SABAEUS*)

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences
at the University of Kentucky

By
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2021

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ABSTRACT OF DISSERTATION

CHARACTERIZATION OF SPONTANEOUS
PREECLAMPSIA IN THE AFRICAN GREEN MONKEY
(*CHLOROCEBUS AETHIOPS SABAEUS*)

Hypertensive pregnancy disorders are a major contribution to maternal and neonatal mortality worldwide. Two of these disorders, preeclampsia and chronic hypertension in pregnancy, affect up to 10% of all pregnancies. These hypertensive disorders of pregnancy are associated with long-term, postnatal risk factors for both mother and offspring. Despite numerous recent advances in preeclampsia research, the underlying mechanisms are still not understood. This could be due to lack of a spontaneous animal model. This dissertation presents the African Green Monkey (AGM; *Chlorocebus aethiops sabaeus*) as the first known spontaneous animal model of preeclampsia and a highly translational model of chronic hypertension in pregnancy. The AGM diverged from the human lineage approximately 29 million years ago and shares close genetic homology to humans. Thus, the AGM is similar to humans regarding gene structure, upright posture, circadian rhythm, organ physiology, and complex familial systems. Chapter 2 characterizes preeclamptic (PE) and normotensive (NT) pregnancies in the AGM, showing that PE animals have higher systolic and diastolic arterial pressure concomitant with elevated proteinuria compared to NT animals. PE animals also had a more rapid first trimester weight gain associated with reduced plasma osmolality. Offspring born to PE mothers have lower birth weight and a higher rate of stillbirth as compared to NT pregnancies. Because low birth weight is associated with increased disease susceptibility in later life, chapter 3 characterizes blood pressure, metabolic function, and renal sufficiency in offspring from NT, PE, and chronic hypertensive (CHT) pregnancies. Overall, this chapter shows that offspring born to PE, but not CHT, pregnancies have reduced glucose tolerance, proteinuria, and kaliuresis with no effect on systolic arterial pressure in early adolescence compared to those from NT pregnancies. Together, these data support the AGM as a novel and highly translational animal model for studying the etiology of preeclampsia and transgenerational disease transmission.

KEYWORDS: Preeclampsia, hypertension, nonhuman primates

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April 15, 2021

Date

CHARACTERIZATION OF SPONTANEOUS PREECLAMPSIA IN THE AFRICAN GREEN MONKEY (*CHLOROCEBUS AETHIOPS SABAEUS*)

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DEDICATION

To Braden--the driving force for this degree. Every time I wanted to quit, I thought of you and it kept me going. I love you more than anything. Work hard, dream big, and choose happiness.

To my sweet niblings: Adrian, Jacob, Eli, Cebastian, and Caleb. This is also for you. There is nothing you cannot do with me by your side.

In loving memory of Elijah Etheridge and Cayce Castelow. I wish you could see this.

ACKNOWLEDGMENTS

Though this dissertation was an individual work, it greatly benefited from the support, insight, and direction of several people. First, my Dissertation Committee Chair, Dr. Jeffrey Osborn, who took a chance on a single mom. Not only have you taught me everything I know about physiology, but you made me feel like I could succeed *every single day.* Your understanding and support made all this possible. I also want to thank my Dissertation Committee: Dr. Julie Pendergast, Dr. Frederique Yiannikouris, Dr. Bruce O'Hara, and external examiner, Dr. Jennifer White. Each of you provided guidance, insight, and support that substantially improved my work and future career. I also want to thank Dr. Jessica Taylor. You were an unexpected mentor that I cannot imagine doing any of this without. I'll remember your support for the rest of my career and hope to pay it forward as I go. Finally, I want to thank Dr. Pete Mirabito. You taught me so much about education and compassion in teaching. The opportunities you gave me and guidance you offered has helped me grow as an educator. I'll be forever grateful.

This dissertation would not exist without the support of my friends, family, and labmates. My son who had to endure life with a single mom in graduate school has shown patience and understanding beyond his years. My labmates, past and current, have helped me grow every step of the way. Megan, I don't know how I would have ever survived the first years of graduate school without your constant support. Brandon, Shea, Maria, and Patrick: you made graduate school a community rather than a competition. You taught me so much and were always there to offer support. I'll never forget that. And Dave. Words would never be enough to thank you. You quite literally are my foundation and biggest cheerleader. When I didn't think I could do this, you reminded me I could.

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CHAPTER 1: PREECLAMPSIA AND THE AFRICAN GREEN MONKEY

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1.1 Introduction

Hypertensive disorders affect 10% of pregnancies worldwide and include gestational hypertension, preeclampsia-eclampsia, chronic hypertension, and chronic hypertension with superimposed preeclampsia. This chapter aims to review current knowledge on preeclampsia in pregnancy and its effects on offspring. It's important to first begin with blood pressure control mechanisms in healthy, nonpregnant persons.

1.1.1 Blood pressure control mechanisms

Numerous systems participate in a coordinated effort to maintain blood pressure within an acceptable range. Blood pressure is regulated short-term through the autonomic nervous system and long-term regulation includes, but is not limited to, the renin-angiotensin-aldosterone system, pressure natriuresis, and molecules such as natriuretic peptides.

Short-term control of blood pressure through the autonomic nervous system involves high-pressure baroreceptors. These mechanoreceptors, located in the aortic arch and the carotid sinus, sense changes in blood pressure via arterial wall stretching and send feedback to the autonomic nervous system (Thrasher 2005). Increases in blood pressure beyond the homeostatic range will elicit a response through the parasympathetic nervous system. The response involves stimulation of the vagus nerve and decreasing arterial sympathetic tone (Thrasher 2005). This leads to reduced heart rate, cardiac contractility,

and peripheral resistance, ultimately reducing blood pressure. Decreases in blood pressure are sensed by the baroreceptors which then elicit a response from the sympathetic nervous system. The corresponding response includes an increase in heart rate and cardiac contractility which ultimately increases blood pressure (Thrasher 2005). However, baroreceptors are involved mostly in acute regulation of blood pressure, and there are other responses that will control blood pressure over the long-term, primarily through regulation of blood volume.

Long-term blood pressure control involves multiple physiological mechanisms including the renin-angiotensin-aldosterone system. Renin is released from the granular cells of the juxtaglomerular apparatus in the kidney in response to reduced sodium delivery to the distal nephron, decreased blood flow to the kidney, or sympathetic stimulation (Poulsen and Fenton 2019). Angiotensinogen, released from the liver, is the substrate for renin, which cleaves angiotensinogen into angiotensin I. Angiotensin I is released into the circulation and this peptide is cleaved into angiotensin II by angiotensin converting enzyme in the lung (Brown 2007). Angiotensin II acts through two types of receptor: angiotensin type 1 receptors (AT1R) and angiotensin type II receptors (AT2R).

When bound by angiotensin II, AT1Rs, located in the vascular smooth muscle, the kidney, and the adrenal cortex, elicit several responses that ultimately increase blood pressure. These responses include vasoconstriction, hypertrophy of smooth muscle and cardiomyocytes, stimulation of the sympathetic nervous system, endothelin secretion, sodium retention, and water reabsorption (Zaman, Oparil, and Calhoun 2002). AT2Rs antagonize the effects of AT1R signaling, resulting in vasodilation. Angiotensin II also promotes the release of aldosterone from the adrenal cortex. Aldosterone then facilitates

the reabsorption of sodium in the distal nephron, which is followed by reabsorption of water. The net result is increased blood volume and thus pressure.

Another mechanism by which the body controls blood pressure long-term is through anti-diuretic hormone (ADH). ADH is produced at the hypothalamus and is released from the posterior pituitary in response to increased thirst or elevated plasma osmolality. If plasma osmolality increases above a certain threshold, ADH is released, and it acts on the kidney to insert aquaporin channels into the luminal membrane of the collecting duct. These aquaporin channels facilitate the reabsorption of water in the collecting tubule, ultimately leading to increased blood volume and pressure. ADH also acts on the thick ascending limb of the loop of Henle to increase sodium reabsorption, which leads to increased water retention. Ultimately, these actions lead to increased blood volume and pressure, along with decreased plasma osmolality.

Various other molecules participate in blood pressure regulation. This includes atrial natriuretic peptide (ANP). This peptide is released from cardiomyocytes in response to atrial stretch due to elevated blood pressure. ANP, as its name implies, promotes sodium excretion, or natriuresis. ANP causes renal afferent arteriole dilation which results in elevated glomerular filtration. ANP also inhibits sodium reabsorption in the nephron, further promoting natriuresis. Prostaglandins also affect blood pressure. These molecules promote vasodilation, increasing glomerular filtration rate and promoting natriuresis (Cannone et al. 2019). Prostaglandins also counter the vasoconstrictor effects of angiotensin II and the sympathetic nervous system.

1.1.2 Preeclampsia: Overview, prevalence, and risk factors

Preeclampsia is defined as new-onset maternal hypertension in the 2nd or 3rd trimester that occurs with signs of end-organ dysfunction. This damage can occur in the form of proteinuria, thrombocytopenia, elevated serum creatinine, increased liver enzymes, pulmonary edema, or neurological symptoms such as new-onset headache or impaired vision (Phipps et al. 2016). While preeclampsia affects up to 8% of pregnancies worldwide, there is a global income disparity to its progression. In Latin America and the Caribbean, pregnant individuals are seven times more likely to develop preeclampsia as compared to higher income nations (Giachini et al. 2017). The rate of maternal and perinatal death due to preeclampsia is higher in lesser-developed countries. According to the World Health Organization, 94% of all maternal deaths occur in the developing world and a significant portion of those deaths are due to hypertensive disorders in pregnancy. However, this problem is not unique to developing nations.

Despite being a high-income country, the United States has the highest rate of maternal death among all developed nations (Creanga et al. 2014). In fact, the rate of preeclampsia diagnosis in the United States has grown rapidly, increasing 26.6% from 2000 to 2014 (MacDorman et al. 2016). In the United States, the rate of maternal death directly attributed to preeclampsia and eclampsia, a severe complication of preeclampsia resulting in seizures, is 9% (American College of Obstetricians and Gynecologists 2013). There are also racial disparities in the development of and death due to preeclampsia, with the Black population being at highest risk (Ross et al. 2019). This may be due to Black patients being disproportionately affected by preeclampsia risk factors, inequalities to

prenatal and postpartum healthcare access, systemic racism in medicine, or a combination of these factors (Bryant et al. 2010; Petersen EE 2019; Tanaka et al. 2007). Ultimately, in the United States, Black patients are three times more likely to die of preeclampsia as compared to white patients (Tucker et al. 2007). While all pregnant people are at risk for developing preeclampsia, there are certain conditions that increase a patient's risk.

Previous history of preeclampsia or eclampsia leads to an increased risk for developing preeclampsia in subsequent pregnancies. Advanced maternal age, multiple gestations, and nulliparity also contribute to an increased risk for developing preeclampsia (Fox et al. 2019). Income and education levels are more predictors of this pathology, with low socioeconomic status being associated with higher risk of preeclampsia (Lindsay M. Silvaa 2008). In fact, higher socioeconomic status attenuates this risk in white, but not Black, patients (Ross et al. 2019). Finally, numerous comorbidities will increase a person's risk of developing preeclampsia or eclampsia, including obesity, type 1 and 2 diabetes mellitus, renal disease, chronic hypertension, and autoimmune disorders (Fox et al. 2019).

1.1.3 Economic burden, morbidities, and mortalities

Medical expenses related to preeclampsia are a major financial burden. A 2019 healthcare cost analysis showed that preeclampsia was associated with a threefold increase in maternal and infant-related healthcare costs as compared to uncomplicated pregnancies (Hao et al. 2019). Preeclampsia is even more a financial burden than chronic hypertension in pregnancy, averaging nearly twice the cost. Some of this is related to the acute

complications associated with preeclampsia. The associated morbidities include, but are not limited to, stroke, placental abruption, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), liver hemorrhage, renal failure, pulmonary edema, and neurological deficits (Nankali et al. 2013). In fact, patients with preeclampsia are more likely to require a cesarean delivery, a risky and expensive surgical procedure (Hao et al. 2019). However, most of the elevated medical expenses associated with preeclampsia are attributed to infant care.

Preeclamptic patients deliver on average 3 weeks earlier than patients with uncomplicated pregnancies and 2 weeks earlier than patients with chronic hypertension (Hao et al. 2019). Preterm delivery following preeclampsia is associated with several infant complications requiring hospitalization. Subsequently, preeclampsia leads to eight times the perinatal cost as those from uncomplicated pregnancies and twice as high as those attributed to infant costs from chronic hypertensive pregnancies (Hao et al. 2019).

While preeclampsia leads to a high economic burden, the largest price of this syndrome is the maternal and perinatal death toll. Over 70,000 women and 500,000 infants die each year from preeclampsia. This equates to one death per minute, nearly the rate of death to heart attacks. Preeclampsia accounts for 10-15% of all maternal deaths worldwide, with most of these deaths occurring in low-income countries (Duley 2009). Most of the deaths secondary to preeclampsia are due to cerebral complications leading to intracerebral hemorrhaging (Iihara and Nishimura 2015). This type of hemorrhage is more common when the pregnant patient develops eclampsia, a rare but severe neurological complication of preeclampsia. Nearly 1% of preeclampsia patients progress to eclampsia (Sutton,

Harper, and Tita 2018). This occurs when preeclamptic individuals develop seizures which are sometimes followed by a coma and may threaten the life of both mother and fetus.

1.1.4 Screening, prevention, and therapeutics

The recommendation from the US Preventive Services Task Force (USPSTF) is to obtain blood pressure recordings throughout a person's pregnancy. Consistent screening can lead to early detection of preeclampsia which is critical for maternal and fetal outcomes. While the USPSTF reports that early detection and treatment of preeclampsia can significantly improve outcomes by reducing maternal and perinatal mortality, they report limited evidence that risk prediction tools such as serum markers and clinical indicators can be effective (USPSTF 2017). Therefore, blood pressure measurements are routinely used to screen for preeclampsia and sphygmomanometry is the suggested method. Sphygmomanometry, the standard blood pressure measurement approach in routine checkups, uses a blood pressure cuff, stethoscope, and manometer to indirectly measure blood pressure through Korotkoff sounds. It is recommended to obtain blood pressure recordings at every prenatal appointment and if blood pressure is elevated, repeat recordings should be performed. However, in many countries where preeclampsia is more prevalent and more deadly, prenatal care is not accessible. Therefore, affordable and accessible screening methods are needed, and prevention is critical.

The main prophylactics in the prevention of preeclampsia are antiplatelet agents such as low-dose aspirin, vitamin supplements including calcium and vitamin D, as well

as lifestyle modification. Because preeclampsia is associated with activation of clotting and subsequently elevated platelet counts, antiplatelet therapy can prevent this response before it occurs. A 2007 meta-analysis showed that administration of low-dose aspirin consistently reduced not only the risk of developing preeclampsia but also the risk of preterm delivery and adverse pregnancy outcomes (Askie et al. 2007). This study also showed that antiplatelet therapy is a safe method of preeclampsia prevention, as there was no risk of maternal postpartum hemorrhage, infant hemorrhage, or infant congenital defects.

Vitamin supplementation has also been shown effective in preventing preeclampsia. There is an established inverse relationship between calcium intake and blood pressure (Khaing et al. 2017). Therefore, it has been recommended that patients from countries with low calcium intake and high-risk groups should begin a calcium supplementation regimen to lower the risk of preeclampsia. A 2017 meta-analysis showed that calcium supplementation was associated with a 50% reduction in preeclampsia risk, particularly in high-risk groups (Khaing et al. 2017). Vitamin D has also been shown effective in prevention of preeclampsia as low vitamin D intake has previously been associated with its pathogenesis (Nema, Sundrani, and Joshi 2019; Mirzakhani et al. 2016). In a randomized study, pregnant patients with sufficient plasma vitamin D levels in early and late pregnancy had a lower incidence of preeclampsia compared with those who did not have sufficient circulating vitamin D concentrations (Khaing et al. 2017). This study also identified genes associated with vitamin D that affect immunological and inflammatory responses related to the pathogenesis of preeclampsia. Therefore, vitamin D

supplementation when necessary may prevent preeclampsia, particularly in patients at high risk for the syndrome.

The only known cure for preeclampsia is delivery of the fetus and placenta, which often occurs preterm. However, many cases of preeclampsia are early onset and delivery may not be the best option for the fetus. Fortunately, there are some therapeutics available, though they largely treat the symptoms rather than the pathophysiology of preeclampsia. The primary treatment for preeclampsia is antihypertensive therapy to prevent severe vascular damage that could lead to renal failure or stroke (Webster et al. 2017). However, the American College of Obstetrics and Gynecologists (ACOG) states that antihypertensives are not recommended for pregnant women with systolic blood pressure less than 160 mmHg or diastolic blood pressure less than 110 mmHg; this is because the risks of antihypertensive treatment do not yet outweigh the benefits until the hypertension becomes severe (≥ 160 mmHg), Once blood pressure exceeds these values, treatment with antihypertensives such as Labetalol or Hydralazine is strongly recommended (Phipps et al. 2016). Other treatments include magnesium sulfate to prevent or treat seizures associated with eclampsia (Vigil-De Gracia and Ludmir 2015). Ultimately, the treatments used in preeclampsia manage the symptoms until a healthy delivery is possible.

1.2 Hemodynamics, angiogenesis, and placentation in normal and preeclamptic pregnancies

1.2.1 Overview of physiological hemodynamics, angiogenesis, and placentation in pregnancy

In a healthy pregnancy, various physiological adaptations take place to support a growing fetus. The cardiovascular system undergoes a profound redistribution of blood flow in pregnancy with cardiac output increasing 30-50% (Tkachenko 2014). This occurs via increases in both stroke volume and heart rate. Stroke volume increases 40% (Chang and Streitman 2012) and heart rate rises 15-20 beats per minute compared to prepregnancy (Sanghavi and Rutherford 2014).

Both plasma volume and red blood cell production increase during gestation, leading to a 40% elevation in total blood volume (Tkachenko 2014). Much of this increased blood volume is attributed to the renin-angiotensin-aldosterone system. As systemic vascular resistance declines in pregnancy, the renin-angiotensin-aldosterone system (RAAS) is activated and contributes to the increased blood volume to support a growing fetus (Tkachenko 2014). Consequently, circulating levels of renin, aldosterone, and angiotensin II are elevated in early pregnancy. Renin is also released from extrarenal sources such as the ovaries and the decidua (Hsueh 1988). Estrogen, produced and released by the placenta, increases angiotensinogen production by the liver which ultimately leads to increases in circulating angiotensin II. Activation of RAAS leads to water and sodium retention, which ultimately increases total plasma volume. However, while total body

volume and total body sodium increase, the plasma sodium concentrations are not elevated in healthy pregnancies. This is because plasma volume expansion is so great due to resets in the thirst threshold (Davison 1995) and because progesterone, which antagonizes aldosterone receptors, is also elevated in pregnancy (Quinkler M 2002). Hormones such as progesterone and relaxin also contribute to decreased renal vascular resistance leading to a 50-80% increase in renal blood flow and glomerular filtration rate (GFR) compared to prepregnancy (Dunlop 1981). Therefore, distal delivery of sodium in the kidney increases, further decreasing the effect of aldosterone. Some sodium is still retained, however, likely due to elevations in angiotensin II and subsequent upregulation of epithelial sodium channels (ENaC) in the distal nephron.

Pregnant individuals also experience a decreased sensitivity to the vasopressor effect of angiotensin II. To achieve similar vasomotor responses, pregnant individuals require twice the level of angiotensin II as nonpregnant individuals (Verdonk et al. 2014) . It has been previously proposed that this decreased sensitivity may be due to the increased circulating progesterone and prostacyclin characteristic of healthy pregnancies (Irani and Xia 2011).

Hypoosmolality, particularly in the 1st trimester, is another characteristic of an uncomplicated pregnancy (Davison 1995). Free water clearance, the amount of solute-free water that is cleared from the plasma and excreted, remains negative throughout pregnancy. This means that water is being retained rather than excreted in the urine. Vasopressin, or antidiuretic hormone, plays a key role in plasma volume expansion in pregnancy, contributing to the water retention and hypoosmolality. It has been previously suggested

that osmoregulation in pregnancy is associated with a reset of the steady state to a lower osmolality (Davison 1984).

Despite the elevated cardiac output and blood volume in pregnancy, blood pressure normally does not change, and sometimes even decreases (Salles et al. 2015). This is because elevated cardiac output and blood volume are matched by a critical decline in total peripheral resistance. This reduction disproportionately increases flow to the uterine circulation. Consequently, uteroplacental perfusion constitutes 20-25% of maternal cardiac output during pregnancy, a nearly 50-fold increase compared to prepregnancy (Metcalfe 1974).

The increased cardiac output and subsequent elevation in blood volume in pregnancy is balanced by a reduction in vascular resistance during the 1st trimester, which is sustained throughout gestation. There are several factors that regulate this decreased vascular resistance including uterine decidualization and placentation. Vascular tissues undergo profound changes to support the needs of the developing fetus beginning at embryo implantation in the decidua. Cytotrophoblasts first proliferate from the blastocyst and then differentiate into an invasive phenotype known as syncytiotrophoblasts (Turco and Moffett 2019). As these syncytiotrophoblasts invade the endometrium, they induce spiral artery remodeling to allow greater maternal blood flow. Ultimately in a hemochorial placenta, such as those in humans and other primates such as the African Green Monkey, a maternal-fetal interface is formed where fetal chorion has contact with maternal blood (Turco and Moffett 2019). This placental vascular remodeling causes blood flow to the placenta to slow, facilitating gas and nutrient exchange between mother and fetus. The uterine vasculature also undergoes remodeling and sustained vasodilation that further

decreases systemic vascular resistance. This uterine decidualization, whereby the endometrium undergoes transformation into a specialized tissue referred to in pregnancy as the decidua, helps promote placental formation by mediating the invasion of the differentiated trophoblast cells (Okada 2018).

In early pregnancy, pro-angiogenic factors and pregnancy hormones such as human chorionic gonadotropin, estradiol, and progesterone contribute to angiogenesis to reduce vascular resistance (Henry 2006). The uterine vasculature lengthens and undergoes outward hypertrophy. The combination of outward hypertrophy, vessel lengthening, and angiogenesis results in a net increase in vessel diameter and subsequently decreased vascular resistance. While the early stages of pregnancy rely on both angiogenesis and vasodilation for adequate uteroplacental flow, the latter half of pregnancy relies mostly on vasodilation. Indeed, vasodilator production must increase throughout the systemic circulation to support a healthy pregnancy. Ultimately, there are a number of regulatory and interactive factors that play a role in the increased vasodilatory state of pregnancy including shear stress, nitric oxide (NO), prostacyclin, sex steroids, and inflammatory hormones (Boeldt 2017).

Because the fetus and placenta are allogeneic tissues, the immune system plays a critical role in placentation. Natural killer cells in the uterus (uNK) make up the majority of inflammatory molecules at the maternal-fetal interface (Moffett 2015). These uNK cells are critical for trophoblast invasion in the uterus by aiding in spiral artery remodeling. uNK cells also secrete angiogenic factors such as vascular endothelial growth factor (VEGF), important for early pregnancy angiogenesis that assists in decreasing peripheral resistance to allow for the elevated blood volume in pregnancy (Lash et al. 2006). Decidual

macrophages are also critical for placentation as they clear apoptotic debris and facilitate invasion of trophoblasts (Schatz et al. 2016). Uterine dendritic cells infiltrate the decidua in the first trimester, accumulating around the trophoblast cells to regulate implantation (Mor et al. 2011). Finally, macrophages, which have angiogenic functions, play a significant role in uterine vascular remodeling and maintaining immunological homeostasis throughout pregnancy as they are abundant in the decidua through the third term (Lash et al. 2016).

1.2.2 Overview of hemodynamics in preeclampsia

While the ultimate etiology of preeclampsia is to be determined, general consensus is that the syndrome has 2 stages: the first begins at implantation with poor placentation; the second is the clinical manifestation resulting in maternal hypertension, an elevated inflammatory response, and endothelial dysfunction. A physiological pregnancy relies heavily on a drop in vascular resistance to support the growing fetus but preeclampsia is characterized by insufficient reduction in vascular resistance, typically in the latter half of pregnancy (Bowyer 2003). Preeclamptic patients experience an increased sensitivity to vasoconstrictors such as angiotensin II. Widespread vasoconstriction in preeclampsia leads to elevated systemic resistance and lower cardiac output. There is also an impairment in vasodilatory capacity associated with preeclampsia, further contributing to the elevated systemic vascular resistance (Lampinen et al. 2006). This reduced vasodilatory capacity comes in the form of reduced NO availability, decreased prostacyclin production, and

increased vascular oxidative stress (Goulopoulou 2017). Indeed, patients with preeclampsia have decreased flow-mediated vasodilation even before their diagnosis (Kublickiene et al. 2000).

Plasma osmolality, which normally decreases approximately 10 mOsm/kg in healthy pregnancies (Davison 1995), decreases further in preeclamptic pregnancies (Santillan et al. 2017). Exaggerated decreases in plasma osmolality are likely due to even more elevated secretion of vasopressin during preeclamptic pregnancies as compared to healthy pregnancies (Santillan et al. 2014).

Preeclampsia is heterogeneous, presenting in two different ways: early onset and late onset. Early onset preeclampsia, associated with greater placental and angiogenic abnormalities, is the more severe form. Due to the longer duration of hypertension during gestation, early-onset preeclampsia is also associated with a greater risk of fetal growth restriction and stillbirth. Late-onset preeclampsia is less severe, presenting with milder symptoms at term, but still poses major risks to mother and fetus. The next section will cover many of the specific abnormalities associated with the pathogenesis of preeclampsia.

1.3 Factors contributing to the pathogenesis of preeclampsia

1.3.1 Placental dysfunction and uterine abnormalities in preeclampsia

Preeclampsia is characterized in part by improper placentation. The cytotrophoblasts, critical for triggering remodeling of spiral arteries in normal pregnancy, fail to develop an invasive phenotype in preeclampsia. Consequently, the cytotrophoblasts do not penetrate as deeply into the myometrium in placentas destined for complication by preeclampsia (Fisher 2015). This shallow placentation leads to incomplete remodeling of the spiral arteries, which causes narrower maternal vasculature. Instead of becoming low resistance, high capacitance vessels as seen in uncomplicated pregnancies, the spiral arteries of preeclamptic patients are high resistance vessels which can ultimately lead to placenta ischemia. Placental blood flow is further compromised as these spiral arteries become prone to acute atherosis. This acute atherosis presents similarly to early-stage atherosclerosis; the vascular changes include fibrinoid necrosis, subendothelial cholesterol-laden macrophages, and perivascular infiltration by inflammatory cells (Kim and Kim 2015).

Atherosis and atherosclerosis are also present in the uterine vasculature of preeclamptic individuals, particularly in the radial arteries that supply blood to the decidua. This decidual vasculopathy is characterized by vessel media hypertrophy, acute atherosis, perivascular lymphocytes, or fibrinoid necrosis in the vessel wall (Hecht et al. 2016). Decidual vasculopathy is associated with extreme clinical outcomes including elevated

diastolic arterial pressure, renal dysfunction, and fetal death (Stevens et al. 2013). Poor placentation is further complicated by the roles of inflammatory molecules such as natural killer cells, dendritic cells, and macrophages.

1.3.2 Immunological contributions to preeclampsia

Natural killer (NK) cells, a type of innate lymphoid cell, are instrumental in placental vascular remodeling. These NK cells, termed uterine NK cells (uNK), exist at the maternal-fetal interface and are the most abundant lymphocyte in the uterus. The amount of uNK cells begins to increase in early pregnancy, reaches a peak near mid gestation, and declines as the pregnancy comes to term. This suggests an important role for uNK cells in establishing and maintaining a pregnancy. Indeed, these uNK cells regulate the depth of placentation, the remodeling of spiral arteries, and the invasion of trophoblasts into the decidua. NK cells express multiple receptors, one of which is the killer immunoglobulin-like receptor (KIR), and the main ligand for KIR, human leukocyte antigen-C (HLA-C), is expressed on the fetal invasive trophoblasts. Certain haplotypes of maternal KIR are associated with increased protection against preeclampsia while others have been implicated in part in its pathogenesis. Furthermore, inhibition of the uNK response in mice leads to defects in spiral artery remodeling, poor implantation, and even fetal growth restriction, all of which are characteristic of preeclampsia (Kieckbusch et al. 2014).

Multiple other immunological factors have been implicated in the pathogenesis of preeclampsia, long considered a pro-inflammatory state. Depletion of these factors in

animal models has been associated with pathophysiological features of preeclampsia. When dendritic cells, important for implantation, are depleted in mice, decidualization is impaired and blastocyst implantation is prevented (Plaks et al. 2008). In mice, depletion of macrophages, critical in uterine vascular remodeling, has also been associated with deficient placental development, decidualization of the uterus, and implantation (Mor et al. 2011).

1.3.3 Endothelial dysfunction and antiangiogenic factors in preeclampsia

Preeclampsia is associated with placental ischemia due to inadequate uterine and placental vascular remodeling. However, the tissue most affected by preeclampsia is the maternal endothelium (Boeldt 2017). The placental ischemia leads to release of soluble factors in the maternal circulation that bring about the common symptoms of preeclampsia, including widespread forms of endothelial dysfunction such as proteinuria, edema, and hypertension. This proteinuria occurs due to renal glomerular endotheliosis in which the endothelial cells within the kidney swell and lose their endothelial fenestrations (Stillman and Karumanchi 2007). Ultimately, this leads to a decrease in glomerular filtration rate and an increase in protein excretion as compared to uncomplicated pregnancies. Consequently, preeclamptic patients are at a higher risk for renal failure. A growing body of evidence suggests that this endothelial dysfunction could be due to alterations in vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and soluble fms-like tyrosine

kinase-1 (sFlt-1). Preeclampsia is associated with decreased PlGF and VEGF signaling concomitant with elevated expression of sFlt-1 (Tomimatsu et al. 2019).

VEGF, an important mediator of angiogenesis and a critical component in the stabilization of endothelial cells, is elevated in normal pregnancy. VEGF is expressed in the glomerular podocytes and there are VEGF receptors on the renal glomerular endothelial cells (Bartlett, Jeansson, and Quaggin 2016). In endothelial cells, VEGF induces nitric oxide (NO) and prostacyclin production, contributing to vasodilation and decreased vascular tone (He et al. 1999). Pregnant mice receiving anti-VEGF therapy experience renal endothelial damage resulting in proteinuria, a common pathology of preeclampsia (Gerber et al.). VEGF also has a role in pathological angiogenesis such as that seen in cancer. In human trials of cancer therapy, use of anti-VEGF antibodies results in endothelial glomerulosis, proteinuria, and hypertension (Zhu et al. 2007). Taken together, this indicates a critical role for VEGF in maintaining endothelial integrity and function.

PlGF, a member of the VEGF family, is expressed in multiple tissues including the heart, lung, liver, and predominantly in the placenta (Chau, Hennessy, and Makris 2017). It plays a vital role in angiogenesis by enhancing the effects of VEGF, particularly in the event of pathological, but not physiological, ischemia. PlGF even influences differentiation of uNK cells (Tayade et al. 2007), critical in placental remodeling and subsequently is upregulated in normal pregnancy, with a steady increase towards a peak at week 30 of gestation (Saffer et al. 2013). However, in preeclampsia, both expression of PlGF and circulating levels of free PlGF are reduced. The former could be due to placental ischemia associated with preeclampsia. PlGF expression decreases in hypoxic environments such as those that occur with ischemia (Gobble et al. 2009). It has been proposed that the reduction

in circulating PlGF is due to binding of sFlt-1, a VEGF and PlGF antagonist (Maynard et al. 2003).

sFlt-1 is a soluble form of the VEGF and PlGF receptor that is produced by alternative splicing of the endothelial VEGF receptor, Flt-1 (FMS-like receptor-1). This receptor is produced by multiple tissues, including the placenta, and is a very potent antagonist of both VEGF and PlGF (Rana et al. 2019). Circulating sFlt-1 levels increase near term of an uncomplicated pregnancy, causing PlGF to bind, likely contributing to the PlGF reduction after week 30. Preeclamptic pregnancies are associated with excess circulating sFlt-1 which causes endothelial damage and proteinuria through antagonization of VEGF and PlGF. Additionally, rats injected with exogenous sFlt-1 develop many of the hallmark characteristics of preeclampsia, including hypertension, proteinuria, and renal glomerular endotheliosis (Maynard et al. 2003).

Previous studies have proposed the ratio of sFlt-1 to PlGF could be an effective early-pregnancy predictor of preeclampsia (Bian et al. 2019). PlGF is low in the 1st trimester for patients who ultimately develop preeclampsia, long before the symptoms present. However, using that value alone has not been shown to be a very sensitive predictor of preeclampsia (Kleinrouweler et al. 2012). When measuring the ratio of PlGF to sFlt-1, along with other clinical parameters such as blood pressure and uterine artery pulsatility index, can be useful in predicting preeclampsia, particularly the early presenting form.

Soluble endoglin (sENG) is another antiangiogenic factor implicated in preeclampsia. sENG inhibits transforming growth factor beta-1 (TGF-β) signaling, an important contributor to vascular homeostasis. sENG is secreted by the placenta and is

elevated in preeclamptic patients before their symptoms present and declines following delivery (Venkatesha et al. 2006).

1.3.4 Renin-angiotensin-aldosterone system in preeclampsia

The renin-angiotensin-aldosterone system (RAAS) plays a key role in the necessary volume expansion in healthy pregnancies, with each component upregulated in gestation. In preeclamptic pregnancies, these three components of RAAS show reduced plasma circulation, in addition to decreased plasma volume, compared to healthy counterparts (Brown, Wang, and Whitworth 1997). Additionally, the vasodilatory ANG-(1-7) is also reduced in preeclamptic plasma (Merill et al. 2002). Plasma renin activity is decreased in preeclampsia, likely due to the decreased substrate. However, while aldosterone is decreased compared to normal pregnancies, there are greater levels of aldosterone in preeclampsia relative to the amount of renin (Shah 2005). This may indicate elevated adrenal sensitivity to angiotensin II.

Despite reduced angiotensin II in preeclampsia, the angiotensin II pressor response is exaggerated, even before the onset of symptoms, compared to the physiologically decreased pressor response in normal pregnancies (Symonds 1988). This may be due to increased expression of angiotensin II type 1 receptors (AT1) associated with preeclampsia (AbdAlla et al. 2001). It is currently unknown whether downregulation of RAAS occurs in response to hypertension or is a contributor to the pathophysiology of preeclampsia.

Preeclamptic patients also release agonistic autoantibodies to the AT1 receptor (AT1-AA) (Wallukat et al. 1999). AT1-AA enhances the effects of angiotensin II in pregnancy and intensifies the downstream effects. AT1-AA concentration has also been shown to correlate with disease severity in preeclamptic patients (Siddiqui et al. 2010). Furthermore, AT1-AA levels remain elevated even after delivery following a preeclamptic pregnancy, which may contribute to its long-term cardiovascular and renal risk factors (Hubel et al. 2007). Infusion of AT1-AA in rats induces preeclamptic symptoms such as hypertension and endothelial dysfunction (Campbell, LaMarca, and Cunningham 2018). Postpartum, these rats are prone to reduce cardiac dysfunction, hypertension, and increased susceptibility of ischemia.

Local RAAS likely plays an important role in the pathogenesis of preeclampsia as well (Shah 2005). Renin, ACE, angiotensinogen, and prorenin, renin's precursor, are all expressed in the human uteroplacental unit. An angiotensinogen variant, Thr235, is associated with abnormal uterine artery remodeling, suggesting that local RAS may play a role in placental remodeling of spiral arteries (Morgan et al. 1999). Previous research has shown elevated angiotensin II type 1 receptors (AT1) in the decidua of preeclamptic patients (Herse et al. 2007). Additionally, local RAS may also be regulated by estrogen and progesterone, as treating decidual cells with progesterone in vitro causes renin release (Shaw et al. 1989).

1.4 Long-term effects of preeclampsia on mother and offspring

1.4.1 Maternal long-term effects of preeclampsia

In addition to the dangerous short term risk factors mentioned previously, preeclampsia is also associated with a multitude of long-term consequences. Preeclampsia is now considered an independent risk factor for cardiovascular disease and stroke, according to the American Heart Association. Experiencing a preeclamptic pregnancy has long been associated with an increased risk of not only developing chronic hypertension (Garovic et al. 2010), but also experiencing it at an earlier age compared to those without a history of hypertensive pregnancy (Heida et al. 2015). Indeed, many people who develop preeclampsia are hypertensive a mere 10 years following delivery (Selvaggi et al. 1988). This hypertension presents itself with much variability, with some experiencing diurnal hypertension, some nocturnal hypertension, and others masked hypertension. One study even showed that only 2 years following delivery, 30% of the persons who had hypertension in pregnancy were now hypertensive (Hermes et al. 2013).

A history of preeclampsia has also been linked to a 60% higher risk of nonpregnancy-related ischemic stroke compared to those without a history of preeclampsia (Brown et al. 2006). Studies have shown an elevated risk for venous thrombosis in patients who previously experienced preeclampsia. Hemorrhagic stroke is also associated with a history of hypertensive pregnancy. Alarmingly, the severity of preeclampsia has also been associated with the severity of subsequent cardiovascular disease. Patients with early-onset

preeclampsia were at an even higher risk of not only developing cardiovascular disease but also dying from it compared to those with the late-onset presentation (Mongraw-Chaffin, Cirillo, and Cohn 2010; Veerbeek et al. 2015).

Preeclampsia has been associated with later relative risk of end-stage kidney disease, with a greater risk associated with more severe forms of preeclampsia (Vikse et al. 2008). Indeed, patients who experienced preeclampsia have albuminuria, a marker of renal damage, years after their pregnancy (McDonald et al. 2013). Preeclampsia patients also experienced proteinuria and reduced glomerular filtration rate up to 1 year postpartum (Kaleta et al. 2016). This end-stage kidney disease presents itself earlier in patients with a history of preeclampsia compared to those without (Vikse et al. 2010). Patients with a previous diagnosis of preeclampsia later have sustained increases in angiotensin II sensitivity and reduced vasodilatory capacity (Stanhewicz et al. 2017). Finally, patients still had signs of endothelial dysfunction with elevated antiangiogenic factors up to 10 years following the end of their preeclamptic pregnancy (Sandvik et al. 2013).

1.4.2 Offspring long-term effects of preeclampsia

Experiencing preeclampsia *in utero* has been linked with a number of risk factors for offspring in later life. Because placental ischemia associated with preeclampsia can lead to intrauterine growth restriction, offspring are often born low birthweight and/or preterm. The offspring also face a much higher risk of being stillborn. Offspring born to preeclamptic patients on average weigh 5% less than those born to uncomplicated

pregnancies (Odegård RA 2000). Intrauterine growth restriction, even without preeclampsia, has been associated with elevated risk of hypertension in postnatal offspring (Barker et al. 1989). And preeclampsia, even without intrauterine growth restriction, is also associated with elevated risk of hypertension in postnatal offspring (Davis et al. 2012). This may be due to the programming of impaired vascular function. Indeed, offspring born to preeclamptic patients show reduced vascular function compared to not only offspring born to uncomplicated pregnancies, but also siblings born to uncomplicated pregnancies, indicating that the preeclampsia itself, rather than just genetics, is a contributing factor (Jayet et al. 2010).

Exposure to preeclampsia also increases the risk of postnatal impairment of kidney function in offspring. Experiencing preeclampsia during development can program offspring for alterations in sodium handling, angiotensin II sensitivity, and total nephron number (Paauw et al. 2017). All these factors can also increase the risk for hypertension later in life.

Placental insufficiency such as that which occurs with preeclampsia has also been associated with reduced glucose tolerance in offspring later in life. Low birth weight, a common complication of preeclampsia, increases the risk of developing type 2 diabetes and obesity (Jornayvaz et al. 2016). There is also a sex difference in this susceptibility to metabolic dysfunction. Adult women, but not men, who were born low birth weight had higher fasting glucose levels (Mogren, Lindahl, and Hogberg 2003).

The major hypothesis surrounding the inheritance of disease following preeclamptic pregnancies centers on Dr. David Barker's hypothesis regarding the developmental origins of disease (Barker 2005; Wadhwa et al. 2009). Dr. Barker proposed

that reduced nutrient and oxygen delivery during gestation, such as that which occurs with uteroplacental ischemia in preeclampsia, could result in changes to gene expression related to metabolism and response to stress. This is particularly important in the case of preeclampsia in humans, as nephrogenesis occurs in the 3rd trimester when preeclampsia is most severe, which could explain the offspring long-term renal consequences of preeclampsia.

1.5 Chronic hypertension in pregnancy

Chronic hypertension is defined by the American College of Obstetrics and Gynecology as a systolic blood pressure greater than 130 mmHg prior to and throughout pregnancy (Bulletins—Obstetrics 2019). Approximately 5% of all pregnancies are affected by chronic hypertension and this prevalence has grown over the past few decades (Seely and Ecker 2014). The prevalence of chronic hypertension in pregnancy has grown over the past decade and this growth is largely attributed to delayed childbearing increasing the average maternal age at childbirth (Yoder, Thornburg, and Bisognano 2009). However, increases in other risk factors such as obesity and metabolic syndrome also contribute to this increasing prevalence, in addition to the growing prevalence of hypertension outside of pregnancy (Mills et al. 2016). This complication disproportionately affects the Black community (Sibai 2002); in the United States, Black patients have twice the prevalence of chronic hypertension in pregnancy compared to white patients (Martin JA 2015). Diagnosis of chronic hypertension in pregnancy can often be masked by the physiological decline in blood pressure associated with pregnancy.

Chronic hypertension in pregnancy can be either primary or secondary. Approximately 90% of all chronic hypertension occurring in pregnancy is primary, or essential hypertension. The remaining 10% is secondary, meaning it occurs as a result of underlying conditions such as kidney disease or endocrine disorders. Patients with chronic hypertension in pregnancy are at an elevated risk of developing superimposed preeclampsia, where their blood pressure rises even higher and occurs with signs of new-onset end-organ damage, such as proteinuria (McCowan 1996). This risk is even greater in

patients with long-term hypertension (over 4 years prior) or in those experiencing preeclampsia in a previous pregnancy (Sibai 1998).

Another potential complication of chronic hypertension in pregnancy is placental abruption. This is when the placenta separates from the wall of the uterus before the pregnancy reaches term, putting both mother and fetus at risk. In fact, pregnant patients with chronic hypertension have a 3-fold higher risk of placental abruption compared to those in uncomplicated pregnancies, although this risk is highest in superimposed preeclampsia (Williams, Mittendorf, and Monson 1991; Ananth et al. 2007). Pregnant patients with chronic hypertension are also at a higher risk for other life-threatening complications. These patients have a higher risk of stroke, hemorrhage, kidney failure, and pulmonary edema, among other complications ('Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy' 2000). Ultimately, chronic hypertension could lead to fetal growth restriction, low birth weight offspring, and a higher risk of stillbirth in offspring.

When compared with preeclampsia, chronic hypertension in pregnancy is associated with near normal placentation (Suranyi et al. 2017). This same study showed that chronic hypertension was associated with fewer detrimental perinatal outcomes compared to preeclampsia. These researchers suggested that there is a compensatory mechanism associated with placentation in chronic hypertension that may not take place in preeclampsia. Another study showed that placentation is impaired in chronic hypertensive mothers who develop superimposed preeclampsia (Panaitescu et al. 2017). However, research comparing preeclampsia and chronic hypertension in pregnancy is limited and typically included under the umbrella of hypertensive disorders of pregnancy.

1.6 Animal models of preeclampsia

All laboratory models of preeclampsia currently used are induced either genetically, pharmacologically, or experimentally. A more commonly used model of preeclampsia is the reduced uterine perfusion pressure (RUPP) rat model. Clips are placed on the abdominal aorta and the uterine arteries of pregnant Sprague-Dawley rats to induce uteroplacental ischemia (Granger et al. 2006). This procedure reduces uteroplacental blood flow by approximately 40%. The RUPP model recapitulates many of the characteristics of preeclampsia, including hypertension, proteinuria, impaired glomerular filtration rate, immune abnormalities, and intrauterine growth restriction. These rats also have reduced NO, enhanced contractility to angiotensin II, elevated AT1-AA, elevated tissue ET-1, increased sFlt-1 and sEng, and reductions in plasma VEGF and PlGF (Li, LaMarca, and Reckelhoff 2012). This clipping procedure has been reproduced in other rodents, dogs, and even nonhuman primates, recapitulating many of the pathophysiological characteristics of preeclampsia (Morton et al. 2019).

When pregnant rats are treated with L-NAME, a nitric oxide synthase (NOS) inhibitor, they develop hypertension, proteinuria, renal vasoconstriction, reduced GFR, and higher maternal and fetal mortality (Molnär and Hertelendy 1992). This model not only confirms the role of NO in pregnancy-induced hypertension, but also provides an effective model for studying the downstream effects of preeclampsia. Endothelial NOS can also be genetically manipulated to produce a preeclampsia model. eNOS knockout mice ($eNOS^{-/-}$ /$Nos3^{-/-}$) have hypertension prior to pregnancy and blood pressure increases in pregnancy concomitant with proteinuria, reduced cardiac output, and fetal growth restriction (Li

2012). However, there are no placental abnormalities, which may be due to major differences in placentation between humans and mice. The reduced fetal growth, therefore, is likely due to reductions in circulating NO leading to reduced fetoplacental blood flow.

Another mouse model of preeclampsia, the BPH/5 model, is an inbred strain generated by mating borderline hypertensive sibling mice (Sones 2018). In pregnancy, these mice experience a further increase in mean arterial pressure by about 50 mmHg along with proteinuria, fetal growth restriction, and renal glomerulosclerosis. The elevated blood pressure is of unknown origin but it returns to prepregnancy values following delivery. One downfall of this model is that the hypertension exists prior to pregnancy, making it more appropriate for studies of chronic hypertension in pregnancy (Marshall et al. 2018).

As the RAAS plays a key role in placentation and maintenance of blood volume in pregnancy, it has been a useful target for creating models of preeclampsia. In a transgenic mouse model generated through mating a male expressing human renin with a female expressing human angiotensinogen, they experience placental secretion of renin into the maternal circulation (Takimoto et al. 1996). These animals experience late-pregnancy elevations in blood pressure as well as proteinuria, placental necrosis, cardiac hypertrophy, and uniform enlargement of glomeruli. When this model was generated using Sprague-Dawley rats, the animals developed numerous pathologies of preeclampsia, including hypertension, proteinuria, and increases in circulating AT1-AAs (Verlohren et al. 2008; Dechend et al. 2005). Furthermore, overexpression of renin and angiotensinogen in mice results in chronic hypertension which develops into superimposed preeclampsia in pregnancy (Falcao et al. 2009). The symptoms in this model include proteinuria without renal pathology, placental necrosis, and elevated sFlt-1. Finally, administration of human

AT1-AAs into pregnant mice leads to hypertension, elevated sFlt-1 and sEng, glomerular endotheliosis, proteinuria, placental abnormalities, and fetal growth restriction (Zhou et al. 2008). This supports a role for AT1-AA in the pathogenesis of preeclampsia.

Anti-angiogenic treatment has been another avenue for generating models of preeclampsia in the laboratory. An adenovirus of rodent sFlt-1 was injected in the tail vein of 2nd trimester pregnant Sprague-Dawley rats, resulting in elevated blood pressure, proteinuria, decreased plasma VEGF and PlGF, and glomerular endotheliosis (Maynard et al. 2003). This process was repeated in mice with similar results as well as significantly reduced offspring birth weight (Szalai 2015). Continuous infusion of sFlt-1 in pregnant Sprague-Dawley rats also recapitulates many preeclampsia pathologies such as hypertension, fetal growth restriction, reduced placental weight, and decreased plasma VEGF (Bridges et al. 2009).

1.7 The African Green Monkey

While these animal models have been incredibly successful in recapitulating many of the downstream effects of preeclampsia, the lack of a spontaneous animal model of this syndrome has left a gap in understanding its etiology. The early mechanisms, including trophoblast invasion and uterine vascular remodeling, cannot be fully investigated without a spontaneous animal model of preeclampsia. Despite the numerous contributions of these animal models to preeclampsia research, much remains unknown regarding its pathology, indicating a major need for a clinically relevant, spontaneous animal model. This dissertation is focused on characterizing a novel, spontaneous animal model of preeclampsia which may reduce the translational gap in our understanding of its etiology.

1.7.1 Overview, behavior, and reproduction

The African Green Monkey (AGM; *Chlorocebus aethiops sabaeus*) is an Old-World monkey that diverged from the human lineage approximately 23 million years ago (Warren et al. 2015). As the name suggests, these animals originated from Africa but were brought to the Caribbean during colonialism. In St. Kitts and Nevis, the AGM has no natural predator aside from humans and carries none of the pathogens associated with the African populations of AGMs (McGuire 1987). This invasive species has become a major pest to farmers in St. Kitts and Nevis, now outnumbering humans with an estimated 50,000-100,000 animals spread between both islands (Dore, Eller, and Eller 2018). This rapid

growth over the past 400 years stemming from a small initial population has, as would be expected, created a bottleneck effect.

In the wild, the average lifespan of AGMs is estimated to be 10-12 years of age due to high predation and limited food availability (Isbell et al. 2009). As expected, this lifespan is extended in captivity, with animals living up to 30 years. The animals are omnivorous by nature but show a strong preference for fruits. The AGM shows sexual dimorphism in size, with males weighing an average 5 kg and females approximately 3-4 kg.

The AGM has a complex social structure within their troops. There is an alpha male as well as an alpha female within the troop. Troops are large, on average consisting of 20-30 members but sometimes up to 75 individuals. These troops include multiple males and females. These troops are also matrifocal and matrilineal (Struhsaker 1967). The females remain in their natal troop for their entire lives whereas males tend to leave upon sexual maturation in pursuit of a new troop (Roberta M. Palmour 1997). The AGMs participate in other complex behaviors such as grooming, scent marking of territory, and parenting. These monkeys cooperate in child rearing, with siblings and unrelated females sharing parental responsibilities with the mother. Older females also appear to teach adolescent female troop members how to parent.

The AGM female reaches sexual maturity around 3 years of age while males sexually mature around the age of 4. They have a 32-day menstrual cycle, close to that of humans. Also similar to humans, ovulation is concealed in the vervet, meaning there are no outward signs such as genital swelling. Upon pregnancy, gestation typically lasts for 23 weeks and the AGM predominantly experiences singleton, or one fetus, pregnancies (Cho et al. 2002). Twins do occur but are very rare. Placentation is also similar to humans, with

the AGM having a hemochorial placenta (Owiti et al. 1986). One important distinction, however, is that the AGM has a bidiscoid (double disc) placenta compared to the discoid (single disc) placenta of humans.

1.7.2 Use as a biomedical model

Nonhuman primates are critical components of biomedical research as they are highly translational due to close relation to humans. The AGM and humans share conservation of gene structure, genome sequence, organ physiology, inflammatory processes, and circadian rhythms, among other levels of biological organization (Jasinska et al. 2013). AGMs also have an upright posture and are diurnal, similar to humans. They also share endocrine characteristics with humans, making them ideal models of hormone effects on disease. The abundance, particularly in the Caribbean, of the AGM also makes it valuable in research. The sequencing of the AGM genome by the International Vervet Genome Consortium has advanced the utility of this animal model (Warren et al. 2015).

Due to these similarities with humans, the AGM has quickly become the most widely used nonhuman primate in biomedical research. They are used in studies of atherosclerosis, insulin resistance, obesity, Alzheimer's disease, and simian immunodeficiency virus. Over the past few decades, the AGM has emerged as a highly translational animal model of spontaneous hypertension.

Hypertension in the AGM was first characterized in the 1980s. Kraft-Schreyer showed the transmission of hypertension through breeding hypertensive AGMs (Kraft-

Schreyer N 1991). It was later shown that infusion of atrial natriuretic peptide or administration of captopril, an ACE inhibitor, can reduce blood pressure in the hypertensive AGM (Martin 1990). Blood pressure in the AGM can be salt-sensitive without any changes in body weight (Srinivasan 1984). However, loop diuretics such as furosemide seem to have no effect on AGM blood pressure (Martin 1990). Finally, Rhoads et al characterized the progression of spontaneous hypertension in the AGM. Dr. Rhoads showed that the AGM experiences spontaneous elevations in blood pressure at a similar prevalence to the human population that increases with age and is associated with renal, glomerular pathologies leading to proteinuria (Rhoads et al. 2017). Hypertensive AGMs also experience elevated heart rates and left ventricular hypertrophy. Together, this supports the unique utility of the African Green Monkey as a highly translational animal model for short- and long-term studies of cardiovascular and renal function.

1.8 Rationale, overall hypothesis, and specific aims

Providing novel, translational animal models of human disease is a critical contribution to biomedical research. When a spontaneous animal model is discovered, it must first be characterized before it can be provided as a tool for innovative research. Such a highly translational tool is of particular use in complex syndromes such as preeclampsia where etiological research in humans can be very limited. The overall hypothesis of this doctoral project is that the African Green Monkey develops spontaneous preeclampsia with pathophysiological similarities to humans. The following aims were developed to test this hypothesis:

Specific Aim I will characterize normal pregnancy and postpartum in the AGM. This will include measuring blood pressure, water balance, renal handling of sodium, potassium, and protein, plasma osmolality, and offspring birth weight and survival. Aim I hypothesizes that the AGM will experience similar physiological adaptations to pregnancy as seen in human pregnancy: elevated water and sodium retention, no differences in urinary protein excretion, decreased plasma osmolality, and normal fetal growth.

Specific Aim II will characterize the spontaneous elevation of blood pressure in preeclamptic AGMs. This will include measuring blood pressure, water balance, renal handling of sodium, potassium, and protein, plasma osmolality, and offspring birth weight and survival. Aim II hypothesizes that spontaneously preeclamptic AGMs will have

elevated systolic and diastolic arterial pressure, an exaggerated decrease in plasma osmolality, elevated urinary protein excretion, and fetal growth restriction resulting in low birth weight offspring and high stillbirth rates.

Specific Aim III will characterize the effect of maternal spontaneous preeclampsia and chronic hypertension on the offspring born to these pregnancies. This will include measuring protein excretion, sodium and potassium excretion, fasting blood glucose, determination of tolerance to an acute glucose load, plasma osmolality and protein concentration, and blood pressure. Specific Aim III hypothesizes that offspring born to preeclamptic and chronically hypertensive mothers will have elevated urinary protein excretion, reduced glucose handling, and elevated systolic and diastolic arterial pressures in pre-adolescence (pre-sexual maturity).

1.9 Figures and Tables

Table 1-1
Summary of major changes in normal pregnancy and preeclampsia

	Normal Pregnancy	**Preeclampsia (relative to normal pregnant)**
Plasma Volume	Increases	Increases in early pregnancy, can decrease in late pregnancy
Glomerular Filtration Rate	Increases	Decreases
Renal Plasma Flow	Increases	Decreases
Cardiac Output	Increases	Decreases
Stroke Volume	Increases	Decreases
Heart Rate	Increases	Can sometimes increase
Hematocrit	Increases	Increases
Prorenin	Increases	Increases
Renin	Increases	Decreases
Angiotensinogen	Increases	Decreases
Angiotensin II	Increases	Decreases
Angiotensin II Sensitivity	Decreases	Increases
Aldosterone	Increases	Decreases
Plasma Osmolality	Decreases in early pregnancy	Decreases in early pregnancy, can increase in late pregnancy
Vascular Resistance	Decreases	Increases
Ang-(1-7)	Increases	Decreases
ANP	Decreases	Increases
VEGF	Increases	Decreases
PlGF	Increases	Decreases
ADH/AVP	Increases	Increases

CHAPTER 2: SPONTANEOUS PREECLAMPSIA IS ASSOCIATED WITH MATERNAL PROTEINURIA AND FETAL GROWTH RESTRICTION IN AFRICAN GREEN MONKEYS (*CHLOROCEBUS AETHIOPS SABAEUS*)

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Submitted: AJP Regulatory, Integrative, and Comparative Physiology

2.1 Introduction

Preeclampsia annually affects 6.8 million pregnancies worldwide, killing 70,000 women and an estimated 500,000 infants (Ghulmiyyah and Sibai 2012). This pathophysiologic syndrome is characterized in humans as *de novo* maternal hypertension at 20 weeks or later in gestation, typically concurrent with signs of renal damage first expressed as early onset proteinuria ('The Preeclampsia Foundation' 2020). Preeclampsia also has long-term, postnatal deleterious effects on both mother and child. Women who develop preeclampsia are at a higher risk for future neurological and cardiovascular events such as stroke and/or myocardial infarction (MCDonald SD 2010; McDonald SD 2008). Reduced uterine blood flow during a preeclamptic pregnancy can lead to fetal morbidities such as growth restriction, premature delivery, and stillbirth (Bokslag et al. 2016; Odegård RA 2000; Temming et al. 2017). Surviving offspring are often born low birth weight or small for gestational age and have a significantly higher risk of cardiovascular and renal disease in adulthood (Cheong et al. 2016; LaMarc 2018; Stojanovska, Scherjon, and Plosch 2016; Chehade et al. 2018). Currently, the only known treatment of preeclampsia is the often-preterm delivery of the placenta and fetus, making diagnostic and therapeutic development essential to worldwide women's health.

Preeclampsia pathologies are multifactorial (Karumanchi and Granger 2016). Although the ultimate etiology of the disease progression is not fully understood, numerous causative factors have been implicated, including excess vasopressin (28, 35), statin administration (Ahmed 2011; Brownfoot et al. 2015; Kumasawa et al. 2011), increased circulating endothelin (George and Granger 2012; George, Palei, and Granger 2012), and immune responses to inflammation (Mark K. Santillan 2014; LaMarca, Cornelius, and

Wallace 2013). Previous family history of chronic hypertension and/or preeclampsia increase the risks for developing *de novo* maternal hypertension (Bezerra et al. 2010; Carr et al. 2005; Boyd et al. 2013). Maternal risk factors include nulliparity, age at time of pregnancy, multiple gestations, and a personal history of previous preeclampsia (Levine 2004; English, Kenny, and McCarthy 2015). Ultimately, the placenta has been shown to play a critical role in the pathogenesis of preeclampsia (Roberts and Escudero 2012; Saito and Nakashima 2014). However, the placenta is a biparental organ (Apicella et al. 2019; Wang et al. 2013) and paternal factors have also been implicated, both immunologically (Galaviz-Hernandez et al. 2018; Redman and Sargent 2010; Tan 2008) and genetically (Galaviz-Hernandez et al. 2016; Galaviz-Hernandez et al. 2018; Zusterzeel 2002), as causative factors leading to the development of preeclampsia.

Currently, effective and representative animal models used to study the etiology of preeclampsia are artificially induced using genetic manipulations, infusion of pharmacological agents, or via surgical manipulations such as physical reduction of uterine blood flow (Podjarny 2004; Santillan et al. 2014; Marshall et al. 2018; McCarthy et al. 2011; Sones and Davisson 2016). Developing a spontaneous, translational model of this significant pathology is critical to understanding the initiating events underlying its pathogenesis. This model would also provide the opportunity for greatly improved diagnostics and therapeutics. In the present study, the African Green Monkey (AGM; *Chlorocebus aethiops sabaeus*) is described as a novel model of spontaneous preeclampsia.

The AGM (common name vervet) is an Old-World Monkey that diverged from the human lineage approximately 29 million years ago (Warren et al. 2015). Given this close evolutionary history, the AGM is very similar to humans with respect to upright posture,

diurnal circadian rhythms, complex social and familial traits, behavior, genome sequence, and reproduction (Jasinska et al. 2013). They undergo a 28-31-day menstrual cycle (Carroll et al. 2007), have bidiscoid, hemochorial placentas (DeMartelly et al. 2012), and give birth annually, typically to one offspring per pregnancy (Palmour 1997). Previously, we have reported that both male and female AGMs develop spontaneous hypertension with a population frequency of approximately 35% (Rhoads et al. 2017). Therefore, we hypothesized that this animal model may also exhibit similar characteristics in spontaneous maternal hypertension, altered renal function, and fetal growth conditions that recapitulate symptoms observed in preeclamptic humans. We propose that the AGM provides a highly translational, nonhuman primate for the study of short and long-term effects of spontaneous preeclampsia in both adult females and their offspring.

2.2 Methods

2.2.1 Animal Care and Housing

All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the animal vivarium, SKN Primates. Animals were maintained in natural environment troop housing at the SKN Primates facility. Animals were fed twice daily with a combination of fresh fruits and vegetables, along with a standard nonhuman primate chow three times per week (Harlan Teklad 8773). Water was provided *ad libitum*. All breeding male and female vervets had been individually quarantined for a minimum of 45 days followed by a full veterinary exam prior to entering the vivarium and provided for future study. All vervets were tested tuberculosis negative and administered Ivermectin (0.5 mg/kg s.c.) twice during quarantine for the elimination of parasites. Colony-bred offspring were kept with their natal troop and mother until 6 months of age at which time they were weaned and placed in a group enclosure of 10-15 other juveniles.

2.2.2 Blood Pressure Measurement

At the time of all measurements, animals were weighed and given a complete health examination. Blood was collected in ACD-B tubes (BD Vacutainer 364816) and fasting glucose obtained using a commercial glucometer (TrueTrack). Blood pressure was measured as previously described in detail (Rhoads et al. 2017). Briefly, animals were sedated with ketamine (15 mg/kg i.m.) and placed in a supine position. Blood pressure was measured via forearm plethysmography, with an inflatable cuff and doppler stethoscope. After confirming occlusion with the cuff, Korotkoff sounds, indicative of systolic arterial

pressure (SAP) and diastolic arterial pressure (DAP), were identified. SAP and DAP were recorded a minimum of 7 times within less than 5% intrameasurement deviation. Following blood pressure determination, heart rate was recorded. Blood pressure measurements were conducted between 9am-11am. Blood pressure and HR measurements were performed prior to pregnancy, in each trimester, and at days 1, 7, 14 and 42 postpartum. Blood pressure measurements and blood collection/glucose monitoring occurred at the same time each day, between 9-11am.

2.2.3 Metabolic Studies

For studies involving water intake, urine flow rate, and urinary protein, sodium, and potassium excretion, animals were sedated with ketamine (15 mg/kg i.m.) for placement in individual, metabolic pens. Animals were maintained on the same diet outlined above and provided 1-liter water bottles for daily water intake measurements. Following a minimum one-week acclimation to the individual housing, water intake and urine flow were measured every 24 hours for 3 consecutive days. Urine samples were collected daily and centrifuged (1000xg) to remove any particulate contaminants. Urine outputs and water intakes were collected prior to pregnancy, during the estimated 2nd and 3rd trimesters, and at 1, 14, and 42 days postpartum.

2.2.4 Breeding and Pregnancy Determination

Nonpregnant females were sedated with ketamine (15 mg/kg i.m.) and a full health examination was performed. SAP, DAP, HR, and body weight were measured. Venous

blood collection and fasting glucose measurements were then obtained. Animals were placed in individual, metabolic pens for metabolic studies as described above. Following completion of urine flow and water intake measurements, animals were placed in a group enclosure with 1 male and 15-25 females, representative of their natural troop dynamic. After mating was initiated, animals were periodically assessed for clinical signs of pregnancy. When animal care staff suspected a pregnancy, females were pulled from the group and abdominal palpations were performed to identify a viable fetus. When a fetus was identified, the crown to rump length was measured externally and recorded. This process was repeated for each trimester. Trimesters were estimated based on fetal crown-to-rump length, with 1st trimester fetuses between 1-6 cm, 2nd trimester between 7-12 cm, and 3rd trimester greater than 12 cm. This guideline was established by monitoring fetal growth over multiple breeding seasons and backtracking after delivery to determine the size of the fetus at each trimester in normal pregnancies. Once a fetus reached the estimated second, females were lightly sedated (ketamine, 15 mg/kg) and blood pressure, HR, fasting glucose were measured followed by systemic venous blood collection as described above. This process was repeated in the third trimester. After the third trimester measurements, pregnant females were moved to single housing for daily maternity monitoring until delivery. After delivery, all mothers and their offspring remained in individual housing for daily monitoring for 42 days at which time both mother and offspring were returned to their original group until weaning at 5-6 months of age.

2.2.5 Analytical Measurements

Following blood collection, plasma was separated and buffy coat was collected for future DNA extraction. Plasma osmolality was measured via freezing point depression (Precision Instruments Micro-Osmette). Urinary sodium and potassium concentrations were measured by flame photometry (Cole-Parmer Item # UX-02655-10). Sodium and potassium concentrations were expressed in mmol/ml and multiplied by the urine flow rate to obtain ion excretion rate: $Ion\ Excretion\ Rate = \left[urinary\ ion\ (\frac{mmol}{ml})\right] * UFR\ (\frac{ml}{day})$.

Urinary protein concentration was measured using a bicinchoninic acid assay (ThermoFisher Scientific Item #23225). Urine was pre-diluted 1:5. Following, 25ul of either bovine serum albumin standard or sample were pipetted into a microplate along with 200ul of working BCA reagent. The plate was incubated at 37 degrees Celsius for 30 minutes. After cooling to room temperature, the absorbance was read at 562nm and a standard curve was generated (r^2>0.99) to determine the protein concentration. Protein excretion rates were calculated as: $Protein\ Excretion\ Rate = \left[urinary\ protein\ (\frac{mg}{ml})\right] * UFR\ (\frac{ml}{day})$.

2.2.6 Newborn Handling

Within 24 hours following delivery, the mother was sedated with ketamine (15 mg/kg i.m.). SAP, DAP, HR, and body weight of the mother were measured. Systemic venous blood was collected and fasting glucose determined. The offspring were weighed, and crown-to-rump length measured. An examination was performed to assess overall health of mother

and offspring. Both were returned to their respective enclosures. This process was repeated at days 7, 14, and 42 postpartum. All pregnancies in this study were singleton pregnancies.

2.2.7 Statistical Analyses

All statistical analyses were performed in JMP Pro 12. A two-way ANOVA followed by a Tukey's post hoc analysis when significant was used to compare phenotype and time interactions. One-way ANOVA with Tukey's post hoc when significant was used to compare time points within a single maternal phenotype. Any comparisons between 2 groups were performed with a student's t-test or Mann-Whitney U in JMP Pro 12. All values are reported as mean ± SEM. The 0.05 level of probability was utilized as the accepted criterion for significance.

2.3 Results

2.3.1 Effect of Pregnancy on Maternal Systolic and Diastolic Arterial Pressure and Heart Rate

In a cohort of 62 pregnancies, 27 were identified as normotensive (NT) before and throughout pregnancy as well as during postpartum. In this group, 35 females were normotensive prior to pregnancy and became preeclamptic in either the second or third trimester of pregnancy. Figure 2.1A illustrates the spontaneous increase in systolic arterial pressure in PE animals compared to normotensive animals. Nonpregnant (NP) mean SAP for NT animals was 103.7 ± 4.7 mmHg and for PE animals 109. 1 ± 4.0 mmHg. In the 1st trimester, SAP did not change in NT (99.3 ± 6.5 mmHg) or PE (100.6 ± 7.5 mmHg) animals. In the 2nd trimester, NT SAP remained unchanged (103.1 ± 6.0 mmHg) but significantly increased with PE (129.1 ± 4.3 mmHg, $p<0.05$ vs NP and 1st trimester). In the 3rd trimester, SAP for NT animals remained the same (99.2 ± 4.4 mmHg) but continued to increase in PE (144.0 ± 4.3 mmHg, $p<0.05$ vs nonpregnant, 1st, and 2nd trimester). SAP remained the same at days 1, 7, and 14 postpartum in NT animals (1: 101.9 ± 5.2 mmHg, 7: 98.6 ± 6.5 mmHg, 14:100.9 ± 6.8 mmHg). With PE, SAP remained elevated at 1 and 7 days postpartum (1: 126.8 ± 4.1 mmHg, 7: 132.7 ± 4.9 mmHg vs nonpregnant and 1st trimester) but decreased by postpartum day 14 (14: 117.5 ± 4.7 mmHg). By 6 weeks postpartum, NT SAP averaged 97.4 ± 6.0 mmHg and PE returned to 114.9 ± 4.6 mmHg, the same as the nonpregnant state.

Diastolic arterial pressure (DAP) followed a similar trend to SAP (Figure 2.1B). Nonpregnant DAP for NT animals was 47.3 ± 3.6 mmHg and for PE animals 53.1 ± 3.0 mmHg. In the 1st trimester, DAP did not change for NT (41.3 ± 4.9 mmHg) or PE animals

(40.8 ± 5.7 mmHg) compared to nonpregnant animals. DAP remained unchanged in the 2nd trimester for NT (46.5 ± 4.6 mmHg) and PE animals (53.0 ± 3.2 mmHg). However, DAP increased from 1st to 2nd trimester for PE animals (1st trimester 40.8 ± 5.7 mmHg vs 2nd trimester 53.0 ± 3.2 mmHg; $p<0.05$). For NT animals, 3rd trimester DAP also remained the same (46.2 ± 3.4 mmHg) but increased with PE pregnancies (64.7 ± 3.2 mmHg; $p<0.05$). DAP remained the same at days 1, 7, and 14 postpartum in NT animals (1: 41.7 ± 3.9 mmHg, 7: 37.2 ± 4.9 mmHg, 14: 40.5 ± 5.2 mmHg). With PE, DAP remained elevated at 1 and 7 days postpartum (1: 55.5 ± 3.1 mmHg, 7: 62.8 ± 3.7 mmHg vs PE NP) but decreased by postpartum day 14 (56.1 ± 3.6 mmHg). By 6 weeks postpartum, NT DAP averaged 39.1 ± 4.6 mmHg and PE returned to 45.1 ± 3.5 mmHg, both equal to the nonpregnant state.

Heart rate (Figure 2.1C) did not change in NT pregnancies (NP: 155.8 ± 5.0 bpm, 1st trimester: 152.0 ± 6.0 bpm, 2nd trimester: 153.4 ± 6.4 bpm, 3rd trimester: 150.0 ± 4.7 bpm), but decreased at 14 and 42 days postpartum compared to nonpregnant (1 day postpartum: 144.6 ± 5.5 bpm, 7 days postpartum: 128.0 ± 6.0 bpm, 14 days postpartum: 125.8 ± 7.2 bpm, 42 days postpartum: 125.4 ± 6.4 bpm). Heart rate did not change in PE pregnancies (NP: 163.6 ± 8.0 bpm, 1st trimester: 163.6 ± 8.0 bpm, 2nd trimester: 158.4 ± 4.5 bpm, 3rd trimester: 160.4 ± 4.5 bpm). Heart rate decreased at 14 and 42 days postpartum for PE AGMs, though postpartum heart rate was higher for PE animals than NT animals (1 day postpartum: 158.7 ± 4.4 bpm^, 7 days postpartum: 149.9 ± 4.2 bpm^, 14 days postpartum: 140.7 ± 5.0 bpm*, 42 days postpartum: 143.1 ± 4.9 bpm*^; *$p<0.05$ vs nonpregnant and each trimester, ^$p<0.05$ vs NT for that timepoint).

2.3.2 Effect of Spontaneous Preeclampsia on Water Balance and Maternal Body Weight

Water intake did not change during pregnancy or postpartum for NT animals (NP: 287.2 ± 40.5 ml/day n=26, 2nd trimester: 244.1 ± 68.8 ml/day n=9, 3rd trimester: 303.3 ± 73.0 ml/day n=8, 1 day postpartum: 384.9 ± 84.3 ml/day n=6, 14 days postpartum: 491.2 ± 78.0 ml/day n=7; 42 days postpartum: 431.0 ± 92.3 ml/day; n=5). Water intake did not change in PE pregnancy, but increased at days 14 and 42 postpartum (NP: 160.0 ± 50.1 ml/day n=17, 2nd trimester: 322.6 ± 68.8 ml/day n=8, 3rd trimester: 215.6 ± 51.6 ml/day n=16, 1 day postpartum: 252.2 ± 65.3 ml/day n=10, 14 days postpartum: 355.8 ± 65.3 ml/day* n=10; 42 days postpartum: 456.2 ± 78.0 ml/day* n=7; *$p<0.05$ vs nonpregnant and each trimester; Figure 2.2A).

Urine flow rate did not change during NT pregnancy or in NT females postpartum (NP: 167.4 ± 24.2 ml/day n=26, 2nd trimester: 169.3 ± 41.2 ml/day n=9, 3rd trimester: 210.6 ± 43.7 ml/day n=8, 1 day postpartum: 257.3 ± 50.4 ml/day n=6, 14 days postpartum: 244.5 ± 46.7 ml/day n=7; 42 days postpartum: 225.8 ± 55.2 ml/day; n=5). With PE, urine flow rate was unchanged throughout pregnancy and into the postpartum period, but increased at 42 days postpartum (NP: 119.0 ± 29.9 ml/day n=17, 2nd trimester: 183.2 ± 41.2 ml/day n=8, 3rd trimester: 158.9 ± 30.9 ml/day n=16, 1 day postpartum: 174.5 ± 39.0 ml/day n=10, 14 days postpartum: 215.4 ± 39.1 ml/day n=10, 42 days postpartum: 331.0 ± 46.5 ml/day* n=7; *$p<0.05$ ANOVA vs NP and 2nd/3rd trimester; Figure 2.2B).

Plasma osmolality did not change in NT pregnancy or in NT females postpartum (NP: 308.5 ± 3.9 mOsm/kg n=13, 2nd trimester: 297.2 ± 6.3 mOsm/kg n=5, 3rd trimester: 304.3 ± 5.8 mOsm/kg n=6, 1 day postpartum: 294.8 ± 6.3 mOsm/kg n=5, 14 days postpartum: 307.8 ± 7.1 mOsm/kg n=5, 42 days postpartum: 301.6 ± 6.3 mOsm/kg; n=5).

With PE however, plasma osmolality decreased in the 2nd and 3rd trimesters, but returned to values not different from pre-pregnancy at 14 and 42 days postpartum (NP: 312.8 ± 4.7 mOsm/kg n=8, 2nd trimester: 286.4 ± 5.4 mOsm/kg* n=6, 3rd trimester: 296.0 ± 3.9 mOsm/kg* n=9, 1 day postpartum: 297.0 ± 4.3 mOsm/kg* n=11, 14 days postpartum: 312.5 ± 5.0 mOsm/kg n=8, 42 days postpartum: 315.0 ± 6.3 mOsm/kg n=6; *p<0.05 ANOVA vs nonpregnant; Figure 2.3).

Female body weight for NT animals (n=14) did not increase until the 2nd and 3rd trimesters (NP: 3.7 ± 0.1 kg, 1st trimester: 3.8 ± 0.2 kg, 2nd trimester: 4.2 ± 0.1 kg^, 3rd trimester: 4.5 ± 0.1 kg^; n=15). Body weight remained elevated at 1 day postpartum (4.0 ± 0.1 kg^) but returned to pre-pregnancy values at 7, 14, and 42 days postpartum (7: 3.8 ± 0.1 kg; 14: 3.7 ± 0.2 kg; 42: 3.7 ± 0.2 kg; ^ indicates p<0.05 vs NT NP). With PE (n=11), animals gained a significant amount of body weight during the 1st trimester (NP: 3.6 ± 0.1 kg, 1st trimester: 4.2 ± 0.1 kg*). Body weight continued to increase in the 2nd and 3rd trimesters (2nd trimester: 4.4 ± 0.1 kg*, 3rd trimester: 4.5 ± 0.1 kg*; n=28). Body weight remained elevated at 1 day postpartum for PE animals (4.0 ± 0.1 kg*) but reached pre-pregnancy values at 7, 14, and 42 days postpartum (7: 3.9 ± 0.1 kg; 14: 3.9 ± 0.1 kg; 42: 3.9 ± 0.1 kg; * indicates p<0.05 vs PE NP). The change in weight between pre-pregnancy and the end of the 1st trimester was greater for PE than NT (NT Δ weight: 114.0 ± 118 g vs PE Δ weight: 568.5 ± 76.4 g; p<0.05 student's t-test). By the 3rd trimester, NT and PE weighed the same (NT 4.5 ± 0.1 kg vs PE 4.5 ± 0.1 kg; p>0.05; Figure 2.4).

2.3.3 Effect of Spontaneous Preeclampsia on Urinary Protein, Sodium, and Potassium Excretion

Protein excretion rate did not change in during NT pregnancy or postpartum (NP: 380.5 ± 39.8 mg/day n=26, 2nd trimester: 408.9 ± 67.6 mg/day n=9, 3rd trimester: 464.6 ± 71.7 mg/day n=8, 1 day postpartum: 461.2 ± 82.8 mg/day n=6, 14 days postpartum: 372.3 ± 76.6 mg/day n=7, 42 days postpartum: 252.0 ± 90.7 mg/day n=5). In PE however, protein excretion increased in the 2nd and 3rd trimester, but declined by 14 days postpartum (NP: 369.9 ± 19.2 mg/day n=17, 2nd trimester: 742.8 ± 71.7 mg/day* n=8, 3rd trimester: 691.7 ± 52.3 mg/day* n=15, 1 day postpartum: 402.9 ± 67.6 mg/day n=8, 14 days postpartum: 401.0 ± 64.1 mg/day n=9, 42 days postpartum: 458.7 ± 76.6 mg/day n=7; *p<0.05 vs NP; Figure 2.5).

Urinary sodium excretion remained the same throughout NT pregnancy and postpartum (NP: 2.2 ± 0.3 mmol/day n=26, 2nd trimester: 2.3 ± 0.5 mmol/day n=9, 3rd trimester: 2.1 ± 0.5 mmol/day n=8, 1 day postpartum: 2.2 ± 0.6 mmol/day n=6, 14 days postpartum: 2.3 ± 0.6 mmol/day n=7, 42 days postpartum: 2.0 ± 0.7 mmol/day n=5). In pregnant females that developed PE, sodium excretion remained the same throughout pregnancy, but increased significantly at days 1, 14, and 42 postpartum relative to nonpregnant and 3rd trimester (NP: 1.2 ± 0.5 mmol/day n=17, 2nd trimester: 2.7 ± 0.5 mmol/day n=8, 3rd trimester: 1.8 ± 0.4 mmol/day n=15, 1 day postpartum: 4.1 ± 0.6 mmol/day* n=8, 14 days postpartum: 3.8 ± 0.5 mmol/day* n=9, 42 days postpartum: 4.2 ± 0.6 mmol/day* n=7; *p<0.05 ANOVA vs NP and 3rd trimester; Figure 2.6A).

Urinary potassium excretion was similar to urinary sodium excretion, with no changes throughout NT pregnancy (NP: 3.0 ± 0.4 mmol/day n=26, 2nd trimester: 4.2 ± 0.7

mmol/day n=9, 3rd trimester: 4.2 ± 0.8 mmol/day n=8) or postpartum (1 day 3.0 ± 1.0 mmol/day n=6, 14 days: 3.3 ± 0.8 mmol/day n=7, and 42 days: 3.0 ± 1.0 mmol/day n=5). With PE, potassium excretion remained unchanged throughout pregnancy, but increased at days 14 and 42 postpartum (NP: 1.3 ± 0.7 mmol/day n=10, 2nd trimester: 3.0 ± 0.8 mmol/day n=8, 3rd trimester: 2.0 ± 0.7 mmol/day n=11, 1 day postpartum: 3.6 ± 0.8 mmol/day n=7, 14 days postpartum: 5.2 ± 0.7 mmol/day* n=9, 42 days postpartum: 5.6 ± 0.8 mmol/day* n=7; *$p<0.05$ ANOVA vs NP and 3rd trimester; Figure 2.6B).

2.3.4 Effect of Spontaneous Preeclampsia on Offspring Survival & Birth Weight

NT pregnancies had a stillbirth rate of 4% (1/27 births) while PE resulted in a 29% stillbirth rate (10/35 births; Figure 2.7A). Surviving offspring born to PE mothers weighed less than those born to NT mothers (NT: 338.6 ± 9.8 g n=14 vs PE: 309.3 ± 8.0 g n=20; $p<0.05$ t-test; Figure 2.7B). There were no differences between male and female birth weights.

2.4 Discussion

Current animal models used to study the pathogenesis of preeclampsia are pharmacologically, surgically, or genetically induced. It is critical to develop a spontaneous animal model that recapitulates early preeclampsia phenotypes to further investigate the initiating events of this condition. We have presented the African Green Monkey as a novel, spontaneous model of preeclampsia that mimics human pathologies.

PE in the AGM leads to a spontaneous increase in SAP and DAP in the 2nd and 3rd trimesters that declines by 6 weeks postpartum. The animals within this colony were not bred for or selected for inclusion based on a particular phenotype, nor are they fed a diet to induce hypertension. In 62 pregnancies, 57% were preeclamptic. This is 7-11 times higher than the estimated prevalence of human preeclampsia (5-8% of pregnancies) (Ananth, Keyes, and Wapner 2013). This may be due to the current breeding paradigm. This colony consists of 3 large breeding groups with 1 male and 14-22 females. Two of the three breeder males are hypertensive (SAP>140 mmHg). It has previously been shown that spontaneous hypertension in the AGM has a genetic component, with parental blood pressure having a significant effect on the development of hypertension in the offspring (Nancy Kraft-Schreyer 1987b). A paternal contribution to the pathogenesis of PE in humans has also been suggested (Takimoto et al. 1996; Lie 1998). It is possible that there is a paternal contribution to the pathogenesis of preeclampsia in the AGM, and this resulted in a higher percentage of PE animals than expected. However, AGMs still develop PE when bred to a normotensive male, suggesting that this phenotype is determined by more than just paternal blood pressure.

It is also possible that the initial colonization event that brought the AGM to the isolated island nations of Saint Kitts and Nevis (McGuire 1987) created a founder effect that might be involved in the high rate of spontaneous PE in this animal model. Because there is clearly a familial component to PE (Angrimsson 1990; Chesley 1986), it reasons that a founder population with reduced heterogeneity might reveal susceptibility of genes for pathologies such as PE (Ober 2001).

PE in the AGM is not associated with any significant differences in heart rate during pregnancy. Both phenotypes (NT and PE) experienced a decline in heart rate following delivery (2 and 6 weeks postpartum). If an AGM pregnancy progresses like that of humans, we would expect an increase in cardiac output, driven by an increase in both stroke volume and heart rate (Mahendru et al. 2014; Sanghavi and Rutherford 2014). However, we did not see any changes in heart rate during an AGM pregnancy. Cardiac output is still expected to increase to accommodate the growing fetus. The decline in heart rate in the postpartum period recapitulates the physiological changes observed following normal human pregnancies (Groer et al. 2013). We postulate that cardiac output may have been increased through stroke volume during pregnancy, and perhaps the heart rate of the animals' declines postpartum to decrease cardiac output until stroke volume returns to pre-pregnancy values. At the time of the current study, functional myocardial data was not obtained. Previous work from our lab has identified HT male and female AGMs with significant left ventricular hypertrophy. A major ongoing goal that has evolved from this work is to conduct a careful evaluation of myocardial function of both NT and PE females before, throughout, and after pregnancy.

The arterial pressure increase associated with PE is concurrent with a significant 1st trimester weight gain and subsequent 2nd and 3rd trimester reduction in plasma osmolality. This occurs despite no differences in water intake or urine flow during these pregnancy stages. However, we were unable to measure 1st trimester water intake and urine flow at this time.

Previous human and animal studies have implicated vasopressin release in the pathogenesis of preeclampsia (Sandgren JA 2015; Santillan et al. 2014). In the AGM, it is possible that a decreased threshold for stimulation of vasopressin release might lead to 1st trimester increases in water intake, reduction in urine flow rate, and ultimately water retention and exaggerated weight gain. This would explain the large decrease in plasma osmolality and rapid weight gain in the 1st and 2nd trimesters of females expressing PE. Future studies will further investigate water balance in the early stages of pregnancy, which would require timed pregnancies and elimination of the natural troop environment.

PE in the AGM is associated with 2nd and 3rd trimester proteinuria, a well-known marker of reduced renal function and a hallmark of preeclampsia in humans (English, Kenny, and McCarthy 2015; Karumanchi and Granger 2016; Phipps et al. 2019). Elevated arterial pressure during pregnancy likely leads to glomerular damage resulting in protein leakage at the glomerular basement membrane. This should be further investigated by measuring albumin to determine whether the damage is specific to the glomerulus.

Sodium and potassium excretion are unchanged, even as blood pressure increases during the 2nd and 3rd trimester of PE. This increase in SAP should lead to a significant pressure natriuretic response in PE AGMs, but sodium excretion did not change until after delivery. A shift in the renal pressure-natriuresis curve has been shown in the reduced

uterine perfusion pressure rat model of preeclampsia (Gilbert et al. 2008). However, evidence of natriuresis is seen in the PE AGM following delivery, as sodium excretion increases at days 1, 14, and 42 postpartum simultaneous with a decline in SAP and DAP, despite being maintained on the same diet. PE in the AGM could be associated with inhibition of pressure natriuresis during gestation. The mechanisms of this phenomenon are yet to be determined, but a reduction in renal nitric oxide synthesis has been suggested in other PE models (Gilbert et al. 2008).

PE pregnancies resulted in a greater percent of stillbirth in the fetus (29% vs 4% in NT pregnancies). Surviving offspring also weighed less following PE pregnancies regardless of sex. Reduced birth weight and stillbirth percentage could indicate either intrauterine growth restriction or premature delivery. While timed pregnancies in the AGM are difficult, we can accurately identify pregnancies within the first trimester. Experienced staff palpate the abdominal area to measure the length of the fetus from crown to rump, allowing us to estimate the delivery date and backtrack to the timing of conception. This indicates that spontaneous elevation of arterial pressure in the African Green Monkey is likely leading to fetal growth restriction, similar to preeclamptic humans and other induced animal models of preeclampsia (Li, LaMarca, and Reckelhoff 2012; Santillan et al. 2014; Marshall et al. 2018; Rasmussen 2003). Elevated arterial pressure and placental abnormalities could result in reduced nutrient and oxygen supply to the developing fetus. This limits fetal growth, leading to reduced birth weight in offspring. As the animals are maintained on a healthy diet of fruits, vegetables, and standard nonhuman primate chow, this growth restriction is not the result of maternal malnutrition, but likely impaired

uteroplacental perfusion. Future studies will investigate uteroplacental pathologies in PE AGMs.

2.5 Acknowledgements

The authors thank the staff of SKN Primates and Primates Plus, LLC for their expertise in animal care and maintenance; specifically, we would like to thank Brea Williams, Dez Henry, and Khaliq James. We thank Gilbert Gordon of SKN Primates for his technical assistance in animal procurement and building of the highly successful breeding colonies, along with Frances Louard for her significant contribution in identifying pregnancies, performing health exams, and her excellence in animal husbandry. We thank Drs. Justin Grobe, Mark Santillan, and Donna Santillan for their expertise and guidance during these studies. The mentorship, support, and scientific expertise of Dr. Jessica Taylor is gratefully acknowledged. We thank our funding sources, including American Heart Association, SKN Primates and Primates Plus, LLC, along with the University of Kentucky Department of Biology. Finally, we would like to acknowledge the continued support of former and current Osborn lab students, particularly Dr. Megan Rhoads, Dr. Brandon Franklin, Shea Sickles, Maria Venegas, and Patrick Rivera.

Grants and Funding: This work was funded in part by an American Heart Association Predoctoral Fellowship awarded to C. C. Weaver (17PRE33670127). Primates Plus, LLC funded the remainder of this project.

2.6 Figures and Tables

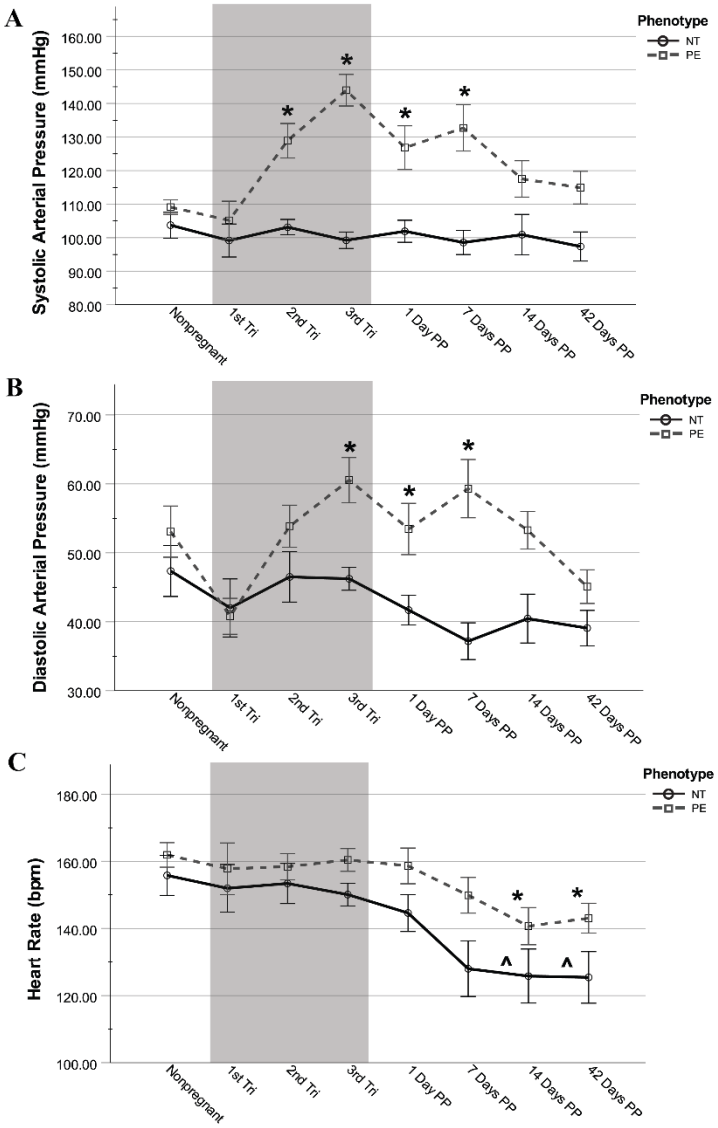

Figure 2-1: Preeclamptic African Green Monkeys have spontaneous elevations in blood pressure during the second and third trimesters

Systolic arterial pressure (SAP; **A**), diastolic arterial pressure (DAP; **B**), and heart rate (HR; **C**) measured via forearm plethysmography for normotensive (NT) and preeclamptic (PE) AGMs prior to pregnancy (NP, n=20 and 29, respectively) in the 1st (n=14 and 11), 2nd (n=19 and 26), and 3rd trimesters (n=23 and 25), and at days 1 (n=20 and 28), 7 (n=11 and 20), 14 (n=11 and 23), and 42 (n=14 and 24) postpartum (PP). * indicates $p<0.05$ versus PE NP with one-way ANOVA; ^ indicates $p<0.05$ versus NT NP with one-way ANOVA. Shading highlights measurements during pregnancy.

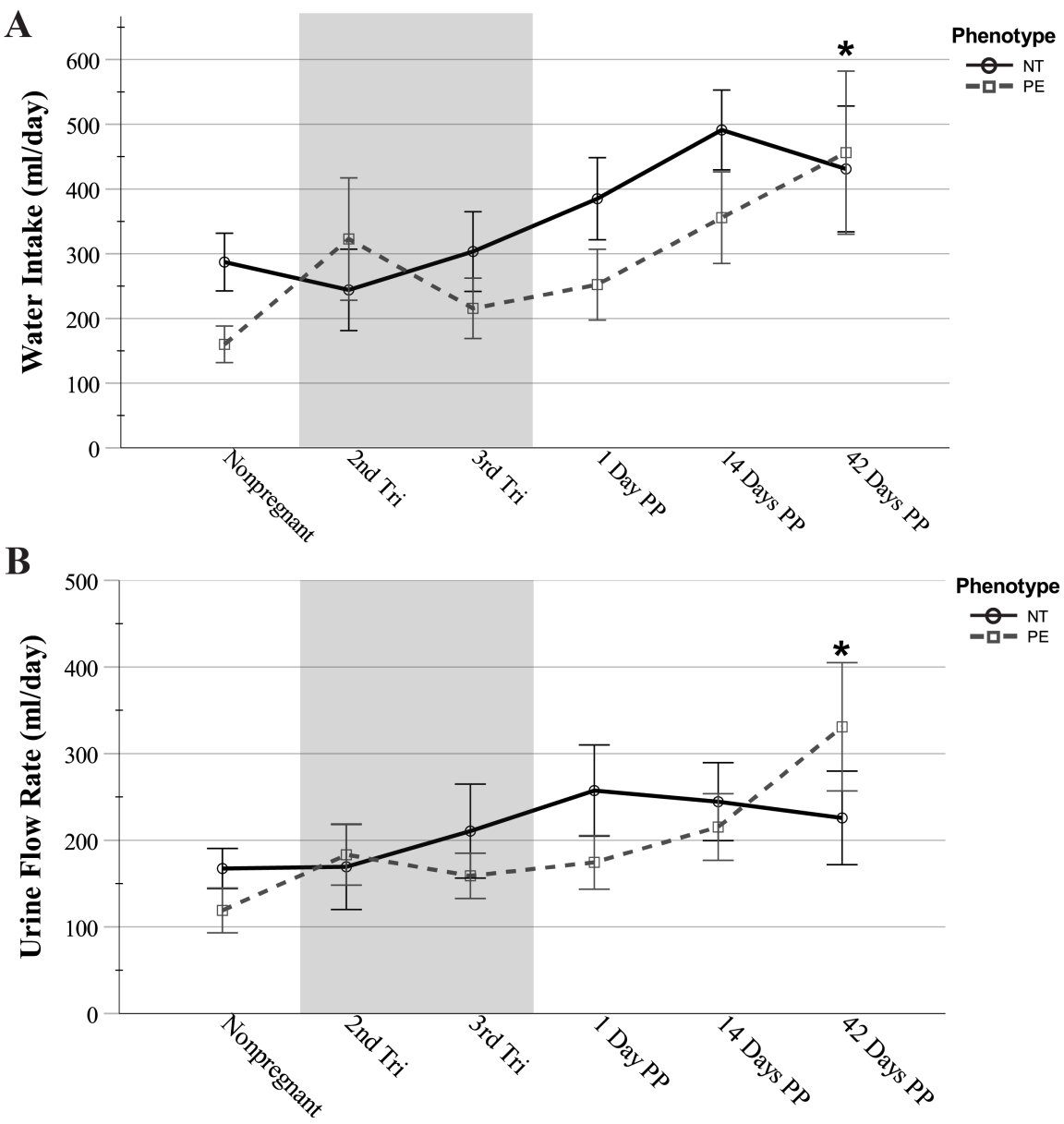

Figure 2-2: Average water intake and urine flow throughout pregnancy and postpartum

Water intake (WI; **A**) and urine flow rate (UFR; **B**) for normotensive (NT) and preeclamptic (PE) African green monkeys prior to pregnancy (NP, n=26 and 17, respectively) in the 2nd (n=9 and 8) and 3rd trimesters (n=8 and 16), and days 1 (n=6 and 10), 14 (n=7 and 10), and 42 (n=5 and 7) postpartum (PP). * indicates $p<0.05$ versus PE NP with one-way ANOVA. WI and UFR did not change in an NT pregnancy or postpartum, but WI and UFR increased at 42 days PP following a PE pregnancy. Values represented as mean ± SEM.

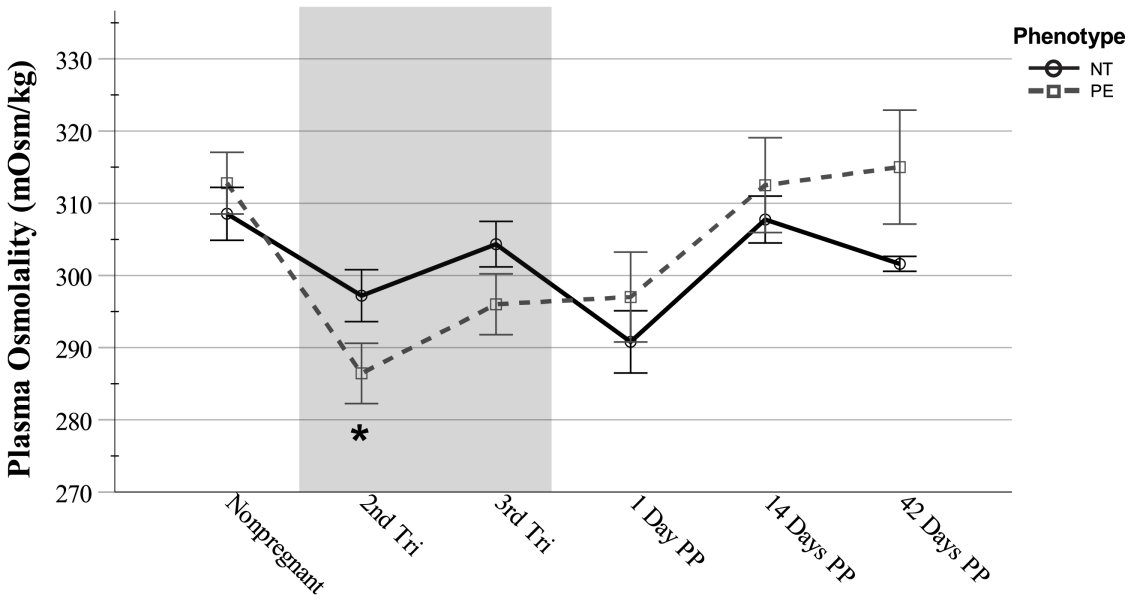

Figure 2-3: Preeclamptic African Green Monkeys have decreased second trimester plasma osmolality

Plasma osmolality measured via freeze-point depression for normotensive (NT) and preeclamptic (PE) African green monkeys prior to pregnancy (NP, n=13 and 8, respectively) in the 2nd (n=5 and 6) and 3rd trimesters (n=6 and 9), and days 1 (n=5 and 11), 14 (n=5 and 8), and 42 (n=5 and 6) postpartum (PP). * indicates $p<0.05$ versus PE NP with one-way ANOVA. Plasma osmolality did not change in an NT pregnancy or postpartum. Plasma osmolality decreased in the 2nd and 3rd trimester of PE pregnancies, remained decreased at 1 day PP, and returned to NP values at 14- and 42-days following delivery. Values represented as mean ± SEM.

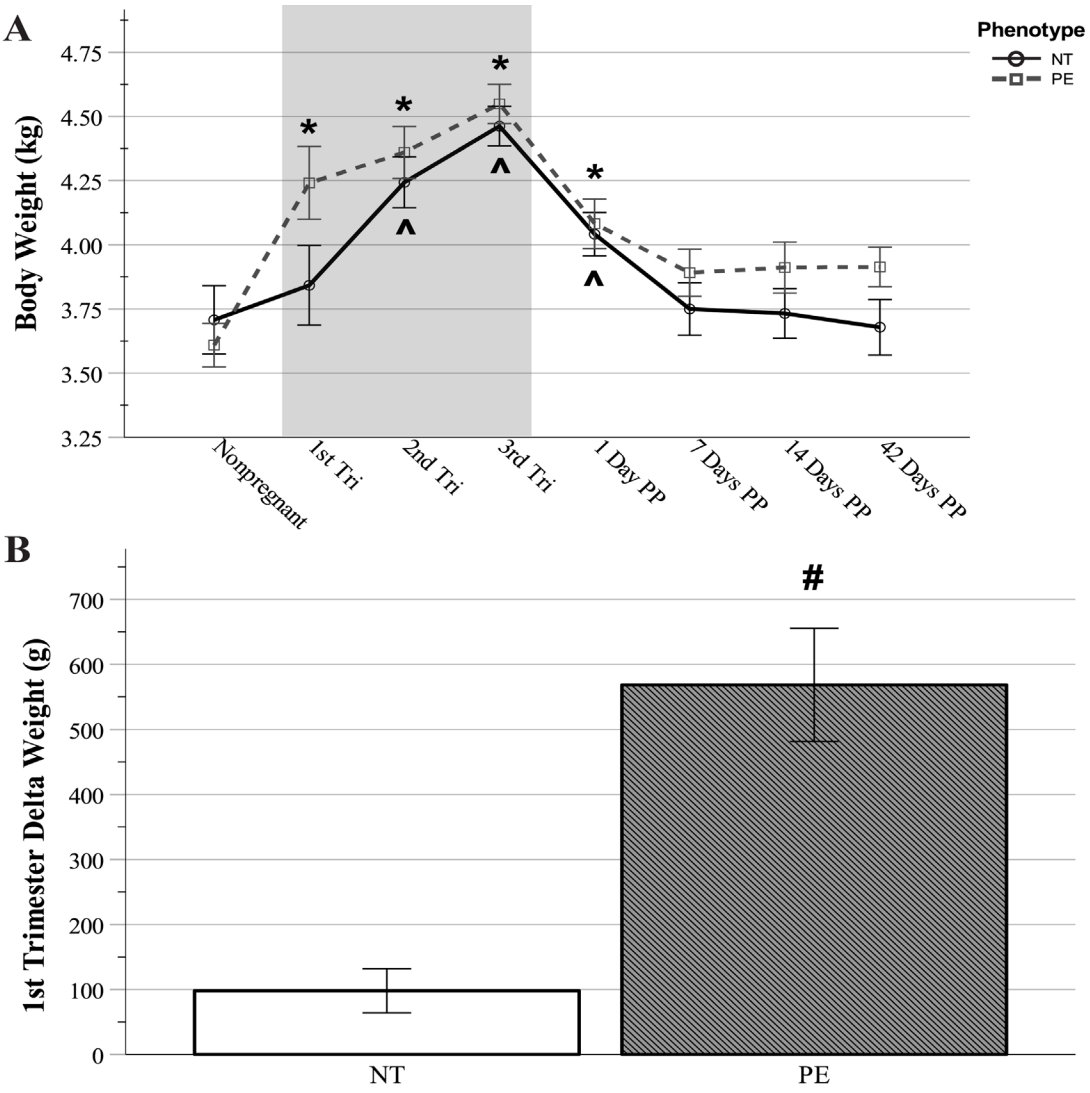

Figure 2-4: Preeclamptic African Green Monkeys gain more weight in the 1st trimester than normotensive monkeys

Total body weight (**A**) for normotensive (NT) and preeclamptic (PE) African green monkeys prior to pregnancy (NP, n=15 and 17, respectively), in the 1st trimester (n=10 and 13), in the 2nd (n=11 and 22) and 3rd trimesters (n=15 and 23), and days 1 (n=15 and 25), 7 (n=10 and 17), 14 (n=9 and 21), and 42 (n=9 and 13) postpartum (PP). * indicates $p<0.05$ versus PE NP with one-way ANOVA and ^ indicates $p<0.05$ versus NT NP with one-way ANOVA. Animals that ultimately developed PE gained an average of 568.5 ± 86.8 g of body weight by the end of the 1st trimester (**B**, # indicates $p<0.05$ compared to NT by two-sample t-test; PE n=12, NT n=10), whereas NT animals' weight did not change until the second trimester. Weight continued to increase for both phenotypes in the 2nd and 3rd trimesters and remained elevated until 7 days postpartum. Values represented as mean ± SEM.

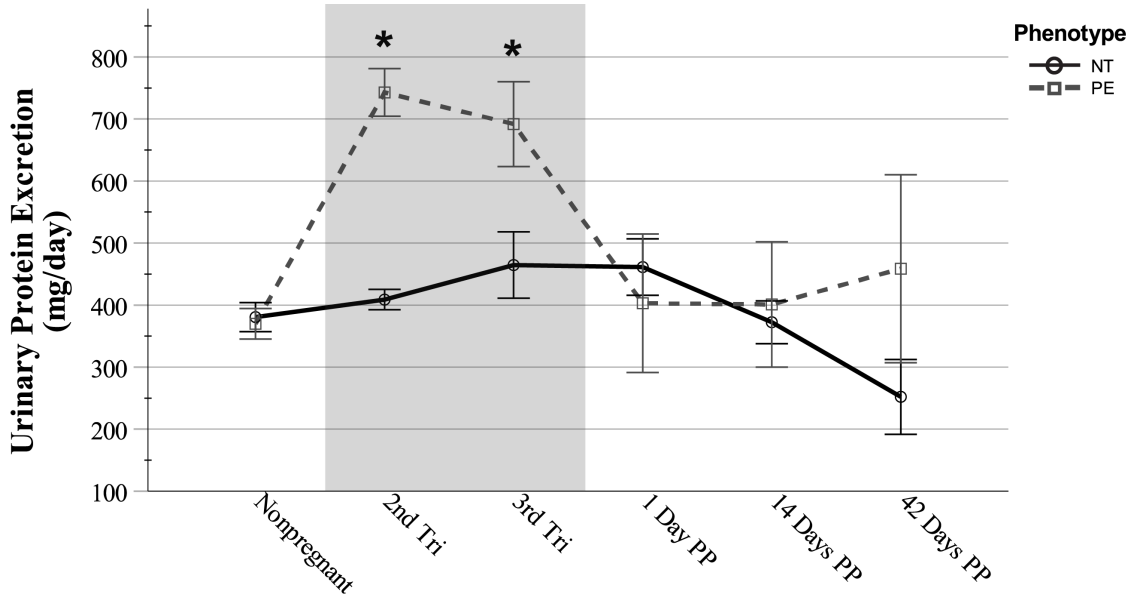

Figure 2-5: Preeclamptic African Green Monkeys have elevated urinary protein excretion in the second and third trimesters

Protein excretion, measured by bicinchoninic acid assay, for normotensive (NT) and preeclamptic (PE) African green monkeys prior to pregnancy (NP, n=26 and 17, respectively) in the 2nd (n=9 and 8) and 3rd trimesters (n=8 and 15), and days 1 (n=6 and 8), 14 (n=7 and 9), and 42 (n=5 and 7) postpartum (PP). * indicates $p<0.05$ versus PE NP with one-way ANOVA. Shading highlights measurements during pregnancy. Protein excretion did not change in an NT pregnancy, but increased in the 2nd and 3rd trimesters of a PE pregnancy, returning to pre-pregnancy values following delivery. Values represented as mean ± SEM.

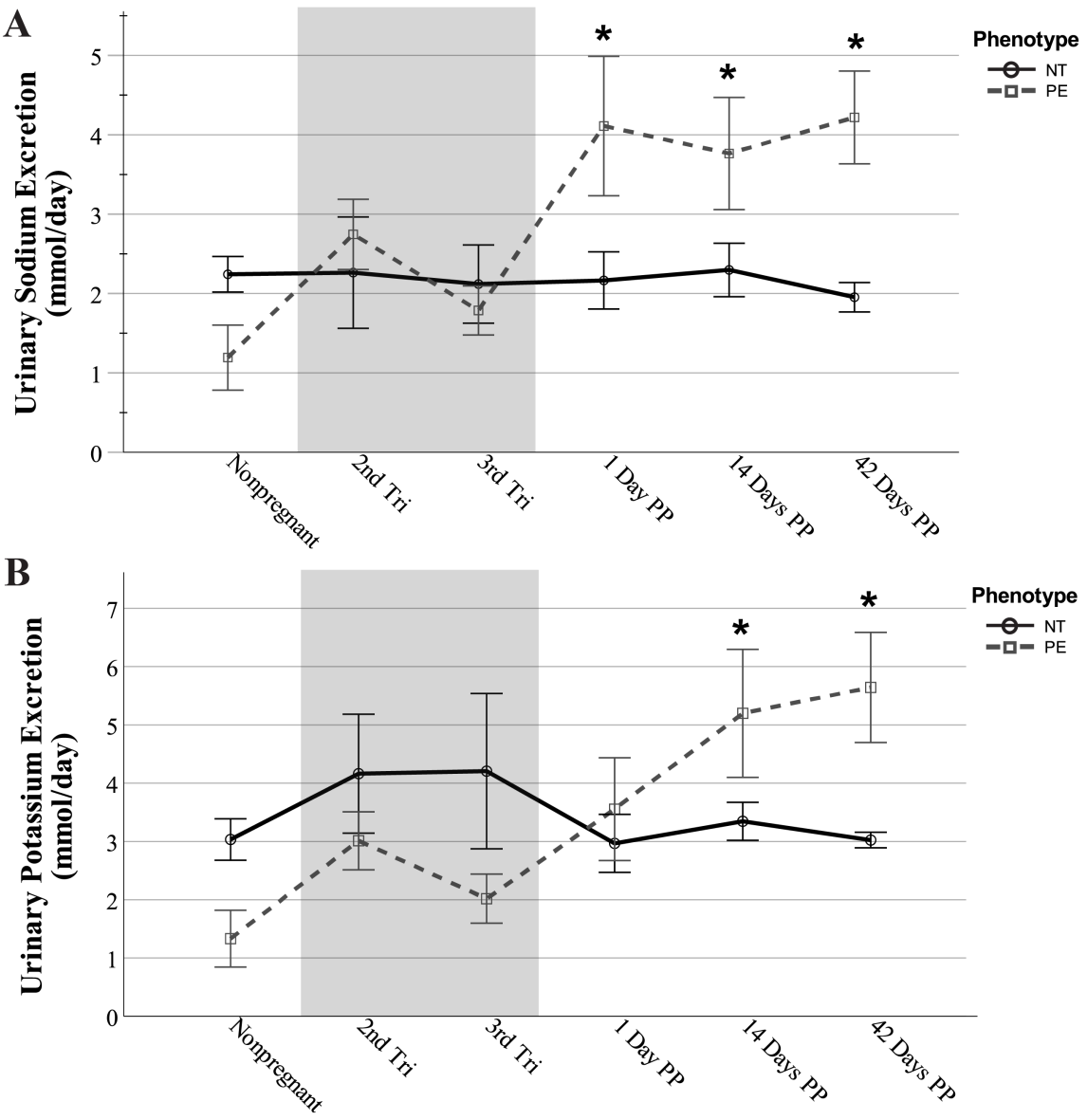

Figure 2-6: Preeclamptic African Green Monkeys have elevated postpartum urinary sodium and potassium excretion

Sodium (**A**) and potassium (**B**) excretion rate measured by flame photometry for normotensive (NT) and preeclamptic (PE) African green monkeys prior to pregnancy (NP, n=26 and 10, respectively) in the 2nd (n=9 and 8) and 3rd trimesters (n=8 and 11), and days 1 (n=6 and 7), 14 (n=7 and 9), and 42 (n=5 and 7) postpartum (PP). * indicates $p<0.05$ versus PE NP with one-way ANOVA. Shading highlights measurements during pregnancy. Sodium and potassium excretion rate did not change in an NT or PE pregnancy, but increased during the postpartum period following a PE pregnancy. This occurs as blood pressure declines. Values represented as mean ± SEM.

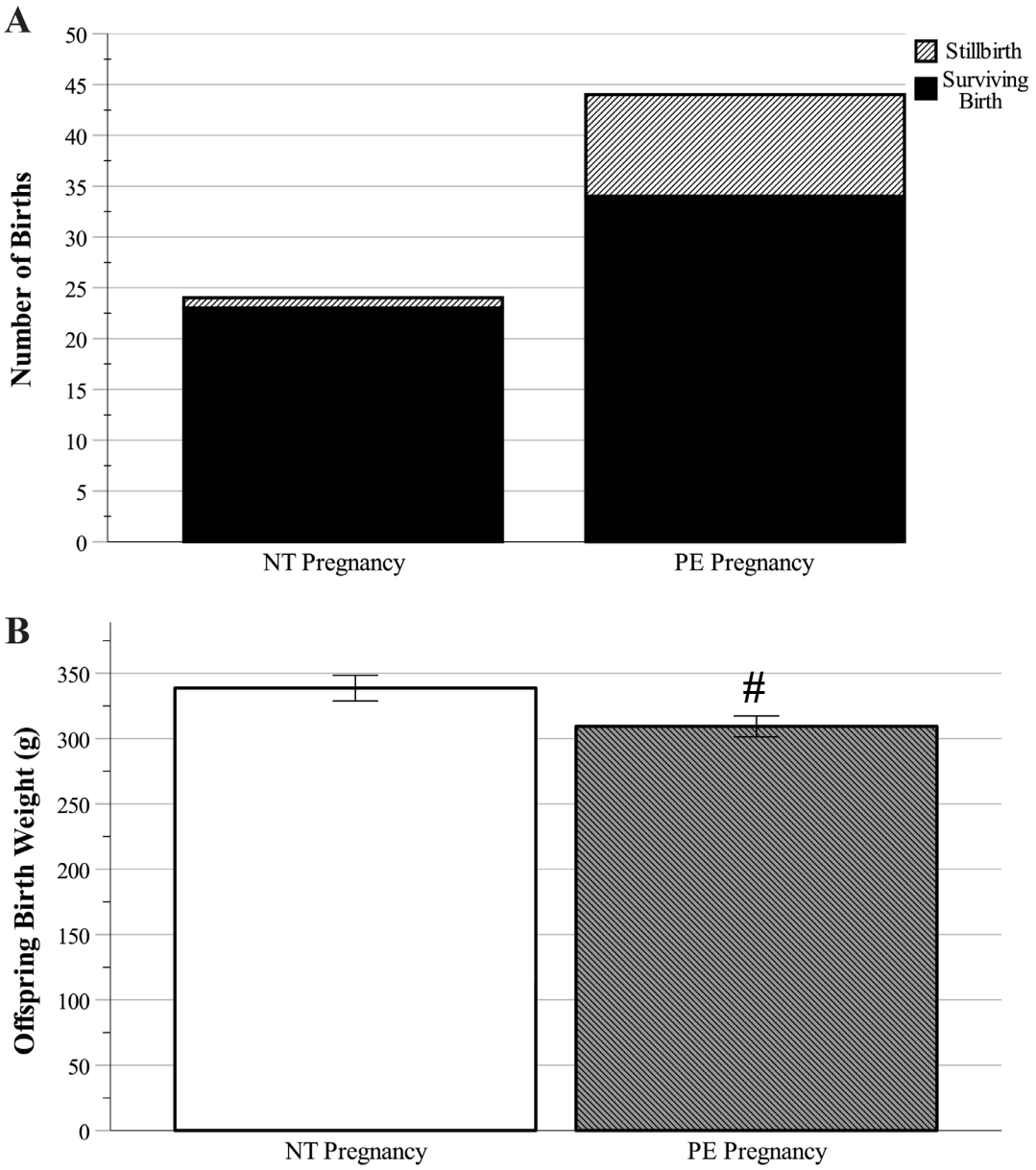

Figure 2-7: Preeclamptic African Green Monkeys have higher rates of stillbirth in offspring and give birth to offspring with reduced birth weight

Number of surviving births in black with stillbirths stacked on top in stripes for offspring born to NT and PE pregnancies (**A**). NT pregnancies resulted in a stillbirth rate of 4% compared to 29% in PE pregnancies. Panel **B** shows the total body weight at birth for offspring born to NT (n=14) and PE (n=20) pregnancies. # indicates $p<0.05$ compared to offspring born to NT pregnancies via two-sample t-test. Together, this indicates fetal growth restriction leading to low-birth weight offspring with higher risk of stillbirth. Values represented as mean ± SEM.

CHAPTER 3: JUVENILE OFFSPRING BORN TO PREECLAMPTIC AFRICAN GREEN MONKEYS HAVE REDUCED GLUCOSE TOLERANCE AND MILD RENAL INSUFFICIENCY

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Submitted: AJP Regulatory, Integrative, and Comparative Physiology

3.1 Introduction

Hypertensive pregnancy disorders are a leading cause of maternal and infant morbidity and mortality worldwide (Sutton, Harper, and Tita 2018). There are four recognized hypertensive pregnancy disorders: gestational hypertension, preeclampsia-eclampsia, chronic hypertension, and chronic hypertension with superimposed preeclampsia (Wilkerson and Ogunbodede 2019). These disorders affect 5-8% of all pregnancies and lead to a high economic burden worldwide (Hao et al. 2019). A large contributor to these growing financial costs is the effect hypertensive pregnancy disorders have on the developing offspring at birth; hypertensive pregnancy disorders can cause reduced uterine blood perfusion, leading to decreased nutrient and oxygen supply for the growing fetus (Bokslag et al. 2016). This results in intrauterine growth restriction which ultimately leads to low birthweight offspring (Bakker et al. 2011).

Preeclampsia is defined as *de novo* hypertension after the 20th week of gestation concomitant with evidence of maternal end-organ damage (Amaral et al. 2017). This damage can occur in the form of new-onset proteinuria, thrombocytopenia, impaired liver function, renal insufficiency, or pulmonary edema (Amaral et al. 2017). Parents who experience preeclampsia have an increased risk for cardiovascular disease and stroke for the remainder of their life (Turbeville and Sasser 2020).

Chronic hypertension, or elevated arterial pressure prior to conception, also leads to increased risk of adverse health outcomes for both mother and offspring (Seely and Ecker 2014). Approximately 15% of chronic hypertensive (CHT) pregnancies result in superimposed preeclampsia, or chronic hypertension with new-onset proteinuria in the latter half of gestation (Bramham et al. 2014). Chronic hypertension in pregnancy is

associated with increased risk for maternal complications such as acute renal failure, pulmonary edema, and stroke (Bramham et al. 2014).

Because hypertension can cause placental ischemia, offspring exposed to hypertensive pregnancies may experience fetal growth restriction as evidenced by having low birth weight or being small-for-gestational-age (SGA) (Proctor et al. 2019). SGA and low birth weight are recognized as indices of fetal and neonatal health that contribute substantially to perinatal mortality (Bukowski et al. 2014). SGA increases the risk of stillbirth up to four-fold and low birth weight infants comprise 60-80% of neonatal deaths worldwide (Bukowski et al. 2014). Exposure to hypertension *in utero* is associated with a lifetime of elevated risk for hypertension, cardiovascular disease, and renal, neurological and metabolic dysfunction (Davis et al. 2012; Imterat et al. 2020; Bokslag et al. 2016).

The reason for this increased disease susceptibility in offspring is not entirely understood. However, Dr. David Barker pioneered the Developmental Origins of Health and Disease theory, which postulates that adverse *in utero* environments could cause alterations in fetal gene expression, leading to changes in stress response and metabolism (Wadhwa et al. 2009). These adverse *in utero* conditions can also lead to reduced fetal nephron number, which is associated with hypertension and reduced renal function in postnatal life (Coats et al. 2019; Moritz et al. 2009; Ruggajo et al. 2016; Wlodek et al. 2008). Ultimately, fetal exposure to hypertensive pregnancy disorders is detrimental to offspring growth and survival. However, the link between these risk factors and hypertensive pregnancy disorders is still not fully understood.

The African Green Monkey (AGM; *Chlorocebus aethiops sabaeus*) is a translational animal model for investigating acute and long-term effects of hypertensive

pregnancy disorders on offspring. This animal model is similar to humans with regards to a hemochorial placenta (Owiti et al. 1986), almost exclusively singleton pregnancies, as well as close evolutionary history (Warren et al. 2015), Therefore, characterization of the effects of maternal hypertension in pregnancy on offspring provides a unique animal model for studying in utero programming of disease susceptibility. We previously reported on the prevalence of spontaneous preeclampsia in adult AGMs (Weaver, Louard, and Osborn 2018). The AGM also develops spontaneous hypertension, as previously characterized (Rhoads et al. 2017). In this paper, we present a chronically hypertensive maternal phenotype in addition to preeclamptic AGMs. This study tested the hypothesis that offspring exposed to preeclampsia *in utero* will exhibit proteinuria, elevated fasting glucose, and insufficient glucose handling as indicated by an oral glucose tolerance test. We also expect that offspring born to preeclamptic and chronically hypertensive pregnancies are more likely to have elevated arterial pressure as they approach sexual maturation.

3.2 Methods

3.2.1 Animal Subjects, Care, and Housing

The Institutional Animal Care and Use Committee (IACUC) of SKN Primates approved all protocols used. Animals were maintained at the SKN Primates/Primates Plus outdoor facility in St. Kitts, West Indies. Animals were fed a combination of nonhuman primate chow (Harland Teklad 8773) (0900) and fresh fruits and vegetables (1400) while provided water *ad libitum*. All wild-caught AGMs were quarantined for a minimum of 45 days followed by a full veterinary health exam prior to entering breeding groups. This included tuberculosis testing and two Ivermectin administrations (0.5 mg/kg s.c.) for parasite elimination. AGMs were housed in breeding groups composed of 1 male and 15-25 females, consistent with their natural troop dynamic. Females were removed group housing, moved to the maternity ward, and singly housed during the second trimester of pregnancy. Females and their offspring then remained in the maternity ward following parturition for approximately 6-7 weeks before returning to group housing. Colony offspring were kept with their natal troop and mother until reaching 6 months of age, the natural weaning time for wild AGMs. At weaning, offspring were removed from their mother's care and placed into a group enclosure with 10-15 juveniles of a similar age.

3.2.2 Blood Pressure Measurements

Blood pressure measurements were performed on mothers and juvenile offspring using forearm plethysmography as previously described (Weaver, Louard, and Osborn 2018). Briefly, overnight-fasted animals were sedated with ketamine (15 mg/kg i.m.) and placed

in a supine position. A doppler scope and inflatable infant cuff were used to listen for Korotkoff sounds indicative of systolic and diastolic arterial pressure (SAP and DAP). A total of seven measurements of SAP and DAP within ≤5% variance were recorded and afterwards heart rate (HR) was recorded using the doppler. All measurements were taken between 0900-1100 to account for circadian variations in blood pressure and heart rate. Maternal blood pressure was measured as previously described (Weaver, Louard, and Osborn 2018); the timepoints for measurement were prior to pregnancy, in each trimester, and at days 1, 7, 14, and 42 postpartum. At the time of blood pressure measurements, offspring were weighed, and crown-to-rump length was recorded. All animals also received an overall health examination. For juvenile offspring, we repeated these measurements twice annually beginning when offspring reached one year of age.

3.2.3 Phenotyping

Offspring were grouped based on their mother's blood pressure phenotype during pregnancy, as previously described (Weaver, Louard, and Osborn 2018). Phenotypic groups include offspring born to normotensive mothers (NT, SAP < 120 mmHg before, during, and after pregnancy), offspring born to pre-eclamptic mothers (PE, maternal SAP < 120 mmHg prior to pregnancy and SAP > 140 mmHg in the 2nd and/or 3rd trimester which returns to < 120 mmHg within 6 weeks following delivery), and offspring born to chronically hypertensive mothers (CHT, SAP ≥ 140 mmHg before, during, and after pregnancy). Data from offspring were also grouped by offspring SAP to investigate the effects of their own blood pressure on the parameters measured (NT SAP < 120 mmHg,

borderline hypertensive, BHT, SAP ≥ 120 mmHg and < 140 mmHg, and HT SAP ≥ 140 mmHg).

3.2.4 Blood and Urine Collection

Venous blood collection occurred under ketamine sedation (15 mg/kg i.m.) with collection into lithium heparin treated tubes (BD Vacutainer 367886). Blood glucose was recorded using a commercial glucometer (OneTouch Ultra) by finger stick after overnight fasting. Animals were housed in individual metabolic cages for a one-week acclimation period prior to urine collection. Following acclimation, urine was collected every 24 hours over 3 consecutive days. Water was provided *ad libitum* in water bottles and daily intake quantified. These collections were repeated annually beginning at one year of age.

3.2.5 Oral Glucose Tolerance Tests

A modified oral glucose tolerance test, the "banana tolerance test", was performed, which allowed for conscious glucose measurements per Crown Biosciences recommended protocol. Pre-adolescent vervets were fasted overnight prior to glucose tolerance studies and blood glucose was measured via finger stick and standard glucometery (OneTouch Ultra). Banana pieces equivalent to 1% of the animal's body weight (equivalent to 1 g glucose/kg body weight) were weighed out and glucose was added to achieve a final concentration of 1.75 g of glucose/kg body weight. Bananas were chosen from the same bunch and of equal ripeness to help control for variations in glucose content. Blood glucose measurements were taken prior to glucose administration (baseline) and at 15, 30, 60, 90,

and 120 minutes after the AGM consumed the entire piece of glucose-loaded banana. Animals remained conscious and upright throughout the protocol and finger pricks were obtained through the openings in the enclosure. Stress to the animals was minimal as animals have been familiarized with staff and handled since birth. Oral-banana glucose tolerance tests were performed on juvenile monkeys between the ages of 1 and 3 years old, which is pre-sexual maturity in the AGM.

3.2.6 Analytical Measurements

After blood collection, plasma and buffy coat were separated through centrifugation. Plasma osmolality was measured via freezing point depression (Precision Instruments Micro-Osmette). Plasma creatinine concentration was measured with a commercial plasma colorimetric creatinine assay kit using the manufacturer's recommended protocol (Cayman Chemical item #700460). A bicinchoninic acid assay was used to measure both plasma and urinary protein concentration per the manufacturer's recommended protocol (Thermo-Fisher Scientific Item #23225). Urinary sodium and potassium ion concentrations were obtained using standard flame photometry (Cole-Parmer Item #UX-02655-10). To obtain urinary protein and ion clearances, the respective urinary product concentration was multiplied by the animals' urine flow rate (UFR; ml/day).

3.2.7 Statistical Analyses

All statistical analyses were performed in IBM SPSS 27. For studies involving glucose handling, time by blood glucose concentration was plotted and area under the curve (AUC)

was calculated in GraphPad Prism. A one-way ANOVA was used to compare offspring from the three presented phenotypes (born to NT, PE, or CHT pregnancies). A one-way ANOVA was also used to evaluate interactions of each parameter with offspring phenotype (based on offspring SAP and grouped as follows: NT SAP <120 mmHg, HT SAP >140 mmHg, and BHT SAP between 120-140 mmHg) and to evaluate the effect of offspring sex on each parameter. A two-way ANOVA was used when analyzing glucose handling over time per phenotype in oral glucose tolerance studies. All ANOVAs with a significant p-value included a Tukey's post-hoc analysis to investigate interactions using a 0.05 alpha level cut-off for statistical significance criteria. Values are reported as mean ± S.E.M.

3.3 Results

We previously published phenotypic data on normotensive and preeclamptic mothers (Weaver, Louard, and Osborn 2018). Chronically hypertensive mothers remained hypertensive from pre-pregnancy (SAP: 154.8 ± 3.3 mmHg, n=17), through gestation (1st trimester: 146.3 ± 7.4 mmHg, n=6; 2nd trimester: 153.6 ± 6.5 mmHg, n=13; 3rd trimester: 146.2 ± 8.8 mmHg, n=10), and during the six-week postpartum period (1 day PP: 142.6 ± 13.1 mmHg, n= 12; 7 days PP: 135 ± 16.4 mmHg, n=7; 14 days PP: 162.4 ± 14.2 mmHg, n=9; 42 days PP: 139.44 ± 7.3 mmHg, n=9; p=0.59; **Figure 3.1**).

3.3.1 Effect of Maternal Phenotype on Offspring Survival and Birth Weight

We previously reported (Weaver, Louard, and Osborn 2018) that offspring born to preeclamptic mothers had reduced birth weight compared to those born to normotensive mothers (**Table 1**). These data also showed that preeclamptic pregnancies resulted in a higher stillbirth rate (NT: 1/27 births vs PE: 10/35 births).

Offspring born to CHT mothers have similar birth weights to those born to normotensive mothers (**Table 3.1**). CHT pregnancies resulted in 2 stillbirths out of 17 total births. For all studies beyond birth weight and stillbirth rate, average age of offspring was 1.6 ± 0.1 years (NT group: 1.4 ± 0.6 years; PE group: 1.8 ± 0.6 years; CHT: 1.7 ± 0.9 years; p=0.50).

3.3.2 Effect of Maternal Phenotype on Offspring Water Balance

Water intake was similar among offspring born to NT, PE, and CHT pregnancies (NT: 193.5 ± 28.2 ml/day, n=11; PE: 199.7 ± 29.5 ml/day, n=11; CHT: 177.6 ± 27.3 ml/day, n=9; p=0.86; **Figure 3.2A**). There was no effect of sex on these data (**Figure 3.2B**; p=0.84). Urine flow rate was also similar between all three groups (NT: 85.3 ± 19.6 ml/day, n=11; PE: 87.7 ± 17.5 ml/day, n=11; CHT: 63.1 ± 15.0 ml/day, n=9; p=0.59; **Figure 3.2C**). Sex had no effect on urine flow rate (**Figure 3.2D**; p=0.57).

3.3.3 Effect of Maternal Phenotype on Offspring Blood Pressure and Heart Rate

Offspring systolic arterial pressure was similar regardless of maternal phenotype (NT: 108.2 ± 6.3 mmHg, n=11; PE: 116.1 ± 7.2 mmHg, n=17; CHT: 114.9 ± 11.0 mmHg, n=9; p=0.76 overall; **Figure 3.3A**). There was an effect between maternal phenotype and offspring sex, where females born to CHT mothers have higher SAP compared to males born to CHT mothers (NT males: 109.6 ± 8.9 mmHg n=8; NT females: 108.0 ± 14.6 mmHg n=3; PE males: 116.9 ± 8.9 mmHg n=8; PE females: 108.3 ± 8.4 mmHg n=9; CHT males: 96.3 ± 10.3 mmHg n=6; CHT females: 152.0 ± 14.6 mmHg n=3 p=0.02 vs CHT males; **Figure 3.3B**)

While diastolic arterial pressure did not reach significance, there was a trend towards an elevated DAP in offspring born to PE and CHT pregnancies (NT: 42.1 ± 3.5 mmHg, n=11; PE: 56.1 ± 4.1 mmHg, n=17, p=0.05 vs NT; CHT: 57.9 ± 4.9 mmHg, n=9, p=0.06 vs NT; **Figure 3.3C**). There was no effect of sex on DAP (**Figure 3.3D**).

Offspring heart rate was similar regardless of maternal phenotype (NT: 174.6 ± 5.6 bpm, n=11; PE: 187.5 ± 7.5 bpm, n=17; CHT: 184.0 ± 8.9 bpm, n=9; p=0.46 overall; **Figure 3.4A**). There was no effect of sex on these data (**Figure 3.4B**)

3.3.4 Effect of Maternal Phenotype on Offspring Glucose Handling

Fasting glucose was measured in juvenile AGMs between the ages of 1 and 3 years old. Offspring born to mothers with preeclampsia trended towards higher fasting glucose in pre-adolescence compared to those born to normotensive mothers, but that value did not reach significance (NT: 71.6 ± 4.8 mg/dL, n=12; PE: 89.1 ± 6.4 mg/dL*, n=15; CHT: 71.1 ± 3.2 mg/dL, n=10; *p=0.06 vs NT; **Figure 3.5A**). There was no effect of offspring sex on these data (**Figure 3.5B**).

Based on these results, oral glucose tolerance tests were performed on these pre-adolescent monkeys (**Figure 3.6A and 3.6B**). The area under the curve (AUC) was calculated as an index of glucose handling over time. Offspring born to PE mothers had a higher AUC compared to those born to NT and CHT pregnancies (NT: 11305 ± 497 min*mg/dL, n=10; PE: 15013 ± 1703 min*mg/dL, n=11; CHT: 10954 ± 523 min*mg/dL, n=8; p=0.04; **Figure 3.7A**). Offspring sex had no effect on AUC (**Figure 3.7B**).

3.3.5 Effect of Maternal Phenotype on Offspring Urinary Protein, Sodium, and Potassium Excretion

Three-day average protein excretion rate in offspring was measured using the same cohort as in the glucose tolerance studies. Offspring born to PE pregnancies had higher protein

excretion rate compared to those born to NT and CHT pregnancies (NT: 166.2 ± 28.1 mg/day, n=10; PE: 354.7 ± 71.6 mg/day, n=11; CHT: 160.3 ± 13.7 mg/day, n=9; p=0.01; **Figure 3.8A**). Offspring sex had no effect on protein excretion rate (**Figure 3.8B**).

Three-day average sodium and potassium excretion rates were also measured in these same offspring. Sodium excretion was not different among maternal phenotypic groups (NT: 15.2 ± 3.5 mmol/day, n=9; PE: 18.0 ± 2.4 mmol/day, n=11; CHT: 12.8 ± 2.4 mmol/day, n=10; p=0.42; **Figure 3.9A**). Offspring sex had no effect on sodium excretion (**Figure 3.9B**). However, potassium excretion was higher in offspring born to PE pregnancies compared to those born to NT and CHT pregnancies (NT: 2.6 ± 0.4 mmol/day, n=9; PE: 5.9 ± 0.9 mmol/day, n=11; CHT: 3.3 ± 0.5 mmol/day, n=10; p=0.01; **Figure 3.9C**). Offspring sex had no effect on potassium excretion rate (**Figure 3.9D**).

3.3.6 Effect of Maternal Phenotype on Offspring Plasma Protein Concentration and Plasma Osmolality

Plasma protein concentration was measured in the offspring and no differences were found among phenotypes (**Table 3.2**; p=0.78). Plasma osmolality was also similar among phenotypes (**Table 3.2**; p=0.51).

3.4 Discussion

We previously reported that preeclampsia (PE) in the AGM is associated with both a reduced birth weight and a high stillbirth rate (Weaver, Louard, and Osborn 2018). PE offspring have nearly an 8% reduction in birth weight and more than a seven-fold higher stillbirth rate than offspring born to normotensive (NT) pregnancies. Here, we have shown that offspring born to chronically hypertensive (CHT) pregnancies have similar birth weights as those born to NT pregnancies. Stillbirth rate, though, was higher with CHT pregnancies compared to NT, but not PE pregnancies (**Table 3.1**). While PE in the AGM is associated with reduced birth weight, offspring born to mothers with CHT seem to have similar fetal growth as those born to NT mothers. The reason for this difference could be due to placental pathologies and fetal growth restriction associated specifically with PE.

The placenta is a vital organ for maternal-fetal circulation, providing oxygen and nutrients to the growing fetus. Abnormal placentation or dysfunction of the developed placenta can lead to a compromised maternal-fetal interface and maternal endothelial dysfunction (Redman 2005; Brosens et al. 2011). This can in turn cause a variety of adverse outcomes for the developing fetus. Placental dysfunction is a main contributor to the pathogenesis of PE, leading to a reduction in uteroplacental flow and contributing to fetal growth restriction (Rana et al. 2019). However, it has been previously shown that CHT in pregnancy is not always associated with significant placental abnormalities (Stanek 2017). Thus, the low birth weights observed with PE, but not CHT, in the AGM suggests that exposure to maternal hypertension alone may not be a severe enough insult to cause significant adverse outcomes for the fetus compared to PE. This highlights the possibility that PE in the AGM has distinct, or more severe, placental dysfunction than CHT. Future

studies are required to fully investigate the placental morphology in AGMs with PE or CHT.

Low birth weight and exposure to preeclampsia are associated with later risk of metabolic and renal dysfunction (Thoulass et al. 2016; Coats et al. 2019). Dr. David Barker first posed the Developmental Origins of Health and Disease, the hypothesis for transgenerational transmission of disease susceptibility, in the 1980s (Wadhwa et al. 2009). According to Barker, fetal malnutrition could cause increased disease susceptibility in later life. This reduced nutrient availability can arise directly from maternal malnutrition or indirectly from poor maternal-fetal nutrient transfer. Reduced uteroplacental perfusion is a hallmark of preeclamptic pregnancies and numerous studies have shown altered placental nutrient transporter expression associated with its pathogenesis (Janzen et al. 2013; Yan et al. 2016). This reduced nutrient and oxygen delivery during organogenesis has been shown to program offspring for impaired glucose metabolism later in life (Gatford et al. 2010). Multiple studies have shown that offspring exposed to PE are also more susceptible to impaired renal function later in life (Turbeville and Sasser 2020; Moritz et al. 2009). In humans and other animal models, offspring consequences of preeclampsia include reduced nephron number, decreased kidney size, and ultimately increased risk for multiple renal diseases, especially in cases of fetal growth restriction (Sehgal et al. 2020; Wlodek et al. 2008; Coats et al. 2019; Intapad et al. 2019; Moritz et al. 2009). This nephron deficit affects excretory capacity and can lead to hypertension. Indeed, previous studies have suggested that low birth weight is associated with increased postnatal systolic and diastolic arterial pressure (Davis et al. 2012; Wlodek et al. 2008; Coats et al. 2019; Intapad et al. 2019). The

reason for this is likely due to fetal compensatory mechanisms to ensure survival amidst hostile *in utero* conditions such as those present during PE.

Offspring born to PE pregnancies in this study trended towards having higher fasting blood glucose compared to those born to NT pregnancies, but this value did not reach significance (p=0.05). When given an oral glucose challenge, offspring born to PE pregnancies had a higher area under the curve (AUC) for blood glucose over time compared to those born to NT mothers. This suggests that pathologies specific to preeclampsia in the AGM lead to impaired glucose tolerance in offspring even prior to sexual maturation. However, a limitation to this study was use of a novel Banana Tolerance Test method, whereby glucose is delivered to conscious offspring via a pre-weighed piece of banana. The sugar content of bananas can vary, making precision difficult. However, this method is more appropriate for the AGM as it allows the animals to remain conscious during the test and any associated error should be spread across phenotypes. Due to the nature of the conscious glucose tolerance test, we were unable to draw plasma samples for measuring insulin at this time, but future studies will include this analysis.

Water intake and urine flow were both similar regardless of maternal phenotype during pregnancy or offspring sex. Plasma osmolality in these animals was also similar among phenotypes. Currently, it appears that hypertension in AGM pregnancies, whether PE or CHT, does not lead to altered water intake, urine flow, or plasma osmolality in prepubescent AGMs. Taken together, this suggests that water balance is similar regardless of offspring sex or maternal blood pressure during pregnancy. Three-day sodium, potassium, and protein excretion in these offspring were also measured. While there was no difference in sodium excretion among offspring, those born to PE pregnancies exhibited

significant increases in three-day potassium excretion compared to offspring from NT and CHT pregnancies. Offspring born to PE pregnancies also had higher protein excretion rates compared to those born to NT pregnancies, while those born to CHT pregnancies had similar protein excretion to the NT group.

While nephrogenesis in the AGM is not yet well documented, in humans and other nonhuman primates, kidney development primarily occurs in the 3rd trimester when PE is most severe (Batchelder et al. 2013; Batchelder et al. 2010; Batchelder et al. 2009). In this animal model, fetal growth restriction during nephrogenesis may be programming these offspring for poor endothelial function, resulting in proteinuria. Potassium excretion was also higher in the PE offspring, but not offspring born to NT or CHT mothers. Alterations in aldosterone secretion could explain this kaliuresis, though this would typically be concomitant with reduced sodium excretion. However, at their current ages, the offspring to PE pregnancies do not exhibit alterations in sodium excretion, indicating that renal function may only be mildly altered, particularly with regards to endothelial function. Exposure to PE is known to alter offspring sodium excretory capacity which ultimately increases blood pressure (Yeung et al. 2018). It is possible that these offspring are in early stages of renal insufficiency whereby they may be reabsorbing some excess sodium, leading to increased potassium excretion. It will be important to monitor these animals as they reach sexual maturity to better understand this imbalance in excretory load. Insulin is a potent stimulator of potassium influx into cells (Palmer and Clegg 2016). The PE offspring have kaliuresis concomitant with reduced glucose tolerance; this may suggest that PE offspring have reduced insulin activity, leading to glucose intolerance and an outward cellular potassium shift . This hyperkalemia would result in kaliuresis to return

plasma potassium concentration to normal values. At this time, we have not assessed plasma potassium or insulin levels in the offspring. Future studies include investigating renal morphology, plasma electrolyte concentration, plasma insulin, and circulating aldosterone levels in offspring born to NT, PE, CHT pregnancies.

At this time, offspring born to CHT mothers do not have altered glucose metabolism or renal endothelial dysfunction. Because placental morphology varies among hypertensive pregnancy disorders, it is possible that maternal-fetal nutrient transfer is only hindered in the PE model. This is supported by the difference in birth weight and stillbirth rate between CHT and PE pregnancies. Offspring born to CHT mothers weigh more than those born to PE mothers and they have a higher live birth rate. This suggests that fetuses exposed to CHT pregnancies do not undergo the same growth restriction as those from PE pregnancies. Therefore, offspring of CHT pregnancies also may not undergo the same genetic adaptations to adverse *in utero* conditions as proposed with PE pregnancies. Our lab plans to investigate placental morphology in future studies to better understand maternal-fetal nutrient delivery in PE and CHT animals and subsequent metabolic and renal consequences.

Blood pressure and heart rate were also measured in these offspring. Systolic arterial pressure (SAP) and heart rate were similar regardless of maternal phenotype during pregnancy. Diastolic arterial pressure (DAP) trended towards an increase in offspring born to PE and CHT pregnancies. When taking offspring sex into account, females born to CHT mothers had higher systolic arterial pressure compared to males from the same cohort, but there was no difference when compared to females from NT pregnancies. This could reflect sample size, as there were only 3 female offspring from CHT pregnancies, all of which had

SAP ranging from 130-180 mmHg. As the colony grows, future studies will include increased sample sizes and post-pubescent offspring measurements. This should reveal a better understanding of sex differences in the pathogenesis of hypertension relative to maternal phenotype in pregnancy. However, with CHT mothers, there is growing evidence for genetic contributions to hypertension that must be considered. In the AGM in particular, Kraft-Schreyer characterized a parent-offspring transmission of elevated blood pressure as early as one year of age (Nancy Kraft-Schreyer 1987a; Kraft-Schreyer N 1991). The trend towards elevated DAP of both sexes and the elevated SAP in females born to CHT mothers indicates that maternal phenotype may influence offspring susceptibility to hypertension in the AGM. However, it is currently unknown whether this effect is due to genetics, adverse intrauterine conditions, or a combination.

At this time, there were no other sex differences and no effect of the offspring's blood pressure on any of the reported outcomes. However, a limited sample size with multiple groups does reduce the power of the study and future work will include more offspring as the colony grows. Our lab plans to reevaluate blood pressure, including any sex differences, in these offspring following puberty.

3.5 Perspectives and Significance

This study characterizes the offspring of a novel, highly translational non-human primate model of spontaneous preeclampsia and spontaneous chronic hypertension in pregnancy. The data presented show that preeclampsia in the African Green Monkey leads to offspring with reduced glucose tolerance and proteinuria without hypertension.

Characterization of the maternal and offspring phenotypes allows for a spontaneous, translational animal model for investigation of structural and physiological alterations causing long-term risk in offspring. Having two maternal phenotypes of hypertension in pregnancy also allows for better understanding of how each phenotype uniquely contributes to offspring risk factors later in life. However, the changes observed occur in offspring prior to puberty and mechanisms mediating these changes in adults may differ. Further studies are needed to clarify the effects of spontaneous preeclampsia and chronic hypertension on offspring, particularly following sexual maturation, including any sex differences that may occur. However, this animal model does provide a very unique opportunity to study the effects of spontaneous, rather than induced, preeclampsia, both on mother and offspring. This study characterized glucose tolerance and renal function in juvenile offspring born to preeclamptic pregnancies, highlighting stark similarities between human and AGM preeclamptic consequences on offspring.

3.6 Acknowledgements

We thank the phenomenal staff of SKN Primates and Primates Plus, LLC for their expertise in animal care and maintenance; Brea Williams, Dez Henry, and Khaliq James were integral in daily animal management. We acknowledge the critical contributions of Frances “Dora” Lourdes in breeding animals, health examinations, and expertise in animal husbandry. We gratefully acknowledge Gilbert “Sully” Gordon of SKN Primates for animal procurement, ground maintenance, and building highly successful breeding colonies. We wish to thank our funding sources; SKN Primates and Primates Plus, LLC, solely funded the work laid out in this study, and we thank University of Kentucky Department of Biology Gertrude F. Ribble foundation for funding travel. This work would not have been possible without the continued support of former and current Osborn lab members, Dr. Megan Rhoads, Dr. Brandon Franklin, Maria Venegas, Patrick Rivera, and Lucas Barrett.

Grants and funding: No grants were awarded to cover this study. All funding was provided by Primates Plus, LLC.

3.7 Figures and Tables

Table 3-1
Offspring birth weight and stillbirth rate

Phenotype	**Birth Weight (g)**	**Stillbirth Rate**
NT (n=14)	335.6 ± 10.0	4% (1 in 27)
PE (n=20)	309.3 ± 8.4*	29% (10 in 35)
CHT (n=11)	328.3 ± 13.0	12% (2 in 17)

*p<0.05 vs NT

African Green Monkey birth weight and stillbirth rate for offspring born to normotensive (NT), preeclamptic (PE), and chronic hypertensive (CHT) pregnancies. Offspring born to PE African Green Monkeys had lower birth weight compared to those born to NT mothers. *p<0.05 denotes significant difference vs. NT group. Values represented as means ± S.E.M.

Table 3-2
Juvenile plasma osmolality and protein concentration

Phenotype	**Plasma Osmolality (mOsm/kg)**	**[Plasma Protein] (g/dL)**
NT (n=8)	324.3 ± 5.	6.0 ± 0.2
PE (n=10)	323.1 ± 4.7	6.1 ± 0.1
CHT (n=11)	317.2 ± 3.7	5.9 ± 0.2

Offspring plasma osmolality as measured by freezing point depression and plasma protein concentration measured by BCA assay. All values were similar regardless of maternal phenotype or sex. Values represented as means ± S.E.M.

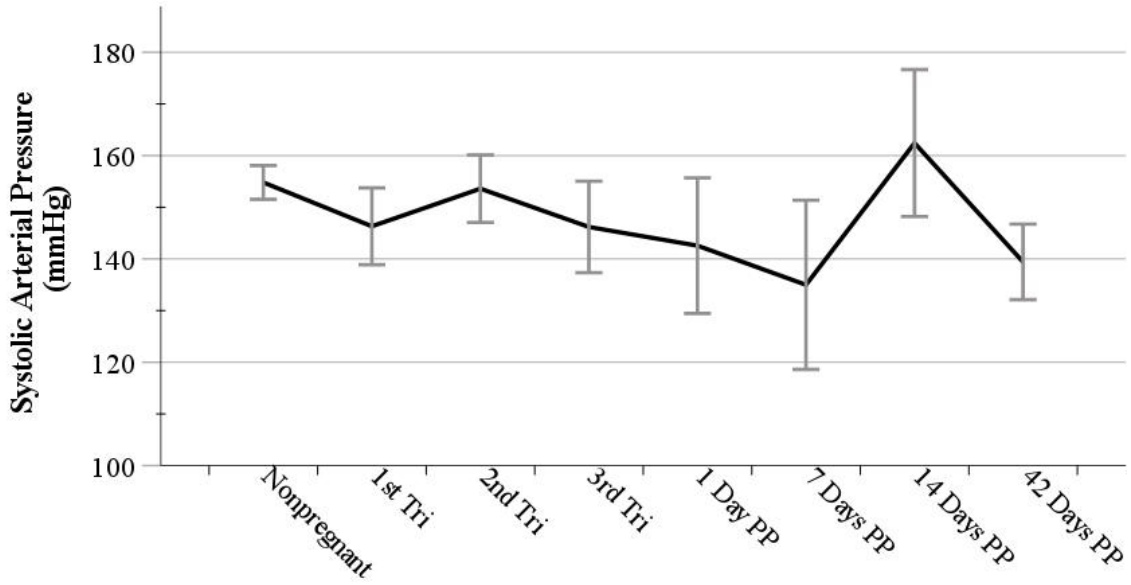

Figure 3-1: Systolic arterial pressure for chronic hypertensive African Green Monkeys throughout pregnancy and postpartum

Systolic arterial pressure measured via forearm plethysmography for chronic hypertensive (CHT) female African Green Monkeys prior to pregnancy (nonpregnant, n=17), in the 1st (n=6), 2nd (n=13), and 3rd trimesters (n=10), and at days 1 (n=12), 7 (n=7), 14 (n=9), and 42 days (n=9) postpartum. SAP remained unchanged in CHT pregnancies and the 6-week postpartum period (p=0.59). Values represented as means ± S.E.M.

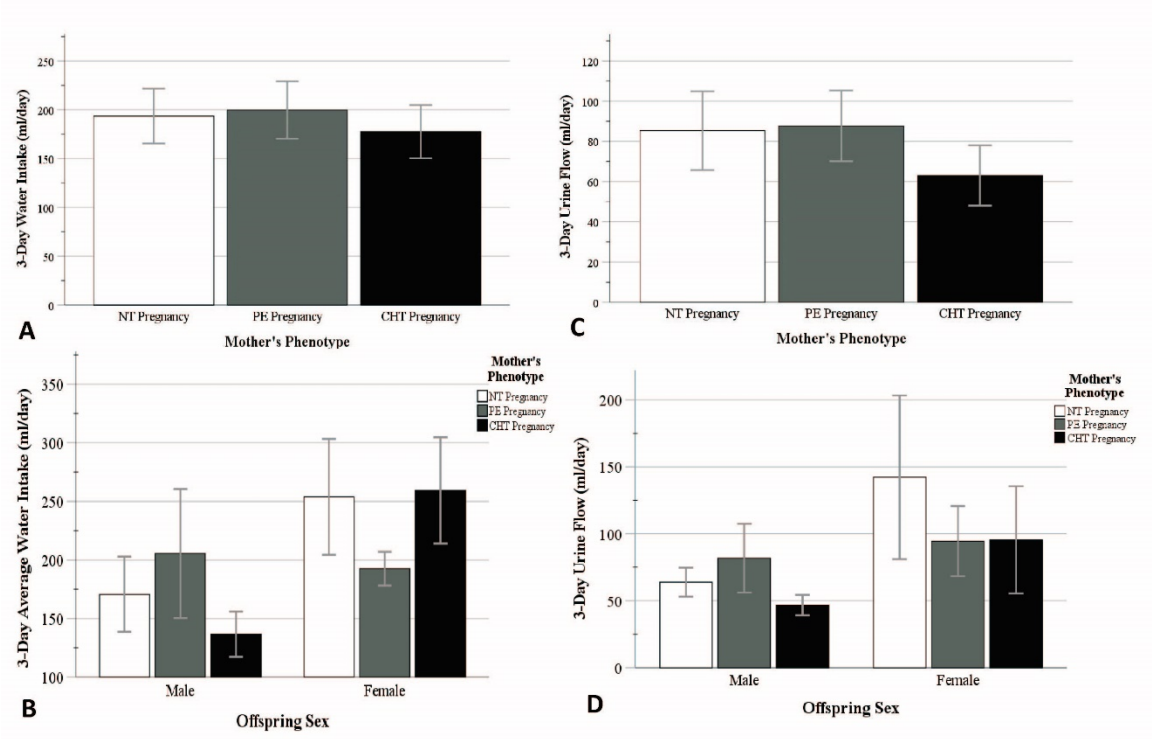

Figure 3-2: Water intake and urine flow are similar regardless of maternal phenotype or offspring sex

A) 3-day average water intake for juvenile offspring born to normotensive (NT; n=11), preeclamptic (PE; n=11), and chronic hypertensive (CHT; n=9) pregnancies. B) 3-day average water intake for juvenile male and female offspring born to NT (n=8 and n=3, respectively), PE (n=6 and n=5), and CHT (n=6 and n=3) pregnancies. These values were similar regardless of maternal phenotype or offspring sex. C) 3-day urine flow rate for juvenile offspring born to normotensive (NT; n=11), preeclamptic (PE; n=11), and chronic hypertensive (CHT; n=9) pregnancies. D) 3-day average urine flow rate for juvenile male and female offspring born to NT (n=8 and n=3, respectively), PE (n=6 and n=5), and CHT (n=6 and n=3) pregnancies. These values were similar regardless of maternal phenotype or offspring sex. Values represented as means ± S.E.M.

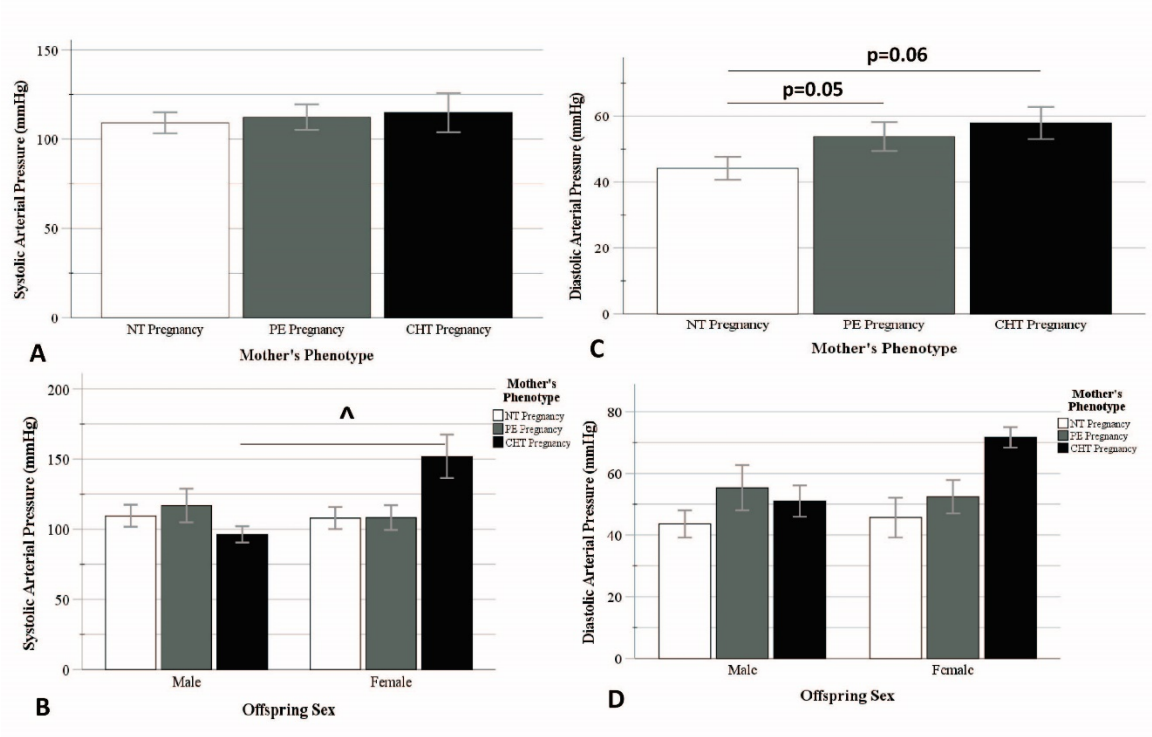

Figure 3-3: Average systolic and diastolic arterial pressure by maternal phenotype and offspring sex

A) Average systolic arterial pressure (SAP) measured by forearm plethysmography for juvenile offspring born to normotensive (NT; n=11), preeclamptic (PE; n=17), and chronic hypertensive (CHT; n=9) pregnancies. B) Average SAP for male and female juvenile offspring born to NT (n=8 and n=3, respectively), PE (n=8 and n=9), and CHT (n=6 and n=3) pregnancies. Maternal phenotype had no effect on offspring SAP overall. However, female offspring born to CHT pregnancies have elevated SAP compared to those born to male counterparts. C) Average diastolic arterial pressure (DAP) measured by forearm plethysmography for juvenile offspring born to normotensive (NT; n=11), preeclamptic (PE; n=17), and chronic hypertensive (CHT; n=9) pregnancies. B) Average DAP for male and female juvenile offspring born to NT (n=8 and n=3, respectively), PE (n=8 and n=9), and CHT (n=6 and n=3) pregnancies. Maternal phenotype had no effect on offspring DAP overall. However, there was a trend to elevated DAP in PE (p=0.05) and CHT (p=0.06) offspring. Offspring sex had no effect on DAP. Values represented as means ± S.E.M. ^ indicates $p<0.05$ vs CHT males.

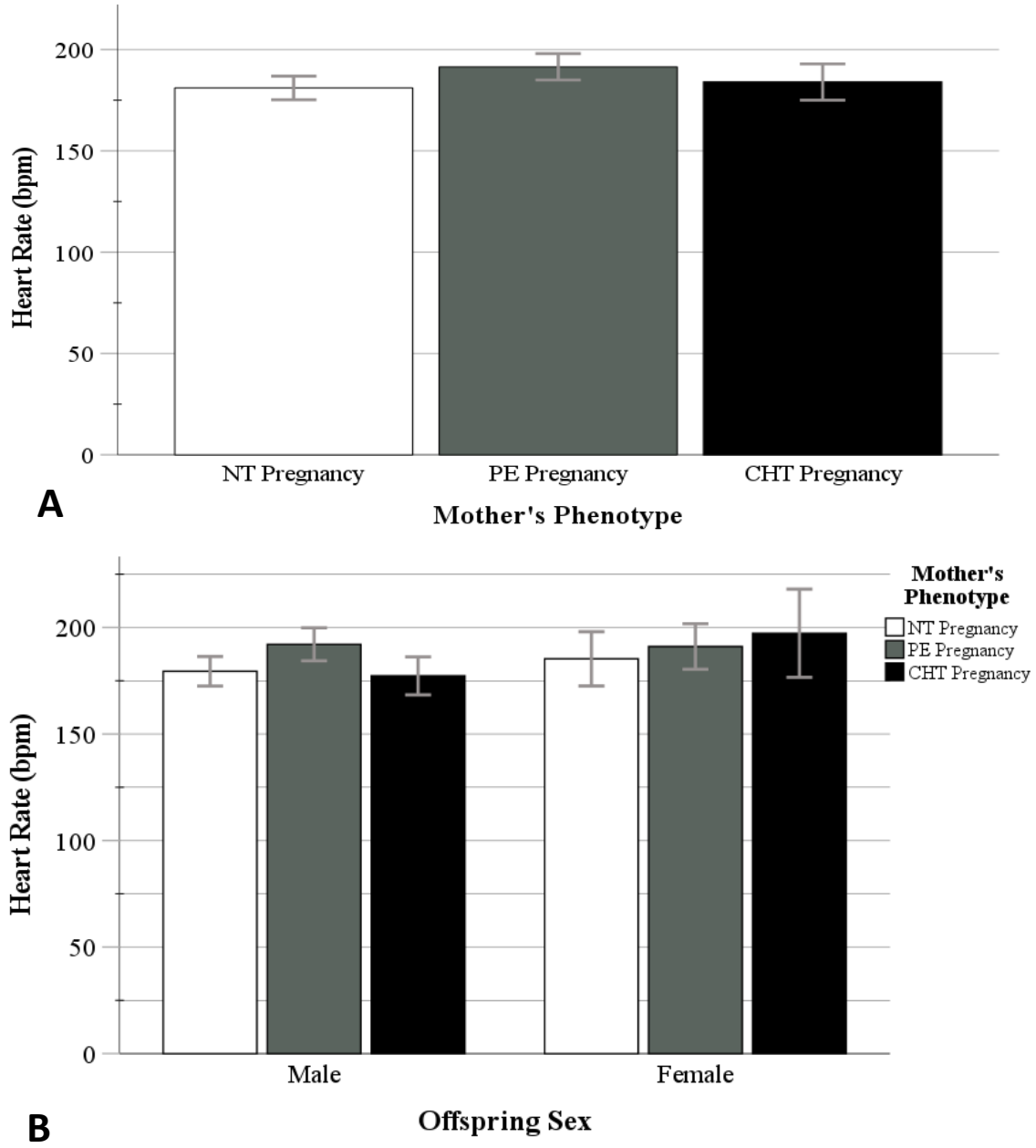

Figure 3-4: Heart rate is similar regardless of maternal phenotype or offspring sex

A) Average heart rate measured by doppler for juvenile offspring born to normotensive (NT; n=11), preeclamptic (PE; n=17), and chronic hypertensive (CHT; n=9) pregnancies. B) Average heart rate for male and female juvenile offspring born to NT (n=8 and n=3, respectively), PE (n=8 and n=9), and CHT (n=6 and n=3) pregnancies. Neither maternal phenotype nor sex influenced offspring heart rate. Values represented as means ± S.E.M.

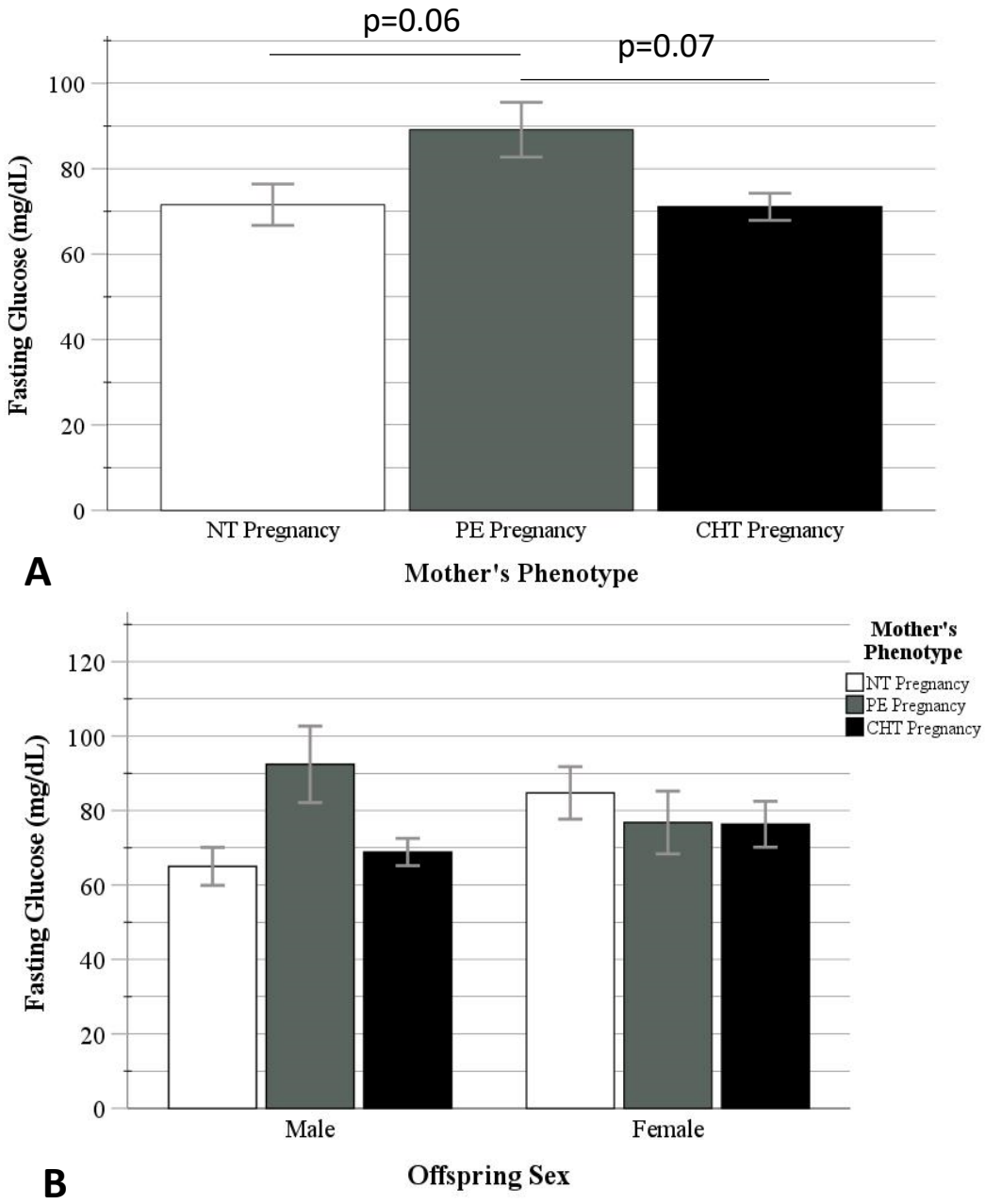

Figure 3-5: Average fasting glucose by maternal phenotype and offspring sex

A) Average fasting glucose as measured by finger stick and glucometer for offspring born to normotensive (NT; n=12), preeclamptic (PE; n=14), and chronic hypertensive (CHT; n=10) pregnancies. B) Average fasting glucose for male and female juvenile offspring born to NT (n=8 and n=4, respectively), PE (n=8 and n=6), and CHT (n=7 and n=3) pregnancies. Fasting glucose trended toward significance when comparing PE offspring to NT (p=0.06) and CHT (p=0.07) offspring. There was no effect of sex on fasting glucose. Values represented as means ± S.E.M.

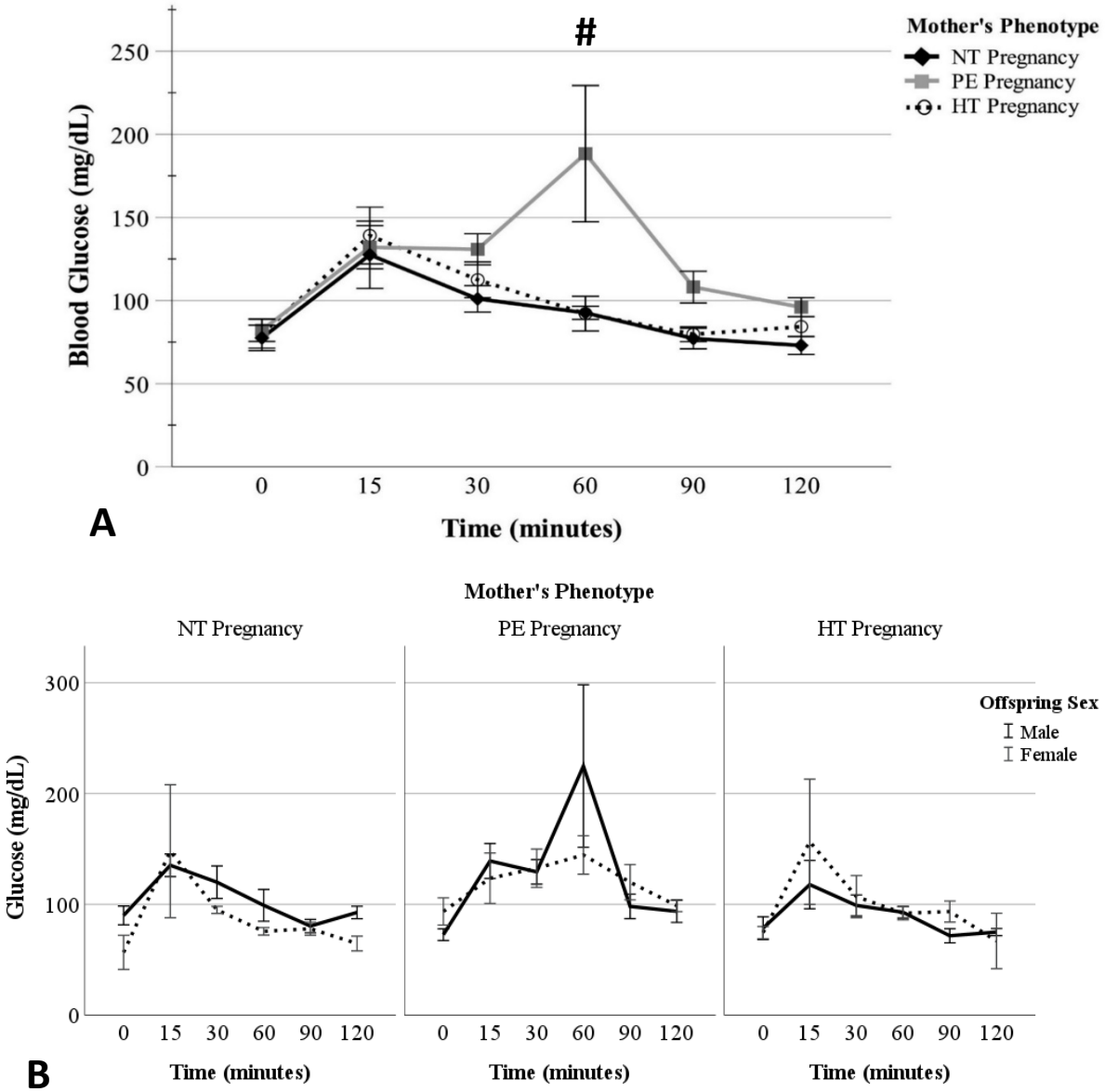

Figure 3-6: Average blood glucose over time for offspring born to normotensive, preeclamptic, and chronic hypertensive pregnancies

A) Blood glucose over time following glucose load in offspring born to normotensive (NT; n=10), preeclamptic (PE; n=11), and chronic hypertensive (CHT; n=8) pregnancies. Blood glucose was higher at 60 minutes following glucose load in PE offspring. B) Average blood glucose over time following glucose load in male (solid line) and female (hashed line) juvenile offspring born to NT (n=7 and n=3, respectively), PE (n=6 and n=5), and CHT (n=6 and n=2) pregnancies. Sex had no effect on blood glucose at any timepoint. # indicates $p<0.05$ compared to all other NT and PE timepoints. Values represented as means ± S.E.M.

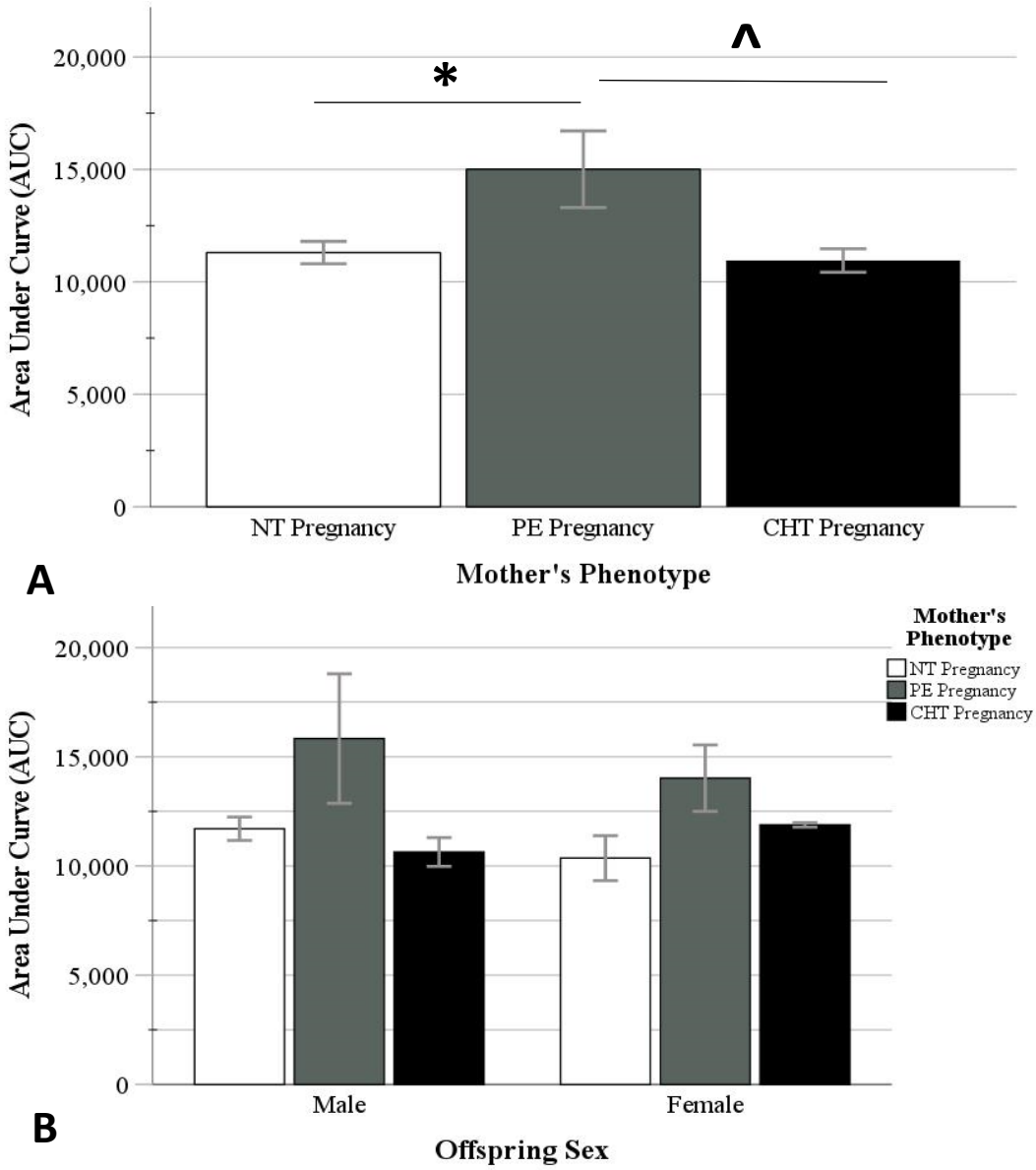

Figure 3-7: Offspring born to preeclamptic pregnancies have elevated Area-Under-Curve for glucose over time

A) Average are under the curve (AUC) in blood glucose over time following a glucose load in offspring born to normotensive (NT; n=10), preeclamptic (PE; n=11), and chronic hypertensive (CHT; n=8) pregnancies. AUC was higher in offspring born to PE pregnancies compared to those born to NT pregnancies. B) AUC in blood glucose over time in male and female juvenile offspring born to NT (n=7 and n=3, respectively), PE (n=6 and n=5), and CHT (n=6 and n=2) pregnancies. Sex had no effect on AUC in any group. * indicates $p<0.05$ vs NT group. ^indicates $p<0.05$ vs CHT group. Values represented as means ± S.E.M.

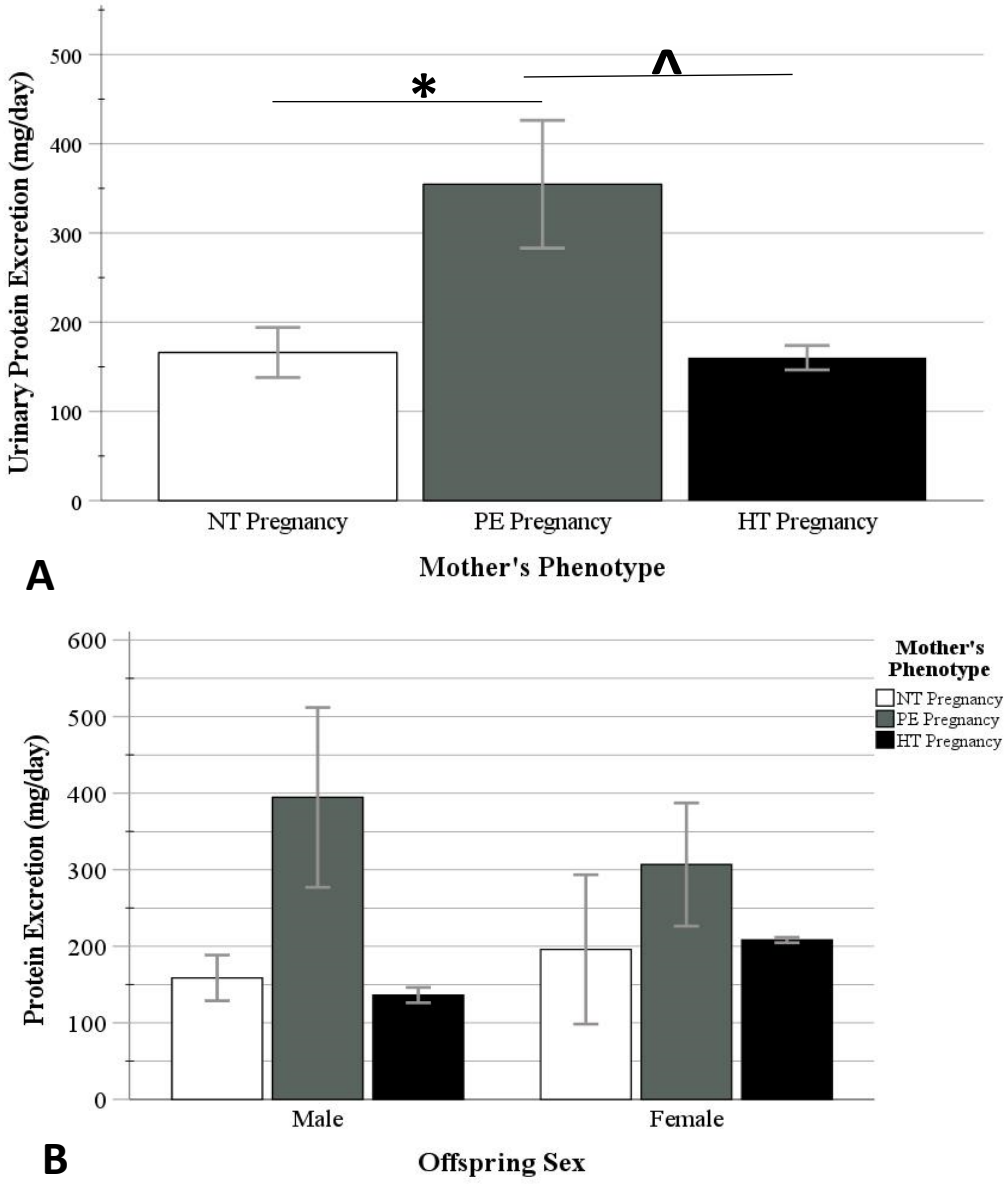

Figure 3-8: Offspring born to preeclamptic pregnancies have elevated urinary protein excretion

A) Average urinary protein excretion rate in offspring born to normotensive (NT; n=10), preeclamptic (PE; n=11), and chronic hypertensive (CHT; n=9) pregnancies. Urinary protein excretion was higher in juvenile offspring born to PE pregnancies compared to those born to NT and PE pregnancies. B) Average urinary protein excretion rate in male and female juvenile offspring born to NT (n=8 and n=2, respectively), PE (n=6 and n=5), and CHT (n=6 and n=3) pregnancies. Sex had no effect of protein excretion rate in any group. * indicates $p<0.05$ vs NT group; ^ indicates $p<0.05$ vs CHT group. Values represented as means ± S.E.M.

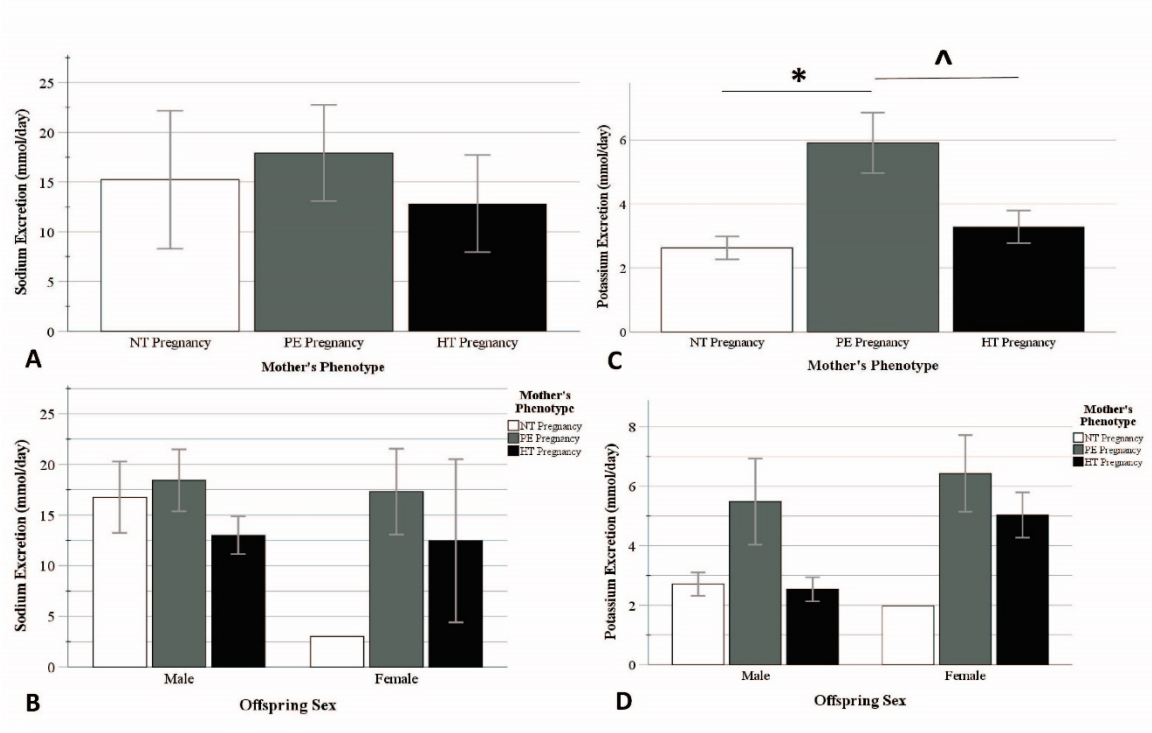

Figure 3-9: Offspring born to preeclamptic pregnancies have elevated urinary potassium, but not sodium, excretion

A) Average sodium excretion rate in offspring born to normotensive (NT; n=9), preeclamptic (PE; n=11), and chronic hypertensive (CHT; n=10) pregnancies. B) Average sodium excretion rate in male and female juvenile offspring born to NT (n=8 and n=1, respectively), PE (n=6 and n=5), and CHT (n=7 and n=3) pregnancies. Neither maternal phenotype nor sex influenced sodium excretion rate in any group. C) Average potassium excretion rate in offspring born to normotensive (NT; n=9), preeclamptic (PE; n=11), and chronic hypertensive (CHT; n=10) pregnancies. Potassium excretion was higher in offspring born to PE pregnancies compared to those born to NT and CHT pregnancies. D) Average potassium excretion rate in male and female juvenile offspring born to NT (n=8 and n=1, respectively), PE (n=6 and n=5), and CHT (n=7 and n=3) pregnancies. Sex had no effect on offspring potassium excretion in any group. * indicates $p<0.05$ vs NT group; ^ indicates $p<0.05$ vs CHT group. Values represented as means ± S.E.M.

CHAPTER 4: DISCUSSION AND CONCLUSIONS

The African Green Monkey (AGM) is a highly translational nonhuman primate for studying cardiovascular and renal disease. Here, it has been shown that the AGM is a novel model of spontaneous preeclampsia with pathophysiological characteristics that recapitulate human preeclampsia. All animal models currently used for studying preeclampsia are induced either genetically, pharmacologically, or surgically. While these animal models have significantly contributed to our understanding of the pathogenesis of preeclampsia, the ultimate etiology is still unknown. A spontaneous animal model would allow for investigations of the initiating events that lead to the clinical manifestation of preeclampsia. Ultimately, this could lead to improved diagnostics and therapeutics to minimize the disparities associated with preeclampsia.

The AGM is ideal for studying cardiovascular disease due to its large size, upright posture, and diurnal rhythm. AGMs also have a close evolutionary history with humans, having diverged from the human lineage 23 million years ago (Warren et al. 2015). This has led to shared gene sequence, gene structure, and organ physiology (Jasinska et al. 2013). The sequencing and subsequent growing annotation of the AGM genome along with the increasing availability of genetic tools supports the utility of this model for studying complex disease. High similarities to human physiology further support their use for pharmacological studies. They also have very similar placentation to humans, with hemomonochorial placentas, whereas mice and rats have hemotrichorial placentas (Hemberger, Hanna, and Dean 2020; Furukawa, Tsuji, and Sugiyama 2019). This indicates that maternal-fetal exchange may be more similar in AGMs and humans than with rodents. An ideal animal model of preeclampsia recapitulates many of the pathologies seen in

human patients and the AGM experiences *de novo* hypertension in the 2nd and 3rd trimester that occurs with proteinuria, similar to humans. Due to the availability of both group and individual housing, animals can be maintained in their natural troop environments with the opportunity for individual collections that allow control of numerous variables. This would allow further characterization of angiogenic factors and placentation in preeclamptic AGMs.

There are limitations with nonhuman primate models such as the AGM, particularly in comparison to murine models. They are more expensive to both procure and maintain compared to rodents, though they are more readily available than other nonhuman primates such as macaques. They have a long gestation, which, though more similar to humans, complicates studies with a longer duration. They also give birth to a single offspring per year and each offspring takes 3-4 years to reach sexual maturity, further complicating long-term studies. Finally, metabolic studies require removal from troop which could initiate a stress response that may confound studies, though level of stress can be highly monitored. Ultimately, the clinical relevance of the AGM in short and long-term studies of spontaneous cardiovascular disease greatly overwhelms these limitations.

In 62 purpose-bred AGMs, 35 previously normotensive females spontaneously developed hypertension in the 2nd or 3rd trimester which normalized by 6 weeks postpartum. This rate is much higher than the prevalence of pregnancy-induced hypertension in humans, which is estimated at 8-10%. The high prevalence among the AGMs could be due to several factors. The first may be because of genetic variability. Relative to the human population, AGMs in the Caribbean have less genetic diversity. The now large population estimated between 50,000-100,000 individuals came from a likely

very small founder population in Central Western Africa approximately 400 years ago when they were brought to the islands via trade ships. It is likely that many did not survive the trip due to stressful conditions. Those who did were the founding population, creating a significant bottleneck effect. This may influence the high rate of preeclampsia in the AGMs from the Caribbean.

Another possible contribution to the increased prevalence could be paternal. The studies presented in this work stemmed from pregnancies out of 3 breeding groups. The breeding groups consist of 1 alpha male and 15-25 females. This represents the natural troop dynamic for AGMs in the wild. Of these 3 breeding groups, 2 of the breeder males were hypertensive (SAP > 140 mmHg). A paternal contribution to human preeclampsia has been previously suggested. First, the placenta, considered to be the setting of the initial pathogenesis of preeclampsia, is an allogeneic tissue, consisting of maternal and fetal tissues. The trophoblast cells, responsible for invading the maternal decidua for implantation, express the paternal allele of the histocompatibility antigen C on their surface. Certain combinations of fetal HLA-C genotypes and maternal killer immunoglobulin receptor genotypes increase the risk of preeclampsia (Hiby et al. 2004). This supports the Immune Maladaptation Hypothesis, whereby the maternal response to fetal antigens (encoded by paternal DNA) contributes to the pathology of preeclampsia. This is further supported by the fact that shorter durations of sexual relationship prior to pregnancy, and therefore less exposure to paternal antigens and subsequent inflammatory responses (Sibai, Dekker, and Kupferminc 2005), increases the risk for preeclampsia (Kho et al. 2009). Furthermore, changing partners can increase a person's risk for developing preeclampsia, even more so if the new partner has fathered a child resulting from a

preeclamptic pregnancy in the past (Tubbergen 1998). Finally, population studies have shown that there is a contribution of both mother and father to the development of preeclampsia (Lie 1998; Esplin 2001). However, preeclampsia still occurs in female AGMs mated to normotensive males, indicating that male phenotype/genotype is not the driving force of preeclampsia in this model.

The AGMs which developed *de novo* hypertension in pregnancy also had elevated protein excretion compared to normotensive counterparts. This recapitulates one of the defining features of preeclampsia in humans. Elevated arterial pressure may be damaging the renal glomerular or tubular endothelium or epithelium respectively, leading to excess protein filtration and/or reabsorption. It should be noted that the scope of this study was to evaluate protein excretion in pregnant AGMs experiencing *de novo* increases in blood pressure. However, not all the preeclamptic AGMs experienced proteinuria, indicating that some AGMs may be experiencing gestational hypertension alone. Now that it has been established that some AGMs develop proteinuria related to elevated blood pressure, urinary protein levels should be used to further distinguish preeclampsia from gestational hypertension. Future studies will also be needed to further assess the level of renal damage, but this finding supports the AGM as a model of preeclampsia with similar pathologies to humans. Examining glomerular morphology will be a necessary next step to further characterize this model.

Preeclampsia in the AGM occurs with an even more exaggerated decline in plasma osmolality, similar to human studies. This occurs with a faster rate of weight gain in the 1st trimester compared to normotensive counterparts. This may be due to excess water retention early in pregnancy, perhaps through elevated ADH release similar to

preeclampsia in humans. However, it is difficult to perform 1st trimester water intake and urine flow evaluations in these AGMs so it is unclear whether urine flow rate decreases in the 1st trimester to contribute to this rapid weight gain. Future studies should evaluate 1st trimester water handling and ADH levels in preeclamptic AGMs.

During the postpartum period, formerly preeclamptic AGMs experience elevated sodium and potassium excretion compared to normotensive AGMs. This occurs with increasing urine flow rate and increasing plasma osmolality. The elevated ion excretion may be an effort to reduce plasma volume and return blood pressure to normal, as blood pressure simultaneously declines. This is a potential mechanistic avenue to explore in future studies.

Preeclampsia results in a higher stillbirth rate (29% vs 4% in normotensive pregnancies) and low birth weight, similar to humans. Taken together, this indicates that preeclampsia in the AGM leads to fetal growth restriction. The next step would be to measure uteroplacental flow to determine whether placental ischemia is occurring. It will also be important to examine placental morphology as this could be contributing to the fetal growth restriction. One limitation, however, is that the AGM, like many primates, consumes the placenta as it is delivered. This makes it challenging to collect enough placentas for a proper analysis. Examining the depth of trophoblast invasion and placental damage is critical to advance this model of preeclampsia. Performing cesarean deliveries would increase the chances of obtaining placenta for future histological analyses.

Because preeclampsia in the AGM is associated with reduced newborn birth weight, we were interested in whether offspring would experience similar long-term effects as those born to preeclamptic pregnancies in humans. When offspring were juveniles

between 1-3 years of age (pre-sexual maturity), we measured blood pressure, water intake, urine flow, protein and ion excretions, fasting glucose, and performed conscious oral glucose tolerance tests.

The first notable finding was that offspring born to preeclamptic pregnancies have elevated urinary protein excretion. This is indicative of compromised renal glomerular or tubular function. This finding is in line with human studies on long-term effects of preeclampsia on offspring. Offspring born to human preeclamptic patients have a higher risk of renal dysfunction and chronic kidney disease. Offspring that are born low birth weight or small for gestational age also have fewer nephron numbers in their kidneys. Future studies should be conducted to further assess the level of renal dysfunction in offspring born to preeclamptic AGMs. It will be important to examine renal morphology in addition to other markers of kidney damage, such as albumin excretion and GFR. It is possible that reduced uteroplacental perfusion during development in preeclamptic AGMs could lead to reduced nephron number in offspring, leading to early life reduced renal function. Performing nephron counting on offspring born to preeclamptic AGMs could lead to a better understanding of the proteinuria exhibited in early life.

Another key finding in the offspring studies was reduced glucose tolerance in juveniles born to preeclamptic pregnancies. The first finding was that offspring born to preeclamptic mothers had elevated fasting glucose. This prompted us to perform oral glucose tolerance tests on conscious offspring using a novel method: the banana tolerance test. These results showed that offspring to preeclamptic mothers had a sharp peak in blood glucose at 60 minutes post-glucose load which resulted in a higher area-under-curve. This suggests that experiencing preeclampsia in utero may be programming these offspring for

increased susceptibility to glucose intolerance in their postnatal lives. This could be through epigenetic modifications to various genes, such as glucose transporters (GLUT4), pancreatic homeobox factor-1, or insulin-like growth factor 2. Performing bisulfite sequencing or chromatin immunoprecipitation sequencing may elucidate whether offspring born to preeclamptic AGMs undergo epigenetic programming.

We could not measure insulin at this time, so it is unclear whether this glucose tolerance occurs via reduced insulin availability or reduced insulin sensitivity. Future recommendations include measuring fasting insulin and examining pancreatic beta cells. The reduced glucose tolerance observed in offspring born to preeclamptic AGMs recapitulates the effects of preeclampsia on human offspring. Exposure to hypertension in utero is associated with significant risks of metabolic dysfunction in postnatal life in humans (Imterat et al. 2020). This further supports the utility of the AGM in studying the short and long-term consequences of spontaneous preeclampsia.

This dissertation has presented evidence supporting the discovery of preeclampsia in the AGM that has pathophysiological similarities to that of humans. This preeclampsia also appears to program offspring for reduced renal and metabolic health in postnatal life. Taken together, this supports the AGM as a highly translational animal model of preeclampsia and its long-term consequences. Future studies will be critical to further establish this model. In particular, it will be important to determine whether AGMs experience the reduced trophoblast invasion characteristic of human preeclampsia. It will also be necessary to evaluate circulating sFlt-1, PlGF, and VEGF to determine whether preeclampsia in the AGM occurs with an imbalance of anti-angiogenic and angiogenic factors. It will also be beneficial to examine both placental and renal morphology in

preeclamptic AGMs. The AGM provides a unique opportunity to investigate early stages of preeclampsia that cannot be performed in human pregnancies, such as timed retrieval of the placenta at specific stages in development. This model also allows for investigation of the safety and efficacy of novel therapeutics for the treatment of preeclampsia. Ultimately, the AGM can bridge the gap in our understanding of the etiology of preeclampsia by providing a highly translational nonhuman primate model that develops spontaneously.

APPENDIX:

An Active-Learning Approach to Teaching Scientific Writing to Undergraduate Physiology Students

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JLO assisted in data analysis and interpretation, manuscript revising and editing.

JCT assisted in data collection and analysis, manuscript revising and editing.

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ABSTRACT

Comprehension and communication of scientific literature are crucial techniques for prospective scientists, yet undergraduate students are rarely exposed to these concepts in their STEM curriculum. This study presents an active learning mini-workshop focusing on abstract composition. Volunteer participants were recruited from a freshman research course and an upper-level physiology course. Students were provided sample data and instructed to write an abstract prior to the workshop. The workshop included a short lecture, including the different components of an abstract, followed by an active-learning activity where the students sorted sentences from a previously-published abstract into the different components. They also participated in an active-learning exercise on brevity by editing sentences from their pre-workshop abstracts. Following the workshop, students were given additional sample data and asked to write another abstract. Both pre- and post-workshop abstracts were blindly scored by two graders. Participation in the workshop led to a 20% increase in grade on average (pre: 38.2 ± 15.6% vs post: 58.2 ± 15.3%; SD; $p<0.05$ t-test). Qualitative analyses from pre- and post-workshop surveys indicated an increase in writing-confidence amongst workshop participants and a better understanding of abstract composition. These results, combined with positive participant feedback, support the workshop as an engaging and effective strategy for preparing novice science students for scientific writing.

INTRODUCTION

Effective comprehension and communication of research are essential in training prospective scientists. These skills are critical not only in peer-review publications but also in dissemination of scientific findings to the general public. Overall, this can lead to

improved scientific literacy in nonscientists (6, 13). One of the most common forms of scientific communication is writing quantitative abstracts and manuscripts. However, students are rarely exposed to these concepts in the standard undergraduate STEM curriculum (4). While they become well-trained in academic English writing through core coursework, undergraduate students are challenged with translating this into their science courses without explicit, science-focused training. It is then up to students to independently integrate core coursework into STEM courses to present quantitative data and support scientific outcomes.

Though teaching of scientific writing is limited in undergraduate curricula, efforts exist to bridge this gap, particularly through teaching the peer review process (3-5, 7). Teaching peer review can facilitate the writing process by providing a set of guidelines for students to follow. Previous research demonstrates utility in using published manuscripts as a guideline for formulating laboratory reports through quantitative instructor feedback (9). However, students are still challenged with de novo production of material in a coherent and concise format.

The present study provides a hands-on, team-based workshop to engage students while teaching them the fundamentals of scientific writing, such as clarity, brevity, and evidentiary support. It is well-established that student-centered, team-based approaches to instruction engage students, leading to higher order skills and success in learning (2, 8). Therefore, we hypothesized that participation in a team-based, active-learning workshop would improve student outcomes on de novo abstract writing and editing.

MATERIALS AND METHODS

Students and study design

Students were recruited from Bio 199, a freshman introductory research course (n=6), or Bio 350, an upper level (junior/senior level) animal physiology class (n=11). Students from Bio 199 participated as a portion of their course and enrollment was dictated by scheduling alone. Participation by Bio 350 students was entirely voluntary. Prior to the workshop, students were sent a worksheet with details on a hypothetical experiment, including fabricated results. Students completed a pre-workshop survey (Appendix A) and submitted an abstract based on the results from the hypothetical experiment worksheet. The following week, students participated in the three-part workshop detailed below (mini-lecture, group activity, and brevity activity). Afterwards, students were given another set of experiments/results. They were allowed 10 days to submit an abstract utilizing the methods learned in the workshop. They were also asked to complete the post-workshop survey upon submission of the abstract (Appendix B).

Survey and blinding

The survey was created and distributed via Google Forms. Students were assigned a pre- and post-abstract identifier number by a third-party individual. This number was used on the Google Form so that grades could be matched after scoring was complete, allowing graders to remain impartial. The last question allowed the students to copy and paste their abstract into the form for submission. Students were incentivized to complete the study with a chance to win a single $25 gift card if they submitted both a pre- and post-workshop abstract. The winner was randomly drawn at the end of the study.

Scoring

Two individual graders blindly scored abstracts before and after the workshop using a scoring rubric (Appendix C), which was not given to any of the students during the study. Scores were on a 10-point grading scale with 100% as the maximum score. The graders met afterwards to compare their rubrics. Scores that varied by no more than 5% were averaged between graders. If scores differed by more than 5%, graders discussed each section of the rubric and regraded the abstract. Intergrader variability of greater than 5% only occurred in 6% of abstracts. All students included in the analysis submitted both a pre- and post-workshop abstract (Bio 199 n=6, Bio 350 n=11).

Mini-lecture

The workshop began with a brief PowerPoint presentation overviewing the following topics: storytelling in science, IMRaD (14) (Introduction, Methods, Results, and Discussion) structure of papers, components of an abstract with example sentences (12), and writing brevity.

Group activity

Students were divided into groups of four to six members and each group was given laminated section headers with the components of an abstract discussed in the mini-lecture above: Title, Introduction, Introduce the Animal Model, Hypothesis, Methods, Results, Discussion, and Conclusion (Figure 1). They were also provided with laminated slips of paper, each containing a single sentence from a previously-published abstract (12). All laminates were given in a random order. Together students read each sentence and determined where it fit within the abstract header outline with the knowledge that multiple

sentences may belong under the same component header. They then categorized each sentence under the appropriate laminated header. After they had all the sentences sorted by components, they worked as a group to align the abstract sentences together in a coherent format. The workshop instructor verified that the abstract was in the correct order before moving on to the brevity activity.

Brevity activity

Sentences selected from each of the students' pre-workshop abstracts were utilized for the brevity activity. Sentences were written anonymously on a white-board in the workshop room to stimulate discussion and urge the students to work in a group, focusing on eliminating unnecessary words and writing concisely.

Statistical analyses

All statistical analyses were performed using JMP 12. Scores were first compared among students in the same course (Bio 199 or Bio 350, as described above) and if no differences were identified, they were combined. A two-sample t-test was used to compare pre- and post-workshop abstract scores as well as percent improvement between courses. An ANOVA was used to determine if there was any interaction between abstract scores and personal factors (self-reported GPA, parental education levels, etc.) with a Tukey's post-hoc comparison. All values are reported as mean ± standard deviation. Qualitative data was assessed by comparing comments from students in pre- and post-workshop surveys. Qualitative responses were converted to numerical values, similar to a Likert scale, for quantitative analysis. The responses ranged from not at all confident (assigned a value of 1) to extremely confident (assigned a value of 5) with not so confident, somewhat

confident, and very confident in between (assigned values of 2, 3, and 4, respectively). Quantitative values were then analyzed using a two-sample t-test to compare pre- and post-workshop responses.

RESULTS

This study included 17 participants (Table 1) spanning two courses at the University of Kentucky: Bio 199 (n=6) and Bio 350 (n=11). Students in the freshman research course, Bio 199, had a higher self-reported GPA than those in the animal physiology course, Bio 350 (3.94 ± 0.14 vs 3.69 ± 0.3, respectively; $p<0.05$ t-test). There were no differences in improvement between the Bio 199 and the Bio 350 cohorts, nor was there an effect of participants' self-reported gender on the results; therefore, all data will be reported together. Additionally, there was no association between parents' highest level of education or employment in a scientific field and the students' initial abstract scores or improvement post-workshop (Figure 2).

Abstract scores were higher after participating in the workshop (pre: 38.2 ± 15.6% vs post: 58.2 ± 15.3%; $p<0.05$ t-test; Figure 3). Overall, the writing workshop led to a 20 ± 18.9% improvement amongst students, with no differences between cohorts.

Qualitative analyses showed a clear shift in confidence in scientific reading and writing following participation in the workshop (Figure 4). When students were asked to rate their confidence in reading and comprehending a scientific abstract, there was no change between pre- and post-workshop responses (pre: 3.6 ± 0.2 vs post: 4.1 ± 0.2; $p=0.10$). However, confidence in ability to identify components of an abstract increased following the workshop (pre: 2.7 ± 0.2 vs post: 4.1 ± 0.2; $p<0.05$). Confidence in ability to write a

good quality abstract also increased (pre: 2.4 ± 0.2 vs post: 3.7 ± 0.2; $p<0.05$). Students also responded positively to survey questions regarding their experiences in the workshop and likelihood of future participation (Table 2).

DISCUSSION

This study assessed the utility of active-learning, a pedagogical method that focuses on engaging students in the classroom, in teaching scientific writing to undergraduate students. The students participated in a brief lecture highlighting the components of an abstract and how to write effectively. This was followed by a student-centered activity detailing the importance of brevity coupled with participation in a group activity putting this concept into practice. Finally, students worked as a group to identify components of an abstract and then arranged them into a coherent, logical order, honing their skills from the previous activities.

The major study finding was that participation in the workshop is associated with student improvement in abstract writing. Specifically, students were able to improve their abstract scores by two full letter grades based on a 10-point scale (20%) after participating in a one-hour workshop. These improvements occurred regardless of cohort, gender, or parental education levels. However, with a small sample size of volunteers, it was difficult to assess the contribution of factors such as gender and parental education level. However, we suggest that this activity will close gaps in scientific writing skills regardless of student academic background. Additionally, women were more likely than men to volunteer and complete both the pre- and post-workshop abstract activity. While this is unsurprising given previous studies investigating the effect of gender on willingness to volunteer (1, 11), it doesn't allow for a thorough evaluation of gender effects on learning outcome.

In addition to the quantitative analyses mentioned previously, this study also allowed for a qualitative overview: 1) Students were asked similar questions prior to and following their participation in the workshop; 2) Students were asked a question on their post-workshop survey specifically regarding their willingness to participate in future workshops or courses, if offered.

Feedback from students was positive with regards to gained confidence in comprehending, identifying components of, and writing an abstract after participating in this hands-on activity. Additionally, post-workshop feedback indicated that all participants left the workshop feeling more confident in and knowledgeable about scientific writing, and all participants would consider taking this workshop if it were offered as part of a full course (Appendix D).

The shift in student confidence, combined with the likelihood that they would participate in this workshop if offered within a course, indicates that students have a desire to learn scientific writing, but perhaps are not given opportunities to do so. With an active-learning approach, students are taught the fundamentals of writing an abstract while engaging with their peers to put these concepts into practice.

It is well recognized that undergraduate students have difficulty translating their core writing skills into scientific writing (10) and guidelines on scientific writing may be too complex for undergraduate students (5). Comprehending and effectively communicating scientific literature is critical to becoming a scientist, yet there is rarely enough time in a typical curriculum to focus heavily on developing these necessary writing skills. This workshop allows an additional level of learning that, if combined with peer-review and draft-revision, could more effectively train undergraduate students in scientific

communication. Thus, our findings support a role for active learning in the instruction and training of scientific writing.

DISCLOSURES

The authors have no disclosures to report.

ACKNOWLEDGMENTS

The authors would like to thank the volunteer students for participating. We also extend our appreciation to undergraduate researchers in the Osborn lab who participated in the workshop as it was being developed; specifically, we thank Lucas Barrett, Madison Webb, Patrick Rivera, Sushovan Dixit, and Emma Bateman. We also extend our appreciation to Osborn lab members for their support and guidance throughout this project; thank you to Shea Sickles, Maria Venegas, and Patrick Rivera.

TABLES AND FIGURES

Table 1: Characteristics of Study Participants

Table 1: Characteristics of Study Participants		
	Bio 199	**Bio 350**
Men, n	2	2
Women, n	4	9
College level	Freshman	Junior/Senior
Number of abstracts previously written, n	1.33 (SD 0.52)	1.91 (SD 2.21)
Number of courses taken that require scientific writing, n	0.67 (SD 0.82)	2.55 (SD 2.07)
Self-reported GPA	3.94 (SD 0.14)	3.69 (SD 0.30)*

Table 2: Post-workshop survey responses to qualitative questions.

Table 2: Post-workshop survey results			
	Yes	**Moderately**	**No**
Do you think this workshop gave you more confidence in your writing ability?	85%	15%	0%
Do you think this workshop made you more knowledgeable about scientific writing?	100%	0%	0%
Would another workshop aimed at writing other components of a scientific paper be beneficial?	100%	0%	0%
If this were a course for credit, would you consider taking it as an elective?	77%	23%	0%

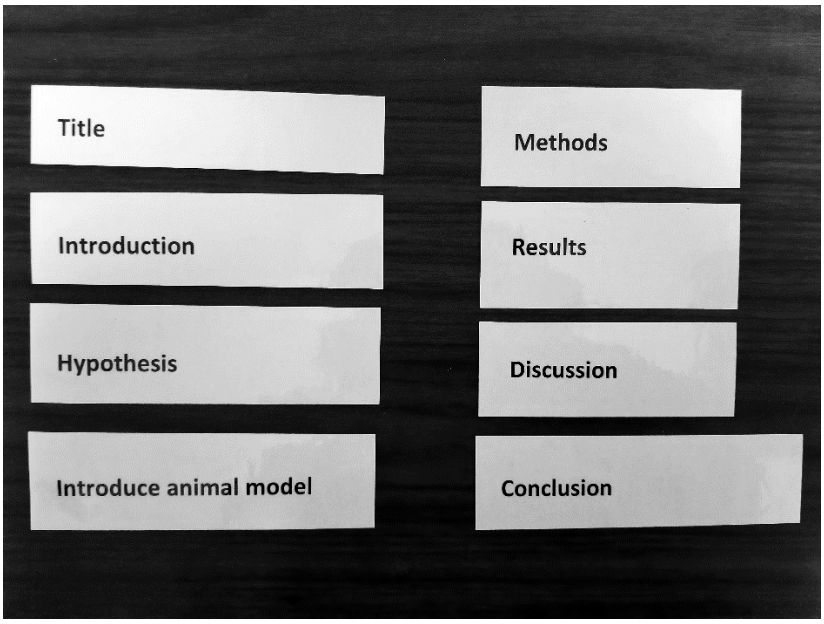

Figure 1: Laminated abstract component headers used in the abstract component sorting activity.

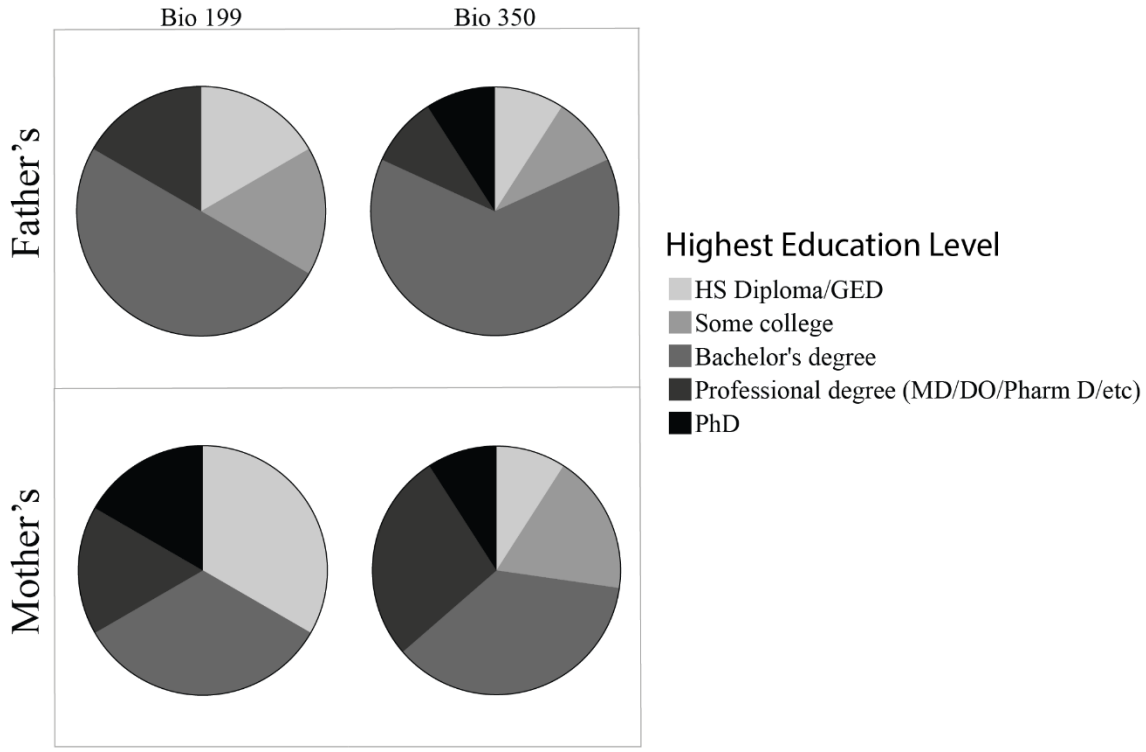

Figure 2: Parental education level for study participants

Top: self-reported father's highest level of education for Bio 199 (left) and Bio 350 (right) participants. Bottom: self-reported mother's highest level of education Bio 199 (left) and Bio 350 (right) participants. Parental education level had no effect on participant abstract score or improvement.

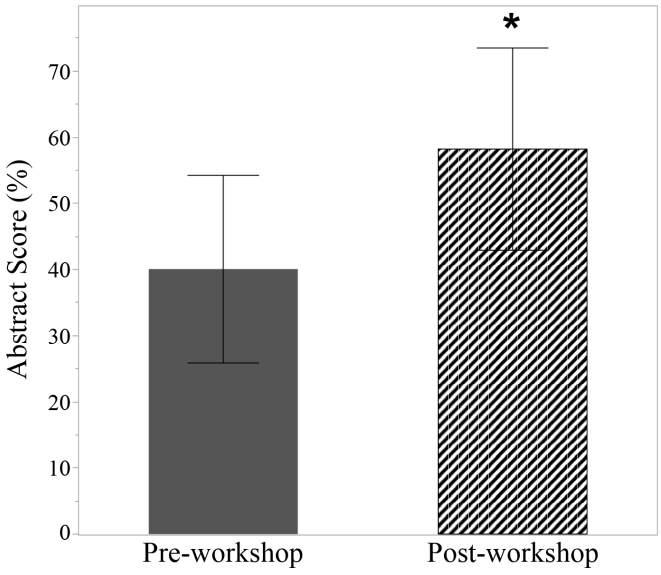

Figure 3: Abstract scores

Abstract score in percent for the pre-workshop (solid, left) abstract and the post-workshop (hashed, right) abstract for all participants (n=17). * indicates $p<0.05$ vs pre-workshop. All values mean ± standard deviation.

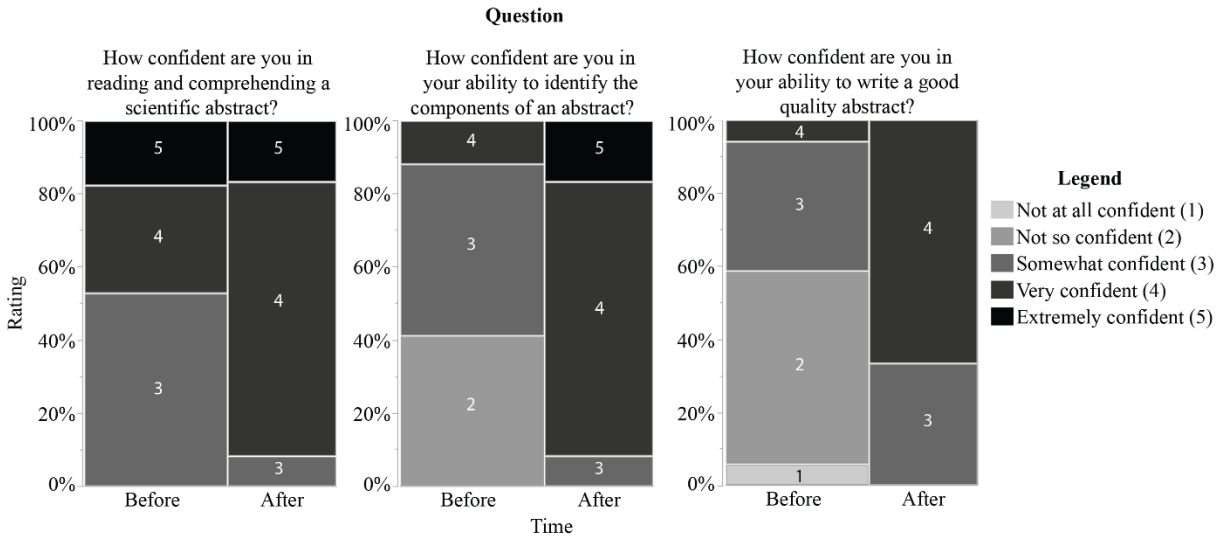

Figure 4: Participant confidence levels

Students' perceived confidence level with regards to reading/comprehending a scientific abstract, identifying the components of an abstract, and ability to write a good abstract. The left stacked bar shows pre-workshop responses and the right stacked bar indicates post-workshop responses. Ratings were assigned a numerical value, 1-5, with 1 corresponding to the lowest rating (Not at all confident) and 5 the highest (Extremely confident).

APPENDIX REFERENCES

1. Einolf CJ. Gender Differences in the Correlates of Volunteering and Charitable Giving. Nonprofit and Voluntary Sector Quarterly 40: 1092-1112, 2010.

2. Freeman S, Eddy SL, McDonough M, Smith MK, Okoroafor N, Jordt H, and Wenderoth MP. Active learning increases student performance in science, engineering, and mathematics. Proc Natl Acad Sci U S A 111: 8410-8415, 2014.

3. Geithner CA and Pollastro AN. Doing peer review and receiving feedback: impact on scientific literacy and writing skills. Adv Physiol Educ 40: 38-46, 2016.

4. Guilford WH. Teaching Peer Review and the Process of Scientific Writing. Adv Physiol Educ 25: 167-175, 2001.

5. Hyatt JK, Bienenstock EJ, and Tilan JU. A student guide to proofreading and writing in science. Adv Physiol Educ 41: 324-331, 2017.

6. Illingworth S. Delivering effective science communication: advice from a professional science communicator. Semin Cell Dev Biol 70: 10-16, 2017.

7. Linton DL, Pangle WM, Wyatt KH, Powell KN, and Sherwood RE. Identifying key features of effective active learning: the effects of writing and peer discussion. CBE Life Sci Educ 13: 469-477, 2014.

8. Michael J. Where's the evidence that active learning works? Adv Physiol Educ 30: 159-167, 2006.

9. Ruscetti T, Krueger K, and Sabatier C. Improving quantitative writing one sentence at a time. PLoS One 13: e0203109, 2018.

10. Sorrell JM, DAEd, RN; Brown, Hazel N, EdD, RNC. Mentoring Students in Writing: "Gourmet Express" Versus "Fast Food Service". Journal of Nursing Education 30: 284-286, 1991.

11. Walter W. Wymer J, and Sridhar Samu. Men's and Women's Volunteering: Gender Differences in the Effects of Employment and Family Characteristics. Journal of Marketing Management: 971-989, 2002.

12. Weaver CC, Grobe JL, Santillan MK, and Osborn JL. Altered Glomerular Function is Associated with Spontaneous Gestational Hypertension in African Green Monkeys. The FASEB Journal 32: 911.919-911.919, 2018.

13. Webb AB, Fetsch CR, Israel E, Roman CM, Encarnacion CH, Zacks JM, Thoroughman KA, and Herzog ED. Training scientists in a science center improves science communication to the public. Adv Physiol Educ 36: 72-76, 2012.

14. Wu J. Improving the writing of research papers: IMRAD and beyond. Landscape Ecology 26: 1345-1349, 2011.

BIBLIOGRAPHY

AbdAlla, S., H. Lother, A. el Massiery, and U. Quitterer. 2001. 'Increased AT(1) receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness', *Nat Med*, 7: 1003-9.

Ahmed, Asif. 2011. 'New insights into the etiology of preeclampsia: identification of key elusive factors for the vascular complications', *Thrombosis Research*, 127: S72-S75.

Amaral, L. M., K. Wallace, M. Owens, and B. LaMarca. 2017. 'Pathophysiology and Current Clinical Management of Preeclampsia', *Curr Hypertens Rep*, 19: 61.

American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. 2013. 'Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy.', *Obstet Gynecol*, 122: 1122-31.

Ananth, C. V., K. M. Keyes, and R. J. Wapner. 2013. 'Pre-eclampsia rates in the United States, 1980-2010: age-period-cohort analysis', *BMJ*, 347: f6564.

Ananth, C. V., M. R. Peltier, W. L. Kinzler, J. C. Smulian, and A. M. Vintzileos. 2007. 'Chronic hypertension and risk of placental abruption: is the association modified by ischemic placental disease?', *Am J Obstet Gynecol*, 197: 273 e1-7.

Angrimsson, R., Bjornsson, S., Geirsson, RT., Bjornsson, H., Walker, JJ., Snaedal, J. 1990. 'Genetic and familial predisposition to eclampsia and preeclampsia in a defined population', *British Journal of Obstetrics and Gynaecology*, 97: 762-9.

Apicella, C., C. S. M. Ruano, C. Mehats, F. Miralles, and D. Vaiman. 2019. 'The Role of Epigenetics in Placental Development and the Etiology of Preeclampsia', *Int J Mol Sci*, 20.

Askie, Lisa M., Lelia Duley, David J. Henderson-Smart, and Lesley A. Stewart. 2007. 'Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data', *The Lancet*, 369: 1791-98.

Bakker, R., E. A. Steegers, A. Hofman, and V. W. Jaddoe. 2011. 'Blood pressure in different gestational trimesters, fetal growth, and the risk of adverse birth outcomes: the generation R study', *Am J Epidemiol*, 174: 797-806.

Barker, D. J. 2005. 'The developmental origins of insulin resistance', *Horm Res*, 64 Suppl 3: 2-7.

Barker, D. J., C. Osmond, J. Golding, D. Kuh, and MEJ Wadsworth. 1989. 'Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease', *Br Med J*, 298: 564-67.

Bartlett, C. S., M. Jeansson, and S. E. Quaggin. 2016. 'Vascular Growth Factors and Glomerular Disease', *Annu Rev Physiol*, 78: 437-61.

Batchelder, C. A., J. L. Keyser, C. C. Lee, and A. F. Tarantal. 2013. 'Characterization of growth, glomerular number, and tubular proteins in the developing rhesus monkey kidney', *Anat Rec (Hoboken)*, 296: 1747-57.

Batchelder, C. A., C. C. Lee, M. L. Martinez, and A. F. Tarantal. 2010. 'Ontogeny of the kidney and renal developmental markers in the rhesus monkey (Macaca mulatta)', *Anat Rec (Hoboken)*, 293: 1971-83.

Batchelder, C. A., C. C. Lee, D. G. Matsell, M. C. Yoder, and A. F. Tarantal. 2009. 'Renal ontogeny in the rhesus monkey (Macaca mulatta) and directed differentiation of

human embryonic stem cells towards kidney precursors', *Differentiation*, 78: 45-56.
Bezerra, P. C., M. D. Leao, J. W. Queiroz, E. M. Melo, F. V. Pereira, M. H. Nobrega, A. K. Jeronimo, L. C. Ferreira, S. M. Jeronimo, and A. C. de Araujo. 2010. 'Family history of hypertension as an important risk factor for the development of severe preeclampsia', *Acta Obstet Gynecol Scand*, 89: 612-7.
Bian, X., A. Biswas, X. Huang, K. J. Lee, T. K. Li, H. Masuyama, A. Ohkuchi, J. S. Park, S. Saito, K. H. Tan, T. Yamamoto, A. Dietl, S. Grill, W. D. J. Verhagen-Kamerbeek, J. Y. Shim, and M. Hund. 2019. 'Short-Term Prediction of Adverse Outcomes Using the sFlt-1 (Soluble fms-Like Tyrosine Kinase 1)/PlGF (Placental Growth Factor) Ratio in Asian Women With Suspected Preeclampsia', *Hypertension*, 74: 164-72.
Boeldt, D. S., Bird, I. M. 2017. 'Vascular adaptation in pregnancy and endothelial dysfunction in preeclampsia', *J Endocrinol*, 232: R27-R44.
Bokslag, A., M. van Weissenbruch, B. W. Mol, and C. J. de Groot. 2016. 'Preeclampsia; short and long-term consequences for mother and neonate', *Early Hum Dev*, 102: 47-50.
Bowyer, L. 2003. 'Forearm blood flow in pre-eclampsia', *BJOG: An International Journal of Obstetrics and Gynaecology*, 110: 383-91.
Boyd, H. A., H. Tahir, J. Wohlfahrt, and M. Melbye. 2013. 'Associations of personal and family preeclampsia history with the risk of early-, intermediate- and late-onset preeclampsia', *Am J Epidemiol*, 178: 1611-9.
Bramham, K., B. Parnell, C. Nelson-Piercy, P. T. Seed, L. Poston, and L. C. Chappell. 2014. 'Chronic hypertension and pregnancy outcomes: systematic review and meta-analysis', *BMJ*, 348: g2301.
Bridges, J. P., J. S. Gilbert, D. Colson, S. A. Gilbert, M. P. Dukes, M. J. Ryan, and J. P. Granger. 2009. 'Oxidative stress contributes to soluble fms-like tyrosine kinase-1 induced vascular dysfunction in pregnant rats', *Am J Hypertens*, 22: 564-8.
Brosens, I., R. Pijnenborg, L. Vercruysse, and R. Romero. 2011. 'The "Great Obstetrical Syndromes" are associated with disorders of deep placentation', *Am J Obstet Gynecol*, 204: 193-201.
Brown, D. W., N. Dueker, D. J. Jamieson, J. W. Cole, M. A. Wozniak, B. J. Stern, W. H. Giles, and S. J. Kittner. 2006. 'Preeclampsia and the risk of ischemic stroke among young women: results from the Stroke Prevention in Young Women Study', *Stroke*, 37: 1055-9.
Brown, M. J. 2007. 'Renin: friend or foe?', *Heart*, 93: 1026-33.
Brown, MA., J. Wang, and JA. Whitworth. 1997. 'The Renin-Angiotensin-Aldosterone System in preeclampsia', *Clin. and Exper. Hypertension*, 19: 713-26.
Brownfoot, F. C., S. Tong, N. J. Hannan, N. K. Binder, S. P. Walker, P. Cannon, R. Hastie, K. Onda, and T. J. Kaitu'u-Lino. 2015. 'Effects of Pravastatin on Human Placenta, Endothelium, and Women With Severe Preeclampsia', *Hypertension*, 66: 687-97; discussion 445.
Bryant, A. S., A. Worjoloh, A. B. Caughey, and A. E. Washington. 2010. 'Racial/ethnic disparities in obstetric outcomes and care: prevalence and determinants', *Am J Obstet Gynecol*, 202: 335-43.

Bukowski, R., N. I. Hansen, M. Willinger, U. M. Reddy, C. B. Parker, H. Pinar, R. M. Silver, D. J. Dudley, B. J. Stoll, G. R. Saade, M. A. Koch, C. J. Rowland Hogue, M. W. Varner, D. L. Conway, D. Coustan, R. L. Goldenberg, Health Eunice Kennedy Shriver National Institute of Child, and Network Human Development Stillbirth Collaborative Research. 2014. 'Fetal growth and risk of stillbirth: a population-based case-control study', *PLoS Med*, 11: e1001633.

Bulletins—Obstetrics, American College of Obstetricians and Gynecologists' Committee on Practice. 2019. 'ACOG Practice Bulletin No. 203: Chronic Hypertension in Pregnancy', *Obstet Gynecol*, 133: e26-e80.

Campbell, N., B. LaMarca, and M. W. Cunningham, Jr. 2018. 'The Role of Agonistic Autoantibodies to the Angiotensin II Type 1 Receptor (AT1-AA) in Pathophysiology of Preeclampsia', *Curr Pharm Biotechnol*, 19: 781-85.

Cannone, V., A. Cabassi, R. Volpi, J. C. Burnett, and Jr. 2019. 'Atrial Natriuretic Peptide: A Molecular Target of Novel Therapeutic Approaches to Cardio-Metabolic Disease', *Int J Mol Sci*, 20.

Carr, D. B., M. Epplein, C. O. Johnson, T. R. Easterling, and C. W. Critchlow. 2005. 'A sister's risk: family history as a predictor of preeclampsia', *Am J Obstet Gynecol*, 193: 965-72.

Carroll, R. L., K. Mah, J. W. Fanton, G. N. Maginnis, R. M. Brenner, and O. D. Slayden. 2007. 'Assessment of menstruation in the vervet (Cercopithecus aethiops)', *Am J Primatol*, 69: 901-16.

Chang, J., and D. Streitman. 2012. 'Physiologic adaptations to pregnancy', *Neurol Clin*, 30: 781-9.

Chau, K., A. Hennessy, and A. Makris. 2017. 'Placental growth factor and pre-eclampsia', *J Hum Hypertens*, 31: 782-86.

Chehade, H., U. Simeoni, J. P. Guignard, and F. Boubred. 2018. 'Preterm Birth: Long Term Cardiovascular and Renal Consequences', *Curr Pediatr Rev*, 14: 219-26.

Cheong, J. N., M. E. Wlodek, K. M. Moritz, and J. S. Cuffe. 2016. 'Programming of maternal and offspring disease: impact of growth restriction, fetal sex and transmission across generations', *J Physiol*, 594: 4727-40.

Chesley, LC., Cooper, DW. 1986. 'Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women', *British Journal of Obstetrics and Gynaecology*, 93: 898-908.

Cho, F., A. Hiyaoka, MT. Suzuki, and S. Honjo. 2002. 'Breeding of African Green Monkeys (Cercopithicus aethiops) under indoor individually caged conditions', *Exp Anim*, 51: 343-51.

Coats, L. E., G. K. Davis, A. D. Newsome, N. B. Ojeda, and B. T. Alexander. 2019. 'Low Birth Weight, Blood Pressure and Renal Susceptibility', *Curr Hypertens Rep*, 21: 62.

Creanga, A. A., C. J. Berg, J. Y. Ko, S. L. Farr, V. T. Tong, F. C. Bruce, and W. M. Callaghan. 2014. 'Maternal mortality and morbidity in the United States: where are we now?', *J Womens Health (Larchmt)*, 23: 3-9.

Davis, E. F., M. Lazdam, A. J. Lewandowski, S. A. Worton, B. Kelly, Y. Kenworthy, S. Adwani, A. R. Wilkinson, K. McCormick, I. Sargent, C. Redman, and P. Leeson. 2012. 'Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review', *Pediatrics*, 129: e1552-61.

Davison, JM., Robertson, GL., Gilmore, EA., Lindheimer, MD. 1984. 'Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy', *AJP Renal*, 246: F105-F09.

Davison, Marshall D Lindheimer and John M. 1995. 'Osmoregulation, the secretion of arginine vasopressin and its metabolism during pregnancy', *European Journal of Endocrinology*, 132: 133-43.

Dechend, R., P. Gratze, G. Wallukat, E. Shagdarsuren, R. Plehm, J. H. Brasen, A. Fiebeler, W. Schneider, S. Caluwaerts, L. Vercruysse, R. Pijnenborg, F. C. Luft, and D. N. Muller. 2005. 'Agonistic autoantibodies to the AT1 receptor in a transgenic rat model of preeclampsia', *Hypertension*, 45: 742-6.

DeMartelly, V., P. Hurley, M. Lawrence, D. E. Redmond, Jr., and J. Rutherford. 2012. 'Comparison of fresh to fixed weights of the vervet monkey (Chlorocebus sabaeus) placenta and its relation to gestational age', *J Med Primatol*, 41: 158-62.

Dore, K. M., A. R. Eller, and J. L. Eller. 2018. 'Identity Construction and Symbolic Association in Farmer-Vervet Monkey (Chlorocebus aethiops sabaeus) Interconnections in St. Kitts', *Folia Primatol (Basel)*, 89: 63-80.

Duley, L. 2009. 'The global impact of pre-eclampsia and eclampsia', *Semin Perinatol*, 33: 130-7.

Dunlop, W. 1981. 'Serial Changes in Renal Haemodynamics During Normal Human Pregnancy', *British Journal of Obstetrics and Gynaecology*, 88.

English, F. A., L. C. Kenny, and F. P. McCarthy. 2015. 'Risk factors and effective management of preeclampsia', *Integr Blood Press Control*, 8: 7-12.

Esplin, MS., Fausett, MB., Fraser, A., Kerber, R., Mineau, G., Carrillo, J., Varner, MW. 2001. 'Paternal and Maternal Components of the Predisposition to Preeclampsia', *New England Journal of Medicine*, 344: 867-72.

Falcao, S., E. Stoyanova, G. Cloutier, R. L. Maurice, J. Gutkowska, and J. L. Lavoie. 2009. 'Mice overexpressing both human angiotensinogen and human renin as a model of superimposed preeclampsia on chronic hypertension', *Hypertension*, 54: 1401-7.

Fisher, S. J. 2015. 'Why is placentation abnormal in preeclampsia?', *Am J Obstet Gynecol*, 213: S115-22.

Fox, R., J. Kitt, P. Leeson, C. Y. L. Aye, and A. J. Lewandowski. 2019. 'Preeclampsia: Risk Factors, Diagnosis, Management, and the Cardiovascular Impact on the Offspring', *J Clin Med*, 8.

Furukawa, S., N. Tsuji, and A. Sugiyama. 2019. 'Morphology and physiology of rat placenta for toxicological evaluation', *J Toxicol Pathol*, 32: 1-17.

Galaviz-Hernandez, C., E. Arambula-Meraz, D. Medina-Bastidas, M. Sosa-Macias, B. P. Lazalde-Ramos, M. Ortega-Chavez, and L. Hernandez-Garcia. 2016. 'The paternal polymorphism rs5370 in the EDN1 gene decreases the risk of preeclampsia', *Pregnancy Hypertens*, 6: 327-32.

Galaviz-Hernandez, C., M. Sosa-Macias, E. Teran, J. E. Garcia-Ortiz, and B. P. Lazalde-Ramos. 2018. 'Paternal Determinants in Preeclampsia', *Front Physiol*, 9: 1870.

Garovic, V. D., K. R. Bailey, E. Boerwinkle, S. C. Hunt, A. B. Weder, D. Curb, T. H. Mosley, Jr., H. J. Wiste, and S. T. Turner. 2010. 'Hypertension in pregnancy as a risk factor for cardiovascular disease later in life', *J Hypertens*, 28: 826-33.

Gatford, K. L., R. A. Simmons, M. J. De Blasio, J. S. Robinson, and J. A. Owens. 2010. 'Review: Placental programming of postnatal diabetes and impaired insulin action after IUGR', *Placenta*, 31 Suppl: S60-5.

George, E. M., and J. P. Granger. 2012. 'Linking placental ischemia and hypertension in preeclampsia: role of endothelin 1', *Hypertension*, 60: 507-11.

George, E. M., A. C. Palei, and J. P. Granger. 2012. 'Endothelin as a final common pathway in the pathophysiology of preeclampsia: therapeutic implications', *Curr Opin Nephrol Hypertens*, 21: 157-62.

Gerber, Hans Peter, Xiumin Wu, Lanlan Yu, Christian Wiesmann, Xiao Huan Liang, Chingwei V. Lee, Germaine Fuh, Christine Olsson, Lisa Damico, David Xie, Y. Gloria Meng, Johnny Gutierrez, Racquel Corpuz, Linda Hall Bing Li, Linda Rangell, Ron Ferrando, Henry Lowman, Franklin Peale, and Napoleone Ferrara. 'Mice expressing a humanized form of VEGF-A may provide insights into the safety and efficacy of anti-VEGF antibodies'.

Ghulmiyyah, L., and B. Sibai. 2012. 'Maternal mortality from preeclampsia/eclampsia', *Semin Perinatol*, 36: 56-9.

Giachini, F. R., C. Galaviz-Hernandez, A. E. Damiano, M. Viana, A. Cadavid, P. Asturizaga, E. Teran, S. Clapes, M. Alcala, J. Bueno, M. Calderon-Dominguez, M. P. Ramos, V. V. Lima, M. Sosa-Macias, N. Martinez, J. M. Roberts, C. Escudero, and Trem Riva. 2017. 'Vascular Dysfunction in Mother and Offspring During Preeclampsia: Contributions from Latin-American Countries', *Curr Hypertens Rep*, 19: 83.

Gilbert, J. S., M. J. Ryan, B. B. LaMarca, M. Sedeek, S. R. Murphy, and J. P. Granger. 2008. 'Pathophysiology of hypertension during preeclampsia: linking placental ischemia with endothelial dysfunction', *Am J Physiol Heart Circ Physiol*, 294: H541-50.

Gobble, R. M., K. A. Groesch, M. Chang, R. J. Torry, and D. S. Torry. 2009. 'Differential regulation of human PlGF gene expression in trophoblast and nontrophoblast cells by oxygen tension', *Placenta*, 30: 869-75.

Goulopoulou, S. 2017. 'Maternal Vascular Physiology in Preeclampsia', *Hypertension*, 70: 1066-73.

Granger, JP., B. Babbette, D. LaMarca, Kathy Cockrell, Mona Sedeek, Charles Balzi, Derrick Chandler, and William Bennett. 2006. 'Reduced Uterine Perfusion Pressure (RUPP) Model for Studying Cardiovascular–Renal Dysfunction in Response to Placental Ischemia', *Methods in Molecular Medicine*, 122: 383-92.

Groer, M. W., C. M. Jevitt, F. Sahebzamani, J. W. Beckstead, and D. L. Keefe. 2013. 'Breastfeeding status and maternal cardiovascular variables across the postpartum', *J Womens Health (Larchmt)*, 22: 453-9.

Hao, J., D. Hassen, Q. Hao, J. Graham, M. J. Paglia, J. Brown, M. Cooper, V. Schlieder, and S. R. Snyder. 2019. 'Maternal and Infant Health Care Costs Related to Preeclampsia', *Obstet Gynecol*, 134: 1227-33.

He, H., V. J. Venema, X. Gu, R. C. Venema, M. B. Marrero, and R. B. Caldwell. 1999. 'Vascular endothelial growth factor signals endothelial cell production of nitric oxide and prostacyclin through flk-1/KDR activation of c-Src', *J Biol Chem*, 274: 25130-5.

Hecht, J. L., Z. K. Zsengeller, M. Spiel, S. A. Karumanchi, and S. Rosen. 2016. 'Revisiting decidual vasculopathy', *Placenta*, 42: 37-43.

Heida, K. Y., A. Franx, B. B. van Rijn, M. J. Eijkemans, J. M. Boer, M. W. Verschuren, M. A. Oudijk, M. L. Bots, and Y. T. van der Schouw. 2015. 'Earlier Age of Onset of Chronic Hypertension and Type 2 Diabetes Mellitus After a Hypertensive Disorder of Pregnancy or Gestational Diabetes Mellitus', *Hypertension*, 66: 1116-22.

Hemberger, M., C. W. Hanna, and W. Dean. 2020. 'Mechanisms of early placental development in mouse and humans', *Nat Rev Genet*, 21: 27-43.

Henry, F., Quatresooz, P., Valverde-Lopez, JC., and Pierard, GE. 2006. 'Blood vessel changes during pregnancy: a review', *Am J Clin Dermatol*, 7: 65-69.

Hermes, W., A. Franx, M. G. van Pampus, K. W. Bloemenkamp, M. L. Bots, J. A. van der Post, M. Porath, G. A. Ponjee, J. T. Tamsma, B. W. Mol, and C. J. de Groot. 2013. 'Cardiovascular risk factors in women who had hypertensive disorders late in pregnancy: a cohort study', *Am J Obstet Gynecol*, 208: 474 e1-8.

Herse, F., R. Dechend, N. K. Harsem, G. Wallukat, J. Janke, F. Qadri, L. Hering, D. N. Muller, F. C. Luft, and A. C. Staff. 2007. 'Dysregulation of the circulating and tissue-based renin-angiotensin system in preeclampsia', *Hypertension*, 49: 604-11.

Hiby, S. E., J. J. Walker, M. O'Shaughnessy K, C. W. Redman, M. Carrington, J. Trowsdale, and A. Moffett. 2004. 'Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success', *J Exp Med*, 200: 957-65.

Hsueh, Willa A. 1988. 'Renin in the female reproductive system', *Cardiovascular Drugs and Therap*, 2: 473-77.

Hubel, C. A., G. Wallukat, M. Wolf, F. Herse, A. Rajakumar, J. M. Roberts, N. Markovic, R. Thadhani, F. C. Luft, and R. Dechend. 2007. 'Agonistic angiotensin II type 1 receptor autoantibodies in postpartum women with a history of preeclampsia', *Hypertension*, 49: 612-7.

Iihara, K., and A. Nishimura. 2015. 'Maternal Death Due to Stroke Associated With Pregnancy-Induced Hypertension', *Circ J*, 79: 1695-6.

Imterat, M., T. Wainstock, D. Landau, A. Walfisch, and E. Sheiner. 2020. 'Maternal Chronic Hypertension Elevates the Risk for Long-Term Selected Endocrine and Metabolic Morbidity in the Offspring, Particularly Childhood Obesity', *Child Obes*, 16: 421-27.

Intapad, S., J. H. Dasinger, J. M. Johnson, A. D. Brown, N. B. Ojeda, and B. T. Alexander. 2019. 'Male and Female Intrauterine Growth-Restricted Offspring Differ in Blood Pressure, Renal Function, and Glucose Homeostasis Responses to a Postnatal Diet High in Fat and Sugar', *Hypertension*, 73: 620-29.

Irani, R. A., and Y. Xia. 2011. 'Renin angiotensin signaling in normal pregnancy and preeclampsia', *Semin Nephrol*, 31: 47-58.

Isbell, L. A., T. P. Young, K. E. Jaffe, A. A. Carlson, and R. L. Chancellor. 2009. 'Demography and Life Histories of Sympatric Patas Monkeys, Erythrocebus patas, and Vervets, Cercopithecus aethiops, in Laikipia, Kenya', *Int J Primatol*, 30: 103-24.

Janzen, C., M. Y. Lei, J. Cho, P. Sullivan, B. C. Shin, and S. U. Devaskar. 2013. 'Placental glucose transporter 3 (GLUT3) is up-regulated in human pregnancies complicated by late-onset intrauterine growth restriction', *Placenta*, 34: 1072-8.

Jasinska, A. J., C. A. Schmitt, S. K. Service, R. M. Cantor, K. Dewar, J. D. Jentsch, J. R. Kaplan, T. R. Turner, W. C. Warren, G. M. Weinstock, R. P. Woods, and N. B. Freimer. 2013. 'Systems biology of the vervet monkey', *ILAR J*, 54: 122-43.

Jayet, P. Y., S. F. Rimoldi, T. Stuber, C. S. Salmon, D. Hutter, E. Rexhaj, S. Thalmann, M. Schwab, P. Turini, C. Sartori-Cucchia, P. Nicod, M. Villena, Y. Allemann, U. Scherrer, and C. Sartori. 2010. 'Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia', *Circulation*, 122: 488-94.

Jornayvaz, F. R., P. Vollenweider, M. Bochud, V. Mooser, G. Waeber, and P. Marques-Vidal. 2016. 'Low birth weight leads to obesity, diabetes and increased leptin levels in adults: the CoLaus study', *Cardiovasc Diabetol*, 15: 73.

Kaleta, T., A. Stock, D. Panayotopoulos, O. Vonend, D. Niederacher, M. Neumann, T. Fehm, W. Kaisers, and M. Fleisch. 2016. 'Predictors of Impaired Postpartum Renal Function in Women after Preeclampsia: Results of a Prospective Single Center Study', *Dis Markers*, 2016: 7861919.

Karumanchi, S. A., and J. P. Granger. 2016. 'Preeclampsia and Pregnancy-Related Hypertensive Disorders', *Hypertension*, 67: 238-42.

Khaing, W., S. A. Vallibhakara, V. Tantrakul, O. Vallibhakara, S. Rattanasiri, M. McEvoy, J. Attia, and A. Thakkinstian. 2017. 'Calcium and Vitamin D Supplementation for Prevention of Preeclampsia: A Systematic Review and Network Meta-Analysis', *Nutrients*, 9.

Kho, E. M., L. M. McCowan, R. A. North, C. T. Roberts, E. Chan, M. A. Black, R. S. Taylor, G. A. Dekker, and Scope Consortium. 2009. 'Duration of sexual relationship and its effect on preeclampsia and small for gestational age perinatal outcome', *J Reprod Immunol*, 82: 66-73.

Kieckbusch, J., L. M. Gaynor, A. Moffett, and F. Colucci. 2014. 'MHC-dependent inhibition of uterine NK cells impedes fetal growth and decidual vascular remodelling', *Nat Commun*, 5: 3359.

Kim, J. Y., and Y. M. Kim. 2015. 'Acute Atherosis of the Uterine Spiral Arteries: Clinicopathologic Implications', *J Pathol Transl Med*, 49: 462-71.

Kleinrouweler, C. E., M. M. Wiegerinck, C. Ris-Stalpers, P. M. Bossuyt, J. A. van der Post, P. von Dadelszen, B. W. Mol, E. Pajkrt, and Ebm Connect Collaboration. 2012. 'Accuracy of circulating placental growth factor, vascular endothelial growth factor, soluble fms-like tyrosine kinase 1 and soluble endoglin in the prediction of pre-eclampsia: a systematic review and meta-analysis', *BJOG*, 119: 778-87.

Kraft-Schreyer N, Kushner H, Angelakos ET. 1991. 'Discriminant Analysis of Transmission of Elevated Blood Pressure in First Generation Offspring of African Green Monkeys', *Clin Exp Hypertens A*, 13: 32-52.

Kublickiene, K. R., B. Lindblom, K. Kruger, and H. Nisell. 2000. 'Preeclampsia: evidence for impaired shear stress-mediated nitric oxide release in uterine circulation', *Am J Obstet Gynecol*, 183: 160-6.

Kumasawa, K., M. Ikawa, H. Kidoya, H. Hasuwa, T. Saito-Fujita, Y. Morioka, N. Takakura, T. Kimura, and M. Okabe. 2011. 'Pravastatin induces placental growth

factor (PGF) and ameliorates preeclampsia in a mouse model', *Proc Natl Acad Sci U S A*, 108: 1451-5.

LaMarc, Mark W. Cunningham Jr. and Babbette. 2018. 'Risk of cardiovascular disease, end-stage renal disease, and stroke in postpartum women and their fetuses after a hypertensive pregnancy', *AJP: Regulatory, Integrative, and Comparative Physiology*, 315: R521-28.

LaMarca, B., D. Cornelius, and K. Wallace. 2013. 'Elucidating immune mechanisms causing hypertension during pregnancy', *Physiology (Bethesda)*, 28: 225-33.

Lampinen, KH., M. Ronnback, RJ. Kaajaa, and P. Groop. 2006. 'Impaired vascular dilatation in women with a history of pre-eclampsia', *Journal of Hypertension*, 24: 751-56.

Lash, G. E., H. Pitman, H. L. Morgan, B. A. Innes, C. N. Agwu, and J. N. Bulmer. 2016. 'Decidual macrophages: key regulators of vascular remodeling in human pregnancy', *J Leukoc Biol*, 100: 315-25.

Lash, G. E., B. Schiessl, M. Kirkley, B. A. Innes, A. Cooper, R. F. Searle, S. C. Robson, and J. N. Bulmer. 2006. 'Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy', *J Leukoc Biol*, 80: 572-80.

Levine, RJ., Maynard, SE., Qian, C., Lim, KH., England, LJ., Yu, KF., Schisterman, EF., Thadhan,i R., Sachs, BP., Epstein, FH., Sibai, BM., Sukhatme, VP., Karumanchi, SA. 2004. 'Circulating Angiogenic Factors and the Risk of Preeclampsia', *NEJM*, 350: 672-83.

Li, F., Hagaman, J. R., Kim, H.-S., Maeda, N., Jennette, J. C., Faber, J. E., Takahashi, N. 2012. 'eNOS Deficiency Acts through Endothelin to Aggravate sFlt-1-Induced Pre-Eclampsia-Like Phenotype', *Journal of the American Society of Nephrology*, 23: 652–60.

Li, J., B. LaMarca, and J. F. Reckelhoff. 2012. 'A model of preeclampsia in rats: the reduced uterine perfusion pressure (RUPP) model', *Am J Physiol Heart Circ Physiol*, 303: H1-8.

Lie, RT., Rasmussen, S., Brunborg, H., Gjessing, HK., Lie-Nielsen, E., Irgens, LM. 1998. 'Fetal and maternal contributions to risk of pre-eclampsia: population based study', *BMJ*, 316: 1343-7.

Lindsay M. Silvaa, Marianne Coolmana, Eric A.P. Steegers, Vincent W.V. Jaddoe, Henriette A. Molle, Albert Hofmand, Johan P. Mackenbach and Hein Raat. 2008. 'Low socioeconomic status is a risk factor for preeclampsia: the Generation R Study', *Journal of Hypertension*, 26: 1200-08.

MacDorman, M. F., E. Declercq, H. Cabral, and C. Morton. 2016. 'Recent Increases in the U.S. Maternal Mortality Rate: Disentangling Trends From Measurement Issues', *Obstet Gynecol*, 128: 447-55.

Mahendru, A. A., T. R. Everett, I. B. Wilkinson, C. C. Lees, and C. M. McEniery. 2014. 'A longitudinal study of maternal cardiovascular function from preconception to the postpartum period', *J Hypertens*, 32: 849-56.

Mark K. Santillan, Donna A. Santillan, Sabrina M. Scroggins, James Y. Min, Jeremy A. Sandgren, Nicole A. Pearson, Kimberly K. Leslie, Stephen K. Hunter, Gideon K.D. Zamba, Katherine N. Gibson-Corley, Justin L. Grobe. 2014. 'Vasopressin in Preeclampsia: A Novel Very Early Human Pregnancy Biomarker and Clinically Relevant Mouse Model', *Hypertension*, 64: 852-59.

Marshall, S. A., N. J. Hannan, M. Jelinic, T. P. H. Nguyen, J. E. Girling, and L. J. Parry. 2018. 'Animal models of preeclampsia: translational failings and why', *Am J Physiol Regul Integr Comp Physiol*, 314: R499-R508.

Martin JA, Hamilton BE, Osterman MJ, Curtin SC, Matthews TJ. 2015. 'Births: final data for 2013', *Natl Vital Stat Rep*, 64: 1-65.

Martin, S., R. M. Palmour, R. Goldwater, J. Gutkowsa, C. Hughes, P. Hamet and F. R. Ervin. 1990. 'Characterization of a primate model of hypertension. The response of hypertensive and normotensive male vervets (Cercopithecus aethiops) to cold pressor stress, captopril administration, and acute bolus of atrial natriuretic factor', *American Journal of Hypertension*, 3: 27-32.

Maynard, Sharon E., Jiang-Yong Min, Jaime Merchan, Kee-Hak Lim, Jianyi Li, Susanta Mondal, Towia A. Libermann, James P. Morgan, Frank W. Sellke, Isaac E. Stillman, Franklin H. Epstein, Vikas P. Sukhatme, and S. Ananth Karumanchi. 2003. 'Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia', *Journal of Clinical Investigation*, 111: 649-58.

McCarthy, F. P., J. C. Kingdom, L. C. Kenny, and S. K. Walsh. 2011. 'Animal models of preeclampsia; uses and limitations', *Placenta*, 32: 413-9.

McCowan, LM., Buist, RG., North, RA., Gamble, G. . 1996. 'Perinatal morbidity in chronic hypertension', *Br J Obstet Gynaecol*, 103: 123-9.

McDonald, S. D., S. Yusuf, M. W. Walsh, E. Lonn, K. Teo, S. S. Anand, J. Pogue, S. Islam, P. J. Devereaux, and H. C. Gerstein. 2013. 'Increased cardiovascular risk after pre-eclampsia in women with dysglycaemia', *Diabet Med*, 30: e1-7.

MCDonald SD, Han Z, Walsh MW, Gerstein HC, Devereaux PJ. 2010. 'Kidney disease after preeclampsia: a systematic review and meta-analysis', *Am J Kidney Dis*, 55: 1026-39.

McDonald SD, Malinowski A, Zhou Q, Yusuf S, Devereaux PJ. 2008. 'Cardiovascular sequelae of preeclampsia/eclampsia: a systematic review and meta-analyses.', *Am Heart J*, 156: 918-30.

McGuire, L. A. Fairbanks and M. T. 1987. 'Mother Infant relationships in vervet monkeys: Response to new adult males', *International Journal of Primatology*, 8: 351-66.

Merill, DC., M. Karoly, K. Chen, CM. Ferrario, and KB. Brosnihan. 2002. 'Angiotensin-(1–7) in Normal and Preeclamptic Pregnancy', *Endocrine*, 18: 239-45.

Metcalfe, J., Ueland, K. 1974. 'Maternal Cardiovascular Adjustments to Pregnancy', *Progress in Cardiovascular Diseases*, XVI: 363-74.

Mills, K. T., J. D. Bundy, T. N. Kelly, J. E. Reed, P. M. Kearney, K. Reynolds, J. Chen, and J. He. 2016. 'Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries', *Circulation*, 134: 441-50.

Mirzakhani, H., A. A. Litonjua, T. F. McElrath, G. O'Connor, A. Lee-Parritz, R. Iverson, G. Macones, R. C. Strunk, L. B. Bacharier, R. Zeiger, B. W. Hollis, D. E. Handy, A. Sharma, N. Laranjo, V. Carey, W. Qiu, M. Santolini, S. Liu, D. Chhabra, D. A. Enquobahrie, M. A. Williams, J. Loscalzo, and S. T. Weiss. 2016. 'Early pregnancy vitamin D status and risk of preeclampsia', *J Clin Invest*, 126: 4702-15.

Moffett, A. Hiby, S. E., Sharkey, A. M. 2015. 'The role of the maternal immune system in the regulation of human birthweight', *Philos Trans R Soc Lond B Biol Sci*, 370: 20140071.

Mogren, I., B. Lindahl, and U. Hogberg. 2003. 'Impaired fasting glucose and impaired glucose tolerance are related to both heredity and low birth weight', *Scand J Public Health*, 31: 382-8.

Molnär, M., and F. N. Hertelendy. 1992. 'N w-nitro-L-arginine, an inhibitor of nitric oxide synthesis, increases blood pressure in rats and reverses the pregnancy- induced refractoriness to vasopressor agents', *Am J Obstet Gynecol*, 166: 1560-7.

Mongraw-Chaffin, M. L., P. M. Cirillo, and B. A. Cohn. 2010. 'Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort', *Hypertension*, 56: 166-71.

Mor, G., I. Cardenas, V. Abrahams, and S. Guller. 2011. 'Inflammation and pregnancy: the role of the immune system at the implantation site', *Ann N Y Acad Sci*, 1221: 80-7.

Morgan, Terry, Catherine Craven, Jean-Marc Lalouel, and Kenneth Ward. 1999. 'Angiotensinogen Thr235 variant is associated with abnormal physiologic change of the uterine spiral arteries in first-trimester decidua', *American Journal of Obstetrics and Gynecology*, 180: 95-102.

Moritz, K. M., M. Q. Mazzuca, A. L. Siebel, A. Mibus, D. Arena, M. Tare, J. A. Owens, and M. E. Wlodek. 2009. 'Uteroplacental insufficiency causes a nephron deficit, modest renal insufficiency but no hypertension with ageing in female rats', *J Physiol*, 587: 2635-46.

Morton, J. S., J. Levasseur, E. Ganguly, A. Quon, R. Kirschenman, J. R. B. Dyck, G. M. Fraser, and S. T. Davidge. 2019. 'Characterisation of the Selective Reduced Uteroplacental Perfusion (sRUPP) Model of Preeclampsia', *Sci Rep*, 9: 9565.

Nancy Kraft-Schreyer, Harvey Kushner, Evangelos T. Angelakos. 1987a. 'Development of a NHP model for spontaneous hypertension', *Hypertension*, 9: III-57-III-63.

———. 1987b. 'Development of a Nonhuman Primate Model for Spontaneous Hypertension', *Hypertension*, 9: 57-63.

Nankali, A., Sh Malek-Khosravi, M. Zangeneh, M. Rezaei, Z. Hemati, and M. Kohzadi. 2013. 'Maternal complications associated with severe preeclampsia', *J Obstet Gynaecol India*, 63: 112-5.

Nema, J., D. Sundrani, and S. Joshi. 2019. 'Role of vitamin D in influencing angiogenesis in preeclampsia', *Hypertens Pregnancy*, 38: 201-07.

Ober, C., Abney, M., McPeek, MS. 2001. 'The Genetic Dissection of Complex Traits in a Founder Population', *Am J Hum Genet*, 69: 1068-79.

Odegård RA, Vatten LJ, Nilsen ST, Salvesen KA, Austgulen R. 2000. 'Preeclampsia and fetal growth', *Obstet Gynecol*, 96: 950-5.

Okada, H., Tsuzuki, T., Murata, H. 2018. 'Decidualization of the human endometrium', *Reprod Med Biol*, 17: 220-27.

Owiti, G.E.O., M. Cukierski, R.P. Tarara, A.C. Enders, and A.G. Hendrickx. 1986. 'Early placentation in the African green monkey (Cercopithecus aethiops)', *Acta Anat*, 127: 184-94.

Paauw, N. D., B. B. van Rijn, A. T. Lely, and J. A. Joles. 2017. 'Pregnancy as a critical window for blood pressure regulation in mother and child: programming and reprogramming', *Acta Physiol (Oxf)*, 219: 241-59.

Palmer, B. F., and D. J. Clegg. 2016. 'Physiology and pathophysiology of potassium homeostasis', *Adv Physiol Educ*, 40: 480-90.
Palmour, RM., Mulligan, J., Howbert, JJ., Ervin, F. 1997. 'Of Monkeys and Men: Vervets and the Genetics of Human-Like Behaviors', *Am J Hum Genet*, 61: 481-8.
Panaitescu, A. M., R. Akolekar, N. Kametas, A. Syngelaki, and K. H. Nicolaides. 2017. 'Impaired placentation in women with chronic hypertension who develop pre-eclampsia', *Ultrasound Obstet Gynecol*, 50: 496-500.
Petersen EE, Davis NL, Goodman D, et al. 2019. 'Racial/Ethnic Disparities in Pregnancy-Related Deaths — United States, 2007–2016', *MMWR Morb Mortal Wkly Rep*, 68: 762-65.
Phipps, E. A., R. Thadhani, T. Benzing, and S. A. Karumanchi. 2019. 'Pre-eclampsia: pathogenesis, novel diagnostics and therapies', *Nat Rev Nephrol*, 15: 275-89.
Phipps, E., D. Prasanna, W. Brima, and B. Jim. 2016. 'Preeclampsia: Updates in Pathogenesis, Definitions, and Guidelines', *Clin J Am Soc Nephrol*, 11: 1102-13.
Plaks, V., T. Birnberg, T. Berkutzki, S. Sela, A. BenYashar, V. Kalchenko, G. Mor, E. Keshet, N. Dekel, M. Neeman, and S. Jung. 2008. 'Uterine DCs are crucial for decidua formation during embryo implantation in mice', *J Clin Invest*, 118: 3954-65.
Podjarny, E., Losonczy, G., Baylis, C. 2004. 'Animal Models of Preeclampsia', *Semin Nephrol*, 24: 596-606.
Poulsen, S. B., and R. A. Fenton. 2019. 'K(+) and the renin-angiotensin-aldosterone system: new insights into their role in blood pressure control and hypertension treatment', *J Physiol*, 597: 4451-64.
'The Preeclampsia Foundation'. 2020. http://www.preeclampsia.org/.
Proctor, L. K., J. Kfouri, L. Hiersch, A. Aviram, A. Zaltz, J. Kingdom, J. Barrett, and N. Melamed. 2019. 'Association between hypertensive disorders and fetal growth restriction in twin compared with singleton gestations', *Am J Obstet Gynecol*, 221: 251 e1-51 e8.
Quinkler M, Meyer B, Bumke-Vogt C, Grossmann C, Gruber U, Oelkers W, Diederich S, and Bahr V. 2002. 'Agonistic and antagonistic properties of progesterone metabolites at the human mineralocorticoid receptor', *European Journal of Endocrinology*, 146: 789-800.
Rana, S., E. Lemoine, J. P. Granger, and S. A. Karumanchi. 2019. 'Preeclampsia: Pathophysiology, Challenges, and Perspectives', *Circ Res*, 124: 1094-112.
Rasmussen, S. 2003. 'Fetal growth and body proportion in preeclampsia', *Obstetrics & Gynecology*, 101: 575-83.
Redman, C. W., and I. L. Sargent. 2010. 'Immunology of pre-eclampsia', *Am J Reprod Immunol*, 63: 534-43.
Redman, CW, Sargent, IL. 2005. 'Latest advances in understanding preeclampsia', *Science*, 308: 1592-94.
'Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy'. 2000. *American Journal of Obstetrics and Gynecology*, 183: s1-s22.
Rhoads, M. K., S. B. Goleva, W. H. Beierwaltes, and J. L. Osborn. 2017. 'Renal vascular and glomerular pathologies associated with spontaneous hypertension in the

nonhuman primate Chlorocebus aethiops sabaeus', *Am J Physiol Regul Integr Comp Physiol*, 313: R211-R18.
Roberta M. Palmour, John Mulligan, J. Jeffry Howbert, and Frank Ervin. 1997. 'Of Monkeys and Men', *Am. J. Hum. Genet.*, 61: 481-88.
Roberts, J. M., and C. Escudero. 2012. 'The placenta in preeclampsia', *Pregnancy Hypertens*, 2: 72-83.
Ross, K. M., C. Dunkel Schetter, M. R. McLemore, B. D. Chambers, R. A. Paynter, R. Baer, S. K. Feuer, E. Flowers, D. Karasek, M. Pantell, A. A. Prather, K. Ryckman, and L. Jelliffe-Pawlowski. 2019. 'Socioeconomic Status, Preeclampsia Risk and Gestational Length in Black and White Women', *J Racial Ethn Health Disparities*, 6: 1182-91.
Ruggajo, P., R. Skrunes, E. Svarstad, R. Skjaerven, A. V. Reisaether, and B. E. Vikse. 2016. 'Familial Factors, Low Birth Weight, and Development of ESRD: A Nationwide Registry Study', *Am J Kidney Dis*, 67: 601-8.
Saffer, C., G. Olson, K. A. Boggess, R. Beyerlein, C. Eubank, B. M. Sibai, and Normals Study Group. 2013. 'Determination of placental growth factor (PlGF) levels in healthy pregnant women without signs or symptoms of preeclampsia', *Pregnancy Hypertens*, 3: 124-32.
Saito, S., and A. Nakashima. 2014. 'A review of the mechanism for poor placentation in early-onset preeclampsia: the role of autophagy in trophoblast invasion and vascular remodeling', *J Reprod Immunol*, 101-102: 80-88.
Salles, G. F., M. M. Schlussel, D. R. Farias, A. B. Franco-Sena, F. Rebelo, E. M. Lacerda, and G. Kac. 2015. 'Blood pressure in healthy pregnancy and factors associated with no mid-trimester blood pressure drop: a prospective cohort study', *Am J Hypertens*, 28: 680-9.
Sandgren JA, Scroggins SM, Santillan DA, Devor EJ, Gibson-Corley KN, Pierce GL, Sigmund CD, Santillan MK, Grobe JL. 2015. 'Vasopressin: the missing link for preeclampsia?', *Am J Physiol Regul Integr Comp Physiol*, 309: 1062-64.
Sandvik, M. K., E. Leirgul, O. Nygard, P. M. Ueland, A. Berg, E. Svarstad, and B. E. Vikse. 2013. 'Preeclampsia in healthy women and endothelial dysfunction 10 years later', *Am J Obstet Gynecol*, 209: 569 e1-69 e10.
Sanghavi, M., and J. D. Rutherford. 2014. 'Cardiovascular physiology of pregnancy', *Circulation*, 130: 1003-8.
Santillan, M., Donna Santillan, Sabrina Scroggins, Mengda Liu, Taryn Nishimura, Alyssa Ray, Jeremy Sandgren, Philip Witcher, Kellie Thomas, Debra Brandt, and Justin Grobe. 2017. 'Pregnancy-associated suppression of plasma osmolality is exaggerated in preeclampsia: a window to novel early pathologic mechanisms', *American Journal of Obstetrics & Gynecology*: S545-6.
Santillan, M. K., D. A. Santillan, S. M. Scroggins, J. Y. Min, J. A. Sandgren, N. A. Pearson, K. K. Leslie, S. K. Hunter, G. K. Zamba, K. N. Gibson-Corley, and J. L. Grobe. 2014. 'Vasopressin in preeclampsia: a novel very early human pregnancy biomarker and clinically relevant mouse model', *Hypertension*, 64: 852-9.
Schatz, F., O. Guzeloglu-Kayisli, S. Arlier, U. A. Kayisli, and C. J. Lockwood. 2016. 'The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding', *Hum Reprod Update*, 22: 497-515.

Seely, E. W., and J. Ecker. 2014. 'Chronic hypertension in pregnancy', *Circulation*, 129: 1254-61.
Sehgal, A., B. T. Alexander, J. L. Morrison, and A. M. South. 2020. 'Fetal Growth Restriction and Hypertension in the Offspring: Mechanistic Links and Therapeutic Directions', *J Pediatr*, 224: 115-23 e2.
Selvaggi, L., G. Loverro, FP. Schenaa, C. Mannoa, and G. and Cagnazzo. 1988. 'Long term follow-up of women with hypertension in pregnancy', *Int J Gynecol Obstet*, 27: 45-49.
Shah, D. M. 2005. 'Role of the renin-angiotensin system in the pathogenesis of preeclampsia', *Am J Physiol Renal Physiol*, 288: F614-25.
Shaw, KJ., Yung S. Do, Siri Kjos, Pam W. Anderson, Tatsuo Shinagawa, and Louis Dubeau, and Willa A. Hsueh. 1989. 'Human Decidua Is a Major Source of Renin', *J Clin Invest*, 83: 2085-92.
Sibai, Baha, Gus Dekker, and Michael Kupferminc. 2005. 'Pre-eclampsia', *The Lancet*, 365: 785-99.
Sibai, Baha M. 2002. 'Chronic hypertension in pregnancy', *High-Risk Pregnancy Series: An Expert's View*, 100: 369-77.
Sibai, BM., Lindheimer, M., Hauth, J., Caritis, S., VanDorsten, P., Klebanoff, M., et al. . 1998. 'Risk factors for preeclampsia, abruptio placentae, and adverse neonatal outcomes among women with chronic hypertension', *New England Journal of Medicine*, 339: 667-71.
Siddiqui, A. H., R. A. Irani, S. C. Blackwell, S. M. Ramin, R. E. Kellems, and Y. Xia. 2010. 'Angiotensin receptor agonistic autoantibody is highly prevalent in preeclampsia: correlation with disease severity', *Hypertension*, 55: 386-93.
Sones, J. L., and R. L. Davisson. 2016. 'Preeclampsia, of mice and women', *Physiol Genomics*, 48: 565-72.
Sones, J. L., Merriam, A. A., Seffens, A., Brown-Grant, D.-A., Butler, S. D., Zhao, A. M., Douglas, N. C. 2018. 'Angiogenic factor imbalance precedes complement deposition in placentae of the BPH/5 model of preeclampsia', *The FASEB Journal*, 32: 2574-86.
Srinivasan, S. R., E. R. Dalferes, R. H. Wolf, B. Radhakrishnamurthy, T. A. Foster and G. S. Berenson. 1984. 'Variability in blood pressure response to dietary sodium intake among African Green Monkeys (Cercopithecus aethiops)', *Am J Clin Nutr*, 39: 792-96.
Stanek, J. 2017. 'Placental pathology varies in hypertensive conditions of pregnancy', *Virchows Arch*, 472: 415-23.
Stanhewicz, A. E., S. Jandu, L. Santhanam, and L. M. Alexander. 2017. 'Increased Angiotensin II Sensitivity Contributes to Microvascular Dysfunction in Women Who Have Had Preeclampsia', *Hypertension*, 70: 382-89.
Stevens, D. U., S. Al-Nasiry, J. Bulten, and M. E. Spaanderman. 2013. 'Decidual vasculopathy in preeclampsia: lesion characteristics relate to disease severity and perinatal outcome', *Placenta*, 34: 805-9.
Stillman, I. E., and S. A. Karumanchi. 2007. 'The glomerular injury of preeclampsia', *J Am Soc Nephrol*, 18: 2281-4.
Stojanovska, V., S. A. Scherjon, and T. Plosch. 2016. 'Preeclampsia As Modulator of Offspring Health', *Biol Reprod*, 94: 53.

Struhsaker, TT. 1967. 'Ecology of Vervet Monkeys (Cercopithecus Aethiops) in The Masai-Amboseli Game Reserve, Kenya', *Ecology*, 48: 891-904.
Suranyi, A., A. Altorjay, L. Kaiser, T. Nyari, and G. Nemeth. 2017. 'Evaluation of placental vascularization by three-dimensional ultrasound examination in second and third trimester of pregnancies complicated by chronic hypertension, gestational hypertension or pre-eclampsia', *Pregnancy Hypertens*, 8: 51-59.
Sutton, A. L. M., L. M. Harper, and A. T. N. Tita. 2018. 'Hypertensive Disorders in Pregnancy', *Obstet Gynecol Clin North Am*, 45: 333-47.
Symonds, EM. 1988. 'Renin and Reproduction', *Am J Obstet Gynecol*, 158: 754-61.
Szalai, G., Romero, R., Chaiworapongsa, T., Xu, Y., Wang, B., Ahn, H., Than, N. G. . 2015. 'Full-Length Human Placental sFlt-1-e15a Isoform Induces Distinct Maternal Phenotypes of Preeclampsia in Mice', *PLoS One*, 10.
Takimoto, E., Ishida J, Sugiyama F, Horiguchi H, Murakami K, and Fukamizu A. 1996. 'Hypertension induced in pregnant mice by placental renin and maternal angiotensinogen', *Science*, 274: 995-8.
Tan, CY., Ho, JF., Chong, YS., Loganath, A., Chan, YH., Ravichandran, J., Lee, CG., and Chong, SS. . 2008. 'Paternal contribution of HLA-G*0106 significantly increases risk for pre-eclampsia in multigravid pregnancies', *Mol Hum Reprod*, 14: 317-24.
Tanaka, M., G. Jaamaa, M. Kaiser, E. Hills, A. Soim, M. Zhu, I. Y. Shcherbatykh, R. Samelson, E. Bell, M. Zdeb, and L. A. McNutt. 2007. 'Racial disparity in hypertensive disorders of pregnancy in New York State: a 10-year longitudinal population-based study', *Am J Public Health*, 97: 163-70.
Tayade, C., D. Hilchie, H. He, Y. Fang, L. Moons, P. Carmeliet, R. A. Foster, and B. A. Croy. 2007. 'Genetic deletion of placenta growth factor in mice alters uterine NK cells', *J Immunol*, 178: 4267-75.
Temming, L. A., J. M. Dicke, M. J. Stout, R. M. Rampersad, G. A. Macones, M. G. Tuuli, and A. G. Cahill. 2017. 'Early Second-Trimester Fetal Growth Restriction and Adverse Perinatal Outcomes', *Obstet Gynecol*, 130: 865-69.
Thoulass, J. C., L. Robertson, L. Denadai, C. Black, M. Crilly, L. Iversen, N. W. Scott, and P. C. Hannaford. 2016. 'Hypertensive disorders of pregnancy and adult offspring cardiometabolic outcomes: a systematic review of the literature and meta-analysis', *J Epidemiol Community Health*, 70: 414-22.
Thrasher, T. N. 2005. 'Baroreceptors, baroreceptor unloading, and the long-term control of blood pressure', *Am J Physiol Regul Integr Comp Physiol*, 288: R819-27.
Tkachenko, O., Shchekochikhin, D., Schrier, R. W. 2014. 'Hormones and hemodynamics in pregnancy', *Int J Endocrinol Metab*, 12: e14098.
Tomimatsu, T., K. Mimura, S. Matsuzaki, M. Endo, K. Kumasawa, and T. Kimura. 2019. 'Preeclampsia: Maternal Systemic Vascular Disorder Caused by Generalized Endothelial Dysfunction Due to Placental Antiangiogenic Factors', *Int J Mol Sci*, 20.
Tubbergen, P., Lachmeijer, A.M.A., Althuisius, S.M., Vlak, M.E.J., van Geijn, H.P., Dekker, G.A. . 1998. 'Change in paternity: a risk factor for preeclampsia in multiparous women?', *Journal of Reproductive Immunology*, 45: 81-88.
Tucker, M. J., C. J. Berg, W. M. Callaghan, and J. Hsia. 2007. 'The Black-White disparity in pregnancy-related mortality from 5 conditions: differences in prevalence and case-fatality rates', *Am J Public Health*, 97: 247-51.

Turbeville, H. R., and J. M. Sasser. 2020. 'Preeclampsia beyond pregnancy: long-term consequences for mother and child', *Am J Physiol Renal Physiol*, 318: F1315-F26.
Turco, M. Y., and A. Moffett. 2019. 'Development of the human placenta', *Development*, 146.
USPSTF. 2017. 'Screening for Preeclampsia US Preventive Services Task Force Recommendation Statement', *JAMA*, 317: 1661-67.
Veerbeek, J. H., W. Hermes, A. Y. Breimer, B. B. van Rijn, S. V. Koenen, B. W. Mol, A. Franx, C. J. de Groot, and M. P. Koster. 2015. 'Cardiovascular disease risk factors after early-onset preeclampsia, late-onset preeclampsia, and pregnancy-induced hypertension', *Hypertension*, 65: 600-6.
Venkatesha, S., M. Toporsian, C. Lam, J. Hanai, T. Mammoto, Y. M. Kim, Y. Bdolah, K. H. Lim, H. T. Yuan, T. A. Libermann, I. E. Stillman, D. Roberts, P. A. D'Amore, F. H. Epstein, F. W. Sellke, R. Romero, V. P. Sukhatme, M. Letarte, and S. A. Karumanchi. 2006. 'Soluble endoglin contributes to the pathogenesis of preeclampsia', *Nat Med*, 12: 642-9.
Verdonk, K., W. Visser, A. H. Van Den Meiracker, and A. H. Danser. 2014. 'The renin-angiotensin-aldosterone system in pre-eclampsia: the delicate balance between good and bad', *Clin Sci (Lond)*, 126: 537-44.
Verlohren, S., M. Niehoff, L. Hering, N. Geusens, F. Herse, A. N. Tintu, A. Plagemann, F. LeNoble, R. Pijnenborg, D. N. Muller, F. C. Luft, J. W. Dudenhausen, M. Gollasch, and R. Dechend. 2008. 'Uterine vascular function in a transgenic preeclampsia rat model', *Hypertension*, 51: 547-53.
Vigil-De Gracia, P., and J. Ludmir. 2015. 'The use of magnesium sulfate for women with severe preeclampsia or eclampsia diagnosed during the postpartum period', *J Matern Fetal Neonatal Med*, 28: 2207-9.
Vikse, B. E., S. Hallan, L. Bostad, T. Leivestad, and B. M. Iversen. 2010. 'Previous preeclampsia and risk for progression of biopsy-verified kidney disease to end-stage renal disease', *Nephrol Dial Transplant*, 25: 3289-96.
Vikse, B. E., L.M. Irgens, T. Leivestad, R. Skjærven, and B.M. Iversen. 2008. 'Preeclampsia and the Risk of End-Stage Renal Disease', *New England Journal of Medicine*, 359: 800-09.
Wadhwa, P. D., C. Buss, S. Entringer, and J. M. Swanson. 2009. 'Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms', *Semin Reprod Med*, 27: 358-68.
Wallukat, G., V. Homuth, T. Fischer, C. Lindschau, B. Horstkamp, A. Jupner, E. Baur, E. Nissen, K. Vetter, D. Neichel, J. W. Dudenhausen, H. Haller, and F. C. Luft. 1999. 'Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor', *J Clin Invest*, 103: 945-52.
Wang, X., D. C. Miller, R. Harman, D. F. Antczak, and A. G. Clark. 2013. 'Paternally expressed genes predominate in the placenta', *Proc Natl Acad Sci U S A*, 110: 10705-10.
Warren, W. C., A. J. Jasinska, R. Garcia-Perez, H. Svardal, C. Tomlinson, M. Rocchi, N. Archidiacono, O. Capozzi, P. Minx, M. J. Montague, K. Kyung, L. W. Hillier, M. Kremitzki, T. Graves, C. Chiang, J. Hughes, N. Tran, Y. Huang, V. Ramensky, O. W. Choi, Y. J. Jung, C. A. Schmitt, N. Juretic, J. Wasserscheid, T. R. Turner, R. W. Wiseman, J. J. Tuscher, J. A. Karl, J. E. Schmitz, R. Zahn, D. H. O'Connor, E.

Redmond, A. Nisbett, B. Jacquelin, M. C. Muller-Trutwin, J. M. Brenchley, M. Dione, M. Antonio, G. P. Schroth, J. R. Kaplan, M. J. Jorgensen, G. W. Thomas, M. W. Hahn, B. J. Raney, B. Aken, R. Nag, J. Schmitz, G. Churakov, A. Noll, R. Stanyon, D. Webb, F. Thibaud-Nissen, M. Nordborg, T. Marques-Bonet, K. Dewar, G. M. Weinstock, R. K. Wilson, and N. B. Freimer. 2015. 'The genome of the vervet (Chlorocebus aethiops sabaeus)', *Genome Res*, 25: 1921-33.

Weaver, Chelsea C, Frances Louard, and Jeffrey L Osborn. 2018. 'Abstract 018: High Stillbirth Rate and Altered Maternal Renal Function and Water Balance are Associated With Spontaneous Gestational Hypertension in Nonhuman Primates', *Hypertension*, 72: A018-A18.

Webster, L. M., F. Conti-Ramsden, P. T. Seed, A. J. Webb, C. Nelson-Piercy, and L. C. Chappell. 2017. 'Impact of Antihypertensive Treatment on Maternal and Perinatal Outcomes in Pregnancy Complicated by Chronic Hypertension: A Systematic Review and Meta-Analysis', *J Am Heart Assoc*, 6.

Wilkerson, R. G., and A. C. Ogunbodede. 2019. 'Hypertensive Disorders of Pregnancy', *Emerg Med Clin North Am*, 37: 301-16.

Williams, MA., R. Mittendorf, and RR. Monson. 1991. 'Chronic hypertension, cigarette smoking, and abruptio placentae', *Epidemiology*, 2: 450-3.

Wlodek, M. E., K. Westcott, A. L. Siebel, J. A. Owens, and K. M. Moritz. 2008. 'Growth restriction before or after birth reduces nephron number and increases blood pressure in male rats', *Kidney Int*, 74: 187-95.

Yan, Y., H. Peng, P. Wang, H. Wang, and M. Dong. 2016. 'Increased expression of fatty acid binding protein 4 in preeclamptic Placenta and its relevance to preeclampsia', *Placenta*, 39: 94-100.

Yeung, K. R., N. Sunderland, J. M. Lind, S. Heffernan, S. Pears, B. Xu, A. Hennessy, and A. Makris. 2018. 'Increased salt sensitivity in offspring of pregnancies complicated by experimental preeclampsia', *Clin Exp Pharmacol Physiol*, 45: 1302-08.

Yoder, S. R., L. L. Thornburg, and J. D. Bisognano. 2009. 'Hypertension in pregnancy and women of childbearing age', *Am J Med*, 122: 890-5.

Zaman, M. A., S. Oparil, and D. A. Calhoun. 2002. 'Drugs targeting the renin-angiotensin-aldosterone system', *Nat Rev Drug Discov*, 1: 621-36.

Zhou, C. C., Y. Zhang, R. A. Irani, H. Zhang, T. Mi, E. J. Popek, M. J. Hicks, S. M. Ramin, R. E. Kellems, and Y. Xia. 2008. 'Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice', *Nat Med*, 14: 855-62.

Zhu, X., S. Wu, W. L. Dahut, and C. R. Parikh. 2007. 'Risks of proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis', *Am J Kidney Dis*, 49: 186-93.

Zusterzeel, PLM., Morsche, RT., Raijmakers, MTM., Roes, EM.,Peters, WHM., Steegers, EAP. 2002. 'Paternal contribution to the risk for pre-eclampsia', *J Med Genet*, 39: 44-45.

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PROFESSIONAL PUBLICATIONS

Manuscripts

1. **Weaver, C.C., and** Osborn, J.L. (2021). Spontaneous Preeclampsia is Associated with Maternal Proteinuria and Fetal Growth Restriction in African Green Monkeys (*Chlorocebus aethiops sabaeus*). *Am J Physiol Regul Integ Comp Physiol. Submitted Feburary 2021.*
2. **Weaver, C.C.,** Osborn, J.L. and Taylor, J.C., (2021). An Active-Learning Approach to Teaching Scientific Writing to Undergraduate Physiology Students. *Am J Physiol Advances in Physiology Education. Submitted February 2021.*
3. **Weaver, C.C., and** Osborn, J.L. (2021). Juvenile Offspring Born to Preeclamptic African Green Monkeys have Reduced Glucose Tolerance and Mild Renal Insufficiency. *Am J Physiol Regul Integ Comp Physiol. Submitted April 2021.*
4. Rivera, P., **Weaver, C.C.**, Osborn, J.L. (2021). Proteinuria and Reduced Renal Function in African Green Monkeys (*Chlorocebus aethiops sabaeus*) with Postpartum Hypertension. *Am J Physiol Regul Integ Comp Physiol. Submitted April 2021.*
5. Osborn, J.L., **Weaver C.C.**, and Rhoads, M.K. Decreased α-myosin Expression is Associated with Left Ventricular Hypertrophy in Hypertensive African Green Monkeys (*Chlorocebus aethiops sabaeus*). *Medical Research Archives. Submitted April 2021.*

Blogs

1. **Chelsea C. Weaver.** 2019, December 19. *Graduate Student Ambassadors: An APS Effort to Increase Involvement in Professional Societies*. https://blog.lifescitrc.org/pecop/2018/12/19/graduate-student-ambassadors-an-aps-effort-to-increase-involvement-in-professional-societies/

Abstracts

1. **Chelsea C. Weaver*,** Patrick R. Rivera, Lin Xiang, Jessica C. Taylor, Jeffrey L. Osborn. "*Open Problem-Based Instruction Improves Student Outcome on Short Answer Critical Thinking Physiology Questions*." FASEB Journal, April 2020.
2. Shea E. Sickles*, **Chelsea C. Weaver**, Jessica C. Taylor, Jeffrey L. Osborn. "*Adolescent Offspring of Preeclamptic Nonhuman Primate Mothers Have Impaired Glucose Tolerance and Proteinuria*." FASEB Journal, April 2020.
3. Lucas D. G. Barrett*, **Chelsea C. Weaver**, Megan K. Rhoads, Jeffrey L. Osborn. "*Hypertension Does Not Enhance Proteinuria in African Green Monkeys that Exhibit One or More Copies of an APOL1-Like Gene Insertion*." FASEB Journal, April 2020.
4. Madison B. Webb*, Shea E. Sickles, **Chelsea C. Weaver**, Jeffrey L. Osborn. "*Increased Pulmonary Function Following Administration of a CBD-Containing Compound NCMB-1 in Fibrotic Lungs of African Green Monkeys.*" FASEB Journal, April 2020.

5. Maria A. Venegas*, Jessica C. Taylor, **Chelsea C. Weaver,** Jeffrey L. Osborn. "*Female African Green Monkeys Exhibit Protection Against Glucose Loading Indicative of Type 2 Diabetes Mellitus*." FASEB Journal, April 2020.
6. Patrick R. Rivera*, **Chelsea C. Weaver,** Jeffrey L. Osborn. "*Postpartum Hypertensive Nonhuman Primates Display Altered Plasma Osmolality Without Proteinuria*." FASEB Journal, April 2020
7. Sushovan Dixit*, **Chelsea C. Weaver,** Eva Gatineau, Audrey Poupeau, Jeffrey L. Osborn, Frederique B. Yiannikouris. "*Proteinuria and Elevated sPRR Are Associated With PreEclampsia in African Green Monkeys*." FASEB Journal, April 2020.
8. **Chelsea C. Weaver*,** Jessica C. Taylor, Jeffrey L. Osborn. "*Elevated Fasting Glucose and Proteinuria in Offspring Born to Gestational Hypertensive African Green Monkeys."* American Heart Association Council on Hypertension Scientific Session, New Orleans, LA September 2019.
9. **Chelsea C. Weaver*,** Jessica C. Taylor, Jeffrey L. Osborn. "*Offspring Born to Gestational Hypertensive Pregnancies Have Increased Fasting Glucose and Proteinuria in Early Adolescence.*" APS/ASN Control of Renal Function in Health and Disease, Charlottesville, VA June 2019.
10. **Chelsea C. Weaver***, Jeffrey L. Osborn. "*African Green Monkeys Born to Mothers with Hypertensive Pregnancy Disorders have Higher Blood Pressures and Altered Water Balance in Early Adolescence.*" Experimental Biology, Orlando FL April 2019.
11. **Chelsea C. Weaver*,** Jessica C. Taylor, Jeffrey L. Osborn. "*An Active Learning and Draft-Revision Approach to Teaching Scientific Writing to Undergraduate Physiology Students.*" Experimental Biology, Orlando FL April 2019.
12. Lucas Barrett*, **Chelsea C. Weaver**, Megan K. Rhoads, Jessica C. Taylor, Jeffrey L. Osborn. "*Insertion in APOL-1-Like Gene is Associated with Increased Blood Pressure but Not Renal Dysfunction in African Green Monkeys.*" Experimental Biology, Orlando FL April 2019.
13. Sushovan Dixit*, **Chelsea C. Weaver**, Jessica C. Taylor, Jeffrey L. Osborn. "*Structured Breeding Groups for the Transmission of Spontaneous Hypertension in African Green Monkeys.*" Experimental Biology, Orlando FL April 2019.
14. **Chelsea C. Weaver***, Frances Louard, Jeffrey L. Osborn. "*High Stillbirth Rate and Altered Maternal Renal Function and Water Balance are Associated with Spontaneous Gestational Hypertension in Nonhuman Primates*." American Heart Association Council on Hypertension Scientific Sessions, Chicago, IL September 2018.
15. **Chelsea C. Weaver***, Justin L. Grobe, Mark K. Santillan, Jeffrey L. Osborn. "*Altered Glomerular Function is Associated with Spontaneous Gestational Hypertension in African Green Monkeys."* Experimental Biology, San Diego, CA April 2018.
16. Emma Bateman*, **Chelsea C. Weaver**, Jeffrey L. Osborn. "*Infant Birth Weight and Postpartum Hypertension in African Green Monkeys."* Experimental Biology, San Diego, CA April 2018.
17. Megan K. Rhoads*, **Chelsea C. Weaver**, Jeffrey L. Osborn. "*APOL1-like Gene Insertion is Associated with Increased Blood Pressure in the African Green Monkey."* Experimental Biology, San Diego, CA April 2018.

18. **Chelsea C. Weaver*,** Mark K. Santillan, Justin L. Grobe, Jeffrey L. Osborn. "*Fetal Growth Restriction is Associated with Spontaneous Gestational Hypertension in African Green Monkeys."* American Heart Association Council on Hypertension Scientific Sessions, San Francisco, CA September 2017.
19. **Chelsea C. Weaver***, Megan K. Rhoads, Justin L. Grobe, Jeffrey L. Osborn. "*Altered Water Homeostasis in a Non-human Primate Model of Gestational Hypertension."* Experimental Biology, Chicago, IL April 2017.
20. Megan K. Rhoads*, **Chelsea C. Weaver**, Jeffrey L. Osborn. "*Sex and Hypertension in the African Green Monkey."* Experimental Biology, Chicago, IL April 2017.
21. **Chelsea C. Weaver***, Jeffrey L. Osborn. "*Pre- Peri- and Post-natal Characterization of Gestational Hypertension in African Green Monkeys*." Cold Spring Harbor Laboratory Computational and Comparative Genomics Course, Cold Spring, NY October 2016.
22. **Chelsea C. Weaver***, Anthony Gutierrez, Jeffrey L. Osborn. "*Decreased* **α***-Myosin Expression is Associated with Left Ventricular Hypertrophy in Hypertensive Caribbean Vervets*" American Heart Association Council on Hypertension, Orlando, FL September 2016.
23. Alex Gutierrez*, Slavina B. Goleva, Megan K. Rhoads, **Chelsea C. Weaver**, William H. Beierwaltes, Jeffrey L. Osborn. *"Altered Renal and Circulating Renin-Angiotensin System Does Not Cause Spontaneous Hypertension in the African Green Monkey"* Experimental Biology Annual Meeting, San Diego, CA April 2016.
24. Anthony Gutierrez*, **Chelsea C. Weaver**, Jeffrey L. Osborn. *"Spontaneous Hypertension in Nonhuman Primate African Green Monkeys Is Associated with Left Ventricular Hypertrophy Without Significant Cardiac or Aortic Fibrosis"* Experimental Biology Annual Meeting, San Diego, CA April 2016.
25. **Chelsea C. Weaver***, Anthony Gutierrez, Jeffrey L. Osborn. *"Left Ventricular Hypertrophy Is Associated with Decreased* **α** *But Not* **β***-Myosin Gene Expression in Spontaneously Hypertensive Nonhuman Primates"* Experimental Biology Annual Meeting, San Diego, CA April 2016.
26. **Chelsea C. Weaver***, Andrea Gonzalez, Justin-Joseph Simpao, Dalton Hampton, Michelle Giedt, and Douglas Harrison. "*The Morphological Characterization of Male Reproductive Aging in Drosophila melanogaster*" National Conference on Undergraduate Research, Lexington, KY April 2014.
27. **Chelsea C. Weaver***, Connor Appelman, Andrea Gonzalez, Megan Weaver, Michelle Giedt, and Douglas Harrison. "*Morphological Characterization of Male Reproductive Aging in Drosophila melanogaster*" Kentucky Academy of Science Annual Meeting, Morehead, KY November 2013.
28. Rebecca Petersen*, Shannon Brady, Casey Clark, Sandra Onekhena, Victoria Thompson, Connor Appelman, Andrea Gonzalez, Megan Weaver, **Chelsea C. Weaver**, Michelle Giedt, and Douglas Harrison. *"A Genome-Wide Association Study to Identify Factors Regulating Reproductive Aging in Male Drosophila melanogaster"* Kentucky Academy of Science Annual Meeting, Morehead, KY November 2013.