Renal Vascular and Glomerular Pathologies Associated with Spontaneous Hypertension in the Nonhuman Primate *Chlorocebus aethiops sabaeus*

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Abstract Hypertension is a complex, multifactorial disease affecting an estimated 78 million adults in the United States. Despite scientific gains, the etiology of human essential hypertension is unknown and current experimental models do not recapitulate all the behavioral and physiological characteristics of the pathology. Researchers should assess the translational capacity of these models and look to other animal models for the discovery of new therapies. Chlorocebus aethiops sabaeus, the African Green Monkey (AGM), is a nonhuman primate that develops spontaneous hypertension and may provide a novel translational model for the study of hypertension and associated diseases. In a randomly selected group of 424 adult AGMs, 37% (157/424) exhibited systolic blood pressures (SBP) >140 mmHg (SBP: 172.0±2.2 mmHg) and were characterized as hypertensive (HT). 44% (187/424) were characterized as normotensive with SBP <120 mmHg (NT, SBP: 99.6±1.0 mmHg) and the remaining 18% (80/424) as borderline hypertensive (BHT, SBP: 130.6±0.6 mmHg). Compared to NT animals, HT AGMs are older (8.7±0.6 vs 12.4±0.7 years, p<0.05) with elevated heart rates (121.3±1.91 vs 34.3 ±2.1 BPM, p<0.05). BHT animals had average heart rates of 138.2±3.1 BPM (p<0.05 compared to NT) and were 11.00±0.9 years old. NT and HT animals had similar levels of angiotensinogen gene expression, plasma renin activity, and renal cortical renin content (p>0.05). HT monkeys exhibit renal vascular remodeling (wall/lumen ratio NT 0.11±0.01 vs HT 0.15±0.02, p<0.05) and altered glomerular morphology (Bowman’s capsular space: NT 30.9±1.9% vs HT 44.4±3.1%, p<0.05). The hypertensive AGM provides a large animal model that is highly similar to humans and should be studied to identify novel, more effective targets for the treatment of hypertension.
Indexing: Hypertension, African Green Monkey, Blood pressure, Caribbean vervet
Introduction

An estimated 78 million adults in the United States suffer from hypertension, a major risk factor for cardiovascular disease and stroke (11). In the vast majority of these adults (90%), the cause of the elevated blood pressure is unknown, or “essential”. In addition, 6.1 million Americans suffer from resistant hypertension, or uncontrolled blood pressure while prescribed ≥ 4 anti-hypertension medications (23). Elevated blood pressure, left ventricular hypertrophy, cardiovascular disease, and end stage renal disease contribute to one out of every three deaths (11) in western developed nations. Though many different pharmacological therapies have been developed over the past 50 years (with varying degrees of effectiveness), the significant proportion of hypertensive patients with unknown etiology and resistance to currently available medication, might suggest that more translational animal models are needed for the discovery of novel therapies and drug targets.

Current experimental models of hypertension can lack the translational capability required to closely mimic the complex behavioral, physiological, and pathological characteristics of human essential hypertension. Differences between experimental and genetic animal models of hypertension and human essential hypertension can include a lack of upright posture, different circadian rhythms, disparate or undefined social structure and hierarchy, genetic diversity, and unique evolutionary histories. These major differences in fundamental behavioral parameters can undermine the ability of cardiovascular researchers to address the basic etiology of human essential hypertension. Thus, there remains a critical need for an animal model that equates to the human
pathophysiology of hypertensive disease allowing the identification of fundamental causal relationships and the translation of therapies for the treatment and long-term prevention of hypertension.

*Chlorocebus aethiops sabaeus*, the African Green monkey (AGM) is a novel nonhuman primate model of spontaneous hypertension. Commonly known as the vervet, this species was derived from founder populations of AGMs in Western Africa and is currently thriving as an invasive species within specific islands in the West Indies. The AGM behaviorally and socially equates to humans since they live within troops containing a well-described social structure and hierarchy. They are a diurnal species with large syntenic chromosomal regions when compared with humans and exhibit complex social behaviors including male/female parenting of offspring, individual and group cooperation within troops, and significant territoriality in both the wild and captivity. In captivity, these primates can survive to approximately 25 to 35 years old and have been used as biomedical models in the study of neurodegenerative disorders, fetal alcohol syndrome, simian immunodeficiency virus, diabetes, obesity, and many other complex pathologies and behaviors. While there are numerous models of experimentally induced hypertension in various primate species ranging from baboons to rhesus macaques, the Caribbean vervet develops spontaneous hypertension in the wild and in captivity without specialized breeding or dietary intervention. Goldwater et al. first identified elevated blood pressures in individual Caribbean vervets over 30 years ago. We now report the existence of significant, spontaneously occurring hypertension in *Chlorocebus aethiops sabaeus* in a large cohort of phenotyped animals with the potentially confounding
consequences of the hypertension in the development of altered renal vascular and
glomerular morphology.

Methods

Animal Care and Housing

Animals were housed in troop enclosures at an outdoor facility in St. Kitts, West Indies. All protocols strictly adhered to the Guide on the Care and Use of Experimental Animals, Guiding Principles on the Care and Use of Experimental Animals by the American Physiological Society. Water was allowed *ad libitum*. All animals were fed nonhuman primate chow 3 days per week (Harlan, Teklad 8773) and a combination of fresh bananas, mangos, carrots and sweet potatoes the other 4 days per week. All animals were individually quarantined for a minimum of 30 days and undergo a full veterinary examination prior to any handling or introduction into the colony troops. Females born into a troop remain with their natal troop until reaching sexual maturity at which time they may remain or be introduced into another troop society group. Males remain with their natal troop until adolescence and are then introduced into a new troop after reaching sexual maturity.

Measurement of Blood Pressures

Animals were lightly sedated with ketamine (15 mg/kg i.m.). All equipment was thoroughly cleaned between animals to eliminate any foreign scent. After ~10 minutes, animals were placed in a supine position on a clean examination table and a newborn or infant blood pressure cuff placed on the left upper arm. The choice of cuff size was determined based upon the upper forearm diameter to assure accuracy of blood pressure
measurements. Cuff pressure was measured using a digital pressure gauge (Vernier Systems, Inc.) and a Doppler stethoscope to identify forearm vascular flows. The blood pressure technique was developed as an adaptation of the recording techniques previously reported for rodents and utilized extensively in our lab(5). The cuff was inflated to a pressure that achieved full radial artery occlusion. Traditional forearm Korotkoff sounds indicative of systolic (Korotkoff sound 1) and diastolic (Korotkoff sound 2) blood pressures were identified as the blood pressure cuff slowly deflated. This process was repeated until 5 arterial systolic and diastolic pressure measurements were recorded within less than 5% deviation for each animal with approximately 30-second full deflation times between each consecutive measurement. After blood pressure was recorded, heart rate was measured by simply counting pulsatile beats for 15 or 30 seconds. Each animal was weighed and provided an abbreviated health assessment before being placed back within its’ respective enclosure. When available, age was determined from the animal’s complete long-term medical records. Both systolic and diastolic pressures were obtained and recorded for each animal, but only systolic pressures were used for determination of each individual animal’s specific phenotype.

Characterizations of blood pressures of sexually mature, adult vervets (n=424) were organized into three groups, based upon systolic blood pressure (SBP) values. SBP was utilized since this pressure provides the most consistent measurement (i.e. least amount of intra-animal variance) within any given individual using forearm plethysmography due to the clear identification of Korotkoff sound 1. Animals with a SBP ≥ 140mmHg were categorized as hypertensive (HT), while animals with SBP ≤ 120mmHg were considered normotensive (NT). Individuals with SBPs of 121 – 139 mmHg were classified as
borderline hypertensive (BHT). Mean arterial pressure (MAP) was calculated as \( \frac{1}{3} \text{SBP} + \frac{2}{3} \text{DBP} \).

After blood pressure measurements, selected animals were fully anesthetized with supplemental ketamine (25 mg/kg, i.m.) and xylazine (100 mg/kg i.m.). Anesthetized animals were then euthanized with an overdose of sodium pentobarbital (Buthenasia\textsuperscript{R}; 150 mg/kg, i.v.). Tissues were then harvested and thinly sliced for tissue fixation or immediately frozen in ultra-cold isopentane (-20 °C) and dry ice.

**Quantitative Real-Time Polymerase Chain Reaction**

Flash-frozen kidney and liver tissue was dissected from animals and transported to Lexington, KY on ice. RNA was extracted using Direct-zol RNA kits (Zymo Research, Irvine, CA) and converted to cDNA using qScript cDNA SuperMix (Quanta BioSciences, Gaithersburg, MD). qPCR was performed using the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) with Power SYBR Green (Thermo Fisher Scientific, Waltham, MA). Relative gene expression was calculated using the \( 2^{-\Delta\Delta C_T} \) method\( ^{19} \). Primers for angiotensinogen were custom designed using Primer3 software (Forward primer 5'-AAGATTGGCAGCCCCCTGAC; Reverse primer 5'-ATCTTCCCTTGAAAATGGACGTAG).

**Renal Cortical Renin Content**

Renal renin content was determined by measuring the Ang I generation capacity of homogenized renal cortical tissue. After removal from the animal during autopsy, kidneys were flash frozen and stored at -20°C. Cortical sections were dissected out on ice, blotted
and weighed, homogenized in buffer, and centrifuged for 20 minutes at 2000 rpm. The supernatant was removed and diluted with MEM buffer at 1:50 ratio. Aliquots (5 μL) were again diluted at 1:20 ratio in buffer. Aliquots of 200 μL peptidase inhibitor (3% PMSF in methanol and 3.8% EDTA, pH 6.5) and 200 μL sheep angiotensinogen (500 ng) were added, the entire mixture was incubated at 37°C for 30 minutes. Samples were placed on ice and 500 μL of 0.015M HCl added to terminate the reaction. Tubes were boiled for 10 minutes and then centrifuged. Supernatant was removed and the generated Ang 1 was measured by radioimmunoassay, as previously described(12).

**Plasma Renin Activity**

Plasma was collected from sedated animals for determination of PRA. Ethylenediaminetetraacetic acid was used as the anticoagulant and blood was collected in chilled tubes. PRA was analyzed by generation of Ang I/h/min using the Gamma Coat RIA kit (DiaSorin, Stillwater, MN) as previously described and according to the manufacturer's instructions(1, 2).

**Tissue Histological Preparation**

For fixation, tissues were sliced into segments 3 cm in length and 3-5 mm thick. Tissue slices were placed into 5% paraformaldehyde (PFA) for 72 hours (4°C) with the PFA changed at 24-hour intervals. The tissues then were placed in phosphate buffered saline for shipping. All fixed tissues were placed in 70% ethanol for long-term storage. Histological sections (4 mm thickness) were dissected from tissue slices and prepared for tissue sectioning using the Histos 5 rapid microwave tissue histoprocessor (Milestone
Medical, Kalamazoo, MI) with the manufacturer’s recommended protocols. After processing, tissues were embedded into paraffin blocks, cut into 5\(\mu m\) sections using a microtome, and placed on glass microscope slides. Digital images of sections were analyzed using Image J software (NIH, Bethesda, MD)(25).

**Digital Analysis of Tissue Sections**

Kidney tissues were sectioned sagittally (5 \(\mu m\)) and stained with Periodic Acid Schiff stain. Glomeruli and blood vessels in the renal outer cortex were identified (400x) using an Olympus IX70 inverted microscope and digital images captured and then blinded for phenotype using a coded numbering system. A minimum of 10 glomeruli from each animal were individually traced and isolated within Image J. Using Image J’s automated thresholding function, the Bowman’s capsular space within the glomeruli is identified and quantified, resulting in a total area and percent area measurement of space within an individual glomerulus.

Vascular wall thicknesses were determined for renal vessels with diameters between 100 and 200\(\mu m\). At minimum, six unique vessels were identified within the renal cortex of each animal. Digital images of the renal arterioles were captured and assigned a random number for blinded analysis. Wall thickness and lumen diameters were measured on 5 different axes around each arteriole. Wall thickness and lumen diameters were averaged for each arteriole examined, decoded and averaged per animal. Wall/lumen ratios were calculated for comparisons between NT and HT animals.
**Statistical Analysis**

Groups were compared using a one-way analysis of variance (ANOVA) with a Tukey’s *post hoc* comparison. In the case of two group comparisons, an unpaired Student’s t-test and t-statistic or a Mann-Whitney U test were utilized. All values are reported as the mean ± standard error of the mean (SEM). The 5% probability level (p<0.05) was used as the criterion of significance.

Kernel density plots were generated using R 3.0.2 using Bioconductor(9). Kernel density plots estimated the probability of the relationship between age and blood pressure.

**Results**

In a group of 424 adult animals, 44% (187/424) were identified as NT (average SBP= 99.6±1.0 mmHg), 18% (80/424) as BHT (average SBP=130.6±0.6 mmHg), and 37% (157/424) as HT (average SBP= 172.0±2.2 mmHg; Figure 1). HT animals also have greater diastolic blood pressure (DBP; 56.2±1.2 vs. 83.1±1.9 mmHg, p<0.05, ANOVA with Tukey’s *post hoc* comparison) and consequently calculated mean blood pressure (MBP; 70.8±1.0 vs. 112.2±1.7 mmHg, p<0.05, ANOVA with Tukey’s *post hoc* comparison) compared to NT animals. BHT animals averaged 68.7±2.2 mmHg and 89.4±1.5 mmHg for DBP and MBP, respectively (Figure 1).

Heart rate of HT animals averaged 137.7±2.2 beats per minute (BPM) which was greater than that of NT animals (125.7±2.0 BPM; p<0.05, ANOVA with Tukey’s *post hoc* comparison). Heart rates of BHT animals averaged 138.2±3.1 BPM and are significantly
higher compared to NT animals (p<0.05, ANOVA with Tukey's post hoc comparison, Figure 2). Heart rates are greater in female animals, regardless of blood pressure phenotype (males: 124.4±1.3 BPM vs. females: 154.1±2.8 BPM, Figure 2B & 2C).

HT animals were older than NT animals (HT = 12.4±0.7 years; n=52 and NT = 8.9±0.6 years; n=42, p<0.05; Figure 3A). BHT animals were 11.00±0.9 years old (n=32) on average, which was similar to both NT and HT animals (p>0.05, ANOVA with Tukey's post hoc, Figure 3A).

Male and female AGMs have similar incidences of hypertension in adulthood (Male: 119/307 or 38.8%; Female: 38/117 or 32.5%). Systolic, diastolic, and mean blood pressures are similar between male and female AGMs (Table 1). Heart rate is significantly higher in females in all three blood pressure groups (p<0.05, Tukey's post hoc).

Comparative qPCR assessed the gene expression of angiotensinogen in the renal cortex, outer medulla and liver of adult male AGMs. Angiotensinogen gene expression was not different between NT (n=17) and HT (n=14) animals in any of the regional kidney tissues (p>0.05, Student's t-test, Figure 4A). Renal cortical renin content also was similar between NT and HT animals (p>0.05, Student's t-test). NT (n=11) animals RCRC averaged 8.94±1.56 μg Ang II/ml/hr/mg protein while HT (n=13) animals RCRC averaged 10.73±2.98 μg Ang II/ml/hr/mg protein (Figure 4B). Plasma renin activity also was similar in NT (3.27±0.36 ng Ang I/ml/hr, n=15) and HT (3.34±0.48 ng Ang I/ml/hr, n=16) animals (p>0.05, Figure 4C).

Renal vascular hypertrophy was evaluated by measuring the adventitial wall thickness of renal vessels in male AGMs. HT animals have larger wall thickness compared to NT
animals (14.41 ± 0.56 μm vs. 10.33±1.27 μm, p<0.05, Figure 5A). Compromised vascular function was assessed using the ratio of adventitial thickness to vessel lumen diameter (i.e. wall/lumen ratio). High vascular wall/lumen ratios were indicative of vascular remodeling and linked to increased blood pressure. Hypertensive AGMs have greater wall/lumen ratios in renal arterioles >100 micrometers in diameter (NT 0.11±0.01 vs HT 0.15±0.02, p<0.05, Figure 5B).

Renal vascular and glomerular structures were assessed in male NT and HT animals. Using color thresholding analysis (ImageJ), Bowman’s capsular space was greater in glomeruli of HT animals compared with NT animals (NT 30.86±1.88% vs HT 44.44±3.14%, p<0.05). Glomeruli of HT animals show an increase in nonvascular or open area space when compared to glomeruli of NT animals (Figure 6).

**Discussion**

This study characterizes a model of spontaneous hypertension in *Chlorocebus aethiops sabaeus*, the African Green Monkey of the Caribbean or vervet. Blood pressure phenotyping was conducted in 424 animals and included both island inhabiting animals (n= 260) as well as captivity bred and raised vervets (n= 164), with no differences in incidence or elevation of blood pressure between the two populations. HT animals have a significantly higher SBP, DBP, and MBP compared to BHT and NT animals (p<0.05).

In this study, 37% of the adult AGMs have SBPs over 140 mmHg while under light ketamine sedation. This high percentage of animals with hypertension mirrors the American Heart Association’s estimation that 33% of Americans have high blood pressure (11). It is important to note that the AGMs in the colony population were not bred for or
specifically selected for inclusion in this study due to the presence of the pathology; nor
were the animals on unique diets to induce hypertension. Instead, this study identifies a
subset of adult AGMs that display hypertension as a spontaneous pathology with no known
etiology.

Similar to humans, age was associated with a higher risk of the development of
hypertension in the AGM. The kernel density estimation in Figure 3 illustrates the different
age distributions of each group in the subset of age-known animals. The age of NT animals
is skewed heavily to younger ages while the density of the HT animals increases as the age
increases. On average, the typical hypertensive monkey is 4 years older than the average
normotensive monkey in this study; however, the sample size of animals with known ages
is smaller than the total cohort. It is already well known that age is an important factor in
the etiology of hypertension, cardiovascular disease, and end stage renal disease in humans
(3, 30). Future longitudinal studies will further assess the development of hypertension
and associated diseases with increasing age in this nonhuman primate model.

Heart rate is higher in the hypertensive AGM. Elevated heart rate has been
associated with cardiovascular mortality in patients with and without hypertension(10,
26). The elevated heart rate in the AGM mimics the pathologies seen in human essential
hypertensive patients with increased sympathetic drive(7). These similarities suggest that
the AGM and human essential hypertensives may share common etiologies in the
development of cardiovascular disease and hypertension.

The renin angiotensin system (RAS) contributes to human hypertension in some but
not all patients. With this knowledge and the contribution of the renin-angiotensin system
to the maintenance of sodium and water homeostasis, we found it imperative to investigate
the contribution of the RAS to elevated blood pressure in the Caribbean vervet.
Angiotensinogen gene expression, renal cortical renin content, and plasma renin activity
were similar between NT and HT male AGMs (Figure 4). This would suggest that the HT
AGM maintains normal RAS activation compared to NT animals, though the authors accept
that the use of only male animals in these measurements are a limitation of this study. Prior
studies have shown that administration of captopril, an angiotensin converting enzyme
inhibitor, lowers blood pressure in hypertensive AGMs(20), although circulating plasma
levels of Angiotensin II have been reported as lower in animals with high blood
pressure(20). Interestingly, a long-term experiment found that elevated dietary sodium
increases blood pressure in adult AGMs, although components of the RAS were not
measured. Animals with low and high mean blood pressures on sodium deficient control
diets (0%) tended to maintain their relative blood pressure ranking throughout the study,
while all mean blood pressures increased with elevated sodium intake(29). This suggests
that the AGM, or perhaps a subset of AGMs, are salt-sensitive, though the exact role of the
RAS and dietary sodium intake in AGMs with spontaneous hypertension will need to be
clarified in future studies.

Hypertension in humans and animal models is often associated with reductions in
lumen diameter and increases in wall/lumen ratios(24). Increases in renal vessel wall
thickness and renal vessel wall/lumen ratio are indicative of renal vascular remodeling in
the AGM. Changes in Bowman’s capsule space suggest glomerular alteration in
hypertensive AGMs, though functional studies are still needed to assess changes in
glomerular filtration. Previous studies conducted in the hypertensive AGM found no
difference in afferent arteriole lumen diameter, AA media cross-sectional area, or
glomerular number or size between 7 NT and 7 HT adult male AGMs(28). However, our
data focuses specifically on renal vessels with diameters larger than 100μm and illustrates
that the HT AGM undergoes pathophysiological changes in renal vasculature and glomeruli
that mimic those of patients with essential hypertension and are associated with a higher
risk of developing chronic cardiovascular and renal disease.

Previous studies utilizing the AGM have shown that the hypertension in these
animals is heritable as parental blood pressure has a significant effect on the blood
pressure of first generation offspring. A mixed breeding paradigm (NT/NT, NT/HT, and
HT/HT) produced a tri-modal distribution of blood pressures for both male and female
AGMs that was detectable as early as year one in progeny(17, 18). These data suggest that
genetic factors play a role in the development of spontaneous hypertension, similar to
human patients. The recent sequencing and annotation of the AGM genome will accelerate
the ability of researchers to utilize this model in combination with advanced genetic
techniques to identify genes that contribute to both the heritability and pathophysiology of
hypertension(8, 15).

Current therapies for the treatment of hypertension can be effective, but the clinical
utilization of these drugs and targets are currently limited to random and subjective
choices for initial patient treatment. The need exists for a large animal model of
spontaneous hypertension that is genetically, behaviorally, and physiologically similar to
human disease. The AGM is a model of spontaneous hypertension that shares many
similarities with human hypertensive patients. In this novel nonhuman primate model,
researchers can take advantage of the highly similar gene sequence, structure, circadian rhythmicity, and social behavior of primates at the basic science level. In addition, the physiological similarities between this experimental animal model and the intended clinical endpoint, patients, should increase the speed and efficacy of the translational application of discoveries. The AGM offers a rare opportunity for researchers to experimentally manipulate and control variables considered unique to primates, such as development, pregnancy, social stressors, diet, and gut microbiota contributions to the development of hypertension. Utilization of this highly translational nonhuman primate model may elicit new, more effective therapies for the treatment of hypertension and associated cardiovascular and renal diseases.

**Perspectives and Significance**

The current study describes renal pathologies associated with spontaneous hypertension in a novel non-human primate model of hypertension, the African Green Monkey. Although numerous models of experimental hypertension in quadripeds have been studied for decades, the AGM provides a unique spontaneous model of hypertension in a biped where baroreceptor reflex controllers undergo continual loading and unloading daily. Furthermore, the AGM provides an animal model that exhibits behavioral and genetic similarities to human essential HT. The sequenced vervet genome indicates that it exhibits ~96% homology to *H. sapiens* and evolutionarily branched from the great apes approximately 27 million years ago. Thus, the spontaneous hypertension in the vervet provides strong phenotypic similarities to human essential hypertension. HT AGMs have renal vascular hypertrophy and glomerular remodeling compared to NT controls, older
animals are more likely to develop HT and it is a non-renin dependent model of spontaneous HT. The characterization of this model of hypertension provides new and highly translational possibilities for studying the etiology and pathogenesis of spontaneous HT in humans.

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**References**


Figure 1: Hypertensive AGMs have higher systolic, diastolic, and mean blood pressures and comprise 37% of the total population studied (n=424). Blood pressures were measured by forearm cuff plethysmography under light ketamine sedation (15mg/kg). NT AGMs (n=187, white) have an average systolic blood pressure of 99.63±1.04 mmHg, an average diastolic blood pressure of 56.20±1.23 mmHg, and an average mean blood pressure of 79.76±1.04 mmHg. BHT AGMs (n=80, hashed lines) have an average systolic blood pressure of 130.64±0.65 mmHg, an average diastolic blood pressure of 68.74±2.22 mmHg, and an average mean blood pressure of 89.35±1.51 mmHg. HT AGMs (n=157, black) have an average systolic blood pressure of 172.04±2.18 mmHg, an average diastolic blood pressure of 83.13±1.91 mmHg, and an average mean blood pressure of 112.18±1.68 mmHg. *p<0.05 when compared to NT; ^p<0.05 when compared to BHT.

Figure 2: Heart rate is elevated in hypertensive and borderline hypertensive AGMs compared to normotensive AGMs. Figure 2A: NT AGMs (n=148, white) have an average HR of 121.93±1.98 bpm, BHT AGMs (n=72, hashed lines) have an average HR of 129.72±2.39 bpm and HT AGMs (n=119, black) have an average HR of 135.87±2.20 bpm. *p<0.05 compared to NT AGMs. Figure 2B: Adult male AGMs (n=281, white dotted) have lower heart rates than female AGMs (n=58, black dotted; 124.81±1.29 bpm vs. 146.24±3.59 bpm). *p<0.05 compared to male HR (Student’s t-test) Figure 2C: Heart rate is higher in hypertensive animals and in females, but no significant interactions exist between phenotype and sex.

Figure 3: Hypertensive AGMs are older than the normotensive cohort. Figure 3A: On average, NT AGMs (n=42, white) are 8.88±0.62 years of age while HT AGMs (n=52, black) are 12.40±0.74 years of age. BHT AGMs (n=32, hashed lines) average 11.00±0.87 years old. Figure 3B: Kernel density estimation representative of the age distribution within each phenotypic population. NT: dotted line; BHT: dashed line; HT: solid line. *p<0.05 compared to NT.

Figure 4: Components of the renin-angiotensin-aldosterone system are similar between normotensive and hypertensive AGMs. Figure 4A: Gene expression of Angiotensinogen (AGT) is similar in the renal cortex, renal outer medulla, and liver of hypertensive and normotensive AGMs. AGT expression in the cortex, NT (n=14) vs. HT (n=14): 1.00±0.37 vs. 1.00±0.45; AGT expression in the outer medulla, NT (n=12) vs. HT (n=11): 1.00±0.19 vs. 0.52±0.24; AGT expression in the liver, NT (n=17) vs. HT (n=14): 1.00±0.14 vs. 1.16±0.16. p>0.05 for all compared by Mann-WhitneyU test. Figure 4B: Renal cortical renin content (RCRC) was similar in NT and HT AGMs. NT AGMs (n=11) have an average RCRC of 8.94±1.56 ug AngII/ml/hr/mg protein while HT AGMs (n=13) have an average of RCRC of 10.73±2.98 ug AngII/ml/hr/mg protein. p=0.62 using Student’s t-test. Figure 4C: Plasma renin activity (PRA) was similar in normotensive (n=15) and hypertensive (n=16) AGMs. Normotensive AGMs had PRA of 3.27±0.36 ng AngI/ml/hour while hypertensive AGMs had PRA of 3.34±0.48 ng AngI/ml/hour. P=0.917 using Student’s t-test.

Figure 5: Renal blood vessel walls are thicker in HT AGMs. Figure 5A: HT AGMs have greater vessel wall thickness in renal vasculature (14.41±0.56μm, n=8) compared to NT AGMs (10.33±1.27μm, n=8). Figure 5B: HT AGMs have larger wall/lumen ratios in renal
arterioles over 100μm in diameter (0.15±0.01, n=8) compared to NT AGMs (0.11 ± 0.01, n=8) **Figure 5C**: Representative histological sections of Periodic Acid Schiff stained renal tissue in NT and HT AGMs. * denotes p ≤0.05 comparing NT vs. HT.

**Figure 6A**: HT AGMs have greater Bowman’s capsular space within glomeruli (44.44±3.14%, n=5) compared to NT AGMs (30.86±1.88%, n=5). Capsular space was measured by thresholding analysis of digital images of Periodic Acid-Schiff stained renal tissue (n=5). *p≤0.05 NT vs. HT. **Figure 6B**: Histological sections of PAS-stained glomeruli before and after ImageJ thresholding.

**Table 1**: Descriptive Statistics of SBP, DBP, MBP, and HR, separated by phenotype and sex. * p<0.05 compared to phenotype-matched opposite sex.
<table>
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<th>Phenotype</th>
<th>Sex</th>
<th>N</th>
<th>Systolic Blood Pressure (mmHg)</th>
<th>Diastolic Blood Pressure (mmHg)</th>
<th>Mean Blood Pressure (mmHg)</th>
<th>Heart Rate (bpm)</th>
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<td>Normotensive</td>
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<td></td>
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<td>81.6±2.3</td>
<td>111.6±2.0</td>
<td>130.3±2.0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>38</td>
<td>173.3±0.9</td>
<td>87.9±3.2</td>
<td>110.6±3.3</td>
<td>162.3±4.0*</td>
</tr>
</tbody>
</table>