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SPONTANEOUS POSTPARTUM HYPERTENSION IN THE AFRICAN GREEN MONKEY

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SPONTANEOUS POSTPARTUM HYPERTENSION IN THE AFRICAN GREEN MONKEY

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Arts and Sciences
at the University of Kentucky

By

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Lexington, Kentucky

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2021

ABSTRACT OF THESIS

SPONTANEOUS POSTPARTUM HYPERTENSION IN THE AFRICAN GREEN MONKEY

Postpartum hypertension (PPHT) is a hypertensive disorder of the puerperium that occurs in women at a rate between 0.8-28% although the exact incidence is unknown due primarily to its transient presentation during a time of reduced medical supervision. The etiology of PPHT is currently unknown with no present experimental animal model. We present the African green monkey (*Chlorocebus aethiops sabaeus*; AGM) as a potentially translational NHP model of PPHT in humans. AGMs were identified as PPHT using Doppler sphygmomanometry and American Heart Association standards of hypertension for systolic blood pressure (systolic blood pressure > 140 mmHg). Disease characteristics were determined utilizing the following measures: water intakes, urinary excretion rates, and plasma osmolalities were used to assess water balance and urinary Na+/K+ and protein excretion rates were measured as assessments of renal function. The results indicated potential roles for the renin-angiotensin-aldosterone system, antidiuretic hormone, or atrial natriuretic peptide-related dysfunction in the development of PPHT. Concomitant proteinuria potentially indicated the presence of postpartum preeclampsia. The AGM model of PPHT recapitulates many of the potential etiologies of human PPHT.

KEYWORDS: Postpartum hypertension, African green monkey, puerperial hypertension

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CHAPTER 1. INTRODUCTION

1.1 Hypertensive Disorders

Historically, hypertensive disorders have been a leading cause of mortality worldwide and rates of hypertension-related disorders and resultant deaths have continued to increase over time (Lawes et al., 2008). From 1990, the global rate of reported systolic blood pressure greater than 140 mmHg has increased from 17.3% to 20.5% with an estimated 14% of deaths in 2015 attributable to hypertensive disorders (Forouzanfar, M. H. et al., 2017). Further, it is the primary cause of mortality during pregnancy for both mother and fetus as gestational hypertensive disorders contribute to over 10% of pregnancy-related mortalities (Creanga et al, 2017). Common life-threatening gestational hypertensive disorders include preeclampsia and hemolysis, elevated liver enzymes, low platelet (HELLP) syndrome. Though both are considered to be gestational hypertensive disorders, they can continue to present symptoms past delivery or develop *de novo* in the puerperium period (Deruelle et al., 2006). Other studies have also reported a subset of *de novo* pregnancy-induced, postpartum hypertensive disorders. This has been defined as postpartum hypertension, transient puerperial hypertension, or postpartum (pre)eclampsia that are comorbid with proteinuria and altered renal function (Walters et al., 1986, Nagai 1997).

These pregnancy-related diseases have been a major topic of study due to their well-documented deleterious effects to both mother and fetus. Despite the focus on these disorders, the etiology and mechanisms responsible largely remain cryptic. Further, it has been documented that there is a subset of previously normotensive mothers who

develop hypertension after delivery (Redman et al., 2019; Al-Safi et al., 2011; Matthys et al. 2004). Postpartum hypertension is a hypertensive disorder characterized by elevated systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥ 90 mmHg any time during the puerperium that typically returns to normotensive levels by 42 days postpartum. It is well known that hypertension is likely the most significant factor in the development and prevalence of cardiovascular disease worldwide and persistent hypertension carrying through peripartum is often both a primary cause and result of separate hypertensive diseases including those previously listed (He, J., & Whelton, P. K., 1999). Further, postpartum hypertension can exacerbate antepartum disorders or result in other comorbidities like HELLP syndrome and postpartum eclampsia *de novo*. There are two major categories of postpartum hypertension discussed in the literature: Postpartum hypertension as a consequence of gestational hypertensive disorders and de novo postpartum hypertension (Goel Arvind et al., 2015; Ghuman et al., 2009; Walters et al., 1986). Due to the transient nature of the expression of the disease during a period of reduced medical supervision, studies on the mechanisms and effects of postpartum hypertension are less common than hypertensive disorders during gestation. Typically, the first medical wellness check after birth is at 6 weeks. As the 6-week postpartum period is the critical period for postpartum hypertension, diagnosis is difficult as many early symptoms of hypertension are mild. Despite these difficulties, one study determined an incidence rate of 18.6% in a cohort of women undergoing cesarean sections for singleton pregnancies (Goel et al., 2015). The lack of diagnoses in moderate to severe cases of postpartum hypertension can lead to potential comorbidities such as stroke, postpartum preeclampsia/eclampsia, or HELLP syndrome. While the relationship

of *de novo* postpartum hypertension to potential outcomes has not been confirmed in any longitudinal studies, persistent postpartum hypertension has been shown to be concomitant with HELLP and preeclampsia in 48% of cases (Podymow et al., 2010).

1.2 The African Green Monkey as a Model of PPHT

The African green monkey (AGM; *Chlorocebus aethiops sabaeus*) is a nonhuman primate model of spontaneous hypertension and cardiovascular disease. The AGM has been shown to spontaneously develop hypertension with age similar to human expressions along with pregnancy-related hypertensive disorders such as gestational hypertension and preeclampsia (Rhoads 2018, Weaver 2021). There are presently no experimental animal models of postpartum hypertension and the phenotype has only been described in humans prior to this work. However, a previous member of our lab observed that some normotensive AGM mothers spontaneously developed hypertension exclusively in the postpartum. We hypothesize that this is an AGM presentation of postpartum hypertension that may recapitulate the disease phenotype in human pregnancy and serve as a translatable model for greater study of the disease.

1.3 Goals of Study

The primary goals of this study are: 1) to determine if there is a significant spontaneous postpartum hypertensive subset of previously, normotensive AGM pregnancies, 2) to investigate the relationship of postpartum hypertension on renal excretory function, and 3) to characterize the effects of fluid and electrolyte balance as a potential mechanism of postpartum hypertension. These goals have led to the hypothesis that spontaneous postpartum hypertension develops in the AGM associated with elevated water retention and renal damage similar to expression of postpartum hypertension in

humans. The results of these experiments could help establish the AGM as a nonhuman primate model of spontaneous, postpartum hypertension.

CHAPTER 2. LITERATURE REVIEW

As pregnancy-induced hypertension as well as preeclampsia have an onset during pregnancy and symptoms that typically are postpartum, the leading hypotheses for pregnancy related hypertension(s) are focused on the placenta (Dekker and Sibai, 1998; Roberts and Escudero, 2012). Current thinking on postpartum hypertension differs as the symptoms have an onset after delivery which reduces the potential for placental contributions (Sibai et al., 2012). Beyond any potential lack of placental contributions, the mechanisms underlying the etiology of postpartum hypertension remain largely unstudied.

2.1 Mobilization of Interstitial Fluid into the Vascular Space

One major hypothesis of the development of postpartum hypertension is that it is caused by the accumulation of interstitial fluid and sodium retention through the course of normal pregnancy within the vascular fluid (Ghuman et al., 2009, Walters et al., 1986). Normal pregnancy is a unique state of arterial underfilling and controlled blood pressure despite elevated sodium and water retention. The primary method of counteracting this elevated water and sodium retention and the subsequent hypertension is the secretion of the hormone relaxin. Relaxin is a potent vasodilator that is secreted during pregnancy primarily from the placenta and corpus luteum of the ovaries. Relaxin mediates a sustained reduction in blood pressure through a nitric oxide-mediated dilation of the vasculature (Conrad, 2011; Bani-Sacchi et al., 1995).

Nitric oxide is a potent vasodilator that reduces arterial blood pressure through the stimulation of cGMP-dependent protein kinase G activating myosin phosphatase which interrupts vascular smooth muscle contractions leading to vasodilation and, therefore,

reduced total peripheral resistance (Ignarro et al., 1981; Palmer et al., 1987). In one study of the temporal dynamics of relaxin in rabbit pregnancy, the concentration of relaxin dropped to one-fifth its peak pregnancy concentration within one day postpartum and by three days postpartum the concentration dropped such that no relaxin was detected in the animals (Marder et al., 1944). It is worth noting that while relaxin is a major factor in this hypotensive state, other factors may become more important as pregnancy continues and may play some role postpartum (Johnson et al., 1996).

Within this same early postpartum period, sodium and water retention is typically returned to pre-pregnancy values primarily through the action of natriuretic peptide (ANP) which is a protein the elicits a potent natriuresis released in response to increased stretch in the atria of the heart. ANP functions directly to reduce blood pressure through a fourfold mechanism 1) an increase in glomerular filtration rate, 2) an increase in excretion rate of sodium and water, 3) vascular smooth muscle relaxation, and 4) increased vascular permeability favoring movement of fluid from the vascular space to the interstitium (Meyer and Huxley, 1990; de Bold et al., 1981). Further, it reduces the rate of renin secretion in response to salt load detected at the macula densa (Villareal et al., 1986). One potential pathway of ANP-mediated hypertension is a reduced concentration of ANP when relaxin returns to prepregnancy levels leading to reduction of all ANP function and therefore an excess of vascular fluid and hypertension. Typical human postpartum is characterized by an elevated concentration of ANP during the first week postpartum (Steegers et al., 1987) and previous studies have displayed that the loss of ANP production leads to increased salt-sensitivity and reduced antagonism of the RAAS (Melo et al., 1998; O'Tierney et al., 2008).

Even with the loss of function in ANP, the typical renal pressure natriuresis would alleviate this excess volume which may also result in the transient hypertension that is characteristic of postpartum hypertension. This pathway is supported by one study of the relationship of ANP and postpartum hypertension that found that ANP in postpartum hypertensive mothers was decreased compared to normotensive controls (Nagai et al., 1997). This makes it a key potential factor in the etiology of postpartum hypertension.

Though the exact nature of the onset of postpartum hypertension is unresolved, there are some known risk factors that have been associated with its development. This leads into the second category of postpartum hypertension which is persistent hypertension through the peripartum period.

2.2 Persistent Hypertension Through the Peripartum

The primary risk factor associated with the onset of postpartum hypertension is the prior presence of other gestational hypertensive disorders and postpartum hypertension is often treated as part of prior gestational hypertensive disorders like HELLP syndrome and particularly, preeclampsia (Al-Safi et al., 2011, Trogstad et al., 2011). Although the symptoms differ on a case-by-case basis due to individual factors such as physician treatment, a subset of patients previously suffering antenatal hypertensive disorders including HELLP and preeclampsia have been observed to maintain an elevated blood pressure past the intrapartum with an average return to prepregnancy blood pressures at 5.4 weeks postpartum (Podymow and August, 2010).

The development of postpartum hypertension as a delayed outcome of preeclampsia is a well-known and considerable health burden in pregnancy.

Preeclampsia develops in the postpartum period in approximately 5.7% of cases (Matthys et al., 2004) and in one analysis of late-onset eclampsia in women from 1996 to 2001, one-third of women who developed eclamptic seizures developed them in the postpartum versus the antepartum (Chames et al., 2002). Of those women, 79% of them developed eclampsia after 48 hours postpartum which is classified as late onset. Interestingly, from this set of women developing late-onset postpartum eclampsia nearly 78% were not diagnosed as preeclamptic though 90% had some symptoms of hypertension in the antepartum (Chames et al., 2002). This could implicate postpartum hypertension as a subset of late-onset gestational hypertension that displays a subclinical presentation until postpartum. In one study of late-onset eclampsia, almost two-thirds of the postpartum eclamptic cohort displayed no antepartum hypertension (Al-Safi et al., 2011).

Preeclampsia is a disease noted for a particularly obfuscated etiology due to two potentially different etiologies for early-onset (<34 weeks gestation) and late-onset (≥34 weeks gestation). There are a multitude of theories about the risk factors and physiological causes underlying preeclampsia. One major avenue of study is the involvement of the placenta in the onset of preeclampsia as delivery of the baby typically alleviates gestational preeclampsia. This potentially could persist through the intrapartum period and be related to postpartum hypertension, although it is functionally disparate from late-onset which is theorized to largely develop in response to maternal factors rather than placental as in early-onset (Trogstad et al., 2011). Though it is known that preeclampsia typically has postpartum complications, the division between the two etiologies of preeclampsia complicates discussion of how preeclampsia leads to postpartum hypertension and postpartum eclampsia.

The potential for a combination of maternal and placental factors makes researching potential pathways linking preeclampsia to postpartum hypertension a complicated process. However, one study found that early-onset and late-onset preeclampsia share similar relationships to known risk factors such as primiparity, chronic hypertension, and obesity (Wójtowicz et al., 2019). Early-onset preeclampsia typically results in higher blood pressure, higher rates of adverse outcomes, and generally an increased severity of outcomes compared to late-onset (Wójtowicz et al., 2019). Potentially important to postpartum hypertension, early-onset preeclampsia also was found to have a greater rate of puerperal complications compared to early-onset (56% vs 41.6%) (Wójtowicz et al., 2019). This difference may be significant, but it is not necessarily a direct relationship as the increased puerperal complications may arise from the generally elevated severity of antenatal complications such as renal damage in early-onset preeclampsia compared to late-onset. Indeed, other research has shown that the presence of proteinuria is associated with an increase in the duration of postpartum hypertension compared to chronic or gestational hypertensive mothers without proteinuria (Stepan et al., 2006). This is not unexpected as proteinuria is a clinical sign of renal damage and normal renal function is the primary controller of body fluid volume and blood pressure (Ruggenenti et al., 1998). As proteinuria is positively associated with greater persistence of antenatal hypertension, differences in the relative severity of the damage from the two forms of preeclampsia such as greater renal insufficiency in early-onset preeclampsia could be the mediator of the differences in the rate of postpartum complications between early-onset and late-onset disease (Wójtowicz et al., 2019). Further, the differences in renal function could result from timing as a late-onset of

preeclampsia inherently implies that there is less time for damage to occur compared to early-onset preeclampsia.

2.3 Potential Involvement of the Renin-Angiotensin-Aldosterone System in Postpartum Hypertension

The renin-angiotensin-aldosterone system (RAAS) is commonly implicated in hypertension as it is a powerful controller of extracellular fluid volumes and, as a result, blood pressure (de Man et al., 2012,). Renin cleaves angiotensin I from angiotensinogen which is then cleaved into the active octapeptide form angiotensin II by angiotensin-converting enzyme primarily in the lungs. Production of angiotensin II results in vasoconstriction, reabsorption of sodium and water in the nephron, and release of antidiuretic hormone (ADH) and aldosterone, which themselves have effects that synergize with angiotensin II to elevate blood pressure. Aldosterone elevates blood pressure by inducing genetic expression of Na+/K+ antiporters that increase sodium reabsorption in the distal tubule in exchange for elevated potassium excretion.

Though there is a logical possibility of a role of the RAAS in the etiology of preeclampsia, any role it potentially plays and the mechanism mediating that role is currently unknown. Renin and aldosterone levels are typically elevated during pregnancy, and this is a major component of the elevated water and sodium retention during the course of normal pregnancy; however, during the course of preeclamptic pregnancies, plasma renin and aldosterone levels have been found to actually be decreased back to nonpregnant levels or lower in severe cases (Brown et al., 1992; Irani and Xia, 2011). One potentially important concurrent finding was that renin activity in these preeclamptic women is reduced to a greater degree than aldosterone activity

(Brown et al., 1992). This results in an elevated aldosterone/renin activity ratio which, assuming that plasma renin activity is equivalent to angiotensin II activity, implies either a greater adrenal cortical sensitivity to angiotensin II compared to normal pregnancy or a RAAS-independent pathway of increasing plasma aldosterone concentration (Brown et al., 1992) Indeed, another study found that pregnancy-induced hypertension in humans results in greater sensitivity to angiotensin II (Saxena et al., 2010).

2.4 Aldosterone-Induced Hypertension

Despite lacking evidence of RAAS causality in postpartum hypertension, there may be a role of aldosterone in de novo postpartum hypertension though few studies have been performed on evaluating this relationship. In a case study from 2000, two women presented with severe postpartum hypertension ostensibly caused by primary aldosteronism (Nezu et al., 2000). To date, these have only been presented as isolated cases and have received little to no further study expansion but if aldosterone remains elevated above pre-pregnancy levels in the postpartum, it could result in hypertension through increased vascular and extracellular fluid volume retention along with vasoconstriction. It is also possible that the primary aldosteronism may be a more prominent cause of postpartum hypertension than is reported but is unknown due to either a more transient expression or typically mild symptoms that reduce the chances of proper diagnosis.

2.5 Anti-Diuretic Hormone-Induced Hypertension

Similar to aldosterone, ADH increases vascular and extracellular fluid volume by stimulating the translocation of aquaporin-2 channels from the basolateral membrane of the collecting duct to the luminal membrane thus increasing the rate of reabsorption of

water in the collecting ducts of the kidney along with stimulating the central neural sensation of thirst. This makes ADH dysfunction a potential contributor to postpartum hypertension (Share and Crofton, 1982; Blessing, 1982). Although RAAS-mediated postpartum hypertension has not been observed, ADH can be directly stimulated through other pathways, primarily through the detection of plasma hypoosmolality by osmoreceptors in the hypothalamus. A similar relationship has been postulated with preeclampsia though few studies have been conducted assessing the possible role of ADH in the postpartum. One study searched for a predictive relationship between copeptin, a more stable byproduct of the production of ADH, and preeclampsia (Yeung et al., 2014). They reported a relationship of elevated copeptin in preeclamptic mothers compared to normotensive pregnancies with the difference increasing as pregnancy progressed. This implicates ADH as a potential mediator of hypertension persisting through the peripartum; however, this group also reported no relationship in any gestational hypertensive disorders lacking renal damage (gestational hypertension and gestational diabetes mellitus) evidenced by proteinuria (Yeung et al., 2014). This implicates renal damage as primary to the etiology of ADH-induced postpartum hypertension (Yeung et al., 2014).

Along with this finding, evidence of a RAAS-independent mechanism contributing to postpartum hypertension has been reported. One study of induced uterine ischemia mimicking preeclampsia in RUPP (Reduced Uterine Perfusion Pressure) rats found that uterine ischemia during pregnancy resulted in elevated salt sensitivity of blood pressure postpartum compared to sham controls (Matsuura et al., 2019). After salt-loading, copeptin concentration was found to be over 1.5x higher in RUPP mothers

compared to control counterparts and RAAS activity was similar between groups throughout. Further, blockade of ADH-receptors abolished the difference between groups in response to salt-loading (Matsuura et al., 2019). Therefore, preeclampsia may also be inducing postpartum hypertension through increased salt-sensitivity from elevated ADH secretion.

2.6 Diagnosis

Diagnosis of postpartum hypertension is a difficult process due to the transient nature of the expression of the disease during a period of reduced medical supervision. As this 6-week period is critical for defining postpartum hypertension, the diagnosis of this disease and its effects is difficult as many potential patients do not go to a medical professional for diagnosis due to typically mild or non-overt symptoms. Despite these difficulties, one study determined an incidence rate of 18.6% in a cohort of women undergoing cesarean sections for singleton pregnancies with almost half of those cases (77/184) developing *de novo* (Goel Arvind et al., 2015). The lack of diagnoses in moderate to severe cases of postpartum hypertension can lead to potential comorbidities such as stroke, postpartum preeclampsia/eclampsia, or HELLP syndrome.

2.7 Risk Factors

Gestational hypertensive disorders persisting past the intrapartum and into the postpartum period represent a powerful risk factor for postpartum hypertension and risk factors from these gestational hypertensive disorders may also serve as risk factors for postpartum hypertension. Risk factors for preeclampsia largely cannot be generalized to postpartum hypertension as they simultaneously represent very broad categories of persons and also are largely too specific to preeclampsia. Currently, the strongest risk

factors for preeclampsia include primiparity, familial/personal history of preeclampsia, African American race, diabetes mellitus, young maternal age, and chronic hypertension (Lisonkova and Joseph, 2013).Many of these have been previously associated with postpartum preeclampsia and preeclampsia itself is known to often result in postpartum complications, so a positive diagnosis of preeclampsia could be considered as a risk factor necessitating further medical follow-up in the postpartum period for postpartum hypertension.

Further, there is evidence of a new-onset late postpartum expression of preeclampsia. Though making up only 1.3 cases per 1000 live births, some early risk factors have been established that may be applicable to postpartum hypertension due to the shared period of expression in the early puerperium. The case-control study found that a maternal age ≥40, black or Latin ethnicity, BMI ≥ 30 at delivery, and gestational diabetes mellitus are all associated with new-onset late postpartum preeclampsia (Bigelow et al, 2014). Of these, all but Latin ethnicity have previously been associated with antenatal preeclampsia which supports that these criteria could potentially serve as predictors of late postpartum preeclampsia as well as postpartum hypertension.

Preeclampsia in the postpartum further adds burden on the medical system as the incidence of postpartum preeclampsia contributes 5.7% of total preeclampsia diagnoses and results in a 66% readmission rate to the hospital particularly due to a relatively high chance of developing into eclampsia in the absence of treatment (16%) (Matthy et al., 2004). Persistent hypertension from preeclampsia is a large contributor of pregnancy-related morbidity as up to 33% of eclamptic seizures actually occur in the postpartum period versus the antepartum as stated earlier (Chames et al., 2002) The high percentage

of postpartum preeclampsia and the resultant eclamptic seizures are demonstrative of the current weaknesses in modern diagnosis and treatment of postpartum hypertensive disorders as it amounts to one-third of eclamptic seizures occurring after sufferers have been cleared for discharge and have been relocated further away from timely administration of critical life-saving physician intervention.

2.8 sFlt/PlGF Ratio as a Risk Factor

One potential factor that has been proposed to assist in the diagnosis of postpartum hypertension is the assessment of angiogenic factors such as sFlt and PlGF as predictors of risk (Verlohren et al., 2017). Elevated sFlt/PlGF ratio has previously been associated with preeclampsia and assays of sFlt/PlGF ratio can be used to positively predict preeclampsia and HELLP syndrome with a diagnostic specificity of 99.4% and a sensitivity of 94.0% in women who would become early-term preeclamptic and diagnostic specificity of 95.4% and sensitivity of 89.5% in women who would become late-term preeclamptic according to one assay on clinical efficacy (Stepan et al., 2016). The concentration of sFlt and the ratio of sFlt to PlGF during pregnancy have also been associated with postpartum hypertension and may be informative in predicting its occurrence similar to preeclampsia (Stepan et al., 2016; Goel Arvind et al., 2015). However, there is some evidence that sFlt/PlGF ratio is not an effective positive predictor of preeclampsia as another study found mixed results with the use of sFlt/PlGF ratio as a predictive factor at a threshold ratio of 38 (Caillon et al., 2018). In a study of mothers with at least one marker of elevated preeclampsia risk, an sFlt/PlGF ratio below the threshold ratio was 100% negatively predictive of the development of preeclampsia within one week; however, the positive predictive rate had a 79% false positive rate

(Caillon et al., 2018). The addition of this as a risk factor for postpartum preeclampsia when considering medical discharge after delivery could increase the confidence of medical personnel in discharging mothers as well as reduce the number of eclamptic seizures that occur outside of the hospital and therefore, further ameliorate the associated deleterious effects. This testing would not only serve to assist in determining the risk of de novo postpartum hypertension but also the risk of persistent hypertension lasting into the postpartum period.

Postpartum hypertension is an acute state of hypertension that can lead to acute, deleterious comorbidities including cerebral hemorrhage and eclampsia. Diagnosis remains difficult as symptoms are typically mild and present during a period of reduced medical supervision. The etiology of postpartum hypertension is presently unknown leading to limited treatment strategies based on symptom management rather than remedying underlying causes. Some correlated risk factors have been discovered but no reliable clinical method of predicting risk of postpartum hypertension has been produced. Postpartum hypertension and postpartum preeclampsia require further study and represent a health risk to new mothers to an extent that is not fully understood.

CHAPTER 3. METHODS

3.1 Animal Care and Housing

All studies adhered to the protocols approved by the Primates Plus/SKN Primates Institutional Animal Care and Use Committee at the University of Kentucky. Animals were group housed at SKN Primates in St. Kitts and Nevis, West Indies. Purpose-bred AGMS were allowed to stay with their birth group and mother until 6 months of age when they were weaned and moved to a group enclosure of 10-15 juveniles until sexual maturity before being assigned to a breeding group. Wild-trapped animals were also utilized. These animals were quarantined for 45 days with a full veterinary workup before introduction to a group enclosure. All AGMs tested negative for tuberculosis and were administered Ivermectin (0.5 mg/kg s.c.) for parasite removal twice before removal from quarantine. Animals were fed standard NHP chow (Harlan Teklad 8773) and fresh, local fruits and vegetables twice daily three times a week with water provided *ad libitum*.

3.2 Measurement of Blood Pressures

Arterial blood pressures were collected through forearm Doppler plethysmography. Animals were lightly sedated with ketamine (15 mg/kg i.m.) before systolic blood pressure measurement through the use of a pressure cuff and Doppler stethoscope for 7 consistent collections within 5% variance. Pressure cuff size was chosen based on upper forearm diameter to ensure measurement accuracy. After full radial arterial occlusion, traditional Korotkoff sounds were used as indicators of systolic (Korotkoff sound 1) and diastolic (Korotkoff sounds 4 and 5) blood pressures in accordance with protocols previously adapted and described (13). Heart rate was measured (in bpm) by counting pulsatile beats with the Doppler stethoscope for 15 or 30

seconds. Animals were weighed and a full health assessment was conducted at the time of measurements. Blood was collected into ACD tubes (BD Vacutainer 364816) and blood glucose was measured by commercial glucometer (TrueTrack). Although both systolic and diastolic blood pressures were collected, only systolic blood pressures were used to characterize individuals.

For this study, individuals were selected based upon their systolic blood pressure (SBP) grouping prepregnancy. Nonpregnant animals were classified in two phenotypic groups by systolic blood pressures. Animals SBP $\leq$ 120 mmHg were classified as normotensive (NT) and animals were first classified as hypertensive at SBP $\geq$ 140 mmHg. Prepregnant hypertensive females were not considered for this study. AGMs could progress to 1 of 3 phenotypic groupings during or after pregnancy. Animals could remain NT through the pregnancy which was classified as an NT pregnancy. Animals could also display SBP $\geq$ 140 mmHg during pregnancy which was classified as gestational hypertensive or preeclamptic when concomitant with proteinuria. Finally, they could display SBP $\geq$ 140 mmHg during the period lasting from delivery through 42 days postpartum before returning to SBP $\leq$ 120 mmHg which we classified as postpartum hypertensive. Measurements were collected prior to pregnancy, during the 3rd trimester, and at days 1, 14, and 42 postpartum.

3.3 Urine Collection and Sample Preparation

Selected normotensive nonpregnant females were single housed in metabolic pens for an acclimation period of no fewer than 1 week followed by 3 consecutive days of urine collection (n = 88). After acclimation, systolic blood pressures were collected as described above and whole blood was collected. Dietary regimen remained the same as the group-housing described above with single-housed individuals receiving two meals of standard NHP chow (Harlan Teklad 8779) and fresh, local fruits and vegetables. Water was provided *ad libitum* in 1-liter bottles and water intakes and urinary volumes were measured daily. Urine was centrifuged (1000 g) to remove large particles and aliquoted into 1.5 mL microtubes to minimize freeze-thaw cycle sample degradation during downstream analysis and stored at -20°C. After they were released back to their breeding groups, they would be regularly checked for pregnancy. After pregnancy was identified through visual examination and confirmed through abdominal palpation, pregnant females were returned for further measurements prior to pregnancy, during the 3rd trimester, and at days 1, 14, and 42 postpartum.

3.4 Pregnancy Determination

Females were placed into group enclosures with 1 male and 15-25 females representative of a natural troop. Staff identified pregnancy through visual inspection. When pregnancy was suspected, the female was pulled from the enclosure and abdominal palpation was performed to confirm a fetus. Upon confirmation, the fetus was measured externally crown-to-rump. Crown-to-rump measurement was used to determine the trimester of the pregnancy. 1st trimester fetuses between 1-6 cm, 2nd between 7-12 cm, and 3rd past 12 cm. This guideline was determined through measuring fetal length and

backtracking from delivery to determine the range of fetal sizes for each trimester in normal pregnancies over multiple breeding seasons. Once the trimester was confirmed, fetal measurement was repeated for the subsequent trimesters. Once fetuses reached second and trimester according to the above guidelines, mothers were pulled for blood pressure, HR, and fasting glucose measurements followed by venous blood collection as described above. Once the 3rd trimester was reached, females were moved to individual housing for daily maternal monitoring until 42 days postpartum after which the mother and offspring were moved back to their original group until weaning.

3.5 Urinary Protein Measurement

Total urinary protein concentration was determined through Pierce BCA Assay (Catalog # 23225, ThermoFisher Scientific, Waltham MA) per the manufacturer's recommended protocol. Samples were diluted to 1:5 and micro-pipetted into a 96-well plate alongside 25 µL albumin standards serially diluted to up to a concentration of 2000 mg/ml. 200 µL working BCA reagent was added to each well and the plates were incubated at 37°C for 2 hours. After cooling to room temperature, absorbance was read at 595 nm. A linear standard curve was produced for each plate ($r^2 \geq 0.99$) and used to determine the unknown sample protein concentrations. Protein excretion rate was calculated as: $Protein\ Excretion\ Rate = [Urinary\ Protein\ Concentration\ (mg/ml)] * UFR(ml/day)$

3.6 Plasma Sample Preparation and Osmolality Measurement

Whole blood was centrifuged down to its component plasma, buffy coat, and hematocrit. Li-heparinized tubes of blood were aliquoted into centrifuge tubes and spun for 15 minutes at 2200-2500 rpm. The plasma, buffy coat, and hematocrit were pipetted

into separate screw-cap tubes for transport then later aliquoted into 1.5 mL microtubes to minimize freeze-thaw cycles downstream. Samples were stored at -20℃ throughout. Plasma osmolality was determined using a freezing point depression osmometer (Precision Instruments Micro-Osmette) using manufacturer's specification.

3.7 Urinary Sodium and Potassium Measurements

Total urinary sodium and potassium concentrations were measured using a dual-channel flame photometer (#02655-15, Cole-Parmer, Vernon Hills, IL) as per manufacturer's instructions. Thawed aliquots were diluted 20x in lithium diluent and measured. The resulting measurements were expressed in mmol/ml and multiplied by urine flow rate to get the total urinary excretion rate for sodium and potassium.

3.8 Statistical Analysis

Results were analyzed using JMP Pro 14 (SAS) with statistical analysis through mixed-model repeated measures analysis of variance (ANOVA) with Student's t *post hoc* or one-way ANOVA with stepwise Newman-Keuls *post hoc* where appropriate. Significance was set at $\alpha = 0.05$ for the cutoff for statistical significance. Values were presented as Mean±SEM.

CHAPTER 4. RESULTS

4.1 Effect of PPHT on AGM Systolic Blood Pressure

A subset of previously normotensive AGM mothers displayed an elevated systolic blood pressure in the postpartum period compared to NT controls that abated by 42 weeks postpartum (Figure 1A). SBP remained similar to nonpregnant SBP through pregnancy and postpartum in the normotensive control group (Figure 1A; NP 104 ± 4.7 mmHg; 3T 99 ± 4.4 mmHg; PP1 102 ± 5.2 mmHg; PP14 101 ± 6.8 mmHg; PP42 97 ± 6.0 mmHg, n=27 per time point). PPHT mothers displayed an increase in SBP compared to NT controls and NP PPHT at 1 day postpartum and decreasing by 42 days postpartum (Figure 1A: NP 114 ± 10.0 mmHg, n=8; 3T 107 ± 10.0 mmHg, n=8; PP1 144 ± 9.5 mmHg*, n=9; PP14 131 ± 9.0 mmHg*, n=10; PP42 117 ± 10.0 mmHg, n=8; * $p \leq 0.05$ vs NP; ANOVA, *post hoc* Stepwise Newman-Keuls).

4.2 Effect of PPHT on Body Weight and Water Balance

PPHT AGM weights (NP 3.9±0.16 kg, n=8; 3T 4.6±0.19 kg, n=6; PP1 4.0±0.15 kg, n=9; PP14 3.7±0.10 kg, n=10; PP42 3.5±0.13 kg, n=8) remained similar to NT control group weights (NP 3.4±0.15 kg, n=22; 3T 4.7±0.17 kg, n=6; PP1 3.6±0.25 kg, n=5; PP14 3.9±0.09 kg, n=5; PP42 3.6±0.16 kg, n=5) throughout pregnancy and postpartum (Figure 3).

PPHT AGMs did not display any significant differences in water intake throughout pregnancy and postpartum (Figure 2A). PPHT AGM water intake (NP 241.7±44.5 mL/day, n=8; 3T 197.1±29.4 mL/day, n=13; PP1 256.9±31.6 mL/day, n=8; PP14 247.7±39.6 mL/day, n=11; PP42 274.3±60.1 mL/day, n=8) remained similar to the

NT control group (NP 270.7±43.0 mL/day, n=28; 3T 229.4 ±49.9 mL/day, n=6; PP1 334.1±83.5 mL/day, n=4; PP14 358.9±63.0 mL/day, n=3; PP42 377.5±225.8 mL/day, n=2) throughout pregnancy and postpartum (Figure 2A).

PPHT AGMs (NP 139.8±26.8 mL/day, n=8; 3T 89.1±19.4 mL/day, n=13; PP1 146.9±28.2 mL/day, n=8; PP14 210.5±28.6 mL/day, n=10; PP42 223.4±48.1 mL/day, n=8) displayed a decreased urine flow rate during the third trimester that returned to normotensive controls (NP 167.9±22.9 mL/day, n=28; 3T 242.7 ±64.4 mL/day, n=6; PP1 190.6 ±24.6 mL/day, n=4; PP14 212.2±25.4 mL/day, n=3; PP42 148.3±63.3 mL/day, n=2) by 1 day postpartum (Figure 2B).

4.3 Effect of PPHT on AGM Plasma Osmolality

NT AGMs displayed no change in plasma osmolality through pregnancy and postpartum (Figure 4; NP 304.7±4.6 mOsm/kg, n=13; 3T 304.3±2.7 mOsm/kg, n=7; PP1 290.8±4.3 mOsm/kg, n=5; PP14 308.8±2.3 mOsm/kg*, n=6; PP42 299.9±2.1 mOsm/kg, n=8;*$p < 0.05$, Stepwise Newman-Keuls).

PPHT AGMs displayed a decrease in plasma osmolality in the 3rd trimester of pregnancy before returning to prepregnant baselines by 1 day postpartum (Figure 4; NP 315±11.0 mOsm/kg, n=4; 3T 289.3±2.9 mOsm/kg, n=8; PP1 297.6±6.4 mOsm/kg, n=10; PP14 309.9±6.2 mOsm/kg *, n=8; PP42 293±4.2 mOsm/kg, n=7;*$p < 0.05$, Stepwise Newman-Keuls).

PPHT AGMs displayed a decrease in plasma osmolality in the 3rd trimester compared to NT AGMs that returns to NT by 1 day PP (Figure 4; 3T 289.3±2.9 mOsm/kg, n=8 vs 3T 304.3±2.7 mOsm/kg, n=7, Student's T *post hoc*).

4.4 Effect of PPHT on AGM Urinary Na+/K+ Excretion

NT AGMs did not display any change in urinary Na+ excretion rate through pregnancy or postpartum (Figure 5A; NP 2.17±0.21 mmol/day, n=28; 3T 2.12±0.49 mmol/day, n=8; PP1 2.17±0.36 mmol/day,, n=6; PP14 2.30±0.34 mmol/day*, n=7; PP42 1.95±0.19 mmols/day, n=5;*$p < 0.05$, Stepwise Newman-Keuls).

PPHT AGMs did not display any change in urinary Na+ excretion rate through pregnancy or postpartum (Figure 5A; NP 2.79±0.43 mmol/day, n=8; 3T 2.53±0.63 mmols, n=9; PP1 3.98±1.15 mmols/day, n=8; PP14 4.22±0.41 mmol/day*, n=10; PP42 3.55±0.80 mmols/day, n=8; *$p < 0.05$, Stepwise Newman-Keuls). PPHT AGM Na+ excretion rate increased compared to NT during 1- and 14-days PP (Figure 5A; NT PP1 2.17±0.36 mmol/day, n=6 vs PPHT PP1 3.98±1.15 mmols/day, n=8; NT PP14 2.30±0.34 mmol/day, n=7 vs PPHT PP14 4.22±0.41 mmols/day, n=10).

NT did not display any change in urinary K+ excretion rate through pregnancy or postpartum (Figure 5B; NP 3.01±0.34 mmol/day, n=8; 3T 4.21±0.1.33 mmols/day,, n=9; PP1 2.97±0.50 mmol/day,, n=8; PP14 3.34±0.33 mmols/day,*, n=10; PP42 3.02±0.13 mmols/day,, n=8;*$p < 0.05$, Stepwise Newman-Keuls).

PPHT did not display any change in urinary K+ excretion rate through pregnancy or postpartum (Figure 5B; NP 4.91±0.99 mmols/day, n=8; 3T 3.32±0.72 mmol/day,, n=9; PP1 5.64±1.30 mmols/day,, n=8; PP14 6.74±1.30 mmols/day,*, n=10; PP42 3.55±0.80 mmol/day,, n=8;*$p < 0.05$, Stepwise Newman-Keuls). PPHT AGMs displayed elevated K+ excretion rate compared to NT at 14 days PP (NT 4.22±0.41 mmol/day, vs PPHT 6.74±1.30 mmol/day, n=10, Student's T *post hoc*).

4.5 Effect of PPHT on AGM Urinary Protein Excretion Rate

PPHT AGMs (NP 421.4±52.6 mg/day, n=8; 3T 354.7±80.8 mg/day, n=8; PP 560.5±42.6 mg/day, n=24; *p < 0.05 compared to NT and 3T, Stepwise Newman-Keuls) displayed a trend toward elevated protein excretion rate during the postpartum compared to NT and 3T that returns to normal by 42 days postpartum (Figure 6). NT AGMs (NP 365.2±24.2 mg/day, n=28; 3T 464.6±53.4 mg/day, n=8; PP 342.1±26.8 mg/day*, n=24; *p < 0.05 Stepwise Newman-Keuls) maintained similar protein excretion rates throughout pregnancy and postpartum (Figure 6). PPHT AGMs displayed elevated protein excretion rates compared to NT during the PP (Figure 6; PP 560.5±42.6 mg/day vs PP 342.1±26.8 mg/day*, n=24, Student's T *post hoc*).

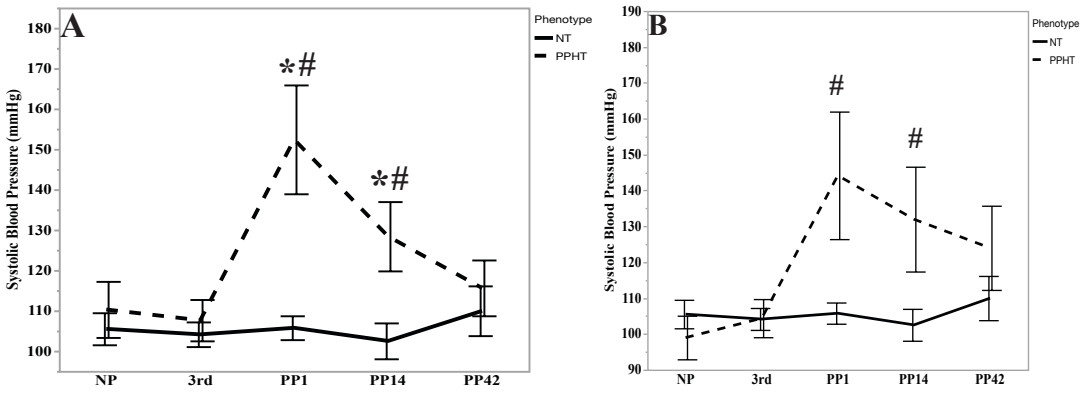

Figure 1 Systolic Blood Pressures of NT and PPHT AGMs

Systolic blood pressure (SBP; **A**) measured via forearm plethysmography for normotensive (NT) and postpartum hypertensive (PPHT) African green monkeys prior to pregnancy (NP, n=37 and 9), in the 3rd trimester (n=17 and 9), and at days 1 (n=15 and 11), 14 (n=11 and 11), and 42 (n=10 and 9) postpartum. **B**) SBP of NT and four PPHT AGMs prior to pregnancy (NP, n=37 and 5), in the 3rd trimester (n=17 and 5), and at days 1 (n=15 and 5), 14 (n=11 and 5), and 42 (n=10 and 5) postpartum. # indicates $p<0.05$ versus NT at same timepoint via mixed-model ANOVA with Student's t *post hoc*. * indicates $p<0.05$ versus NP timepoint within the same group via one-way ANOVA *post hoc* Student Newman-Keuls. SBP remains unchanged in NT pregnancy, but SBP increases by 1 day postpartum in a PPHT pregnancy and remains elevated past 14 days postpartum.

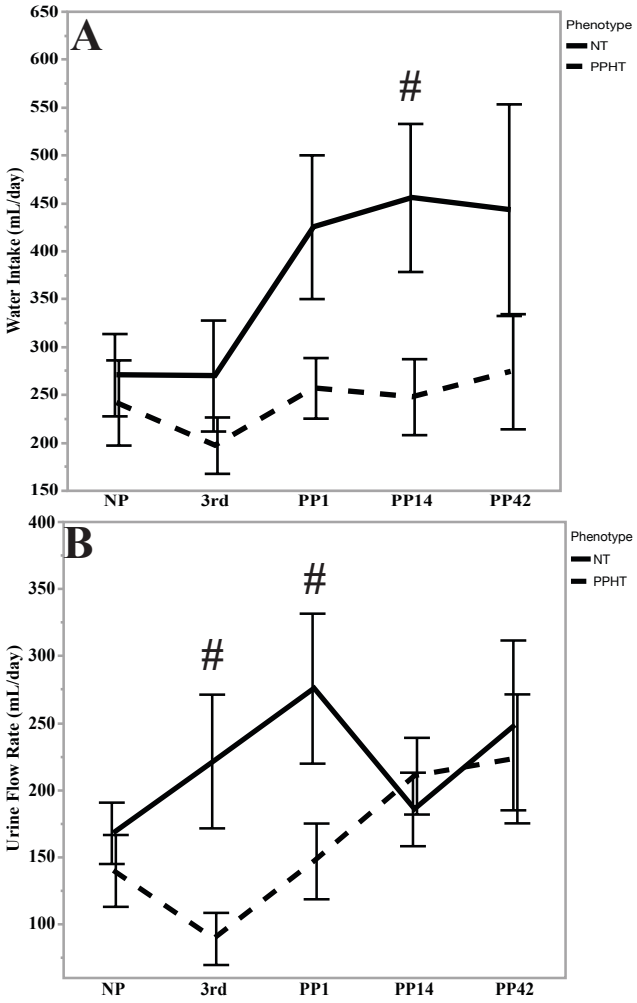

Figure 2 Water Intake and Urine Flow Rate of NT and PPHT AGMs

Water intake (WI; **A**) and urine flow rate (UFR; **B**) for normotensive (NT) and postpartum hypertensive (PPHT) African green monkeys prior to pregnancy (NP, n=28 and 8), in the 3rd trimester (n=8 and 13), and days 1 (n=10 and 8), 14 (n=5 and 11), and 42 (n=4 and 8) postpartum. # indicates $p<0.05$ versus NT at same timepoint by mixed-model ANOVA with Student's t *post hoc*. WI increased 14 days postpartum in PPHT compared to NT. UFR decreased during the 3rd trimester and at 1 day postpartum compared to NT pregnancy.

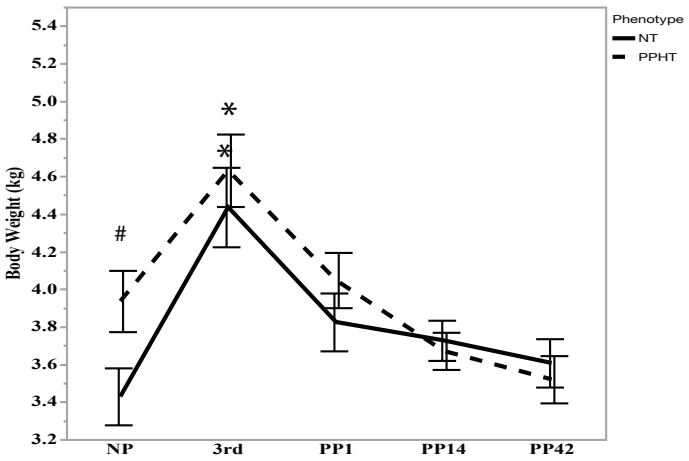

Figure 3 Body Weight of NT and PPHT AGMs

Weight of normotensive (NT) and postpartum hypertensive (PPHT) African green monkeys prior to pregnancy (NP, n=22 and 8), in the 3rd trimester (n=9 and 6), and days 1 (n=10 and 9), 14 (n=7 and 10), and 42 (n=7 and 8) postpartum. # indicates $p<0.05$ versus NT at same timepoint via mixed-model ANOVA with Student's t *post hoc*. * indicates $p<0.05$ versus NP timepoint within the same group via one-way ANOVA *post hoc* Student Newman-Keuls. PPHT weight was elevated nonpregnant compared to NT but changed similarly throughout pregnancy and postpartum.

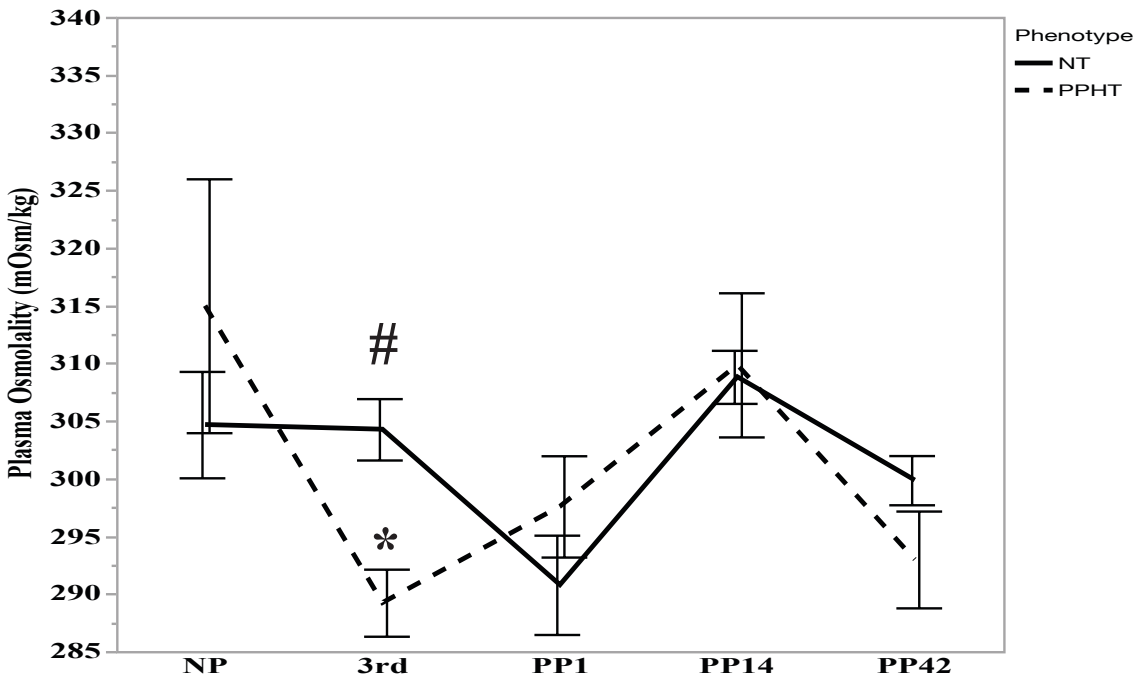

Figure 4 Plasma Osmolality of NT and PPHT AGMs

Plasma osmolality, measured by freezing-point depression, for normotensive (NT) and postpartum hypertensive (PPHT) African green monkeys prior to pregnancy (NP, n=13), in the 3rd trimester (n=7), and days 1 (n=5), 14 (n=6), and 42 (n=8) postpartum. # indicates $p<0.05$ versus NT at same timepoint via mixed-model ANOVA with Student's t *post hoc*. * indicates $p<0.05$ versus NP timepoint within the same group via one-way ANOVA *post hoc* Student Newman-Keuls. Plasma osmolality decreased in the 3rd trimester compared to NP and returned to NP levels by 1 day postpartum. Plasma osmolality was decreased compared to NT at 3rd trimester.

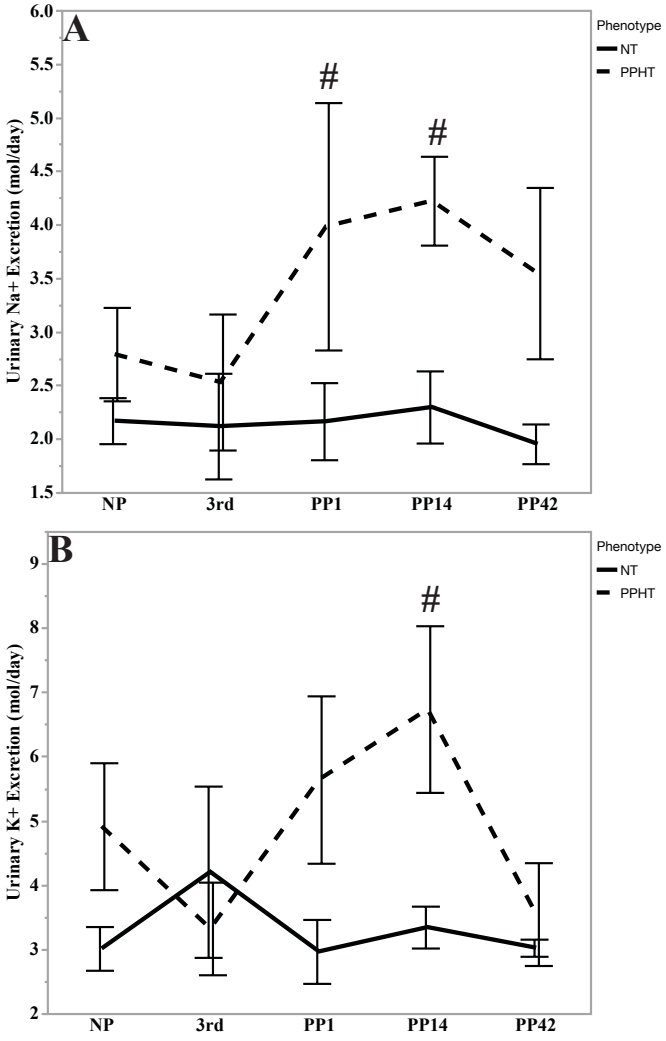

Figure 5 Urinary Na+/K+ Excretion Rates of NT and PPHT AGMs

Urinary sodium excretion rate (A) and urinary potassium excretion rate (B), measured by flame photometry, for normotensive (NT) and postpartum hypertensive (PPHT) African green monkeys prior to pregnancy (NP, n=28 and 8), in the 3rd trimester (n=8 and 9), and days 1 (n=6 and 8), 14 (n=7 and 10), and 42 (n=5 and 8) postpartum. # indicates $p<0.05$ versus NT at the same timepoint via mixed-model ANOVA with Student's t *post hoc*. Urinary sodium excretion rate increased compared to NT at 1 and 14 days postpartum. Urinary potassium excretion rate increased compared to NT at 14 days postpartum.

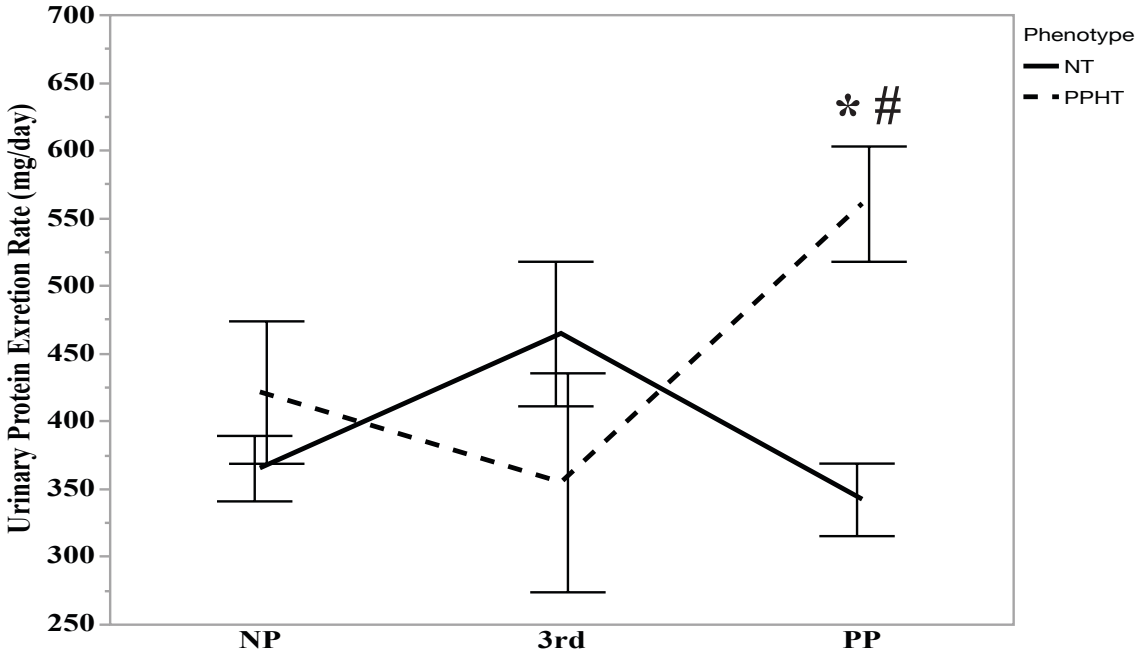

Figure 6 Urinary Protein Excretion Rate of NT and PPHT AGMs

Urinary protein excretion rate, measured by bicinchoninic acid assay, for normotensive (NT) African green monkeys prior to pregnancy (NP, n=28), in the 3rd trimester (n=8), and from 1 to 42 days postpartum (n=24) and postpartum hypertensive (PPHT) African green monkeys prior to pregnancy (NP, n=8), in the 3rd trimester (n=8), and from 1 to 42 days postpartum (n=24). # indicates $p<0.05$ versus NT at same timepoint via mixed-model ANOVA with Student's t *post hoc*. * indicates $p<0.05$ versus NP timepoint within the same group via one-way ANOVA *post hoc* Student Newman-Keuls. Urinary protein excretion rate increased compared to 3rd trimester during postpartum.

CHAPTER 5. DISCUSSION

The purpose of this study was to identify the existence of a postpartum hypertensive population of African green monkeys and identify the renal and fluid balance characteristics in both normotensive and postpartum hypertensive phenotypes. Our results show the presence of a spontaneously postpartum hypertensive subset of African green monkeys. Further, our results collectively show a complex syndrome tending toward fluid retention during late pregnancy through an insufficient urine flow rate despite elevated Na+ excretion and elevated K+ excretion rate by 14 days postpartum potentially implicating hyperaldosteronism in the postpartum hypertensive animals. Further, proteinuria was observed in the postpartum compared to NT controls indicating renal damage. We present the African Green Monkey as a spontaneous model of *de novo* postpartum hypertension and, potentially, postpartum preeclampsia.

The AGM model of postpartum hypertension presented here allows researchers to further identify possible factors that may underlie the disease. The AGM displayed the greatest elevation in blood pressure at one day postpartum that continued through at least fourteen days postpartum. The highest average blood pressures were recorded at one day postpartum (Figure 1A) similar to the timeframe in humans where over 95% of spontaneous postpartum hypertensives develop within the first five days (Goel Arvind et al., 2015). Comparing only PPHT individuals where we have continuous data through all timepoints to NT, the significant one-way difference between 1- and 14- days postpartum compared to NP PPHT was lost (Figure 1B). This may indicate a timeline of postpartum hypertension shorter than two weeks where animals developed hypertension that abated

by the next measurement. However, the significant elevation of SBP compared to NT at 1- and 14- days postpartum was observed as in the entire data set.

Elevated urinary excretion is one of the most well-recognized symptoms of typical pregnancy and is an expected comorbidity of pregnancy starting in the early pregnancy period and resolving during the early puerperium (Thorp et al., 1999; Risberg et al., 2015). Interestingly, in the AGM PPHT cohort, urinary volume did not change during pregnancy and postpartum and NT counterparts had elevated urine excretion rates compared to PPHT in the third trimester and one day postpartum (Figure 2B). Combined with no change in water intake over these timepoints (Figure 2A), this is a potential indicator of an increased tendency toward water retention. Decreased plasma osmolality during the third trimester compared to NT AGMs supports this possibility (Figure 4). Although this could be achieved by loss of osmotically active particles, urinary excretions of Na+, K+, and protein remained similar through the third trimester displaying no evidence for this mechanism (Figures 5A, 5B, 6).

Typical human pregnancy is a complex state of extreme vascular volume overload without an increase in arterial pressure. Relaxin, a hormone produced in the corpus luteum during pregnancy, induces a potent vasodilation, and therefore, hypotension in both animal and human models (Debrah et al., 2006, Dschietzig et al., 2009). The resulting drop in renal arterial plasma flow stimulates increased renin-angiotensin-aldosterone system (RAAS) function and ADH secretion leading to an overall sodium and fluid retention. The week immediately postpartum where relaxin concentrations decrease to prepregnancy levels serves as a critical period of vascular realignment and stress vulnerable to postpartum hypertension. This first week is when the majority of

postpartum hypertension occurs (Goel Arvind et al., 2015) and where the peak systolic blood pressure in our PPHT AGMs occurs (Figure 1A).

The RAAS has previously been implicated in human hypertension and multiple levels remain primary targets in the treatment of hypertension (Weir, 1999). The typical elevated urinary Na+ excretion in the postpartum is not indicative of RAAS dysfunction; however, the elevated postpartum K+ excretion rate in the postpartum hypertensive AGMs implicates primary aldosteronism as a potential causative factor in the etiology (Figures 5A and 5B). Primary aldosteronism has been previously reported as a potential cause of PPHT, but these cases remain uncommon (Nezu et al., 2000).

Elevated natriuresis is an expected response to remedy excess vascular volume and in many forms of chronic hypertension this natriuresis is blunted (Hall et al., 1990); however, the lack of an increase in urine flow rate in response to the elevated Na+ excretion indicates the natriuresis is being counteracted through a sodium-independent mechanism of water reabsorption (Figure 5A). We postulate the PPHT AGM may display an exaggerated ADH response to typical pregnancy vasodilation as displayed by the decreased 3rd trimester plasma osmolality and urine flow rate compared to NT (Figures 4 and 2B). This, combined with a normal postpartum decrease in relaxin production, may be a causative factor in the transient hypertension in the postpartum. In the absence of any change in water intake compared to NT, this implies a potential positive water balance preceding vascular volume overload. It is worth noting that we have observed that AGMs typically present a highly variable water intake in all conditions including an average percent deviation from the mean of 84% in NP NT AGMs (Table 1). This high

baseline variance between animals may be obscuring potential differences in water intake between the PPHT and NT groups.

Atrial natriuretic peptide (ANP) is the primary counterpart of the RAAS that mediates natriuresis-diuresis through actions described above (Meyer and Huxley, 1990; de bold et al., 1981; Villareal et al., 1986). Reduced levels of ANP have previously been implicated in essential hypertension (Macheret et al., 2012), as well as postpartum hypertension in humans (Nagai et al., 1997). These same decreased ANP postpartum mechanisms may be present in the PPHT AGM.

PPHT in the AGM is associated with elevated proteinuria across the postpartum compared to NT controls (Figure 6) which is indicative of the presence and severity of renal damage in human models (Lei et al., 2021; Peterson et al., 1995). The comorbidity of hypertension and proteinuria during the postpartum indicates that a subset of the PPHT AGM cohort may display postpartum preeclampsia. Postpartum preeclampsia has been associated with both acute and chronic comorbidities including high rates of eclampsia- 15.9% of readmissions due to postpartum preeclampsia in one study (Matthys et al., 2004)- as well as increased odds of hypertension past the postpartum period including an increased rate of diagnosis of chronic hypertension (Redman et al., 2019).

There are currently no animal or experimental models of *de novo* postpartum hypertension. Clinical study of human mothers can often only provide observational findings about symptoms of the disease with little insight as to the etiology or mechanisms leading up to the pathology progression. It is critical that a model of postpartum hypertension be developed to investigate the pathology of this disease further.

The African green monkey offers a spontaneous, large, non-human primate model of postpartum hypertension that recapitulates many of the observed features of the disease in human cases. The model will allow for long-term observation of the disease under controlled conditions and in-depth study of many of the complex set of potential structural factors involved that would otherwise remain inaccessible. In summary, we have established the existence of a spontaneous postpartum hypertensive population of AGMs. This model has recapitulated a multitude of the potential factors in the etiology of postpartum hypertension in humans although we are not certain of the relative contribution of each of the potential mechanisms. We hope that the use of this model will allow for greater understanding of this disease and ensure that sufferers are receiving the most effective treatment possible.

Table 1 Percent Deviation in Water Intakes in NT and PPHT AGMs

	NP	3rd trimester	PP1	PP14	PP42
NT	84.0%	60.73%	55.80%	37.92%	49.87%
PPHT	52.04%	53.80%	34.81%	53.08%	61.98%

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