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CHARACTERIZATION OF SPONTANEOUS HYPERTENSION IN CHLOROCEBUS AETHIOPS SABAEUS, THE AFRICAN GREEN MONKEY

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CHARACTERIZATION OF SPONTANEOUS HYPERTENSION IN CHLOROCEBUS AETHIOPS SABAEUS, THE AFRICAN GREEN MONKEY

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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Lexington, Kentucky

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2018

ABSTRACT OF DISSERTATION

CHARACTERIZATION OF SPONTANEOUS HYPERTENSION IN CHLOROCEBUS AETHIOPS SABAEUS, THE AFRICAN GREEN MONKEY

Hypertension is a complex multifactorial pathology that is a major risk factor for the development of cardiovascular disease, stroke, and end stage renal disease. In the United States, hypertension affects over 1 in 3 adults and comprises an annual cost of over $58 billion in the healthcare industry. While remarkable strides in the diagnosis and treatment of hypertension have been made since the pathology was first treated in the 1960s, a remarkable 13% of patients with elevated blood pressures are classified as resistant hypertensive, where blood pressure remains uncontrolled while on three or more classes of anti-hypertensive drugs. This treatment gap suggests that researchers need to develop and utilize translational models that recapitulate the pathologies seen in patient populations. Non-human primates (NHP) are highly similar to humans in terms of physiology, circadian rhythmicity, behavior, and gene sequence and structure. Development of NHP models that spontaneously develop pathologies, like spontaneous hypertension, provide novel and vital tools to studying disease. Overall, this dissertation is a comparative analysis of the mechanisms that drive the development of spontaneous hypertension in *Chlorocebus aethiops sabaeus*, an Old World non-human primate, and known mediators of essential hypertension in human populations. Chapter 2 presents how hypertensive (HT) African Green Monkeys (AGMs) are older, with elevated heart rates, increased renal vascular wall/lumen ratios, and altered glomerular morphologies compared to normotensive (NT) controls. Chapter 3 describes metabolic studies performed in a large cohort of animals that identified elevated proteinuria and ion excretion in HT AGMs compared to NT animals. Chapter 4 focuses on the contribution of sympathetic nervous system to the development of hypertension in this animal model and describes the significant left ventricular hypertrophy and elevation of adrenergic receptor mRNA in HT AGMs. Chapter 5 examines how age affects hypertension and renal function in the NT and HT AGMs. Together these data provide a foundational basis for the development of spontaneous hypertension in the AGM and provide a novel translational model for the study of cardiovascular disease.

KEYWORDS: Hypertension, Blood Pressure, African Green Monkey

Megan Kathleen Rhoads

August 31, 2018

Date

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“You can never be overdressed or overeducated.”

Oscar Wilde

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CHAPTER ONE

HYPERTENSION AND THE AFRICAN GREEN MONKEY

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Keywords: blood pressure control; hypertension; African green monkey;

1.1 Introduction

Hypertension is a complex, multifactorial disease that affects over 85 million adults in the United States and an estimated 972 million people worldwide (Benjamin, Blaha et al. 2017). Hypertension is a major risk factor for the development of cardiovascular disease, stroke, and chronic kidney disease and treatment for hypertension alone is estimated to exceed $200 billion in direct medical costs by the year 2030 (Heidenreich, Trogdon et al. 2011). Multiple risk factors have been identified in the development of hypertension including but not limited to: age, race/ethnicity, family history of high blood pressure, genetic factors, excess weight, decreased physical activity, tobacco usage, stress, diet, education, and socioeconomic status (Benjamin, Blaha et al. 2017). In short, hypertension is a major global disease burden that adversely affects millions of people and is the predominant modifiable risk factor associated with cardiovascular mortality.

The American Heart Association (AHA) has historically defined hypertension as systolic blood pressure (SBP) greater than 140 mmHg or diastolic blood pressure (DBP) greater than 90 mmHg. A recent report from the AHA in conjunction with the American College of Cardiology recommends that the clinical benchmark for the diagnosis of hypertension be lowered to SBP greater than 130 mmHg or DBP greater than 80 mmHg, due to marked differences in mortality(Whelton, Carey et al. 2018).

While the etiology of essential hypertension is still unknown, the pathological consequences that occur due to hypertension have real effects on human health and life expectancy. Hypertension primarily contributed to the death of 78,000 people in 2015

and is considered the most important modifiable risk factor against cardiovascular disease, stroke, and chronic kidney disease (Benjamin, Virani et al. 2018). In addition, organ damage from hypertension in humans is particularly complex due to the comorbidities and variations in severity of hypertension.

1.2 Blood pressure control mechanisms

1.2.1 Cardiovascular function and pathologies

Discussion of normal cardiovascular function must begin with the physiological application of Ohm's Law (V= I * R) to the calculation of blood pressure. BP is defined as the pressure exerted on the vessel walls by circulating blood. BP is also the mathematical product of cardio output (CO) multiplied by total peripheral resistance (TPR), the sum of the resistance of all vessels in the body. CO is the mathematical product of stroke volume (SV) and heart rate (HR). The primary determinates of SV are ventricular end-diastolic volume (EDV), and myocardial contractility. EDV, the volume reached by the ventricular chamber prior to contraction, is determined by venous pressure, which is related to both blood volume and venous smooth muscle tone. Changes in any one of these variables will result in alterations in total blood pressure or a compensatory response in another variable.

The Frank-Starling law of the heart governs the relationship between SV and EDV. SV increases in response to increased EDV, or preload, allowing cardiac output to be synchronized with venous return without extrinsic regulation. This mechanism of the heart also couples left and right ventricular output to maintain balance between the pulmonary and systemic circulations. Increases in afterload, or TPR, lower the Frank-Starling curve of the heart, decreasing both stroke volume and left ventricular EDV, as the atrial value cannot open until ventricular pressure exceeds pressure in the circulation. As a consequence of increased afterload and decreased SV, preload is subsequently increased due to the blood remaining in the ventricle after contraction.

Afterload increases when systemic vascular resistance increases, by stenosis in the aorta, or by ventricular dilation. Chronic increased afterload can promote hypertrophy of the myocardial wall in order to generate more force against circulatory pressure. Hypertrophy allows more sarcomere units to share the increased wall stress caused by increases in afterload and thus can be considered a physiological compensatory response to increased BP.

Changes in HR can also affect BP. HR is set by the activity of pacemaker cells in the primary sinoatrial (SA) node and the secondary atrioventricular (AV) node. Action potentials are spontaneously generated by the SA node and travel through gap junctions to the myocardial cells, beginning their depolarization and eventual action potential associated contraction. The AV node is depolarized after the SA node, and transmits action potentials to the ventricular cardiomyocytes. While the aptly termed 'pacemaker' cells are capable of spontaneous generation of action potentials, their timing is often augmented by activity from the sympathetic and parasympathetic nervous systems. Stimulation of the SA node by norepinephrine from the sympathetic nervous system increases the heart rate while parasympathetic activation via acetylcholine decreases the firing rate of the SA node through hyperpolarization.

Left ventricular hypertrophy (LVH) has long been established as a risk factor for the development of chronic heart failure and a pathological consequence of hypertension. Pathological LVH increases the oxygen demand of the cardiac tissue, decreases mechanical efficiency via reduced compliance and can impair filling, leading to heart failure. Studies in human hypertensive patients have demonstrated associations between LVH and increased cardiac sympathetic outflow (Schlaich, Kaye et al. 2003). In addition,

the reduction in LVH observed in two-kidney one-clip hypertensive Wistar rats after administration of β1-adrenergic antagonists suggests that sympathetic activity is crucial to the development of LVH (Rizzi, Guimaraes et al. 2014).

TPR can be mediated by changes in vascular compliance, hormonal systems such as the renin-angiotensin system, or local vasoactive effectors produced by the endothelium or vasculature. Poiseuille's law describes the inverse relationship between pressure gradients and resistance in tubular flow (Poiseuille 1846). Factors such as vessel length, viscosity, and radius all affect resistance in blood vessels; however, resistance is governed primarily by radius size due to the exponential relationship found in Poiseuille's equation, revealing that any mediator which alters vessel radius can have profound effects on TPR and thus blood pressure. This law in particular illustrates the immense power of the vasoconstriction and vasodilation and the crucial importance of systems that can affect vessel radius in the regulation of blood pressure.

Increased BP also has detrimental effects on the vasculature. Increased wall stress on the vessels can lead to vascular remodeling, inducing hypertrophy and fibrosis. This remodeling can lead to reduced arterial compliance, arterial stenosis, and tissue ischemia (Intengan and Schiffrin 2001). Increased BP and increased turbulent flow due to changes in vessel radius can also damage the vasoactive vascular endothelium, leading to inflammatory cascades and decreases in nitric oxide and prostacyclin availability, which typically oppose the vasoconstrictive adrenergic tone of the vessels (Luscher 1990, Kang 2014), creating a feed-forward mechanism that promotes increased BP.

1.2.2 Renal function and pathologies

The kidneys have long been considered critical to the maintenance of blood pressure due their role in the regulation of fluid homeostasis, an idea introduced by Claude Bernard and refined by Walter B. Cannon(Cooper 2008). Increases in blood volume would increase preload, SV, and CO, resulting in increased BP. The kidney, as the main excretory organ in the body, controls extracellular fluid volume and thus, blood volume. Blood enters the kidney through the renal artery and traverses down the arterial tree to the main filtering unit of the kidney, the nephron. In the renal corpuscle, composed of the glomerular capillaries surrounded Bowman's capsule, blood is filtered through the capillary fenestrations, glomerular basement membrane, and filtration slits created by podocytes. Plasma proteins remain in the blood due to the size and charge of the filtration membranes. Once the filtrate is created, it moves down the tubule of the nephron towards the collecting ducts of the kidney to ultimately become urine. Throughout the nephron, diffusion and ion gradients are utilized to concentrate urine and maintain appropriate extracellular concentrations of water, proteins, and ions. As filtrate moves down the tubular lumen, key proteins, ions, and water are reabsorbed, secreted, and diffused through multiple mechanisms using both active and passive transport.

The variables that determine glomerular filtration rate (GFR) can be described mathematically by the following equation: $GFR = K_f \times (P_G - P_B - \Pi_G + \Pi_B)$; where K_f is the filtration constant, P_G is glomerular pressure, P_B is the pressure within Bowman's capsule, Π_G is glomerular oncotic pressure, and Π_B is the oncotic pressure of Bowman's capsule. GFR is a clinical measure of kidney function and important to the regulation of extracellular fluid volume.

Renal damage and hypertension have long been associated. It's logical that increased levels of renal perfusion pressure would result in larger forces being enacted on the nephron. Chronic elevation of blood pressure would have detrimental effects on the morphology of the glomerulus and the ability to create filtrate from urine. In normal healthy adults, GFR is approximately 125 mL/min and is estimated by measuring serum creatinine and the use of an estimation formula such as the Cockcroft-Gault or Modification of Diet in Renal Disease equation. It is worth noting that high levels of intra- and inter-individual variation in GFR estimation have called into question the use of serum creatinine as a reliable marker of GFR, however, most clinical measurements still utilize creatinine(Shemesh, Golbetz et al. 1985). GFR declines normally with aging, decreasing by approximately 10 mL/min/decade after the age of 40(Glassock and Winearls 2009). GFR is typically decreased in patients and experimental animals models of hypertension and chronic renal failure compared to normal controls (Baylis 1994, Ofstad and Iversen 2005, Astor, Matsushita et al. 2011).

Sympathetic outflow to the kidney has been shown to increase tubular sodium reabsorption, active the RAAS, promote sclerosis of the glomerular capillaries, and increase renal vascular resistance (Schlaich, Socratous et al. 2008). When pathologically elevated and coupled with the volume expanding effects of the RAAS, this can create a feed-forward mechanism that promotes vascular hypertrophy and glomerular damage. As mechanical forces increase in the glomerulus, driven by increased blood volume and/or pressures (P_G), mesangial cells and podocytes undergo hypertrophy and eventual failure(Nagata and Kriz 1992). Failure and atrophy of these cells leads to a lack of filtration (K_F) in the glomerulus allowing large proteins to move into the tubular lumen.

Ang II is a potent vasoconstrictor that has been shown to promote fibrotic growth in tubular, mesangial, and interstitial cells, and promote the activation and recruitment of inflammatory cells (Mezzano, Ruiz-Ortega et al. 2001). The inflammatory and fibrotic changes in the nephron induced by Ang II can promote proteinuria, tubular atrophy, and renal failure. Studies in the SHR suggest that tubular and glomerular damage develop in parallel in the context of elevated blood pressure(Ofstad and Iversen 2005).

1.2.3 Acute control of blood pressure: Baroreceptors

BP control mechanisms include concerted effects by multiple organ systems to maintain adequate blood flow to the entire body. This integrated effort requires the use of both short- and long-term mechanisms to regulate BP. Short-term, or acute, mechanisms of BP control are rapid mechanisms that induce changes in blood vessel diameter, heart rate, or cardiac contractility. Acute mechanisms of BP control include the baroreceptors, chemoreceptors, and atrial receptors, as well as the integration and response of these signals in the brain. Long-term, or chronic, mechanisms of BP control are slow-acting mechanisms that focus primarily on the regulation of blood volume. Chronic mechanisms of BP control center around the actions of the kidney and natriuresis in response to changes in BP.

Baroreceptors are spray-type nerve endings found in the arterial walls of the carotid sinus and the aortic arch (Guyton and Hall 2006). The carotid sinus baroreceptors are high-pressure mechanoreceptors and are stimulated by pressures above 60 mmHg up to a maximum stimulation of 180 mmHg. Comparatively, aortic baroreceptors are stimulated by blood pressures higher than 90 mmHg. Baroreceptors sense increases in arterial pressure via distention in the arterial wall, which then induces signaling to the nucleus tractus solitarius (NTS) where interneurons inhibit the sympathetic preganglionic neurons and excite the parasympathetic neurons. This integrated response causes a decrease in arterial pressure by two distinct mechanisms: a decrease in heart rate due to stimulation of the vagus nerve and a decrease in peripheral resistance by lowering sympathetic tone in the arterial wall. This is clearly demonstrated when a person moves from a supine to standing position. The act of standing tends to cause a drop in arterial pressure in the

head and upper body. This could lead to a loss of consciousness, however, the drop in BP at the baroreceptor elicits a strong sympathetic discharge to preserve BP in the head and upper body and prevent fainting from under perfusion of the brain.

While it is well accepted that the baroreceptor reflex is important to short-term regulation of BP control, some believe that the baroreceptor reflex may also be involved in the long-term regulation of BP via control of sympathetic nerve activity (SNA) (DiBona 2004, Guyenet 2006). Studies have shown that unloading of the baroreceptors by placement of ligature distal to the carotid sinus caused sustained increases in mean arterial pressure (MAP), heart rate (HR), and plasma renin activity (PRA) while decreasing urinary sodium excretion(Thrasher 2002). These changes are indicative of increased renal sympathetic nerve activity in response to a lowered pressure in the carotid sinus, indicating that the baroreceptors can play a role in the long-term regulation of BP.

Similar to the high-pressure mechanoreceptors in the carotid sinus and aorta, low-pressure baroreceptors in the atria and pulmonary arteries help to regulate BP in the low pressure sections of the circulation. Increased pressures in these low-pressure areas are caused by increases in blood volume, so the end effects of these reflexes are geared to quickly sensing and correcting those increases. The Bainbridge reflex is activated when the atrial stretch receptors are stimulated. Afferent signals are transmitted via the vagal nerve to the NTS and travel to the paraventricular nucleus (PVN) to elicit efferent stimulation of the sympathetic nervous system and inhibition of the vagal nerve to increase heart rate and contractility(Coote 2005, Guyton and Hall 2006). This reflex prevents retention of blood in the atria, veins, and pulmonary circulation.

Activation of the atrial baroreceptors can elicit a volume reducing response in the kidneys. Excitation of the PVN stimulates GABA-nergic interneurons that synapse and inhibit the renal sympathetic nerves (Coote 2005). This concomitant excitation of one arm of the sympathetic nervous system (SNS) while inhibiting other arm of the SNS is likely due to a hard-wired neuronal network in the PVN that is specifically activated by activation from the cardiopulmonary reflex(Coote 2005). Inhibition of RSNA induces dilation of the afferent arterioles in the kidney, increasing glomerular capillary pressure and glomerular filtration rate (GFR) to increase sodium and fluid excretion. Other signals from the hypothalamic neurons decrease the secretion of anti-diuretic hormone (ADH), encouraging natriuresis and volume contraction.

Atrial distention also causes the release of atrial natriuretic peptide (ANP) from the atrial granules in the heart (Dietz 2005). Once released, ANP travels to the kidneys and causes minor increases in GFR and decreases Na+ reabsorption in the collecting ducts. This natriuretic effect helps to decrease volume overload in the circulation. Infusion of exogenous ANP inhibits the secretion of both renin and aldosterone and inhibits plasma aldosterone in response to angiotensin II (Chopra, Cherian et al. 2013).

1.2.4 Acute control of blood pressure: Chemoreceptors

Chemoreceptors are another type of sensor found in the arterial wall, however, these receptors sense changes in oxygen, carbon dioxide and hydrogen ion levels. They reside in carotid bodies in the common carotid artery and near the aorta. Carotid bodies are supplied with blood flow via a small nutrient artery. This allows constant contact with the

blood supply. Should blood flow fall below critical levels, the diminished flow in the small artery activates the chemoreceptors to signal to the vasomotor center and stimulate sympathetic vasoconstriction to increase arterial pressure (Guyton and Hall 2006). Hypoxemia, hypercapnia, and acidemia all initiate signaling of chemoreceptors. Chemoreceptor activation also increases ventilation, which combined with SNS activation can increase blood oxygen content and conserve oxygen use by the tissues(Dampney, Coleman et al. 2001). However, it's important to note that the chemoreceptors are not powerful controllers of BP until pressure falls to <80 mmHg.

Recent attention to the role of the chemoreceptors and the sympathoexcitatory chemoreflex described above has led to the postulation that removal of the carotid chemoreceptors may be a viable treatment for hypertensive patients. Studies in the SHR showed that denervation of carotid bodies attenuated hypertension in animals with both developing and established hypertension, with no changes in blood pressure in normotensive animals(Abdala, McBryde et al. 2012). HT patients and patients with normal blood pressures with a family history of hypertension have increased ventilatory response to hypoxia, suggestive of an increased chemosympathetic drive that may contribute to the development of hypertension(Izdebski, Izdebski et al. 2006). In addition, a few human studies have reported reductions in BP after carotid body removal, suggesting that carotid body denervation may be future therapeutic tool in the treatment of hypertension(Paton, Sobotka et al. 2013).

1.2.5 Chronic control of blood pressure: Pressure natriuresis

Chronic control of blood pressure deals with primarily the regulation of body fluid volumes via the kidney. The kidney must regulate body fluid homeostasis by ensuring the balance between fluid intake and output. This seemingly simple task involves the coordination of multiple nervous system and hormonal signals, as well as local systems within the kidney itself. Simply, increased extracellular fluid in the body causes fluid redistribution and an increase in blood volume, which in turn increases the arterial pressure. When the arterial pressure rises, blood flow through the kidneys increases. This creates increased filtration through the glomeruli of the kidney and increased filtrate into the tubule of the nephron. The ultrafiltrate in the tubule moves through the proximal and distal tubules to the collecting ducts and finally to the bladder, where it is excreted.

Figure 1.1 shows the relationship between intake and renal output of water and salt and the relationship to arterial pressure. The kidneys strive to operate at the "equilibrium point" shown here. If body fluid volume and arterial pressure increase, renal outputs of water and salt will increase. This loss of salt and water will decrease body fluid volume and arterial pressure until the pressure returns to the equilibrium point. Should arterial pressure fall below the equilibrium point, the kidneys will retain salt and water until equilibrium is reached again. This concept is known as the "infinite gain" of the renal function curve. These mechanisms provide the kidney with the ability to sense and control arterial pressure in the body. The kidney must be able to sense extracellular fluid volume as well as respond to renal perfusion pressure. As extracellular fluid volume is regulated by the concentration of sodium in the body, the kidney utilizes a set of specialized cells located in the cortical thick ascending limb, known as the macula densa,

to sense sodium levels. The macula densa resides in the juxtaglomerular apparatus and can sense tubular flow and NaCl concentration.

Based on this information, there are two major ways to manipulate the long-term control of body pressure: shifting of the renal output curve or changing the level of the salt and water intake line. It is important to note that a permanent or long term change in either of these curves can cause a new equilibrium point to be created – and this point will act as the new set point for the renal regulation of BP.

This concept demonstrates the importance of salt and water intake to the long-term regulation of BP. Increased water intake doesn't affect arterial pressure greatly, as pure water is normally excreted through the kidneys as rapidly as it is ingested. In contrast, excess salt intake can greatly influence arterial pressure. As salt is ingested, it enters the extracellular fluid (ECF) and increases ECF osmolality. Subtle 2-3% changes in the osmolality can stimulate a thirst response in humans (Stachenfeld 2008), causing increases in ECF, blood volume, and BP. Increases in osmolality also stimulate the hypothalamic-posterior pituitary axis to release ADH to stimulate water reabsorption in the renal tubules. This water reabsorption increases the extracellular fluid volume and lowers osmolality. The strong influence of sodium intake on control of BP is part of the reason that hypertensive patients are often encouraged to partake in a low sodium diet.

1.3 Hormonal regulation of blood pressure

Extrinsic controllers of blood pressure such as hormones are crucial for the body's ability to respond to changes in tissue energy needs. Endocrine factors that can affect blood pressure include, but are not limited to, the renin-angiotensin-aldosterone system (RAAS),sex hormones such as estradiol and testosterone, atrial natriuretic peptide, and vasopressin.

1.3.1 Renin-angiotensin-aldosterone system

A key mechanism of chronic BP regulation is the RAAS, a hormonal cascade with local and systemic effects on body fluid volumes, BP and renal function. Figure 1.2 shows the major classical RAAS pathways as well as pharmacological targets and associated molecules. The first molecule in the pathway is angiotensinogen, a 453-amino acid (aa) peptide synthesized by the liver. This α-2 globulin is the classical substrate for the renin enzyme. Interestingly, other enzymes such as tonin, cathepsin G, and trypsin have been found to synthesize angiotensin II directly from angiotensinogen (Belova 2000).

Prorenin is synthesized and stored in the juxtaglomerular cells found in the wall of the afferent arteriole next to the glomerulus. When arterial pressure falls, cellular mechanisms not well understood cause the cleavage of prorenin into renin and release the activated form into the circulation (Kurtz 2011). Renin acts upon angiotensinogen to cleave angiotensin (Ang) I, a 10-aa peptide with slight vasoconstrictor properties that travels in the systemic circulation. Once Ang I reaches the lungs, angiotensin converting

enzyme (ACE) cleaves two aa off to form angiotensin II. Other enzymes can perform this step - chymase, which is produced in mast cells, can also catalyze this reaction.

Ang II is a potent vasoconstrictor with effects in multiple tissues but a short active half-life. Effects of Ang II are mediated through two unique receptors, angiotensin type 1 receptor (AT1R) and angiotensin type 2 receptor (AT2R). Binding of Ang II to an AT1R causes vasoconstriction (and thus increases arterial pressure by increasing peripheral resistance), sodium retention, water retention, suppresses renin release, induces hypertrophy in cardiomyocytes and smooth muscle, stimulates vascular and myocardial fibrosis, stimulates superoxide formation, activates the SNS, and increases endothelin secretion (Zaman, Oparil et al. 2002). AT1Rs are found in vascular smooth muscle, the adrenal cortex, and the kidney while AT2Rs are expressed in the kidney, uterus, brain, adrenal medulla, and adrenal cortex (Bonnardeaux, Davies et al. 1994). Binding of Ang II to the AT2R induces vasodilation, cell differentiation, tissue repair, apoptosis, and anti-proliferative effects (Zaman, Oparil et al. 2002).

In the adrenal cortex, Ang II induces the release of the mineralocorticoid aldosterone into the circulation. Aldosterone, a steroid hormone, acts primarily on receptors in the distal tubule and cortical collecting ducts to stimulate the reabsorption of sodium and water from the tubular filtrate. This increases ECF volume, blood volume, and consequently, arterial pressure. When dysregulated, aldosterone can be pathogenic and can contribute to the development of cardiovascular and renal through primary aldosteronism, which is characterized by hypertension with hypokalemia.

Ang II contributes to the long-term control of BP by directly and indirectly (via aldosterone) causing salt and water retention in the kidneys. This powerful hormonal cascade shifts the renal output curve to the right – meaning that a higher arterial pressure is required to excrete salt and water from the body (Cowley 1992). Ang II is a major player in increasing BP after it falls in response to rapid hemorrhage. RAAS also allows a person to ingest large amounts of salt and maintain relatively normal blood pressure. The increased ECF and blood flow through the kidneys inhibits the secretion of renin leading to increased renal excretion of water and salt and return of the ECF and BP to the normal equilibrium point.

Recent interest in the non-classical components of the RAAS has discovered other biologically active peptides in the pathway that are relevant to blood pressure regulation. Ang (1-9) can be converted from Ang I through the actions of angiotensin-converting enxyme 2 (ACE2), found in the endothelium of coronary vessels and renal tubules. Ang 1-9 binds to the AT2R and is important in local renin-angiotensin activity in the heart and kidney (Donoghue, Hsieh et al. 2000). Ang (1-7) can be converted from Ang I by the enzymes nephrilysin or thimet oligopeptidase. Ang (1-7) can also be hydrolyzed from Ang II by ACE2. Ang (1-7) binds to the GPCR Mas, which has vasodilatory and anti-oxidant effects (Santos, Simoes e Silva et al. 2002). Ang III is metabolized from Ang II by aminopeptidase A and binds to both the AT1R and AT2R. Ang III is thought to have similar physiological effects to Ang II, as they utilize the same receptors, but some research suggests Ang III may have unique effects in the central nervous system (Yugandhar and Clark 2013). Ang IV is converted from Ang III by aminopeptidase N and binds to the insulin-regulated aminopeptidase (IRAP)(Vanderheyden 2009). The

functionality of IRAP in the context of blood pressure regulation is unknown, but it has been shown to be involved in pro-inflammatory cascades in vascular smooth muscle cells (Esteban, Ruperez et al. 2005).

1.3.2 Sex hormones

Sexual dimorphism in the prevalence of high blood pressure in human populations is a well-described phenomenon. Men have higher rates of hypertension compared to age-matched women until women reach the age of menopause, at which point women equal or even exceed men in rates of hypertension (Dubey, Oparil et al. 2002). Additionally, in the male spontaneously hypertensive rat (SHR), the pressure-natriuresis curve is blunted and castration attenuates this reduction, providing a strong case for the role of androgens in the development of elevated blood pressure (Reckelhoff, Zhang et al. 1998). In addition, administration of testosterone to ovariectomized females produced a similar pressure-natriuresis relationship to that of the male SHR, suggesting that testosterone could affect renal function in women post-menopause. Overall, blood pressure is higher in male SHR, DOCA-salt, and Dahl salt-sensitive rats compared to female animals (Dubey, Oparil et al. 2002).

Sex hormones may influence arterial blood pressure through a number of physiological mechanisms, including, but not limited to endothelin-1, catecholamine synthesis, and activation of the RAAS. Endothelin-1 has been described as the most potent vasoactive peptide produced by the human body and has vasoconstrictive, pro-oxidant, pro-inflammatory, and mitogenic effects. Plasma endothelin-1 levels are

elevated in men compared to women and female-to-male transitioning patients treated with testosterone have increases in plasma endothelin-1 (Polderman, Stehouwer et al. 1993). Men and male experimental animals have a more potent vasoconstrictor response to endothelin-1 compared to women and female animals (Tostes, Fortes et al. 2008). In porcine coronary artery rings, testosterone incubation potentiated the contractile effect of endothelin-1 while incubation with 17β-estradiol attenuated the contraction (Toeh, Quan et al. 2000).

There are multiple sex differences in the expression and functional responses to the classical and non-classical RAAS pathways. Males display elevated expressions of the components of the classical RAAS pathway while females have greater expression of the non-classical RAAS pathways (Sullivan 2008). 17β-estradiol has been shown to promote the production of Ang 1-7, showcasing the possibility of the female hormonal environment to favor the vasodilatory actions of the RAAS (Brosnihan, Li et al. 1997). Glomerular binding of AT1R is significantly reduced in female Sprague-Dawley rats compared to male rats and removal of the ovaries returns glomerular AT1 binding to levels similar to males (Rogers, Mitchell et al. 2007). In humans, ACE inhibitors are less effective in conferring cardiovascular benefits in women compared to men, whereas are angiotensin receptor blockers have been associated with better survival rates in women but not men (Sullivan 2008).

Together, this evidence provides a strong argument that sex, and sex hormones, can have significant effects on the regulation of arterial pressure. It is worth noting that many other systems and pathways, such as inflammation, oxidative stress, and nitric oxide, have all been identified and implicated in the relationship between hypertension

and sex; however, a full review of all of these mechanisms is outside the scope of this current work.

1.3.3 Other hormones

Atrial natriuretic peptide (ANP) is secreted from the atrial wall of the heart in response to increased volume in the atrium. ANP has natriuretic effects through increasing GFR, inhibiting renin secretion and renal sympathetic nerve activity, and decreasing sodium reabsorption in the distal convoluted tubule and cortical collecting duct. Together these actions result in a reduction of ECF and blood volume, returning blood pressure to normal levels(Brenner, Ballerman et al. 1990).

Vasopressin, or antidiuretic hormone, is synthesized in hypothalamic neurons in response to hyperosmolality. Vasopressin promotes the reabsorption of solute-free water in the kidney by promoting the transcription and integration of aquaporin channels in the renal collecting duct cells. It also has mild vasoconstrictive properties to increase arterial pressure, though it is not thought to be causal in the development of essential hypertension, as patients with the syndrome of inappropriate antidiuretic hormone secretion do not develop hypertension (Holt and Haspel 2010).

1.4 Neural regulation of blood pressure

The kidney auto-regulation theory developed by Guyton (Guyton 1991) has been a source of controversy for many years, particularly since the theory disregards the contribution of TPR in the development of hypertension. A major determinate of TPR is vascular smooth muscle contraction induced by the autonomic nervous system. The autonomic nervous system is divided into two major arms, the sympathetic and parasympathetic nervous systems. These two parallel arms consist of cholinergic neurons that synapse to efferent motor neurons and other peripheral targets. Parasympathetic neural control of the circulation operates through the parasympathetic neurons that innervate the heart, while sympathetic control is mediated by efferent neurons that innervate blood vessels, the adrenal medulla, the kidneys, and the heart (Guyenet 2006). Anatomical placement of the autonomic nervous system allows it to affect crucial variables like CO and arterial resistance, strengthening the argument that the autonomic nervous system is key to the regulation of BP.

1.4.1 Sympathetic nervous system

Neuronal networks in the rostral ventrolateral medulla, spinal cord, hypothalamus, and NTS control sympathetic outflow. Sympathetic nerve activity is stimulated through signals from baroreceptors, chemoreceptors, and afferent sensory nerves from organs such as the kidney, heart, and vasculature. After integration and processing in the central nervous system, the efferent motor neurons transmit signals to the appropriate tissues and

organ systems. The majority of these efferent motor neurons are adrenergic, utilizing catecholamines as the primary neurotransmitter. Catecholamines, such as norepinephrine (NE) and epinephrine (E), bind strongly to a class of receptors known as the adrenergic receptors, a group of G-protein coupled receptors (GPCRS). Adrenergic receptors are categorized in two major groups, α- and β- adrenoreceptors, and further divided into various subtypes, the α1-, α2-, β1-, β2-, and β3- adrenoreceptors. In addition, subtypes of the α1- and α2- classes have been identified as the α1A-, α1B-, and α1D-adrenoreceptors, and the α2A-, α2B-, and α2C- adrenoreceptors, respectively. As GPCRs, ligand binding to adrenoreceptors initiates a cascade of downstream signaling events that involve the G-proteins, however, each class of receptors has distinct intracellular pathways. α1-adrenoreceptors utilize the G_q pathway, activating phospholipase C to increase intracellular calcium and signal the activation of protein kinase C. α2-adrenergic receptors initiate the signaling of the G_i protein, which inhibits adenylyl cyclase and activates inwardly rectifying potassium channels attached to G-protein coupled receptors. β-adrenergic receptors stimulate the G_s pathway, which activates adenylyl cyclase to produce cyclic adenosine monophosphate (cAMP). Increases in intracellular cAMP activate the cAMP-dependent kinase, protein kinase A.

While adrenergic receptors are clearly important to the effector arm of the sympathetic nervous system, reports of peripheral adrenergic receptor numbers in experimental models of hypertension vary greatly (Michel, Brodde et al. 1990). However, the effectiveness of sympatholytic drugs, such as α- and β- adrenergic receptor blockers, in the treatment of hypertension suggests a sympathetic component to the development and/or maintenance of hypertension. Patients with essential hypertension, particularly

younger patients, have increased sympathetic outflow and NE spillover to the heart, kidney, and skeletal muscle vasculature (Esler 2000). However, markers such as systemic plasma NE are not strongly correlated with hypertension, suggesting that specific regional sympathetic activation is more important to the development of hypertension than general increased sympathetic outflow(Esler 2000).

1.4.2 Renal sympathetic nerve activity

The efferent renal nerves innervate the kidney in association with the renal artery and vein. The nerves terminate in vascular and tubular structures throughout the kidney, including the interlobar, arcuate, and interlobular arteries; the afferent and efferent arterioles; nephron segments, such as the proximal tubule, loop of Henle, distal convoluted tubule, cortical collecting duct; and juxtaglomerular apparatus (DiBona and Kopp 1997, Johns and Abdulla 2013). Efferent renal nerves are postganglionic and the majority of them are adrenergic, releasing NE from their terminals.

Differential distribution of each of the adrenergic receptor subtypes within the kidney has major effects on renal function. α1-adrenergic receptors are found mainly in the renal cortex and outer medulla and mediate both vascular and tubular responses to sympathetic activation. α1A-adrenoreceptors are the major mediator of the vasoconstrictor response to NE, as α1A- activation causes constriction of the afferent and efferent arteriole, which reduces both renal blood flow and glomerular filtration rate (DiBona and Kopp 1997). α1B- and α1D-adrenergic receptors are also located on vascular smooth muscle, albeit in lower densities than α1A adrenoreceptors

(Salomonsson, Oker et al. 2001). α1B-adrenoceptors are highly expressed in the inner medulla on tubular cells and mediate sodium and water reabsorption (Elhawary and Pang 1994). α2- adrenoreceptors are primarily expressed in vasculature and involved in vasoconstriction (Civantos Calzada and Aleixandre de Artinano 2001). β1-adrenergic receptors are found in the granular cells of the afferent arteriole and mediate the secretion of renin elicited by renal nerve activation (Osborn, DiBona et al. 1981). β2-adrenergic receptors are expressed mainly in medullary tubules and regulate sodium transport (Healy, Munzel et al. 1985, Singh and Linas 1996). β3-adrenoceptors are located in thick ascending limb, thick ascending limb, distal convoluted tubule and cortical collecting duct of the kidney. Recent research in mice suggests that tubular β3-adrenoceptors may have an antidiuretic effect when stimulated (Procino, Carmosino et al. 2016).

Much of the evidence supporting the contribution of renal sympathetic nerve activity to the development of hypertension has arisen from renal denervation studies in experimental models and patient populations. Renal denervation, ablation of the renal nerves, was shown to delay the progression of hypertension or lower blood pressure in many experimental models, including the Dahl salt-sensitive rat (Foss, Fink et al. 2013), the spontaneously hypertensive rat (Norman Jr and Dzielak 1982), DOCA-salt model of hypertension (Jacob, Clark et al. 2005), and angiotensin II induced hypertension in rats (Hendel and Collister 2006). Work from John W. Osborn's group has identified the specific ablation of renal afferent nerves in multiple experimental models of hypertension as a key component to successful reduction in BP in denervation studies(Foss, Wainford et al. 2015). Early studies showed translational success with renal denervation in resistant hypertensive patients (Krum, Schlaich et al. 2009, Symplicity, Esler et al. 2010). It is

worth noting that the results from catheter-based renal denervation studies have been controversial. Some studies have reported lowered blood pressures (Townsend, Mahfoud et al. 2017) while others have found no significant differences in patient blood pressures (Bakris, Townsend et al. 2014, Bhatt, Kandzari et al. 2014). Renal denervation trials in human patients are still ongoing and controversial(DiBona and Esler 2010).

1.5 *Chlorocebus aethiops sabaeus*, the African Green Monkey

Hypertension affects approximately one in three adults in the United States and is a major modifiable risk factor for the development of cardiovascular disease, stroke, and chronic kidney disease. While major strides in the treatment of hypertension have been made in the last 60 years, a remarkable 13% of patients in the United States still suffer from resistant hypertension, defined as uncontrolled blood pressure while on three or more classes of anti-hypertensive medications (Persell 2011). This treatment gap suggests that new, novel models need to be investigated to determine the etiology of essential hypertension. While rodent models have contributed greatly to our understanding of regulation and dysregulation of blood pressure, key differences between humans and rodents may hinder the translational capacity of treatments developed primarily in those models. The research continued in this doctoral thesis focuses on the characterization of a non-human primate model of spontaneous hypertension that may be uniquely suited to help bridge the translational treatment gap in hypertension research and medicine.

1.5.1 Phylogeny and history

The African green monkey (AGM) is an Old World Monkey belonging to the genus *Chlorocebus*. The *Chlorocebus* genus is estimated to have diverged from human lineage approximately 23 million years ago (Jasinska, Schmitt et al. 2013). There are multiple recognized subspecies of the AGM, which are phenotypically distinct and thought to have diverged approximately 600,000 years ago from each other (Pfeifer 2017). The six subspecies include: *aethiops*, *cynosuros*, *hilgerti*, *pygerythrus*, *sabaeus*,

and *tantalus*, and each are geographically isolated, though significant admixture occurs between subspecies (Svardal, Jasinska et al. 2017).

The AGM is native to Africa and is highly adaptable, occupying nearly every ecosystem in Africa from swamps to savannahs to mountainside slopes (Palmour, Mulligan et al. 1997). AGMs were introduced to the Caribbean islands of St. Kitts, Nevis, and Barbados during the colonial era. It is thought that they were bought from West Africa as pets with the shipping industry(Van Der Kuyl, Dekker et al. 1996) and once introduced, quickly became an invasive species (Jasinska, Schmitt et al. 2013). By the late 1700s, the monkeys had become a large pest species. One account, attributed to Lady Andrews who was visiting from England in 1774, suggests that the AGM population tormented visitors and locals alike.

"Their frolics are mischievous, their thefts dexterous. They are subtle enemies and false friends. When pursued they fly to the mountain and laugh at their pursuers, as they are little ashamed of a defeat as a French admiral or general. In short, they are the torment of planters; they destroy whole cane pieces in a few hours and come in troops from the mountain, whose trees afford them shelter. No methods [sic] *to get the better of them has yet been found out."* (Ervin 1993)

Currently, the Caribbean AGM population is estimated to number between 50,000 to 100,000 individuals (Jasinska, Lin et al. 2012). Recent genome-wide analysis work by Svardal and colleagues has found that the St. Kitts AGM population was founded by *C. sabaeus* animals found in the Gambian region of West Africa (Svardal, Jasinska et al. 2017). Meanwhile, the Nevis population of animals was descended from St. Kitts derived

animals, as identified by the decrease in genetic diversity, while animals on nearby Barbados were derived from a separate introduction of Gambian vervets. Thus, the St. Kitts vervet population consists of a bottleneck population descended from animals in the Gambian region of West Africa, making it a novel population in which to study genetic diversity, disease, and environmental adaptation.

1.5.2 Behavior and reproduction

The average lifespan of the AGM is approximately 10-12 years of age in wild due to high levels of predation and limitations on food/water (Isbell, Young et al. 2009). However, in captivity, there have been reports of AGMs surviving upwards of 25 years of age (Schmitt, Service et al. 2017). Male animals typically weigh 5-6 kilograms in adulthood, while adult females are slightly smaller, averaging 3-4 kilograms. AGMs are omnivorous, but prefer to eat fruit or flowers when available (Harrison 1983). Observational studies have shown that AGMs tend to first feed early in the morning and again in the late afternoon. Inactivity and grooming, an important social behavior, typically occurs in the middle of the day, when temperatures peak (Baldellou and Adan 1998).

AGMs have unique social structures and participate in complex behaviors like parenting, cooperation, and significant territoriality in both the wild and captivity. Their social structure is female philopatric, wherein females remain with their natal troop and males typically disperse after they reach sexual maturity. Troops have been reported to be as large as 75 members in the wild (McGuire 1974) and are typically multi-male and

multi-female. Within a troop, female siblings and relatives often share parenting responsibilities of young infants (Fairbanks 1990). Compared to other primates, AGMs have been observed exhibiting cooperation and altruism with non-kin individuals, particularly if those individuals have interacted positively with them in the past (Seyfarth and Cheney 1984).

Menstrual cycles last approximately 29-30 days, similar to both humans and rhesus macaques (Kundu, May et al. 2013). The follicular phase, lasting from the onset of the menstruation through the gonadotropin surge, typically lasts 12 days and is characterized by elevation of follicle-stimulating hormone (FSH) and late (~ day 8) increases in estradiol which peak simultaneously with the luteinizing hormone (LH) and FSH surge. The luteal phase, which begins after the gonadotropin surge and ends at onset of the next menstruation, lasts approximately 16 days and displays increasing levels of progesterone and low to undetectable levels of FSH, LH, and estradiol (Molskness, Hess et al. 2007). Similar to humans, the pituitary gonadotropin (LH and FSH) surge mid-cycle signals the transition from the follicular to luteal phase and promotes oocyte maturation, ovulation, and development of the corpus luteum.

Reproductive senescence (menopause) is highly relevant to women's health and risk of disease in humans. Female AGMs undergo some form of reproductive senescence as they age, with estimates of reproductive life ending around 20 years of age. Atkins *et al* found that serum anti-Müllerian hormone, follicule number, and fecundity were negatively correlated with age while cycle length and probability of menses cessation both increased with age (Atkins, Willson et al. 2014).

Female AGMs can reproduce as early as 3 years of age, but the majority have their first offspring around 4 years of age (Fairbanks and McGuire 1986). Gestation in the AGM lasts between 163 and 165 days in captivity. Fecundity is increased in females who live with their mother compared to females who did not live in the same troop as their mother, suggesting that mothers provide social support long after their female progeny have reached adulthood, which in turns relates directly to reproductive and social success (Fairbanks and McGuire 1986). In Africa, vervets exhibit distinct seasonality in birthing, typically giving birth early in the year between January and March (Dunbar 1974). However, in captivity, this seasonality largely disappears (Kushner, Kraft-Schreyer et al. 1982).

Of particular interest is the evolution of concealed ovulation in the female AGM population. Like humans, female AGMs show no outward signs of sexual receptivity or ovulation. Comparatively, other primate species can show obvious outward signals of ovulation such as swelling of the perineum or overt behavioral signals like head shudders or grimaces (Andelman 1987). Andelman postulates that the lack of outward signals of ovulation in females decreases male-male competition and reduces the probability of infanticide in multi-male troops in the wild. In addition, phylogenetic analysis of concealed ovulation in multiple primate species similarly suggests that the evolution of this trait may promote positive male behavior toward offspring due to parental confusion (Sillen-Tulberg and Moller 1993).

Male reproductive success in the AGM is largely determined by dominance rank. In one study of captive AGMs, the highest ranked male, based on behavioral observational data collected in a 3.5 year period, had more reproductive success than

subordinate males in the first mating season. However, as the study progressed, reproductive success actually decreased for the alpha male as his tenure increased (siring 100% of offspring in the first mating season and only 56% of offspring in the third). By the fourth season, a younger male, who then sired all offspring born by the conclusion of the study, replaced the alpha male (Weingrill, Willems et al. 2010). The authors attribute this to either increased competition between the males or female preference for younger males. Steklis *et al* found that plasma testosterone was not correlated to dominance rank, though it was associated with initiation of aggressive behavior in dominant, but not subordinate, males (Steklis, Brammer et al. 1985). Together this suggests that while plasma testosterone does not directly relate to dominance ranking, it may influence behavior and thus reproductive success in males, though future studies will be required to address this exact relationship.

1.5.3 Use as a biomedical model

Non-human primate models are important and essential tools for the biomedical research community. As a group, NHPs have much greater levels of conservation with humans at multiple levels of biology in comparison to rodent and murine models. NHPs are diurnal and have gene sequences and gene structures that are highly similar to humans. NHPs can also be employed in longitudinal or invasive studies that are not suitable or infeasible for human research. Primate research has led to critical health advances in cancer, obesity, AIDS, and Alzheimer's research (Research 2016). In short, primate research is critical for continued improvements in the health and lives of people all over the globe. Knowledge of how primates express and display common, complex

disease states will not only improve treatment and care, but may provide important insights into the etiology and development of pathologies.

AGMs are widely used as biomedical models in the fields of virology, immunology, neurology, and physiology. A PubMed search reveals over 14,500 citations for the AGM in the last decade, compared to 10,000 for the rhesus macaque, an endangered but widely utilized NHP in biomedical research. While the AGM is similar to the rhesus macaque in both physiology and behavior, the AGM is less expensive, more accessible, and poses fewer health and safety risks due to its disease-free status (Jasinska, Schmitt et al. 2013). Due to the concerted efforts of the International Vervet Genome Consortium, an annotated reference genome for the AGM was published in 2015 (Warren, Jasinska et al. 2015) and genetic analysis tools are becoming more widely available for the species (Huang, Ramensky et al. 2015). AGMs have a higher chromosome number (n=30) and their genome has undergone 12 rearrangements compared to the human genome. Extensive synteny with the human genome has been found, with nine human chromosomes displaying full colinearity with AGM chromosomes (Jasinska, Service et al. 2007).

The *Chlorocebus* genus, like many animals of African origin, acts as a natural host for the simian immunodeficiency virus (SIV) and vervets likely have interacted with the lentivirus for thousands of years (Worobey, Telfer et al. 2010). Natural hosts, when infected with SIV, do not progress to immunodeficiency; instead they retain healthy amounts of $CD4^+$ T cells and low immune activation, despite significant viremia. Caribbean AGMs are naturally SIV-free, however, once infected, they do not progress to immunodeficiency and their virological and immunological profiles are very similar to

African-born AGMs infected with SIV (Pandrea, Apetrei et al. 2006). This makes the AGM, in particular the Caribbean AGM, uniquely suited to studying the progression of SIV in a natural host and may provide insights into the pathology of the closely related human immunodeficiency virus.

The high level of similarity between the AGM brain and the human brain has led to the use of the AGM in studies of neurobiology and neurodegeneration. AGMs (greater than 20 years of age) develop amyloid-β plaques in the frontal and temporal cortex that are associated with gliosis and neuritic dystrophy. Immunization with amyloid β in aged animals successfully decreased cortical plaque formation and has been proposed as possible treatment against Alzheimer's disease (Lemere, Beierschmitt et al. 2004). Genetic linkage analysis of amyloid β markers in the cerebral spinal fluid of aged AGMs has recently identified loci on vervet chromosomes 4 and 12 that may mediate neurodegenerative changes (Chen, Fears et al. 2018).

AGMs, like many Old World monkeys, can develop atherosclerosis, insulin resistance, and obesity when fed a typical Western diet and longitudinal studies have shown that these factors are heritable in the species (Kavanagh, Fairbanks et al. 2007, Schmitt, Service et al. 2017). Genetic analysis of obesity in the AGM reveals, that similar to human, obesity and obesogenic growth are likely complex, polygenic traits with both genetic and environmental influences. While other models exist to study these multifactorial interactions, the AGM is unique in that investigators can experimentally manipulate all of these variables in a highly translational model organism.

Elevated blood pressures in the African Green Monkey were first reported by Kraft-Schreyer and Angelakos in the 1980s (Kraft-Schreyer and Angelakos 1985). Angelakos attempted to create a strain of AGMs that would inherit spontaneous hypertension by breeding animals with normal and elevated blood pressures (Kushner, Kraft-Schreyer et al. 1982). First generation offspring with two HT parents had significantly elevated mean blood pressures compared to offspring born from NT parentage. In addition, blood pressure increased in all F1 animals with age, however, HT offspring had the greatest slope of any group(Kraft-Schreyer, Kushner et al. 1987). This suggested a larger contribution of age to elevation of blood pressure that was dependent on genetic factors in the AGM. Angelakos speculated that a HT strain of the AGM could be developed through selective breeding and showed significant differences in blood pressure distributions in offspring as early as the first generation (Kraft-Schreyer, Kushner et al. 1991).

Once described, research scientists began to utilize AGMs in experimental studies involving blood pressure regulation. Pavel Hamet reported that both acute and chronic infusion of atrial natriuretic factor, a diuretic, natriuretic, and vasoactive peptide, lowered blood pressure in HT(Hamet, Tremblay et al. 1986) and NT monkeys (Hamet, Testaert et al. 1989). Frank Ervin's group published an initial characterization of spontaneous hypertension in adult male AGMs, showing that acute administration of captopril (4 mg/kg), an ACE inhibitor, significantly lowered blood pressure in HT, but not NT, animals. Infusion of furosemide (3.5 mg/kg), a loop diuretic, had no effect on blood pressure in either NT or HT AGMs (Martin, Palmour et al. 1990).

Blood pressure in the AGM was shown to be salt-sensitive in studies by Srinivasan et al. Adult male AGMs were exposed to graded increases of dietary sodium, 0%, 3%, 4.5%, and 6% NaCl diets for 45, 139, 37, and 90 days, respectively. Body weight did not differ between any of the diets, but mean systolic and diastolic pressures were elevated as NaCl intake increased (Srinivasan, Dalferes et al. 1984).

Together this data support the development of the AGM as a model of spontaneous hypertension and associated cardiovascular and kidney diseases. This doctoral thesis will expand upon the body of research available and attempt to determine probable mechanisms crucial to the development and maintenance of hypertension in this specific animal model.

1.6 Rationale, overall hypothesis, and specific aims

The development of highly translational models is crucial to innovative and groundbreaking research. In addition, the ability to assess and examine spontaneously occurring disease pathologies in a closely related species presents a unique opportunity for researchers to study and determine etiology of complex diseases like hypertension. The overall hypothesis of this doctoral thesis is that the African Green Monkey develops spontaneous hypertension with strong translational parallels to human hypertension and provides a novel model to study the etiology of hypertension and its related pathologies. In service of this hypothesis, the following specific aims were examined:

Specific Aim 1: To determine the prevalence of hypertension in a research colony comprised of wild-caught African Green Monkeys and to examine pathologies in cardiac and renal tissues crucial to the regulation of blood pressure.

Specific Aim 2: To assess the interaction between elevated blood pressures and sex in renal function, with a particular focus on glomerular filtration rates, and urinary excretion of protein, sodium, and potassium in African Green Monkeys.

Specific Aim 3: To determine the effect of the sympathetic nervous system on hypertension in the African Green Monkey

Specific Aim 4: To elucidate the effect of age on the development of elevated blood pressure and renal function in the African Green Monkey

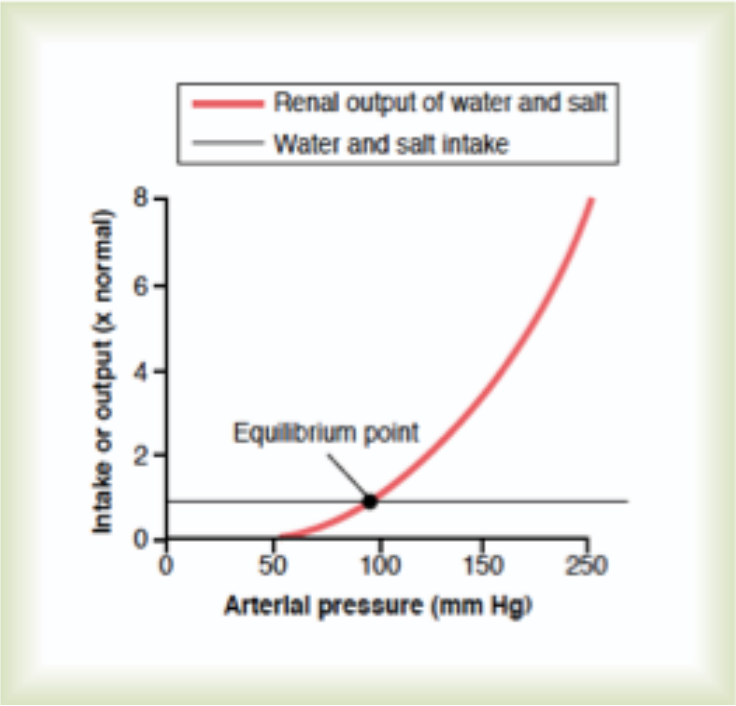

Figure 1.1 The renal function curve

The renal function curve, adapted from Guyton and Hall, 2006

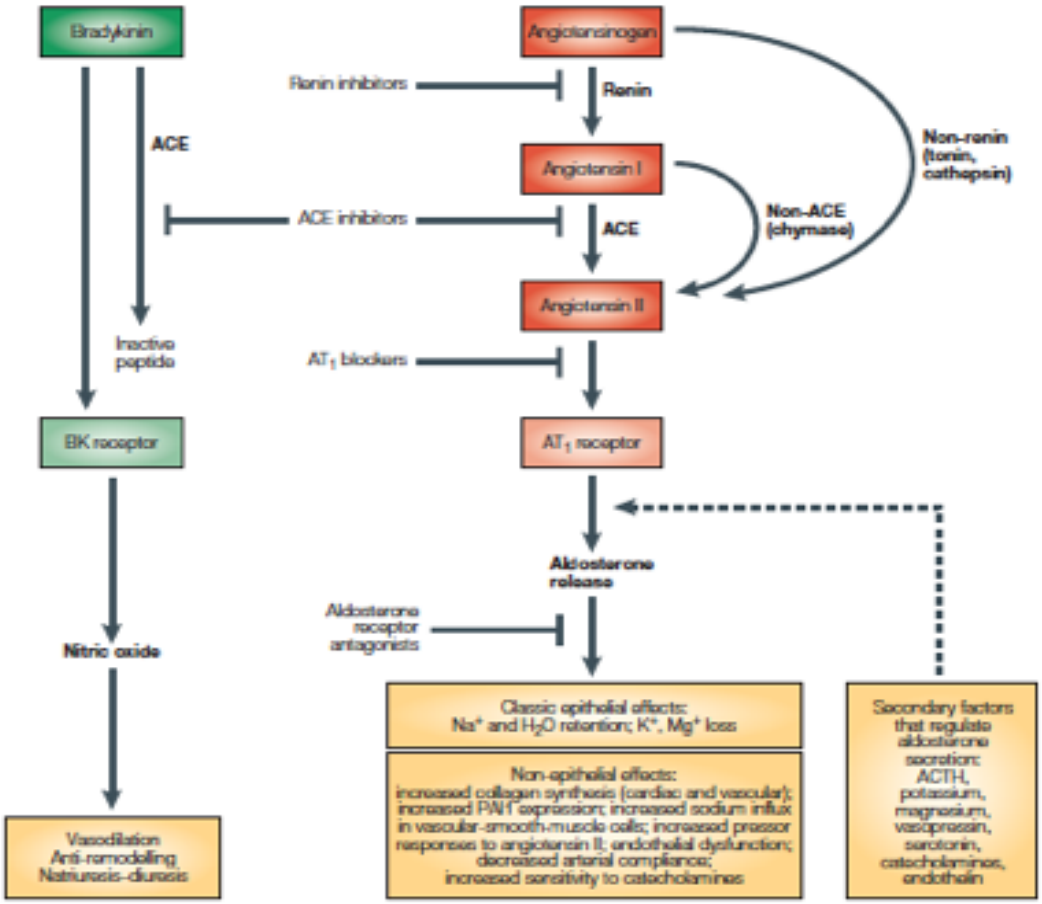

Figure 1.2 The classical renin-angiotensin-aldosterone system

The renin-angiotensin-aldosterone system, adapted from Zaman, Oparil et al. 2002.

CHAPTER TWO

RENAL VASCULAR AND GLOMERULAR PATHOLOGIES ASSOCIATED WITH SPONTANEOUS HYPERTENSION IN THE NON-HUMAN PRIMATE *Chlorocebus aethiops sabaeus*

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2.1 Introduction

An estimated 78 million adults in the United States suffer from hypertension, a major risk factor for cardiovascular disease and stroke (Go, Mozaffarian et al. 2014). 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(Persell 2011). Elevated blood pressure, left ventricular hypertrophy, cardiovascular disease, and end stage renal disease contribute to one out of every three deaths(Go, Mozaffarian et al. 2014) 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(McGuire 1974, Van Der Kuyl, Dekker et al. 1996). The AGM behaviorally and socially equates to humans since they live within troops containing a well-described social structure and hierarchy(Dunbar 1974). They are a diurnal species with large syntenic chromosomal regions when compared with humans(Palmour, Mulligan et al. 1997, Jasinska, Schmitt et al. 2013) 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(Shively, Willard et al. 2012) 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 (Palmour, Mulligan et al. 1997, Jasinska, Schmitt et al. 2013). While there are numerous models of experimentally induced hypertension in various primate species ranging from baboons to rhesus macaques (Cody, Rodger et al. 1982, Jannetta, Segal et al. 1985), 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(Hamet, Tremblay et al. 1986). 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.

2.2 Methods

2.2.1 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.

2.2.2 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(Collett, Hart et al. 2013). 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. Forearm cuff plesythmography systolic measurements were verified by both direct arterial catheter measurement (n=2) and radiotelemetry (n=4) on separate animals (Supplemental Figure 1).

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 1/3 SBP + 2/3 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^{R}; 150 mg/kg, i.v.). Tissues were then harvested and thinly sliced for tissue fixation or immediately frozen in ultra-cold isopentane (-20 ^{0}C) and dry ice.

2.2.3 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(Livak and Schmittgen 2001). Primers for angiotensinogen were custom designed using Primer3 software (Forward primer 5'-AAGATTGGCAGCCCCCTGAC ; Reverse primer 5'-ATCTTCCCTTGGAAATGGACGTAG)

2.2.4 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(Haber, Koerner et al. 1969).

2.2.5 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(Atchison, Ortiz-Capisano et al. 2010, Atchison, Harding et al. 2011).

2.2.6 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 ^{0}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 5μm sections using a microtome, and placed on glass microscope slides. Digital images of sections were analyzed using Image J software (NIH, Bethesda, MD)(Schneider, Rasband et al. 2012).

2.2.7 Digital analysis of tissue sections

Kidney tissues were sectioned sagittally (5 μm) 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 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.

2.2.8 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(Gentleman, Carey et al. 2004). Kernel density plots estimated the probability of the relationship between age and blood pressure.

2.3 Results

2.3.1 Blood Pressure is Elevated in 37% of Adult African Green Monkeys

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 2.1). 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 2.1).

2.3.2 Heart Rates are Elevated in Hypertensive and Borderline Hypertensive Animals

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 2.2). 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 2.2B & 2.2C).

2.3.3 Hypertensive Animals are Older than Normotensive Controls

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 2.3A).

Male and female AGMs have similar incidences of hypertension in adulthood (Male: 119/307 or 38.8%; Female: 38/117or 32.5%). Systolic, diastolic, and mean blood pressures are similar between male and female AGMs (Table 2.1). Heart rate is significantly higher in females in all three blood pressure groups ($p<0.05$, Tukey's *post hoc*).

2.3.4 Components of the Renin-Angiotensin System are Similar in Normotensive and Hypertensive Groups

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 2.4A). 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 2.4B). 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 2.4C).

2.3.5 Renal Vascular Hypertrophy and Wall/Lumen Ratios are Larger in Hypertensive Animals

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 2.5A). 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 2.5B).

2.3.6 Hypertensive Animals Have Altered Glomerular Morphology

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).

2.4 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 (Go, Mozaffarian et al. 2014). 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 (Sun 2015, Buford 2016). 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(Gillman, Kannel et al. 1993, Seccareccia, Pannozzo et al. 2001). The elevated heart rate in the AGM mimics the pathologies seen in human essential hypertensive patients with increased sympathetic drive(Esler 2000). 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(Martin, Palmour et al. 1990), although circulating plasma levels of Angiotensin II have been reported as lower in animals with high blood pressure(Martin, Palmour et al. 1990).

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(Srinivasan, Dalferes et al. 1984). 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(Renna, de Las Heras et al. 2013). 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(Skov, Hamet et al. 2001). 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(Kraft-Schreyer, Kushner et al. 1987, Kraft-Schreyer, Kushner et al. 1991). 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(Jasinska, Lin et al. 2012, Flicek, Amode et al. 2014).

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. HT AGMs exhibit vascular hypertrophy and glomerular remodeling compared to NT controls and older animals are more likely to develop high blood pressure in this species. The characterization of this model of hypertension opens new possibilities for researchers to study the etiology of this disease.

2.5 Acknowledgements

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Table 2.1: Descriptive Statistics of SBP, DBP, MBP, and HR, separated by phenotype and sex.

Phenotype	Sex	N	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean Blood Pressure (mmHg)	Heart Rate (bpm)
Normotensive	Male	130	99.3±1.2	53.5±1.4	68.8±1.2	117.3±2.0
	Female	57	100.4±1.8	62.3±2.6	75.3±3.9	144.9±3.9*
Borderline Hypertensive	Male	58	130.9±0.7	66.2±2.6	87.7±1.8	128.2±2.5
	Female	22	130.0±1.3	75.6±4.0	93.7±2.6	164.5±6.3*
Hypertensive	Male	119	171.6±2.4	81.6±2.3	111.6±2.0	130.3±2.0
	Female	38	173.3±4.9	87.9±3.2	114.0±3.3	162.3±4.9*

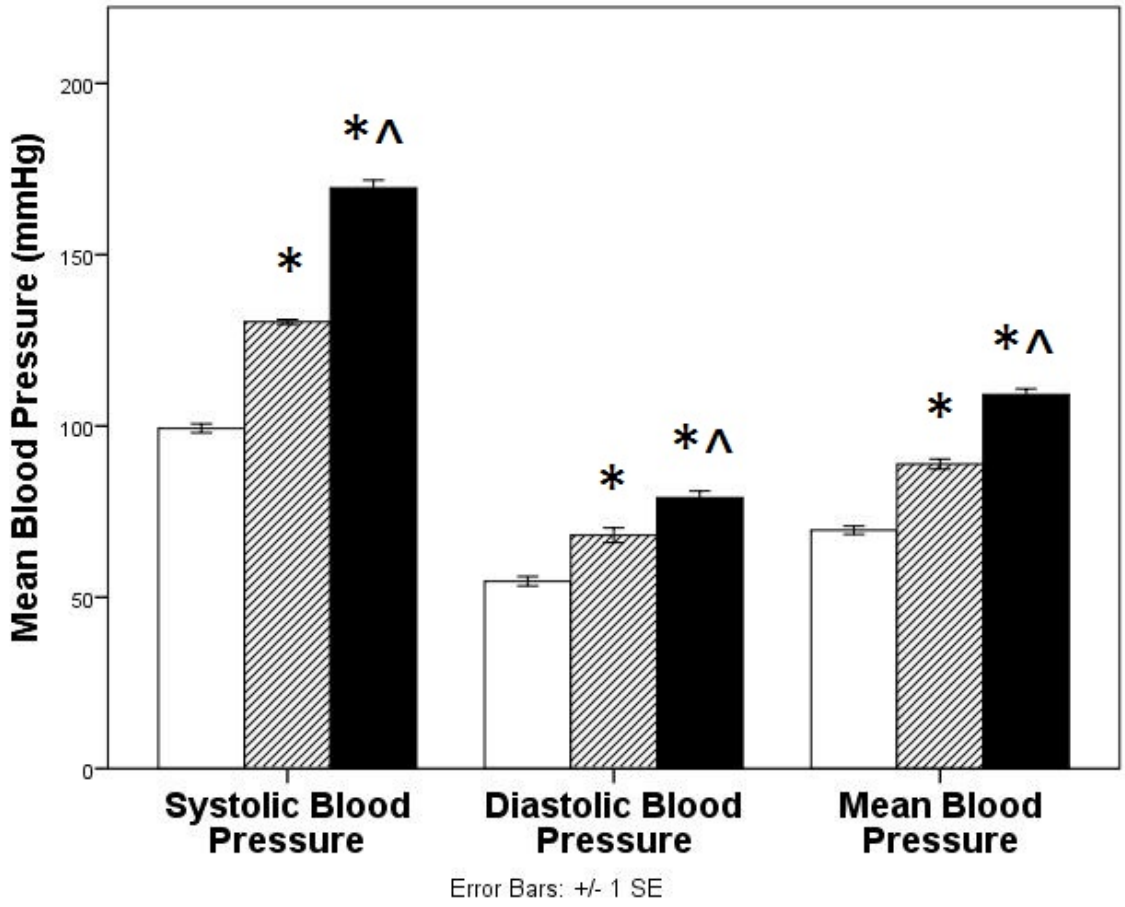

Figure 2.1: Hypertensive AGMs have higher systolic, diastolic, and mean blood pressures and comprise 37% of the total population studied.

Animal blood pressures (n=424) 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±2.22mmHg, and an average mean blood pressure of 89.35±151 mmHg. HT AGMs (n=157, black) have an average systolic blood pressure of 172.04±2.18 mmHg, an average diastolic blood pressure of83.13±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

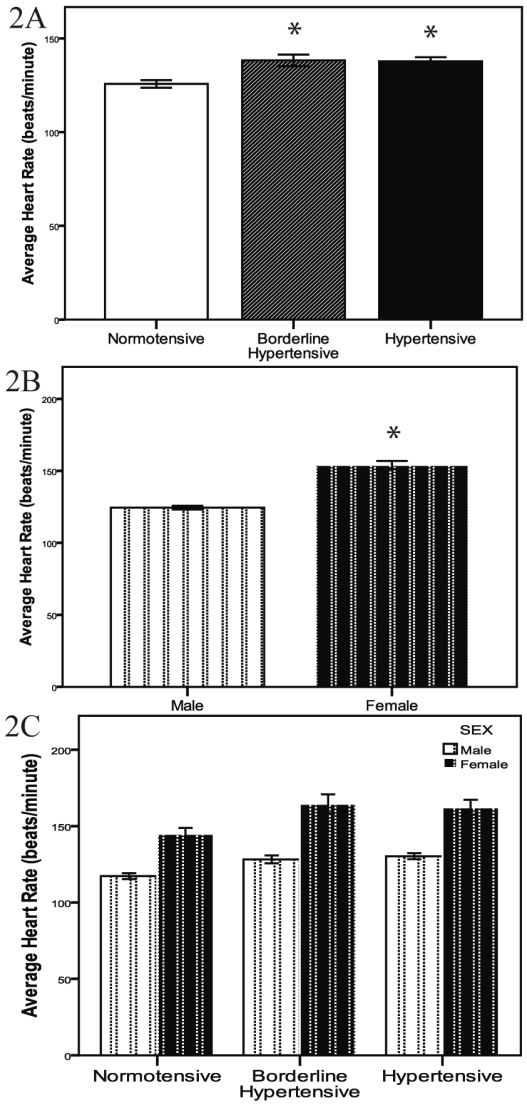

Figure 2.2: Heart rate is elevated in hypertensive and borderline hypertensive AGMs compared to normotensive AGMs.

Figure 2.2A: 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±2.39bpm and HT AGMS (n=119, black) have an average HR of 135.87±2.20 bpm. *$p<0.05$ compared to NT AGMs. Figure 2.2B: 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 2.2C: Heart rate is higher in Hypertensive animals and in females, but no significant interactions exist between phenotype and sex.

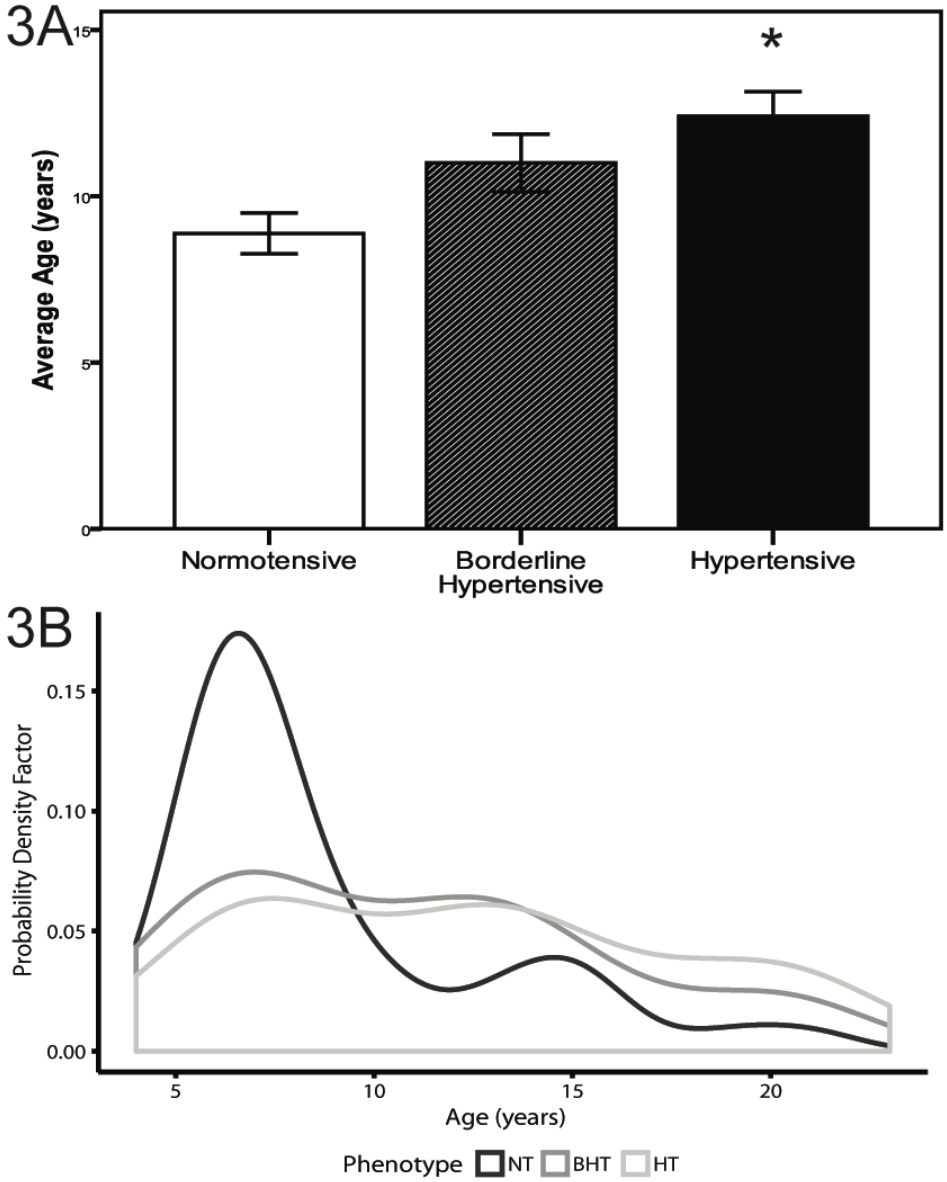

Figure 2.3: Hypertensive AGMs are older than the normotensive cohort.

Figure 2.3A: 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 2.3B: 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.

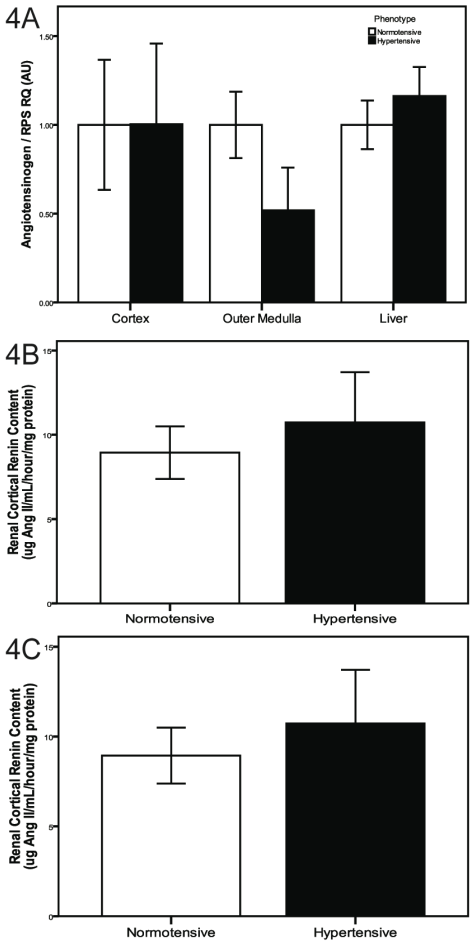

Figure 2.4: Components of the renin-angiotensin-aldosterone system are similar between normotensive and hypertensive AGMs.

Figure 2.4A: 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 2.4B: 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 2.4C: 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.

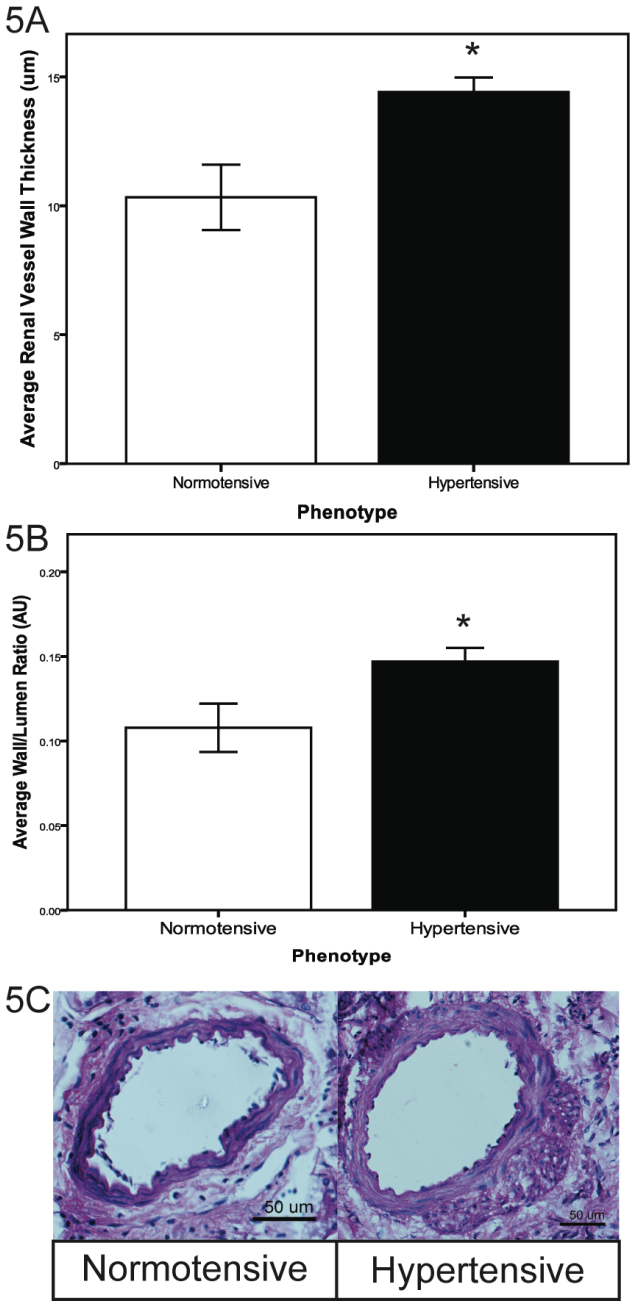

Figure 2.5: Renal vascular hypertrophy is displayed in HT AGMs.

Figure 2.5A: 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 2.5B: 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 2.5C: Representative histological sections of Periodic Acid Schiff stained renal tissue in NT and HT AGMs. * denotes p ≤0.05 comparing NT vs. HT.

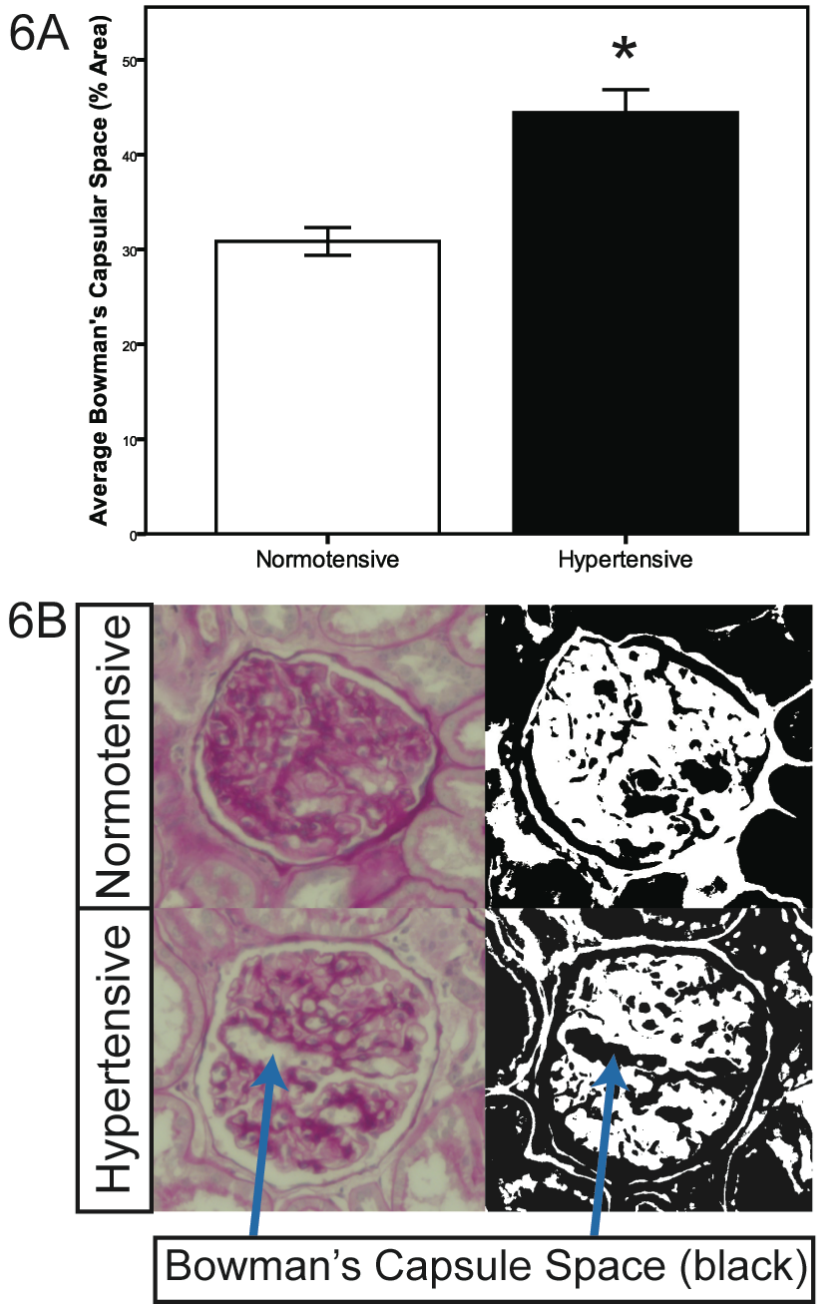

Figure 2.6: Altered glomerular morphology identified in HT AGMs.

Figure 2.6A: 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 2.6B: Histological sections of PAS-stained glomeruli before and after ImageJ thresholding.

CHAPTER THREE

PROTEINURIA IS ASSOCIATED WITH ELEVATED SYSTOLIC BLOOD PRESSURE IN THE AFRICAN GREEN MONKEY, A NON-HUMAN PRIMATE MODEL OF SPONTANEOUS HYPERTENSION

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3.1 Introduction

Hypertension is a major risk factor for cardiovascular disease, affecting an estimated 86 million adults and costing over $51 billion annually in the United States (Benjamin, Blaha et al. 2017). The fact that hypertension is a complex, multifactorial disease with numerous co-morbidities makes treatment difficult and expensive. While many strides have been made in the treatment and prevention of cardiovascular and renal disease, an estimated 13.7% of patients still have uncontrolled blood pressure (defined as systolic blood pressure over 140 mmHg while on three or more classes of anti-hypertensive medications)(Benjamin, Blaha et al. 2017). Historically, hypertension research has been conducted largely in rat and more recently murine models of hypertension that are either selected for genetically (Okamoto, Tabei et al. 1966, Dahl, Knudsen et al. 1967, Rapp and Dene 1985, Bachmann, Peters et al. 1992, Shimokama, Haraoka et al. 1998) or induced by pharmacological, dietary, or surgical interventions (Arnal, Warin et al. 1992, Navar, Zou et al. 1998, Cosenzi, Bernobich et al. 2002, Zimmerman, Lazartigues et al. 2004). Despite this research, there remain significant numbers of hypertensive patients that are resistant to current therapies. This gap suggests that new models and techniques need to be studied in order to identify possible targets and novel therapies to deal with this cardiovascular pathology. Recently, our group has focused on the African Green Monkey as a large animal model of spontaneous hypertension that may offer new mechanisms of the disease and bridge this translational gap (Rhoads, Goleva et al. 2017).

Studies have demonstrated that hypertension is sexually dimorphic and age-related in patient populations. Women have lower blood pressure compared to age-matched men until menopause(Cheng, Xanthakis et al. 2012). After the onset of menopause, women

develop hypertension at rates equal to or greater than that of males. Women and men even respond to anti-hypertensive medications differently: men have greater decreases in ambulatory blood pressure in response to ACE inhibitors, while women have greater decreases in blood pressure in response to diuretics, angiotensin receptor blockers, and calcium channel blockers(Zimmerman and Sullivan 2013). These sex-specific responses to different classes of anti-hypertensive medications underscore the compelling need for translational cardiovascular research that considers sex as a crucial biological variable.

The kidney is a critical mediator of long-term blood pressure control and body fluid homeostasis. Parallel shifts in the pressure-natriuresis curve are associated with the development and maintenance of hypertension in both patients and experimental animal models(Hall, Mizelle et al. 1990). In normal conditions, increases in arterial blood pressure are associated with increased urinary sodium and water excretion, allowing extracellular fluid volumes to decrease and blood pressure to return to normal levels. The parallel resetting of the pressure-natriuresis curve occurs in pathological conditions and thus inhibits the feedback cycle of body fluid homeostasis, allowing the chronic elevation of blood pressure to continue without compensatory increases in sodium and water excretion. Clearly, multiple neurogenic, hormonal, renal and immunological systems can alter the sensitivity of the pressure-natriuresis curve(Akita, Sacks et al. 2003).

Urinary protein excretion across species is a clinical marker for renal damage. In patients, proteinuria is correlated with the rate of decline in glomerular filtration rate (GFR) and thus the progression of renal damage in hypertension and associated renal pathologies. In the spontaneously hypertensive rat (SHR), urinary protein excretion

increases with blood pressure and age(Feld, Van Liew et al. 1977) and differs between age-matched male and female SHR(Reckelhoff, Zhang et al. 2000).

Thus, knowledge of critical clinical markers, with careful regard of sex and blood pressure phenotypes, are needed to further identify causal mechanisms that contribute to the pathogenesis of hypertension in the AGM. This study expands upon the prior characterizations of the model (Kraft-Schreyer, Kushner et al. 1987, Martin, Palmour et al. 1990, Skov, Hamet et al. 2001, Rhoads, Goleva et al. 2017)and provides relevant renal function data in both male and female AGMs with normal and elevated blood pressures. In this work, we test the hypothesis that elevations in urinary protein excretion are related to elevation of arterial pressure in the HT AGM.

3.2 Methods

3.2.1 Animal care and housing

Animal protocols strictly adhere to both the NIH *Guide on the Care and Use of Laboratory Animals*(Animals 2011) and "Guiding Principles on the Care and Use of Experimental Animals" published by the American Physiological Society. Protocols were reviewed and approved by the Institutional Animals Care and Use Committee (IACUC) of the Biomedical Sciences Research Group and SKN Primates in Basseterre, St. Kitts and Nevis, West Indies. Animals were housed in the long term in troop enclosures in a large outdoor facility in St. Kitts, West Indies. Animals were fed nonhuman primate chow (Teklad 8773, Harlan) daily and a seasonal combination of fresh fruit and vegetables (bananas, mangos, sweet potatoes, carrots and squash) on alternating days. Water was provided *ad libitum.* Quarantine and husbandry protocols were maintained as previously described (Rhoads, Goleva et al. 2017).

3.2.2 Blood pressure measurements

Animals were sedated (ketamine, 15mg/kg i.m.) and blood pressure measured as described previously in detail (Rhoads, Goleva et al. 2017). After sedation, animals were placed in a supine position and a blood pressure cuff placed on the left upper arm. A Doppler stethoscope was used to confirm total occlusion and identify Korotkoff sounds indicative of systolic (SBP) and diastolic blood pressures. Animals were characterized as normotensive (NT, SBP $\leq$ 120 mmHg), pre-hypertensive (PHT, SBP between 120 and

140 mmHg), or hypertensive (HT, SBP ≥ 140 mmHg) based on repeated measurements of SBP.

3.2.3 Urine collection and sample preparation

Prior to study, animals were moved from group enclosures into singly housed metabolic pens with 1 L water bottles for the duration of the studies (n=120). The dietary regimen remained the same and animals were fed non-human primate chow (Teklad 8773, Harlan), fresh fruits, and vegetables as described above. Female animals were monitored and examined for pregnancy, and if found to be pregnant, were removed from this cohort. After a minimum 7-day acclimation period, blood pressures were measured as described above and whole blood collected in Li-heparinized tubes. Urine was then collected for 3 consecutive, 24-hour periods. Water intake and urine volume were recorded daily. Urine samples were centrifuged (1000x*g*) to remove any large particulate contaminants. Samples were aliquoted into 1.5 mL tubes to minimize freeze-thaw cycles during further downstream analysis and stored at -20°C.

3.2.4 Urinary protein measurement

Total protein concentration was measured by Pierce BCA Assay (Catalog # 23225, ThermoFisher Scientific, Waltham MA) per the manufacturer's recommended protocol. Briefly, urine samples were diluted 1:4 and 25 µL of bovine standard albumin or thawed samples were pipetted in a microplate. Working BCA reagent (200 ul) was

added to each well and the plate incubated at 37°C for 2 hours. After cooling to room temperature, absorbance was read at 595nm. A linear standard curve was generated ($r^2 \geq 0.98$) and used to determine protein concentration for the unknown samples.

3.2.5 Urinary and plasma creatinine measurements/estimation of glomerular filtration rate

Urinary creatinine was measured by a modified Jaffe reaction(Husdan and Rapoport 1967) or by plasma creatinine assay following the manufacturer's recommended protocol (Item #: 700460, Cayman Chemical, Ann Arbor, MI). Urine samples were diluted 20x. Briefly, known creatinine standard or thawed, diluted urine samples (50 ul) were combined with 100 µL of alkaline picrate (12.5 mL of 0.13% picric acid and 2.5mL of 1 M NaOH). After 30-minute room temperature incubation, absorbance was read at 490 nm. A linear standard curve was generated ($r^2 \geq 0.98$) and used to determine creatinine concentration for the unknown samples. Estimated glomerular filtration rate (eGFR) was calculated by the equation:

$$eGFR = \frac{Urine\ Flow\ Rate\ x\ Urine_{Creatinine}}{Plasma_{Creatinine}}$$

3.2.6 Urinary sodium and potassium measurements

Urinary sodium and potassium concentrations were measured by dual-channel flame photometry (Dual-channel flame photometer #02655-10, Cole-Parmer, Vernon Hills, IL). Thawed urine samples were diluted 20X in lithium diluent and concentrations

multiplied by dilution factor, molecular weight, and the appropriate urine flow rate (UFR) to obtain ion excretion rates.

3.2.7 Statistical analysis

Data was analyzed using SPSS 21.0 (IBM) and groups were compared by ANOVA with Tukey's post-hoc analysis or Student's t-test when appropriate. Values are presented as mean±SEM and $p<0.05$ used as the criterion of significance.

3.3 Results

3.3.1 Cohort blood pressure

Blood pressure was measured in 119 adult AGMs (male: n=50) under light sedation. NT animals (n=40) averaged SBPs of 106.3±2.1 mmHg, diastolic blood pressures (DBP) of 41.5±2.2 mmHg, and mean blood pressures (MBP) of 84.7±1.8 mmHg. PHT animals (n=33) averaged SBPs of 130.5±1.0 mmHg, DBPs of 53.6±3.6 mmHg, and MBPs of 104.9±1.5 mmHg, while HT animals (n=46) averaged 166.0±4.2 mmHg, 68.1±4.8 mmHg, and 133.4±3.3 mmHg for SBP, DBP, and MBP, respectively. SBP, DBP, and MBP are all significantly different between all groups ($p<0.05$, Figure 3.1).

3.3.2 Daily water intake and urine flow rate

Daily water intake is similar in NT (231.9±32.2 mL/day, n=40), PHT (209.6±22.2 mL/day, n=33), and HT (241.9±26 mL/day, n=46; $p>0.05$; Figure 3.2A) AGMs and does not differ between male (200.2±22.2 mL/day, n=50) and female (250.9±22 mL/day, n=69; Figure 3.2B) animals. Daily water intake is also similar between the sexes in each of the blood pressure groups (NT females: 258.4±42.7 mL/day, n=28; NT males: 170.1±36.2 mL/day, n=12; PHT females: 221.7±27.4 mL/day, n=24; PHT males: 177.2±35.5 mL/day, n=9; HT females: 279.9±40.6 mL/day, n=17; HT males: 219.7±33.6 mL/day, n=29; Figure 3.2C).

Urine flow rates are similar in NT (137.7±17.1 mL/day, n=40), PHT (152.4±16.4 mL/day, n=33), and HT (165.6±16.1 mL/day, n=46; $p>0.05$; Figure 3.2D) groups and do not differ between male (138.0±12.7 mL/day, n=50) and female (163.1±13.7 mL/day, n=69; Figure 3.2E) animals. Urine flow rate is also similar between the sexes in each of the blood pressure groups (NT females: 159.1±22.5 mL/day, n=28; NT males: 87.7±15.2 mL/day, n=12; PHT females: 146.9±18.7 mL/day, n=24; PHT males: 167.0±35.4 mL/day, n=9; HT females: 192.3±32.4 mL/day, n=17; HT males: 150.0±16.8 mL/day, n=29; Figure 3.2F).

3.3.3 Estimated glomerular filtration rate (eGFR)

eGFR is similar in NT (3.5±0.4 mL/min, n=32), PHT (4.3±0.5 mL/min, n=25), and HT (4.6±0.5 mL/min, n=36; $p>0.05$) animals (Figure 3.3A) and does not differ between sex (males: 4.3±0.5 mL/min, n=39; females: 4.0±0.3 mL/min, n=54; Figure 3.3B). eGFR is also similar between the sexes of each blood pressure group (NT females: 3.6±0.5 mL/min, n=23; NT males: 3±0.8 mL/min, n=9; PHT females: 4.3±0.4 mL/min, n=20; PHT males: 4.4±2.1 mL/min, n=5; HT females: 4.2±0.8 mL/min, n=11; HT males: 4.7±0.6 mL/min, n=25; Figure 3.3C).

3.3.4 Urinary protein excretion

Protein excretion is elevated in HT and PHT AGMs (HT: 457.7±22.4 mg/day, n=46; PHT: 424.2±29.6 mg/day, n=31) compared to NT animals (NT: 336.2±19.5

mg/day, n=40; $p<0.05$; Figure 3.4A). Protein excretion is similar between male (427.4±23.3 mg/day, n=50) and female (392.3±17.8mg/day, n=67) AGMs (Figure 3.4B). Protein excretion is also similar between sexes of each blood pressure phenotype group (NT females: 359.9±24.2 mg/day, n=28; NT males: 281±27 mg/day, n=12; PHT females: 410.9±34.4 mg/day, n=22; PHT males: 456.7±59.3 mg/day, n=9; HT females: 421.7±34.5 mg/day, n=17; HT males: 478.9±28 mg/day, n=29; Figure 3.4C).

3.3.5 Urinary sodium and potassium excretion

Sodium excretion is elevated in HT AGMs (3.6±0.3 mmol/day, n=46) compared to NT animals (2.2±0.2 mmol/day, n=40; $p<0.05$). PHT animals excrete 3.0±0.3 mmol/day (n=33) of sodium and are not significantly different from either NT or HT animals (Figure 3.5A). Male AGMs (3.4±0.2 mmol/day, n=50) excrete higher amounts of sodium daily compared to female AGMs (2.7±0.2 mmol/day, n=69; $p<0.05$, Figure 3.5B). Sodium excretion is similar between sexes within phenotypic groups (NT females: 2.2±0.2 mmol/day, n=28; NT males: 2.4±0.3mmol/day, n=12; PHT females: 3±0.4 mmol/day, n=24; PHT males: 3.4±0.5 mmol/day, n=9; HT females: 3.2±0.2 mmol/day, n=17; HT males: 3.8±0.3 mmol/day, n=29; Figure 3.5C).

Potassium excretion is elevated in HT AGMs (4.0±0.4 mmol/day, n=46) compared to NT AGMs (2.7±0.3 mmol/day, n=40, $p<0.05$). PHT animals excrete 3.7±0.4 mmol/day of potassium (n=32) and are not significantly different from either NT or HT animals (Figure 3.5D). Female and male AGMs have similar levels of daily potassium excretion (Female: 3.5±0.3 mmol/day, n=68; Male: 3.4±0.3 mmol/day, n=50, Figure

3.5E). Daily potassium excretion is similar between sexes within phenotypic groups (NT females: 2.8±0.3 mmol/day, n=28; NT males: 2.4±0.6 mmol/day, n=12; PHT females: 3.7±0.4 mmol/day, n=23; PHT males: 3.7±0.6 mmol/day, n=9; HT females: 4.5±0.8 mmol/day, n=17; HT males: 3.7±0.4 mmol/day, n=29; Figure 3.5F).

3.4 Discussion

We have previously reported that 37% of adult AGMs have SBPs greater than 140 mmHg in our cohort (n=424) and that HT animals are typically older and have increased heart rates compared to normotensive controls, as well as renal vascular and glomerular pathologies (Rhoads, Goleva et al. 2017). In the current study, water intake and urine flow rates were similar in NT, PHT, and HT groups ($p>0.05$), suggesting that water balance is similar in all groups, regardless of blood pressure phenotype or sex (Figure 3.2). On average, animals consume 229.6 ± 15.9 mLs of water daily and have an average urine flow rate of 152.5 ± 9.6 mL/day. It's crucial to consider that movement of animals from troop enclosures to single housing may cause an elevated stress response due to removal from the troop, though single housing is necessary to accurately collect urine and measure water intake (Kurien, Everds et al. 2004). Animals are still able to see, smell, and hear their troop while in single housing and great efforts are made to reduce time in single housing as much as possible. Animals acting as though under severe duress would be removed immediately from the study, however, the altered living environment is a consideration in the interpretation of the current work.

Declining eGFR is an established marker of kidney function in hypertensive patient populations and decreases as disease states progress. Here we report no significant differences in baseline eGFR in NT, PHT, and HT AGMs, with eGFR averaging 4.1±0.3 mL/min in the entire cohort. However, it is worth noting that the rate of eGFR decline varies in different patient populations, regardless of SBP load and other risk factors(Davidson and Hix 2006) . In addition, the well-documented age-related decline of eGFR has been shown to have large amounts of individual variability in humans

(Glassock and Winearls 2009, Weinstein and Anderson 2010). Imai *et al*. found that proteinuria and lowered eGFR may be risk factors for future declines in eGFR and diagnosis of disease states, such as end stage renal disease(Imai, Horio et al. 2007). Future longitudinal analysis of eGFR in this AGM cohort will provide further information on the association between changes in eGFR, sex, age, and severity of hypertension progression in this model species.

Comparatively, eGFR is 10.4 ml/min in the rhesus macaque(Davies and Morris 1993), a non-human primate of similar size. To date, no one has reported spontaneous elevation of blood pressure in the rhesus macaque. The varied habitats of the rhesus macaque compared to the Sub-Saharan savannah habitat of the AGM may have evolutionarily selected for the AGM to exist in a more hypovolemic state compared to the rhesus macaque. This proposed perpetual dehydration may explain lower eGFRs in comparison to the rhesus macaque and may contribute to the development of spontaneous hypertension in the AGM.

Hypertensive AGMs exhibit elevated urinary protein, sodium and potassium excretions compared to normotensive controls (Figure 3.4). In conjunction with our previously published data that HT AGMs display altered glomerular morphology(Rhoads, Goleva et al. 2017), excess protein excretion is likely indicative of renal damage at some level correlated with chronic elevation of blood pressure, similar to patients with hypertension, chronic kidney disease(Jafar, Stark et al. 2003), cardiovascular disease(Irie, Iso et al. 2006), diabetes(Borch-Johnsen, Andersen et al. 1985), and hypertensive pregnancy disorders(Murakami, Saitoh et al. 2000).

Urinary protein excretion is not just a marker of renal damage, but may also be responsible for the exacerbation during the pathogenesis of renal disease. High levels of protein in the glomerular filtrate increase protein reabsorption in proximal tubular cells to maintain glomerular-tubular balance, which then accumulate abnormal levels of protein in the endolysosomes and endoplasmic reticulum. This abnormal protein accumulation can initiate the transcription of many oxidative stress, inflammatory, remodeling, and vasoactive genes, including (but not limited to) nuclear factor kappa beta, monocyte chemoattractant protein-1, and endothelin-1(Remuzzi, Ruggenenti et al. 1997). Future studies will focus on localization of the renal damage as well as pathogenic consequences of this increased protein load to the kidney in this novel model of spontaneous hypertension.

Sodium and potassium excretion is higher in HT animals compared to NT and PHT animals (Figure 3.5). While all animals have access to the same amount of dietary sodium and potassium (estimated 6.5 mmol/day and 15.4 mmol/day, respectively), it is possible that HT animals consume more food and thus elevate filtered load and electrolyte excretion. It is also possible that prior to single housing, HT animals competitively obtained more food in the troop enclosure and retained excess sodium from this time period. It's important to note that while dietary intake has been estimated based on the non-human primate chow formulation, actual intake varies for each animal and is dependent on a number of factors currently outside of experimental control (including group behavior, personality, weather, etc). The authors are currently pursuing options to more accurately determine food intake for individual animals but do recognize this factor as a limitation to the current work.

Srinivasan and colleagues found that a small group of AGMs (n=14) had elevation of systolic and diastolic blood pressures in response to long-term increases in dietary NaCl.(Srinivasan, Dalferes et al. 1984). Baseline blood pressures were obtained while AGMs were fed a 0% NaCl diet for 45 days. Then, dietary NaCl was increased to 3%, 4.5% and 6% over varied time periods (139, 37, and 90 days, respectively). Animals with low or high baseline blood pressures tended to maintain their ordinal positioning within the cohort while on the control and high salt diets, suggesting that individual animals have other unknown factors that contribute to their higher blood pressures. AGMs, as a species, have blood pressure control mechanisms that respond to increased dietary NaCl similarly to the 60% of patients with salt-sensitive hypertension(Haddy 2006) and like humans, hypertension in the AGM can be mediated by genetic and environmental factors. Since the AGM has not been specifically bred for the presence of hypertension, and yet the phenotype persists, the species represents a novel opportunity to study these complex factors in a laboratory setting.

Sodium excretion is higher in male AGMs compared to female animals (Figure 3.5B) but there are no differences in water intake, urine flow, protein excretion, or potassium excretion. In humans, sex hormones, such as estrogen affect the pressure-natriuresis relationship, which may contribute to the 'protection' from the prevalence of hypertension seen in pre-menopausal women compared to age-matched men(Pechere-Bertschi and Burnier 2004). It's likely that the result seen here is simply a factor of sexually dimorphic body size in the species, as males tend to be larger than females.

This work identifies key physiological parameters crucial to blood pressure control in a novel model of spontaneous hypertension, the African Green Monkey.

Similar to humans, animals with chronic elevation of blood pressure have proteinuria compared to normotensive controls. Identification of the mechanisms that contribute to the development and maintenance of this elevated blood pressure and associated protein excretion in this non-human primate may lead to the creation of new, more effective interventions for the treatment of hypertension in human patients. In addition, this work adds to the characterization of hypertension and associated pathologies in the AGM, creating a foundational baseline to address new research questions in this model species.

Perspectives and Significance

With the increasing focus on translational science that can be quickly adapted to clinical practice, it is critically important that basic science researchers utilize appropriate model organisms to address their research questions. The African Green Monkey represents a novel large animal model organism highly relevant to the study of hypertension and cardiovascular disease with pathological similarities to patient populations and the presence of hypertension in lieu of specialized breeding paradigms or experimental interventions. With this unique model species, researchers can address the complex interactions between disease pathologies, genetic factors, social dynamics, and environmental contributions to the development and maintenance of hypertension, which we hope will provide new targets and more effective therapies for the treatment of patients, particularly those individuals that are resistant to current antihypertensive interventions.

3.5 Acknowledgements

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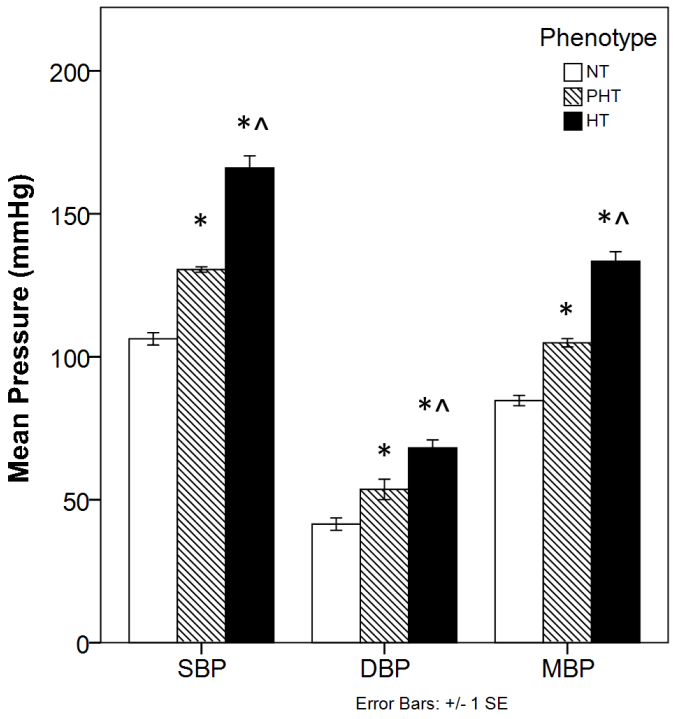

Figure 3.1: Average systolic (SBP), diastolic (DBP), and mean blood pressure (MBP) of the 119 adult African Green Monkeys in the metabolic cohort.

SBP, DBP, and MBP are significantly different in each blood pressure phenotype group, ($p<0.05$, ANOVA with Tukey's post hoc comparison). * denotes $p>0.05$ compared to normotensive; ^ denotes $p<0.05$ compared to pre-hypertensive.

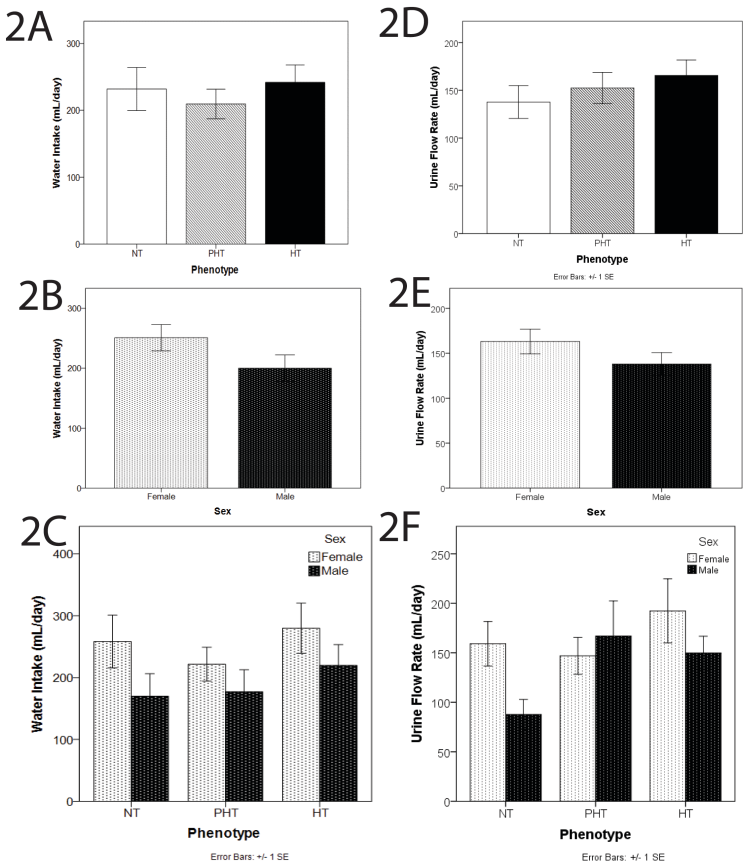

Figure 3.2: Average daily water intake and urine flow rate are similar in the metabolic cohort.

Average values obtained during the 3 day metabolic pen experiments are shown by blood pressure phenotype (2A & 2D), sex (2B & 2E) and sex/phenotype groups (2C & 2F). There are no significant differences in water balance in any of the comparisons ($p>0.05$, ANOVA with Tukey's post hoc comparisons).

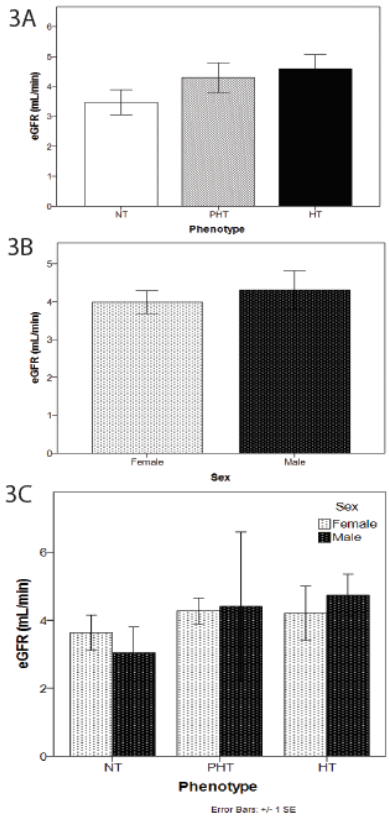

Figure 3.3: eGFR is similar between all groups in the metabolic cohort.

Estimated glomerular filtration rate (eGFR; creatinine clearance) was similar between all groups, including blood pressure phenotype (3A), sex (3B), and sex/phenotype groups (3C; $p>0.05$, ANOVA with Tukey's post hoc comparison).

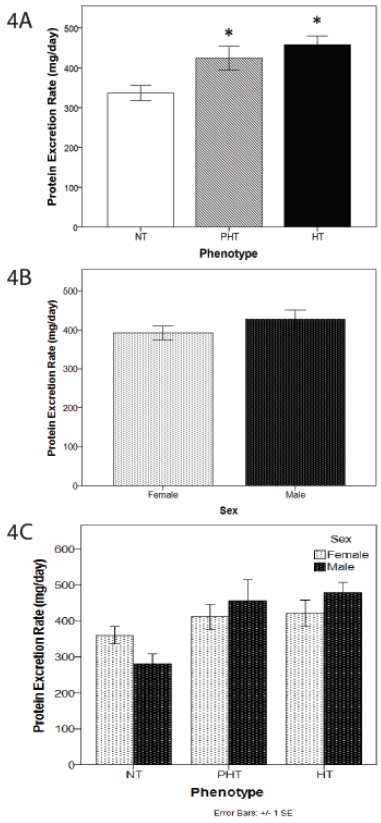

Figure 3.4: Hypertensive and pre-hypertensive animals have elevated urinary protein excretion

Hypertensive and pre-hypertensive animals have increased urinary protein excretion rates compared to normotensive animals ($p<0.05$, ANOVA with Tukey's post hoc comparison) (4A). Urinary protein excretion is similar between the sexes (4B) and sex/phenotype groups (4C). *denotes $p<0.05$ compared to normotensive group

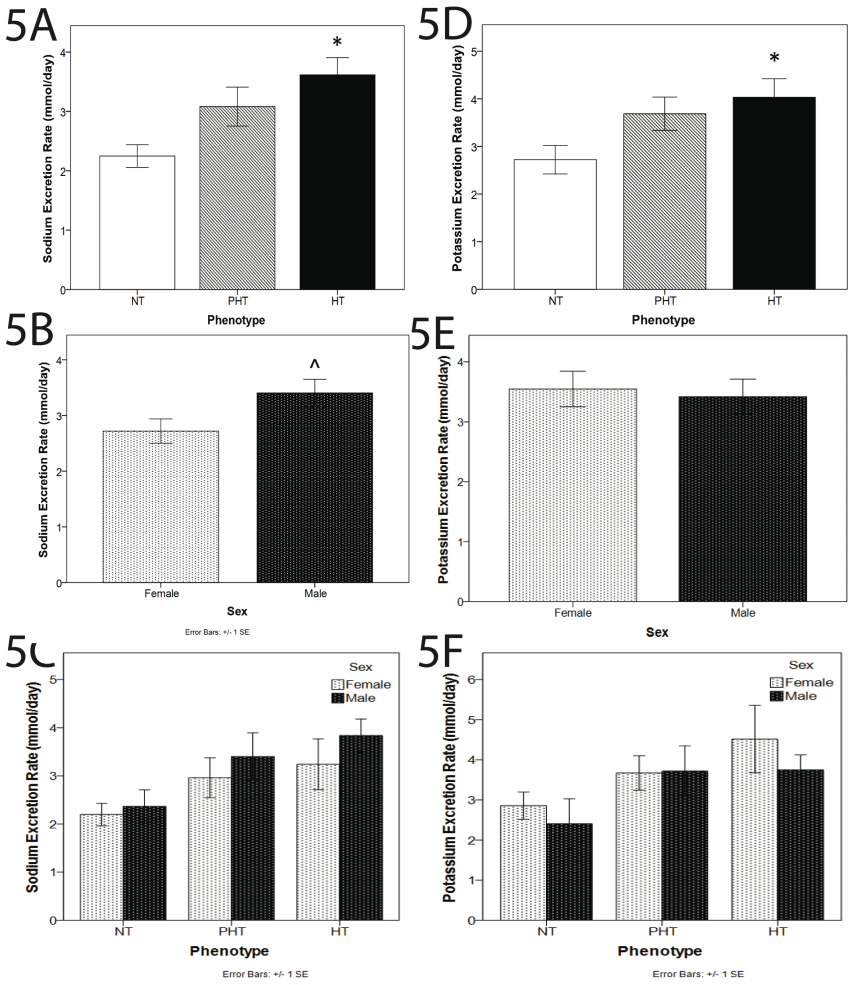

Figure 3.5: Hypertensive animals have elevated urinary sodium and potassium excretion.

Urinary sodium (5A) and potassium (5D) excretion are increased in hypertensive animals compared to normotensive animals ($p<0.05$, ANOVA with Tukey's post hoc comparison). On average, males excrete more sodium than females (5B; $p<0.05$, Student's t-test), but no other differences exist between the sexes or sex/phenotype groups in ion excretion. * denotes $p<0.05$ compared to normotensive group. ^ denotes $p<0.05$ compared to female group.

CHAPTER FOUR

SYMPATHETIC CONTRIBUTION TO SPONTANEOUS HYPERTENSION IN THE AFRICAN GREEN MONKEY

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4.1 Introduction

It is well established that the sympathetic nervous system (SNS) contributes to the development and maintenance of hypertension and cardiovascular disease. As one arm of the autonomic nervous system, the SNS regulates a myriad of physiological processes known as the "flight or fight" response, including, but not limited to, blood vessel constriction, acceleration of heart rate, pupil dilation, inhibition of digestion, and secretion of epinephrine and norepinephrine (NE) from the adrenal cortex. Evolutionarily, sympathetic outflow allows an organism to quickly respond to threats and ensure survival. However, sympathetic nerve activity (SNA) is not limited to responding to external threats and stimuli, it's also an important regulator of arterial blood pressure and extracellular fluid volumes. Sympathetic outflow can quickly alter the level of vasoconstriction in the arterial vessels, which in turn changes blood flow to organs. However, elevation of sympathetic outflow can have pathophysiological effects and combined with a sedentary lifestyle, stress, obesity, and Western diet can contribute to the development of many diseases in modern man.

Sympathetic outflow originates in the central nervous system and acts in the periphery through the release of catecholamines on adrenergic receptors (ADR). ADRs all belong to the superfamily of G-coupled protein receptors, all of which contain seven transmembrane domains with alternating intracellular and extracellular loops (Storsberg 1993). ADRs are categorized into three major groups: α1 ADRs, which includes the subtypes: $\alpha 1_A$, $\alpha 1_B$, and $\alpha 1_D$; α2 ADRs, which has three subtypes: $\alpha 2_A$, $\alpha 2_B$, and $\alpha 2_C$; and the β ADR group, which includes the β_1, β_2, and β_3 subtypes. Though these receptors all respond to the same endogenous catecholamines, they activate different intracellular

signaling cascades, suggesting that the response of an organ to SNA is based on the quantity and types of ADRs present. In the heart, β ADRs are the primary ADR group expressed, with β1 ADRs outnumbering β2 ADRs in an approximate 3:1 ratio, respectively (Iaccarino, Barbato et al. 2001). Activation of cardiac ADRs can lead to changes in contractile rate and force, blood pressure, and intracellular calcium signaling, and has been shown to shown to induce cardiac hypertrophy in transgenic mice overexpressing β_1 ADRs in the myocardium (Barki-Harrington, Perrio et al. 2004).

While cardiac hypertrophy occurs in part due to the increased afterload that the heart muscle must contract against, genetic variation accounts for a significant portion of the hypertrophic variability observed in both patients (Verhaaren, Schieken et al. 1991, Fu, Wang et al. 2008) and animal models of hypertension (Tanase, Yamori et al. 1982). Schlaich and colleagues found that hypertensive patients with left ventricular hypertrophy (LVH) had excess cardiac NE spillover as a direct result of increased cardiac sympathetic stimulation. Interestingly, nonhypertrophic hypertensive patients did not display cardiac NE spillover but had similar amounts of renal NE spillover and plasma NE compared to the LVH hypertensive patients (Schlaich, Kaye et al. 2003). This data suggests that cardiac NE spillover, and the subsequent adrenergic activation, is necessary for the development of LVH in hypertensive patients. In addition, both α- and β- adrenergic receptor blockade has been shown to decrease cardiac hypertrophy, confirming that SNA is crucial to the maintenance of LVH (Rizzi, Guimaraes et al. 2014).

Many lines of evidence have shown that renal sympathetic nerve activity is critical to the development of primary hypertension. The kidney is highly innervated and the majority of these nerves are adrenergic. α1 ADRs are found in the renal vasculature,

nephrons, and proximal tubules. The distribution of the α2 ADRs is similar to the α1 ADRs, but α2 ADRs are additionally located in the collecting ducts. Renal β1 ADRs are located in the juxtaglomerular cells, distal tubules, and collecting ducts, while β2 ADRs can be found in the proximal tubules, distal tubules, and collecting ducts. Urinary NE and sympathetic nerve activation is increased in human hypertensive patients as well as the spontaneously hypertensive rat (Esler and Kaye 2000, Pinto, Amaral et al. 2011). Renal denervation prevents or delays the onset of hypertension in the multiple animal models of hypertension (DiBona and Esler 2010) and has been shown to be effective in the treatment of resistant hypertensive patients in some (Esler, Bohm et al. 2014), but not all human trials, as reported in the controversial results of SYMPLICITY HTN-3 trial (Bakris, Townsend et al. 2014). In addition, renal SNA has been shown to increase renin secretion rate, promote sodium retention in the tubules, and decrease renal blood flow through vasoconstriction of the renal vessels. Together, these data paint a convincing picture of the importance of SNA and the renal ADRs in the development and maintenance of hypertension.

The African green monkey (AGM) is a novel large animal model of spontaneous hypertension that displays renal vascular and glomerular pathologies in conjunction with elevated heart rate (Rhoads, Goleva et al. 2017). This work tests the hypothesis that sympathetic outflow contributes to hypertension in the AGM by measuring indirect indices of sympathetic activation, such as cardiac hypertrophy and gene expression of ADRs in tissues critical to the regulation of blood pressure in conjunction with elevated systolic blood pressures in adult male AGMs.

4.2 Methods

4.2.1 Blood pressure measurement

Systolic (SBP) and diastolic (DBP) blood pressures were measured and phenotype categories assigned as previously described (Rhoads et al. 2017). Briefly, animals were lightly sedated and blood pressures measured using a forearm cuff and Doppler stethoscope. Animals were categorized as normotensive (NT; SBP > 120 mmHg), borderline- or pre-hypertensive (BHT; SBP between 120 and 140 mmHg) or hypertensive (HT; SBP <140 mmHg). Heart rate was determined by counting pulsatile beats for 30 seconds while animals were sedated. Mean blood pressure (MBP) was calculated using the equation $MBP = \frac{SBP+(2*DBP)}{3}$

4.2.2 Tissue collection, shipment, and storage

After blood pressure measurement, selected animals (n=18) were fully anesthetized with supplemental ketamine (25 mg/kg i.m.) and xlyazine (100 mg/kg i.m.), then euthanized with a sodium pentobarbital overdose (150 mg/kg, i.v.; Buthenasia). Tissues were perfused, collected, sliced into sections, and sections alternatively frozen or fixed. For fixation, tissues were sliced into sections 3 cm in length and 3-5 mm thick. Sections were fixed using 5% paraformaldehyde (PFA) for 72 hours with the PFA changed at 24 hour intervals. Fixed tissues were placed in phosphate buffered saline for transport and shipping and then placed in 70% ethanol for long term storage. Frozen

tissues were placed in ultra-cold isopentane (-20°C) on dry ice for flash freezing. Samples were shipped on dry ice and placed in -20°C freezer for storage.

4.2.3 Histological sample analysis

Histological samples (approximate 4 mm thickness) from NT (n=6) and HT (n=6) animals were dissected from fixed tissue sections, as previously described in detail (Rhoads, Goleva et al. 2017). Samples were paraffin embedded and sectioned using a microtome at 5 μm thickness. After drying, slides were stained using hemotoxylin and eosin. Slide images were digitally captured using an Olympus IX70 inverted microscope and analyzed in ImageJ (Schindelin, Rueden et al. 2015). A minimum of 5 images of each ventricular section from each animal were obtained and analyzed. The cross-sectional area of all cells contained in the viewing field were measured and averaged for each animal. Analysis was performed in a blinded fashion.

4.2.4 Quantitative real-time polymerase chain reaction

Frozen left ventricular tissue sections (n=7) were dissected into endocardium, myocardium, and epicardium. Frozen renal sections (n=18) were dissected into cortical, medullary (outer and inner), and papillary regions. RNA was extracted from the myocardium, renal cortex, renal outer medulla, and renal inner medulla using the Direct-zol RNA kit (Zymo Research, Irvine CA), following the manufacturer's recommended

instructions. After extraction, cDNA was created using qScript cDNA SuperMix (Quantabio, Beverly MA) and qPCR performed using SYBR Green (Invitrogen, Carlsbad CA) in a StepOnePlus Real Time PCR System (ThermoFisher Scientific, Waltham MA). Forward and reverse primer sequences can be found in Table 4.1. RPS13A was used as the housekeeping gene in the renal cortex and myocardium, while RPS32 was used as the housekeeping gene in the renal medulla.

4.2.5 Statistical analysis

qPCR RQs were calculated using the comparative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) and normalized to the normotensive cohort. Data is presented as mean ± standard error. Groups were compared using Mann-Whitney U test, or ANOVA with Tukey's post hoc comparison with $p<0.05$ as the level of probability.

4.3 Results

Blood pressure was measured in 18 NT and 18 HT adult male AGMs selected for the qPCR and histology cohort. NT animals averaged 96.6±3.2 mmHg, 54.6±3.9 mmHg, and 68.6±3.7 mmHg for SBP, DBP, and MBP, respectively. HT animals averaged 169.2±6.9 mmHg, 94.5±5.1 mmHg, and 119.4±3.0 mmHg for SBP, DBP, and MBP ($p<0.05$ compared to normotensive, Figure 4.1).

Cardiomyocyte cross-sectional area (CSA) was assessed in the left and right ventricle of 7 normotensive and 7 hypertensive adult male AGMs. In the right ventricle, NT animals had an average CSA of 932.9±179.7 μm^2 while HT animals had an average CSA of 1133.5±298 μm^2 ($p>0.05$). In the left ventricle, NT animals averaged a CSA of 791±180.6 μm^2 and HT animals displayed a significant elevation of cardiomyocyte area with an average CSA of 1658.4±210.3 μm^2 ($p<0.05$, Figure 4.2).

Left ventricular myocardial gene expression of ADRα1A (NT 1±0.2 vs. HT 1.2±0.3), ADRα1D (NT 1±0.2 vs HT 1.2±0.3), ADRα2A (NT 1±0.4 vs HT 0.8±0.5), ADRα2C (NT 1±0.3 vs HT 0.4±0.1) and ADRβ2 (NT 1±0.2 vs HT 1.8±0.4) is similar between NT and HT groups. ADRβ1 left ventricular gene expression is elevated in HT animals compared to NT animals (NT: 1±0.2 vs HT 1.9±0.4, $p<0.05$, Figure 4.3A & 4.3B)

Renal cortical gene expression of ADRα1A (NT 1±0.1 vs. HT 1.1±0.1), ADRα1D (NT 1±0.1 vs HT 1±0.2), ADRα2A (NT 1±0.1 vs HT 0.8±0.1), ADRα2C (NT 1±0.2 vs HT 1±0.2), ADRβ1 (NT 1±0.1 vs HT 0.9±0.1) and ADRβ2 (NT 1±0.1 vs HT 0.9±0.1) is similar between NT and HT groups (Figure 4.4A & 4.4B).

Renal outer medullary gene expression of ADRα1A (NT 1±0.4 vs. HT 2.3±0.5), ADRα1D (NT 1±0.3 vs HT 2.2±0.3), ADRα2A (NT 1±0.2 vs HT 1.9±0.2), ADRα2C (NT 1±0.2 vs HT 1.6±0.1), ADRβ1 (NT 1±0.2 vs HT 1.9±0.2) and ADRβ2 (NT 1±0.2 vs HT 1.9±0.2) is elevated in HT AGMs compared to NT animals ($p<0.05$, in all NT vs. HT comparisons). (Figure 4.5A & 4.5B).

Renal inner medullary gene expression of ADRα1A (NT 1±0.3 vs. HT 1.5±0.4), ADRα1D (NT 1±0.3 vs HT 1.7±0.4), ADRα2A (NT 1±0.2 vs HT 1.4±0.3), ADRα2C (NT 1±0.2 vs HT 1.3±0.3), ADRβ1 (NT 1±0.3 vs HT 1.4±0.3) is similar between NT and HT groups. ADRβ2 gene expression is elevated in the renal inner medulla of HT animals compared to NT animals (NT 1±0.3 vs HT 1.6±0.1, $p<0.05$, Figure 4.6A & 4.6B)

4.4 Discussion

AGMs with elevated blood pressures display elevated heart rates (Rhoads, Goleva et al. 2017), LVH, and select upregulation of alpha- and beta- ADR subtype mRNA in the left ventricular myocardium, renal outer medulla, and the renal inner medulla. Together these data suggest that activation of the SNS is a crucial contributor to the development and maintenance of hypertension in this novel large animal model.

Elevation of heart rate is a risk factor for the development of heart failure, atherosclerosis, and myocardial infarction; it is also associated with increased cardiovascular mortality and excess sympathetic outflow (Palatini and Julius 1996, Custodis, Reil et al. 2013). Analysis of the Framingham Heart Study identified higher heart rates as associated with adverse prognoses, increased risk of cardiovascular events, and higher all-cause mortality (Gillman, Kannel et al. 1993, Ho, Larson et al. 2014). Aronow *et al* found that an increase in heart rate of 5 beats/min created 1.14x higher probability of new coronary events in men and women older than 60 years of age (Aronow, Ahn et al. 1996), while other studies have shown that in adolescents, elevated resting heart rate is associated with cardiovascular risk factors (Farah, Christofaro et al. 2015, Sarganas, Schaffrath Rosario et al. 2017). These studies suggest that rapid heart rate is an independent risk factor for the development of cardiovascular mortality and increase the translational value of a non-human primate model that presents increased heart rate as part of the pathology of spontaneous hypertension. Longitudinal studies that determine the predictive value of elevated heart rates in spontaneously HT AGMs would provide a unique pre-clinical model for scientific research.

β1 adrenergic receptor gene expression is also upregulated in the left ventricular myocardium of HT AGMs, similar to the spontaneously hypertensive rat (SHR), a well-described model of hypertension (Iemitsu, Miyauchi et al. 2001). Ietmitsu et al also found that mRNA expression of the β1- adrenergic receptor kinase, which mediates downstream signaling and adrenoreceptor desensitization, was also upregulated in the SHR heart, suggesting that the signal transduction of this G-protein coupled receptor may be impaired in disease states in hypertrophied cardiac tissue. Furthermore, the impairment and uncoupling of β ADRs in chronic heart failure is a well-described phenomenon. Future studies in the HT AGMs should focus on the gene transcription of downstream signaling events in the myocardium to determine if β1- adrenergic receptor kinase signaling is also impaired in this model organism. In addition, polymorphisms in the β1-adrenergic receptor have been linked to left ventricular hypertrophy in patients with essential hypertension(Fu, Wang et al. 2008). The contribution of genetic polymorphisms in the ADRs in the development of hypertension in the AGM is currently unknown.

The contribution of renal sympathetic nerve activation to hypertension is well documented. Sympathetic nerves innervate the renal circulation, traveling through the cortex and outer medulla while decreasing in number toward the inner medulla (McKenna and Angelakos 1968), and regulate regional renal blood flow. In particular, studies from Dr. Allen W. Cowley Jr.'s group have shown that the renal outer medulla responds to changes in perfusion pressure in a high-salt environment. In Dahl SS rats on a 4% sodium diet, Mori *et al* used a servo-control system to maintain renal perfusion pressure in the left normotensive kidney while allowing the right kidney to be exposed to hypertensive perfusion pressures. Comparison of microarray data between the two kidneys identified

distinct differences in outer medullary gene expression after both 14 and 7 days respectively (Mori, Polichnowski et al. 2008, Evans, Dayton et al. 2018), suggesting that the renal outer medulla is sensitized to changes in perfusion pressure and that molecular pathways mediate this response. Thus, the renal outer medulla has been identified as a pathological 'first responder' region in response to changes in renal blood flow.

Gene expression of α- and β -ADR subtypes are up-regulated in the renal outer medulla of the hypertensive AGM, although whether this observation is causal or a consequence of the elevated blood pressure is still unknown. In regards to the functional consequences of this observation, two possible explanations must be explored in further investigations regarding the sympathetic contribution to hypertension in this model. First, it's possible that the outer medulla of the HT AGM is hyper-sensitized to sympathetic outflow due to an increased ADR density. Experiments that assess the effects of sympathetic activation on regional blood flow in the kidney would provide important information on the role of the ADRs in the HT AGM. However, a limitation to the current study is the well-documented fact that mRNA levels are not necessarily equal to protein levels, due to factors such as mRNA decay, microRNA interactions, post-translational modifications, and protein degradation. In addition, the documented non-specificity of adrenergic receptor antibodies (Hamdani and van der Velden 2009, Jensen, Swigart et al. 2009) requires that ADRs be quantified using other approaches, such as radio-ligand competitive binding assays. An additional limitation to this study is the fact that mRNA was isolated from tissue homogenate and thus, multiple cell types. Future studies should assess receptor density in in isolated or micro-dissected renal tissues of NT and HT AGMs to confirm if protein levels of ADRs are also elevated. Since reports of tissue adrenergic

receptor number and density in both patients and hypertensive animal models are inconsistent (Michel, Brodde et al. 1990), so addition of such data in a non-human primate model would increase the model's translational capacity in the development of novel therapies and interventions to treat hypertension.

Secondly, it is also possible that hypertension in the HT AGM has led to higher levels of ADR desensitization and thus increased transcription of these receptors as a compensatory response. In human studies, β – ADR sensitivity, as measured by the cardiac response to isoproterenol, is decreased in older HT patients compared to age-matched NT controls (Bertel, Buhler et al. 1980). Aging itself is a major risk factor for hypertension, and both human and animal studies have identified pathological changes due to aging in renal sodium handling and the response to renal sympathetic activation (Frame and Wainford 2018). Furthermore, GPCR desensitization is a well-described occurrence and many studies have identified impaired function of the β ADRs in hypertensive patient cohorts, suggesting that the reduced function of the vasodilatory β ADRs promotes hypertension via increased vasoconstriction (Ferguson and Feldman 2014). Experiments that can elucidate the functional contribution of renal ADRs, particularly in the context of aging, are needed in this model organism.

While our data suggests that the renal outer medulla in the HT AGM may be sensitized to SNA in the context of chronically elevated blood pressure, *in vivo* experiments that measure renal blood flow, oxidative stress, and sympathetic activation are needed to provide more insight to the contribution of the SNS to hypertension in this model. Since the blood pressure in the AGM has been shown to be sensitive to dietary sodium (Srinivasan, Dalferes et al. 1984), like the Dahl SS rat, elucidating the roles of the ADRs,

SNA, and blood pressure in the presence and absence of high sodium could also play an important role in understanding the etiology of hypertension.

In conclusion, the HT AGM is a highly translational large animal model of spontaneous hypertension that may provide unique clues to the etiology of essential hypertension in humans. In light of the American Heart Association's new recommendations that will drastically increase the number of patients being treated for hypertension, novel animal models represent an opportunity to identify new targets and expand treatment options. Our work suggests that spontaneous hypertension in the AGM may be driven by excess SNA and altered ADR function and provides evidence to support exploration of this hypothesis. Sympathetic tone, response of blood pressure and oxidative stress to adrenergic blockade, and the effects of renal denervation should be studied in this translational non-human primate model of spontaneous hypertension to further test this hypothesis.

4.5 Acknowledgements

The authors would like to acknowledge the contribution of Slavina B. Goleva, who was instrumental to the development and validation of the qPCR data in this manuscript.

Table 4.1. Forward and Reverse Primer Sequences

Gene	Primer	Sequence
ADR α1A	Forward	5’-TCA TGT ACT GCC GCG TCT AC-3’
	Reverse	5’-CGA GAA GTG CGT CTT GGT CT-3’
ADR α1D	Forward	5’-TGA GAA ACA CTG CCC CAT CC-3’
	Reverse	5’-ACT GGG TTA GCC GAC CAA AG-3’
ADR α2A	Forward	5’-AGA GGA TTA CCT AGC CCT GG-3’
	Reverse	5’-GCT GCC CTG AGT GGG TTT TA-3’
ADR α2C	Forward	5’-TAC TGG TAC TTC GGG CAG GT-3’
	Reverse	5’-CGA TCG ACG AGG TGC AAA AC-3’
ADR β1	Forward	5’-CGC CCC GGA ACG ACT T-3’
	Reverse	5’-TCA GAC GAG GAT TGT GGG CT-3’
ADR β2	Forward	5’-CCC TTA TCT ACT GCC GGA GC-3’
	Reverse	5’-TGT GTT GCC ATT GCT GTT GC-3’
RPS 13A	Forward	5’-CCT GGA GGA GAA GAG GAA AGA GA-3’
	Reverse	5’-TTG AGG ACC TCT GTG TAT TTG TCA A-3’
RPS 32	Forward	5’-CAA CAT TGG TTA TGG AAG CAA CA-3’
	Reverse	5’-TGA CGT TGT GGA CCA GGA ACT-3’

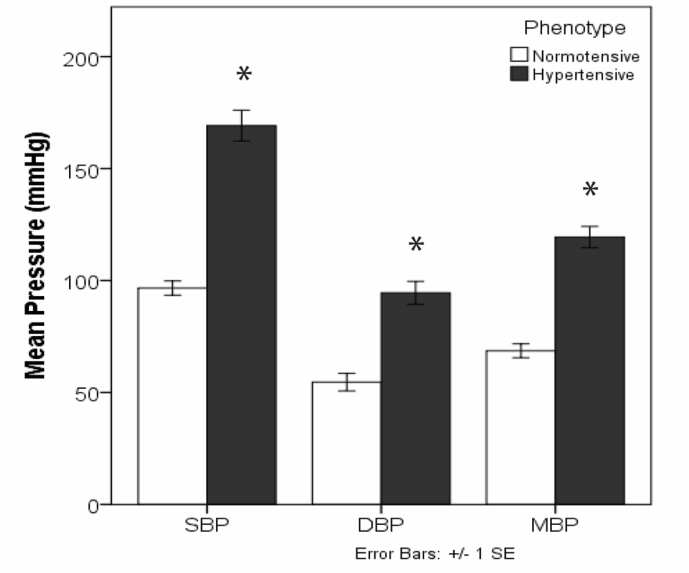

Figure 4.1: Average blood pressures of animals used in the qPCR cohort.

Systolic (SBP), diastolic (DBP), and mean blood pressures (MBP) are elevated in the hypertensive (n=18) animals in the qPCR and histology cohort compared to the normotensive animals. * denotes $p<0.05$ compared to normotensive group using ANOVA with Tukey's post hoc analysis.

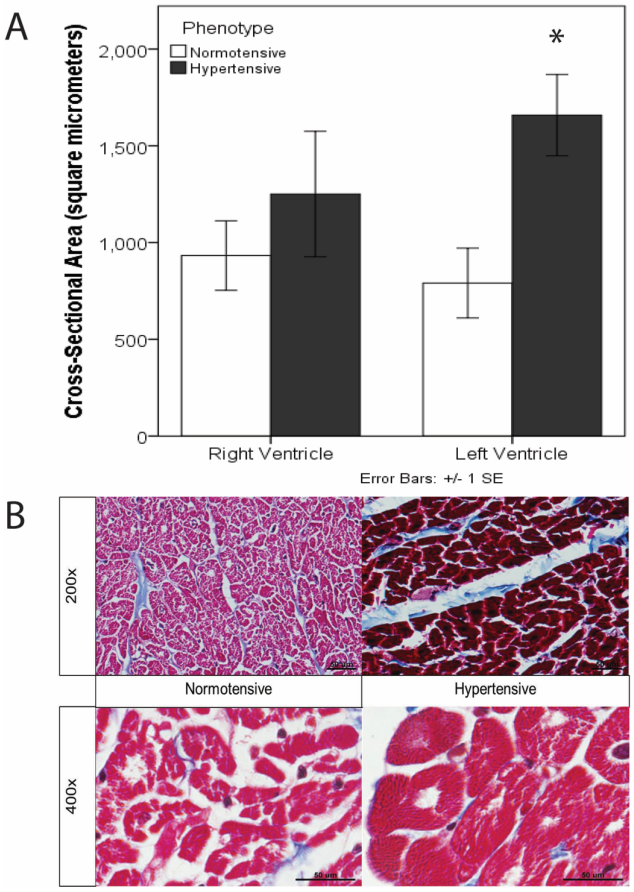

Figure 4.2: Hypertensive animals display left ventricular hypertrophy compared to normotensive animals.

Figure 4.2A: Cardiomyocyte cross-sectional area is elevated in the left ventricle of hypertensive African green monkeys. Cross sectional area is similar in the right ventricle between normotensive and hypertensive groups. Figure 4.2B shows representative cardiac histological images from each group. * denotes $p<0.05$ compared to normotensive group using ANOVA with Tukey's post hoc analysis.

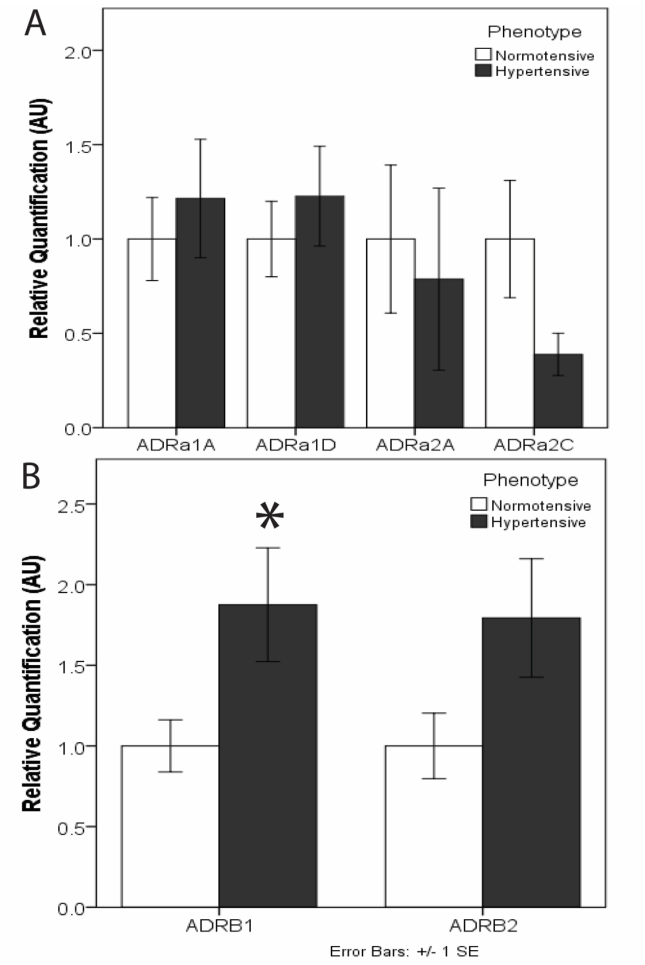

Figure 4.3: β1 adrenoreceptor mRNA expression is elevated in the left ventricular myocardial tissue of hypertensive African green monkeys.

ADRα1A, ADRα1D, ADRα2A, ADRα2C (A), and ADRβ2 (B) gene expression was similar in normotensive and hypertensive left ventricular myocardium. * denotes $p<0.05$ compared to normotensive animals using Mann-Whitney U test.

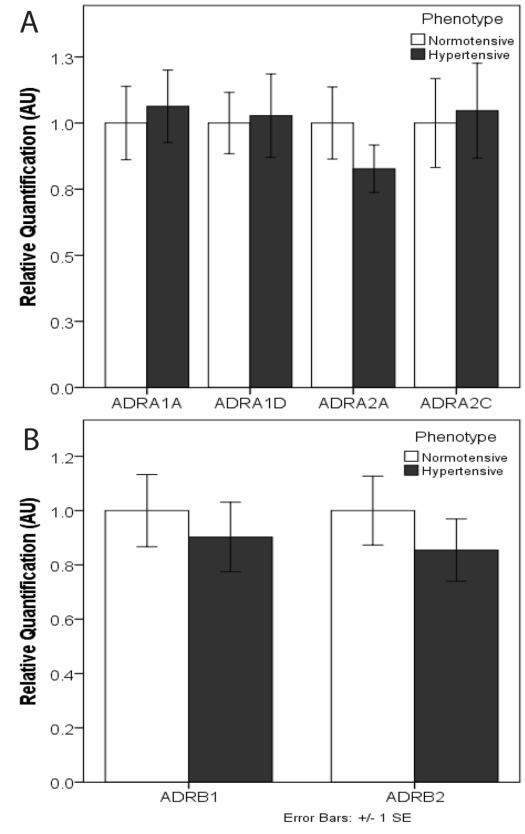

Figure 4.4: Renal cortical adrenergic receptor gene expression in HT and NT AGMs.

Renal cortical gene expression of ADRα1A, ADRα1D, ADRα2A, ADRα2C (A), ADRβ1 and ADRβ2 (B) is similar in normotensive and hypertensive African green monkeys.

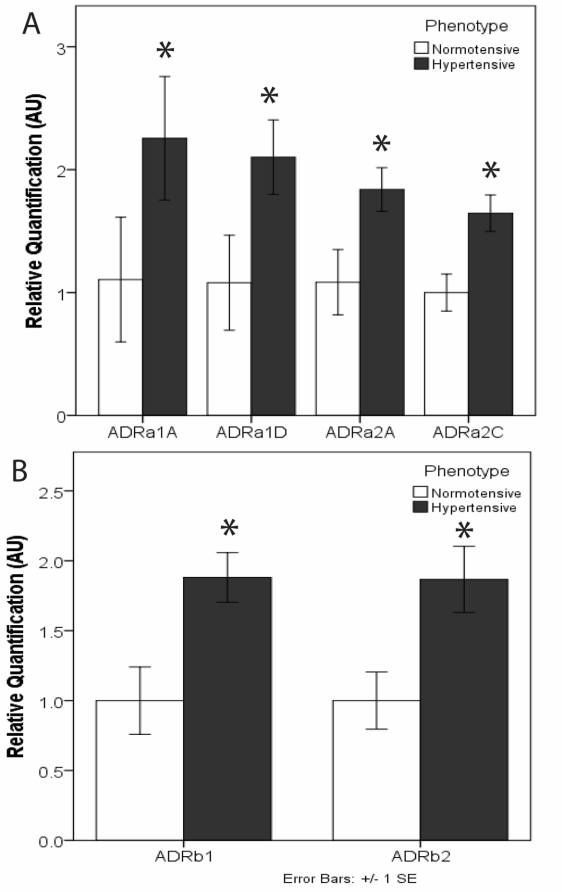

Figure 4.5: Adrenergic receptor gene expression is elevated in the renal outer medulla of HT AGMs.

Renal outer medullary gene expression of all assessed α- and β-adrenoreceptor subtypes is elevated in hypertensive African green monkeys compared to normotensive controls. * denotes $p<0.05$ compared to normotensive animals using Mann-Whitney U test.

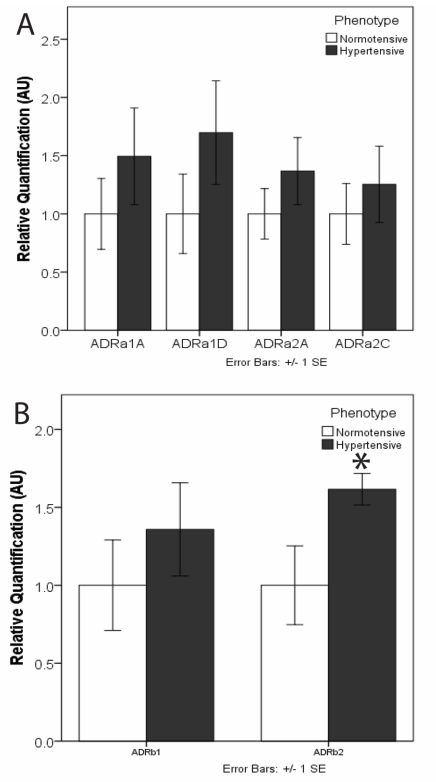

Figure 4.6: Renal inner medullary ADRβ2 expression is elevated in HT AGMs.

Renal inner medullary gene expression of ADRα1A, ADRα1D, ADRα2A, ADRα2C (A), and ADRβ1 (B) is similar between normotensive and hypertensive African green monkeys. ADRβ2 gene expression is elevated in the renal inner medulla of hypertensive African green monkeys compared to normotensive controls. * denotes $p<0.05$ compared to normotensive animals using Mann-Whitney U test.

CHAPTER FIVE

PREVALENCE OF HYPERTENSION IS AGE-RELATED IN THE AFRICAN GREEN MONKEY

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Keywords: Age; Hypertension; African Green Monkey

5.1 Introduction

Aging has long been identified as a risk factor for the development of hypertension, cardiovascular disease, stroke, chronic kidney disease, and other cardiovascular pathologies. Globally, 31.1% (95% confidence interval, 30.0 -32.2%) of the adult population has hypertension, as defined by systolic blood pressure > 140mmHg, diastolic blood pressure >90 mmHg, or use of antihypertensive medications (Mills, Bundy et al. 2016). In the United States, the prevalence of hypertension increases from 7.3% in adults aged 18-39 years to 64.9% in adults over 60 years old (Yoon, Frayer et al. 2015). Currently, the annual direct and indirect healthcare costs of hypertension are estimated to be $53.2 billion and are projected to reach $220.9 billion by 2035 (Benjamin, Virani et al. 2018). As larger numbers of the US population age, increased healthcare burdens will need to be met with new techniques and approaches to treat disease pathologies that are exacerbated by aging (Knickman and Snell 2002). To this end, the authors believe that basic science researchers need to be highly cognizant of age as a key biological variable in the study of the etiology of hypertension. This work seeks to elucidate the effect of age on the development of hypertension and renal dysfunction in the spontaneously hypertensive AGM.

Aging has multiple effects on organ systems, organ function, and the response of individual structures and cells within each organ. In the kidney, renal cortical mass and glomerular filtration rate (GFR) decrease with age in healthy human volunteers (Karam and Tuazon 2013). Classical inulin clearance studies found that GFR decreases 8 mL/min/1.73 m^2 each decade after 30 years of age, though there are substantial individual variations in the age-related declines of GFR (Weinstein and Anderson 2010). Autopsy

analysis of adult kidneys (all presumably healthy without diagnosed renal disease) indicates that glomerular number decreases with age, likely due to increased numbers of sclerosed glomeruli (Hoy, Douglas-Denton et al. 2003). While glomerular number decreases with age, unaffected nephrons may undergo hypertrophy as a compensatory mechanism with increased tuft cross-sectional area, thickening of the basement membrane, and mesangial cell expansion (Denic, Glassock et al. 2016). Changes in renal hemodynamics, such as reductions in renal blood flow and GFR, have been observed in healthy, aged subjects. One study found that in young healthy subjects (ages 19-32), maximal renal vasodilation, as induced by concurrent infusion of amino acids and dopamine, results in a significant increase in both renal blood flow and GFR, known as the renal hemodynamic and functional reserves, respectively. However, in healthy, older subjects (ages 65-76), renal hemodynamic and functional reserves are diminished in comparison to the younger age group (Fuiano, Sund et al. 2001).

Changes in urinary protein excretion and renal electrolyte handling are also associated with increased age (Frame and Wainford 2018). Proteinuria, a clinical marker of renal damage, is associated with increased rates of all-cause mortality, cardiovascular disease, and congestive heart failure in the United States and other developed nations (Muntner, He et al. 2002, Irie, Iso et al. 2006). The activity of Na^+-K^+-ATPase, a key driver of tubular sodium transport, is differentially distributed throughout the nephron during aging in Sabra rats. Na^+-K^+-ATPase activity increases in the proximal tubule and cortical collecting duct while decreasing in the medullary thick ascending limb and distal convoluted tubule as animals age (Scherzer, Gal-Moscovici et al. 2015).

Aging is associated with the impairment of vascular endothelial function, as measured by the vasodilatory response to acetylcholine, in both normotensive and essential hypertensive patients (Taddei, Virdis et al. 1995). This study found that normal aging without the presence of cardiovascular pathologies was associated with alterations in the response to cholinergic stimulation. Tanaka *et al* found that central arterial compliance, a measure of the cardiothoracic circulation's ability to expand and recoil with cardiac pulsation, decreased with age even in healthy, physically active men (Tanaka, Dinenno et al. 2000). These key structural changes that occur with healthy aging greatly increase the possibility of renal damage with aging that is compounded with elevated blood pressure.

This work seeks to determine the effect of age on the prevalence of hypertension in the African green monkey (AGM), a novel non-human primate model of spontaneous hypertension. Wild-caught animals residing in a research colony underwent comprehensive physical examinations and behavioral observations to assign highly confident age estimates. Next, 24 hour urine collection studies were conducted to assess glomerular filtration rates in young and aged AGMs with both normal and elevated blood pressures. Finally, urinary protein and electrolyte excretion rates were compared to determine effects of age and blood pressure on renal function.

5.2 Methods

5.2.1 Animal care and housing

Animal protocols strictly adhere to both the NIH *Guide on the Care and Use of Laboratory Animals*(Animals 2011) and "Guiding Principles on the Care and Use of Experimental Animals" published by the American Physiological Society. Protocols were reviewed and approved by the Institutional Animals Care and Use Committee (IACUC) of the Biomedical Sciences Research Group and SKN Primates in Basseterre, St. Kitts and Nevis, West Indies. Animals were housed in the long term in troop enclosures in a large outdoor facility in St. Kitts, West Indies. Animals were fed nonhuman primate chow (Teklad 8773, Harlan) daily and a seasonal combination of fresh fruit and vegetables (bananas, mangos, sweet potatoes, carrots and squash) on alternating days. Water was provided *ad libitum.* Quarantine and husbandry protocols were maintained as previously described (Rhoads, Goleva et al. 2017).

5.2.2 Blood pressure measurements

Animals were sedated (ketamine, 15mg/kg i.m.) and blood pressure measured as described previously in detail (Rhoads, Goleva et al. 2017). After sedation, animals were placed in a supine position and a blood pressure cuff placed on the left upper arm. A Doppler stethoscope was used to confirm total occlusion and identify Korotkoff sounds indicative of systolic (SBP) and diastolic blood pressures (DBP). Animals were characterized as normotensive (NT, SBP $\leq$ 120 mmHg), pre-hypertensive (PHT, SBP

between 120 and 140 mmHg), or hypertensive (HT, SBP ≥ 140 mmHg) based on repeated measurements of SBP. Mean blood pressure (MBP) were calculated by the equation: $MBP = \frac{SBP+(DBP*2)}{3}$

5.2.3 Age estimations

Highly confident age estimates (n=304) were determined by animal husbandry staff with over 50 years of combined experience working with AGMs. Estimates took into account animal behavior, weight, incisor length, fur coarseness, crown-rump ratio, tail stiffness, and troop hierarchy positioning to determine a reasonable approximation of the animal's age. Adult animals were binned into one of four age categories: "juvenile" estimated 0-4 years old; "young adult" estimated 5-10 years old; "adult" estimated 11-15 years old; or "mature adult" estimated 16+ years old. Animals whose age could not be confidently estimated were removed from analysis.

5.2.4 Urine collection and sample preparation

Prior to study, animals were moved from group enclosures into singly housed metabolic pens with 1 L water bottles for the duration of the studies (n=112). The dietary regimen remained the same, with animals being fed non-human primate chow (Teklad 8773, Harlan), fresh fruits, and vegetables as described above. Female animals were monitored and examined for pregnancy, and if found to be pregnant, were removed from this cohort. After a minimum 7-day acclimation period, blood pressures were measured

as described above and whole blood collected in Li-heparinized tubes. Urine was then collected for 3 consecutive, 24-hour periods. Water intake and urine volume were recorded daily. Urine samples were centrifuged (1000x*g*) to remove any large particulate contaminants. Samples were aliquoted into 1.5 mL tubes to minimize freeze-thaw cycles during further downstream analysis and stored at -20°C. Juvenile animals were not used in 24-hour urine collection experiments as the effect of sexual maturation on renal function in the AGM is unknown and outside the scope of this work.

5.2.5 Urinary protein measurement

Total protein concentration was measured by Pierce BCA Assay (Catalog # 23225, ThermoFisher Scientific, Waltham MA) per the manufacturer's recommended protocol. Briefly, urine samples were diluted 1:4 and 25 µL of bovine standard albumin or thawed samples were pipetted in a microplate. Working BCA reagent (200 ul) was added to each well and the plate incubated at 37°C for 2 hours. After cooling to room temperature, absorbance was read at 595nm. A linear standard curve was generated ($r^2 \geq 0.98$) and used to determine protein concentration for the unknown samples.

5.2.6 Estimation of glomerular filtration rate

Urinary creatinine was measured by a modified Jaffe reaction(Husdan and Rapoport 1967) or by plasma creatinine assay following the manufacturer's recommended protocol (Item #: 700460, Cayman Chemical, Ann Arbor, MI). Urine

samples were diluted 20x. Briefly, known creatinine standard or thawed, diluted urine samples (50 ul) were combined with 100 µL of alkaline picrate (12.5 mL of 0.13% picric acid and 2.5mL of 1 M NaOH). After 30-minute room temperature incubation, absorbance was read at 490 nm. A linear standard curve was generated ($r^2 \geq 0.98$) and used to determine creatinine concentration for the unknown samples. Estimated glomerular filtration rate (eGFR) was calculated by the equation

$$eGFR = \frac{Urine\ Flow\ Rate\ x\ Urine_{Creatinine}}{Plasma_{Creatinine}}$$

5.2.7 Urinary sodium and potassium concentration

Urinary sodium and potassium concentrations were measured by dual-channel flame photometry (Dual-channel flame photometer #02655-10, Cole-Parmer, Vernon Hills, IL). Thawed urine samples were diluted 20X in lithium diluent and concentrations multiplied by dilution factor, molecular weight, and the appropriate urine flow rate (UFR) to obtain ion excretion rates.

5.2.8 Statistical analysis

Data was analyzed using SPSS 23 (IBM) and groups were compared by two-way ANOVA with Bonferroni adjustment. If no interactions were found between the two independent variables, a one-way ANOVA with Tukey's post hoc comparison was used for comparison. 95% confidence intervals were calculated using the Wilson score method and compared using a Chi square test of independence. Values are presented as mean ± SEM and $p<0.05$ used as the criterion of significance.

5.3 Results

Young adult animals (n=86) display lower systolic (129.1±3.3 mmHg), diastolic (51.4±1.9 mmHg), and mean blood pressures (77.3±2.1 mmHg) compared to both adult (n=21; SBP: 151±7.2 mmHg, DBP: 66.6±4.4 mmHg, MBP: 94.7±4.5 mmHg) and mature adult (n=5; SBP: 164.2±23 mmHg, DBP: 72.8±14.4 mmHg, MBP: 103.3±17.1 mmHg) AGMs ($p<0.05$, ANOVA with Tukey's post hoc comparison, Figure 1). There were no significant interactions between age and phenotype for systolic, diastolic, or mean blood pressures ($p>0.05$, two-way ANOVA).

Adult animals (5.3±0.9 kg) weighed significantly more than young adult animals (4.3±0.9 kg, $p<0.05$ by Tukey's post hoc comparison). Mature adult animals had an average weight of 5.0±1.2 kg and were similar to both young adult and adult groups (Figure 2A). No significant interactions were found for weight between age and phenotype groups (Figure 2B). Values for subgroups can be found in Table 1, while values for phenotypic groups have already been discussed in Chapter 3.

The average prevalence of hypertension in the entire AGM cohort (n= 424) is 37% as previously reported (Rhoads, Goleva et al. 2017). Only animals with known ages or highly confident age estimates were included in the prevalence analysis (n=304). In the juvenile age group (n=15), 20% (95% CI, 7.05 to 45.19%) of AGMs display SBPs greater than 140 mmHg, while 31.34% (25.33 to 38.06%) of the young adult (n=201) animals are considered HT. In the adult (n=60) group, 48.33% (36.18 to 60.69%) of the AGMs are HT and prevalence increases to 60.7% (42.41 to 76.43%) in the mature adult (n=28) group. The prevalence of hypertension is significantly increased in the adult and

mature adult age groups compared to the juvenile and young adult age groups (Figure 3 & Table 2, $p<0.05$).

There were no significant differences in water intake between any age, phenotype, or age/phenotype subgroups (Figure 4A & 4B). Young adult animals (n=86) drank on average 224.8±19.0 mL/day, while adult animals (n=21) drank 223.6±32.5 mL/day. Mature adult animals (n=5) drank 322.7±126.9 mL/day of water. Urine flow rate (UFR) was similar between all age groups, phenotype groups, and subgroups (Figure 4C & 4D). Young adult animals had an average UFR of 152.7±11.7 mL/day. Adult animals had an average UFR of 162.5 mL/day while mature adult AGMs had a mean urine volume of 137.5±37.8 mL/day.

eGFR was similar between all comparisons of age and phenotype (Figure 5A & 5 B). Young adult animals (n=63) had a mean eGFR of 3.9±0.3 mL/min while adult animals (n=17) had an estimated GFR of 4.9±0.7 mL/min. Mature adult animals (n=5) had an average eGFR of 4.4±1.5mL/min.

Young adult animals had an average daily protein excretion rate of 396.5±16.1 mg/day, while adult animals had an average protein excretion rate of 458.2±36.8 mg/day. Mature adult animals excreted 427.1±36.8 mg/day of protein (Figure 6A). There was a statistically significant interaction between phenotype and age on protein excretion rate, $F(4, 101)=3.735$, $p = 0.007$, partial $\eta^2=0.129$. Thus, an analysis of simple main effects was performed for age with a Bonferroni adjustment for multiple comparisons. A significant difference was found between blood pressure phenotypes for young adult animals, $F(2,101)=3.664$, $p=0.029$, partial $\eta^2=0.068$; adult animals, $F(2,101)=4.739$,

p=0.011, partial η^2=0.086; and mature adult animals, $F(2,101)=5.348$, p=0.006, partial η^2=0.096. Within the young adult AGM group, HT animals have significantly elevated protein excretion rates compared to NT animals (NT 346.5±21 mg/day vs. HT 440.7±30.0 mg/day, p=0.037). In the adult group, HT animals have significantly elevated protein excretion rates compared to BHT AGMs (BHT 347.5±52.9 mg/day vs. HT 540.9±44.2 mg/day, p=0.023). In the mature adult group, the BHT animal had elevated protein excretion (810.9 mg/day) compared to the NT animal (176.5 mg/day, p=0.006) and the HT group (382.6±39.2 mg/day, p=0.031, Figure 6B).

Sodium excretion rates (Figure 7A & 7B) were significantly elevated in adult animals (3.9±0.4 mmol/day) compared to young adult animals (2.7±0.2 mmol/day, p=0.006). Daily sodium excretion in mature adult animals was not significantly different compared to either age group (2.2±0.5 mmol/day). Potassium excretion rates were similar in all age, phenotype, and subgroup comparisons. Young adult animals excreted 3.3±0.3 mmol/day of potassium, while adult AGMs excreted 3.8±0.4 mmol/day of potassium. Mature adult AGMs had an average potassium excretion rate of 3.6±1.1 mmol/day.

5.4 Discussion

A major finding in this work is that the prevalence of hypertension increases with age in the African green monkey. 20% of juvenile animals display SBPs >140mmHg and the prevalence of hypertension increases to 31.3%, 48.3%, and 60.7% in young adult, adult, and mature adult animals, respectively (Table 2). The adult and mature adult groups have a significantly elevated prevalence of hypertension compared to the younger two groups, demonstrating that age may contribute to the development of hypertension in this non-human primate model. In addition, average SBP, DBP, and MBPs are higher in the adult and mature adult age groups compared to the young adult group, suggesting that blood pressure increases with age in this model species. Data from both human studies and experimental animal models illustrates that many pathophysiological changes occur with aging that promote the development of hypertension, including, but not limited to, reduced arterial compliance, endothelial dysfunction, reductions in renal blood flow, alterations in GFR, glomerular morphology, and renal sodium handling, as previously described.

Weight is increased in the adult age group compared to the young adult age group. While not a novel finding, this increased body mass may explain the increased sodium excretion rate between the same two groups. Larger animals should, in theory, consume more food and thus excrete more sodium. However, as previously discussed, this interpretation is limited until more precise methods of estimating food and sodium intake in this model organism are available. The lack of significant differences in water intake and UFR between age groups suggests that AGMs are able to maintain water balance regardless of age.

eGFR decreases normally with age in human populations and is considered predictive of mortality, development of cardiovascular disease, and a key clinical indicator of chronic kidney disease. However, eGFR was similar in all age groups in this study and in prior studies that investigated blood pressure phenotype and sex. Inter-individual variation in eGFR is common in patient populations and some variation is expected in an outbred population of animals. However, the usage of creatinine for estimation of GFR has been called into question (Lamb, Wood et al. 2005). Some studies have shown that creatinine is limited in estimation of GFR since creatinine serum levels can vary widely with disease states, muscle mass, and nutritional status (Hoek, Kemperman et al. 2003). In addition, creatinine can be secreted from tubules in glomerulopathic disease states, causing unpredictable estimates of GFR (Shemesh, Golbetz et al. 1985). It is entirely possible that the methods used in this study may not accurately estimate GFR in the AGM. Future studies in the AGM should utilize other methods of eGFR measurement, such as FITC-sinistrin, serum cystatin C, or classical inulin clearance, in order to confidently determine the effect of age, sex, and blood pressure on eGFR.

Urinary protein excretion is elevated in young adult HT animals compared to age-matched NT controls. In the adult age group, HT animals had elevated protein excretion compared to age-matched BHTs. Interestingly, average protein excretion was nearly identical in both NT and BHT adults, however, the small group size of the NT adult AGMs (n=3), likely interfered with statistical comparison of the age/phenotype groups. More NT animals in the 11-15 year old age group, as well as more mature adult animals, should undergo 24-hour urine collections in order to accurately draw conclusions on the

effect of age and elevation of blood pressure on renal damage in the AGM. Proteinuria is an important clinical marker of renal damage in patients. In aging male Wistar rats, protein excretion remains relatively stable until senescence, approximately 38 months of age, where it greatly increases. However, albumin excretion increases with age as early as 12 months of age, suggesting of increased glomerular permeability at a relatively young age(Alt, Hackbarth et al. 1980). Future studies that assess albumin excretion, and thus indirectly glomerular permeability, in the context of age and elevated blood pressure would provide crucial information regarding the pathogenesis of hypertension in the AGM.

A major limitation in this study is the small sample size of the mature adult group (n=5). Animals that are 16+ years old are rare to find in the St. Kitts habitat, perhaps due to territorial disputes between troops, hunting by man, and natural causes of death. Currently, the authors are working with the local government and trappers to procure additional animals for study. In addition, the authors are also breeding and maintaining animals that will be used to directly assess the longitudinal effects of age and hypertension in a larger cohort of AGMs, as well as track the development of hypertension throughout the lifespan of the species in captivity.

In conclusion, hypertension prevalence increases with age in the African green monkey, similar to human populations, without specialized diets, breeding paradigms, or experimental interventions. Urinary protein excretion, a clinical marker of renal damage, is increased in young adult hypertensive animals compared to age-matched normotensive controls. While the exact mechanisms that promote the development of hypertension in young and old AGMs are currently unknown, future studies will attempt to determine key

differences in normotensive and hypertensive animals within all age groups. The African green monkey represents a unique model system for studying the effect of age on the development and maintenance of hypertension and associated cardiovascular diseases with a high translational capacity.

Perspectives and Significance

The recent blood pressure guidelines from the American Heart Association and the American College of Cardiology lower target systolic and diastolic blood pressures for the diagnosis of hypertension to 130 mmHg and 80 mmHg, respectively (Whelton, Carey et al. 2018). These new guidelines will result in an estimated 46% of adult in the U.S. being diagnosed with high blood pressure, though 'newly diagnosed' Stage I hypertensive patients are recommended for non-pharmacological therapy in most circumstances. Analysis of the effects of age and sex in the development of hypertension in a highly translational model organism will give researchers new tools to study and facilitate the identification of newer and more effect treatments and targets as the healthcare burden of hypertension increases.

5.5 Acknowledgements

The authors wish to thank Chelsea C. Weaver for her invaluable technical assistance and advice in the data collection and manuscript preparation process.

Table 5.1: Mean and SEMs for each age/phenotype group

Age Group	Phenotype	n	Water Intake (mL/day)	Urine Flow Rate (mL/day)	eGFR (mL/ min)	Protein Excretion Rate (mg/ day)	Sodium Excretion Rate (mmol/day)	Potassium Excretion Rate (mmol/day)
Young adult: 5-10 Years	NT	34	244.8±37.8	143.1±19.6	3.5±0.5	346.6±21.0	2.3±0.2	2.7±0.3
	BHT	25	200.5±23.9	150.2±17.4	3.8±0.5	419.2±32.2	2.9±0.3	3.6±0.4
	HT	27	222.7±31.6	167.2±23.3	4.4±0.6	440.7±30.0	3.1±0.4	3.8±0.6
Adult: 11-15 Years	NT	3	178.9±28.0	144.5±32.2	2.3±0.7	348.9±77.5	3.2±0.3	4.5±0.8
	BHT	6	177.5±43.2	135.8±54.5	5.6±1.5	347.5±52.9	2.6±0.4	2.4±0.5
	HT	12	257.8±51.6	180.4±30.8	5.1±1.0	540.9±44.2	4.8±0.6	4.3±0.6
Mature adult: 16+ Years	NT	1	78.3	40.2	1.0	176.5	0.6	0.8
	BHT	1	308.3	203.3	6.1	810.9	3.7	6.3
	HT	3	408.9±199.9	148.1±49.0	4.9±2.3	382.6±39.2	2.2±0.4	3.6±1.2

Table 5.2: Prevalence of hypertension in the African Green Monkey by age

Age Group	**Prevalence (95% CI)**
0-4 years	20% (7.05 to 45.19)
5-10 years	31.34% (25.33 to 38.06)
11-15 years	48.33% (36.18 to 60.69)
16+ years	60.7% (42.41 to 76.43)

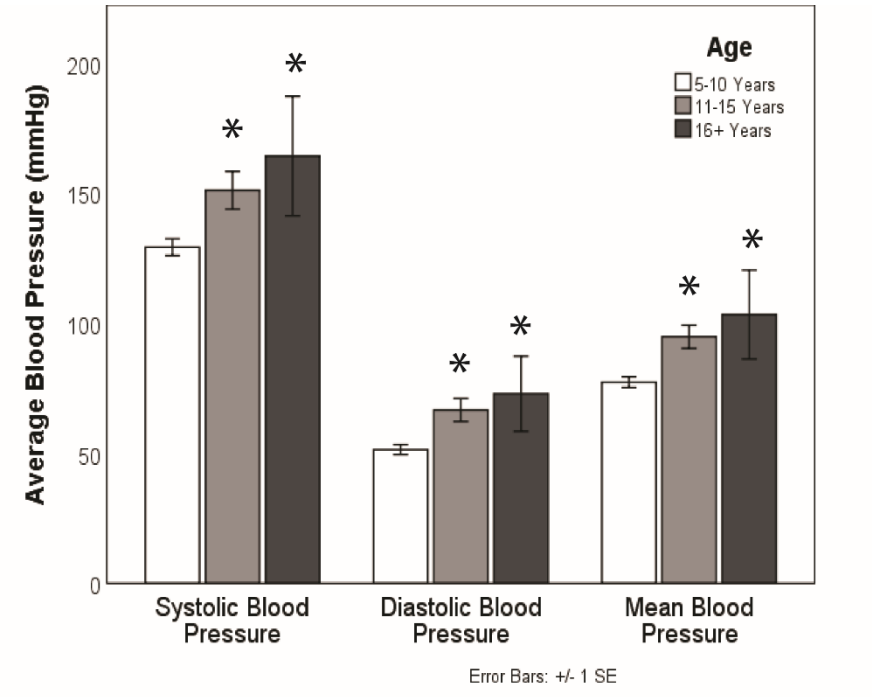

Figure 5.1:Blood pressure by age in the AGM.

Young adult (aged 5-10 years) have lower systolic, diastolic, and mean blood pressures compared to adult (aged 5-10 years) and mature adult (16+ years) groups ($p<0.05$, ANOVA with Tukey's post hoc comparison).

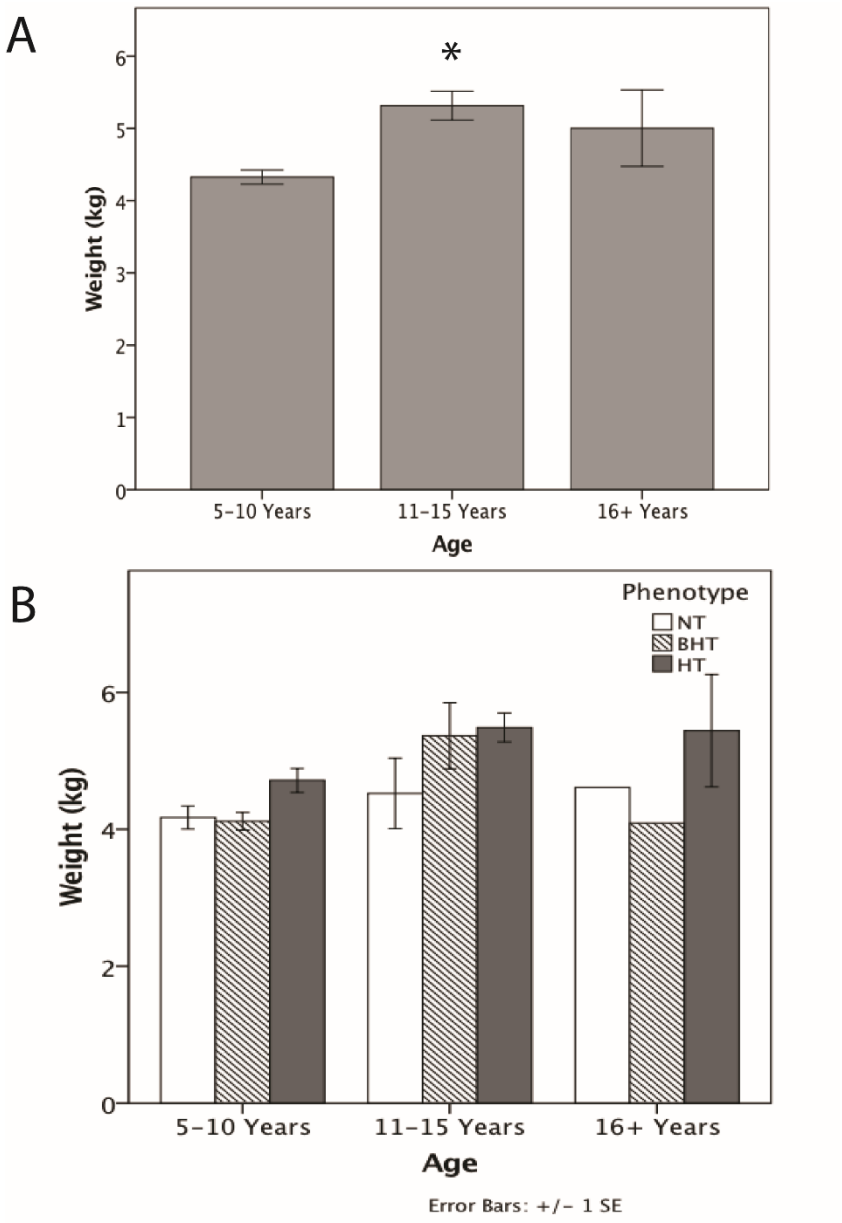

Figure 5.2: Adults animals are larger than young adult animals.

Adult animals (aged 11-15 years) are significantly larger than young adult animals (aged 5-10 years, $p<0.05$, ANOVA with Tukey's post hoc comparison). Mature adult animals (aged 16+ years) had weights similar to the other age groups. No significant interactions between age and phenotype were found (2B).

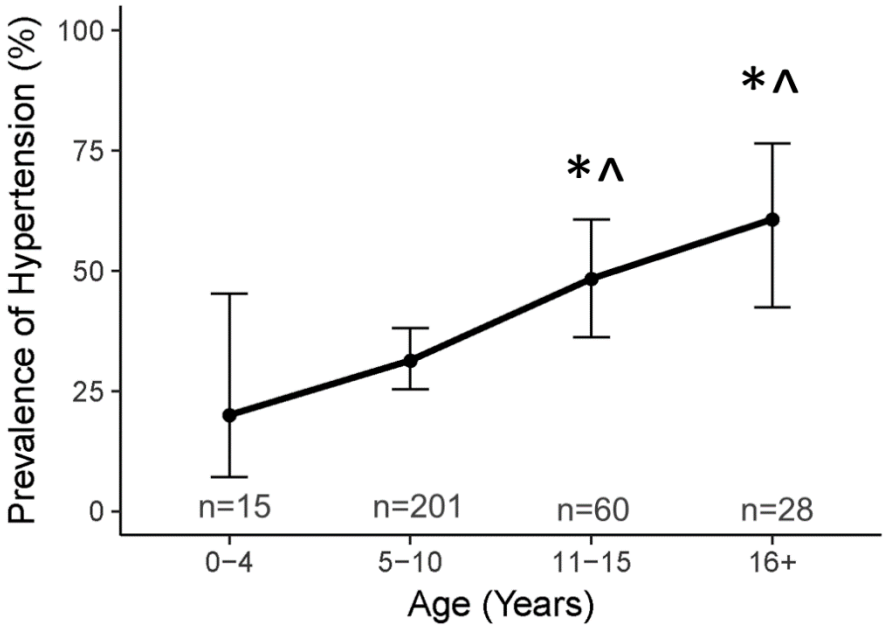

Figure 5.3: Hypertension prevalence increases with age in the African Green Monkey.

Adult (aged 11-15 years) and mature adult (aged 16+ years) animals have significantly increased rates of hypertension compared to juvenile (aged 0-4 years) and young adult (aged 5-10 years) animals.

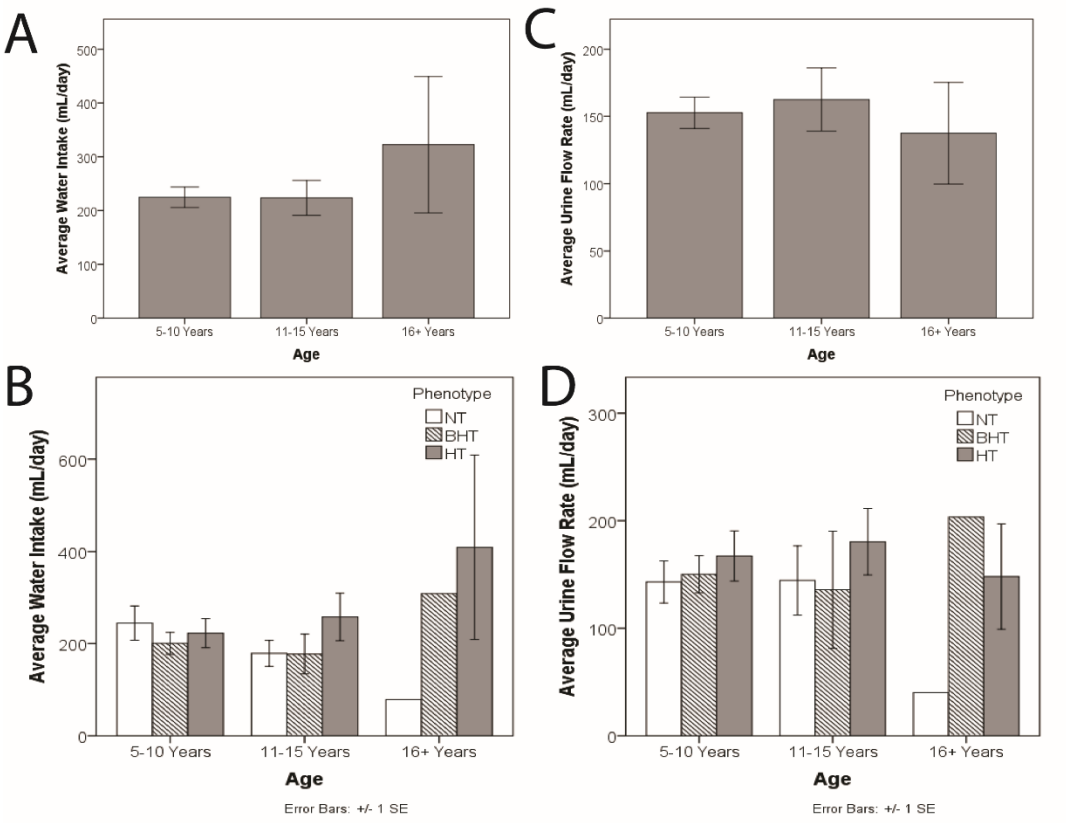

Figure 5.4: Water balance is similar between all age groups.

Water intake and urine flow rates are similar between age groups and age/phenotype groups ($p>0.05$, ANOVA with Tukey's post hoc comparison) and no significant interactions exist between age and phenotype group ($p>0.05$, two-way ANOVA).

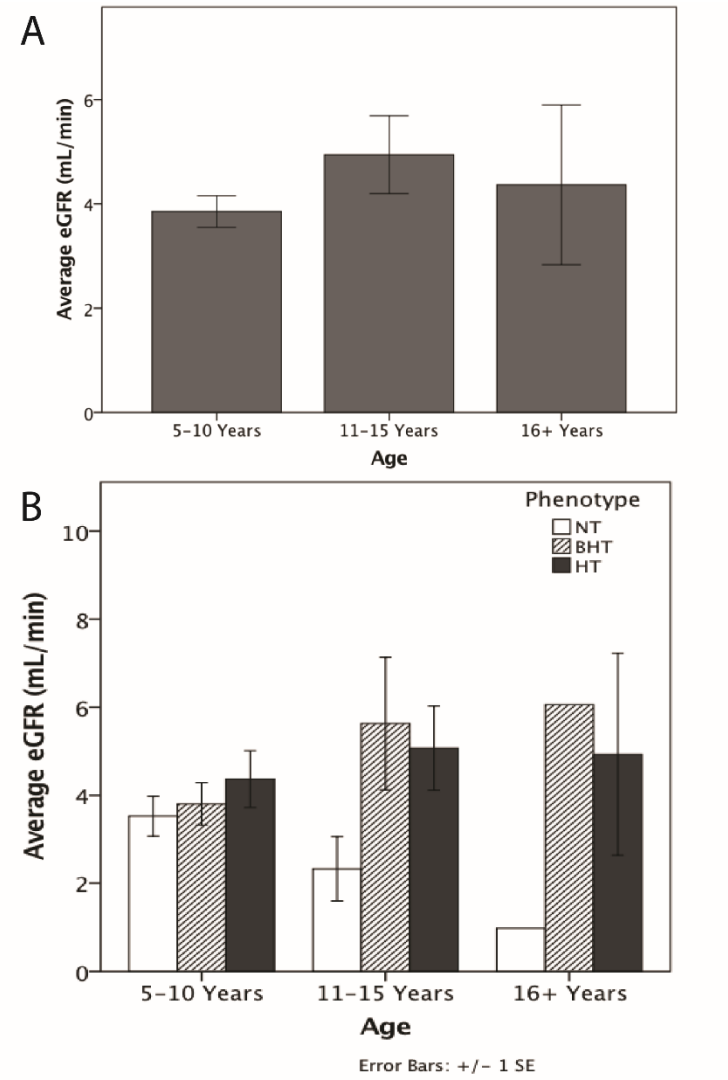

Figure 5.5: eGFR is similar between all age groups.

Estimated glomerular filtration rates are similar between age groups and age/phenotype groups ($p>0.05$, ANOVA with Tukey's post hoc comparison) and no significant interactions exist between age and phenotype group ($p>0.05$, two-way ANOVA).

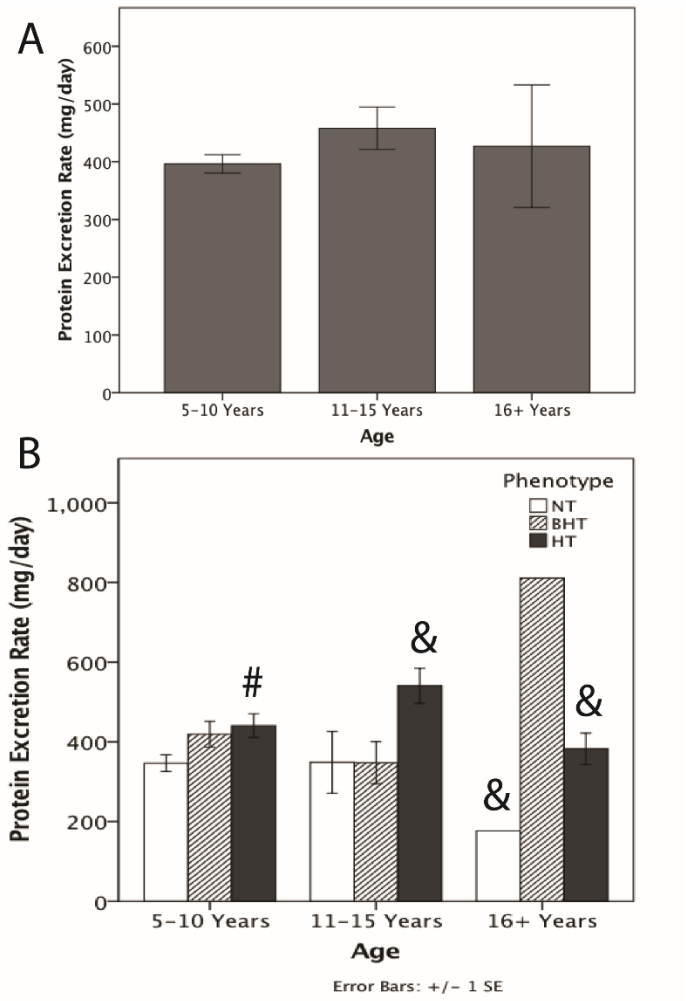

Figure 5.6: Urinary protein excretion is altered by age and phenotype in the AGM.

Protein excretion is significantly affected by age and blood pressure phenotype ($p<0.05$, two-way ANOVA). In the young adult age group, protein excretion is elevated in HT animals compared to NT animals. In the adult age group, HT animals have elevated protein excretion compared to BHT animals. In the mature adult group, the BHT animal has significantly elevated protein excretion compared to both the NT animal and the HT animals. # denotes $p<0.05$ compared to age-matched NT; & denotes $p<0.05$ compared to age-matched BHT

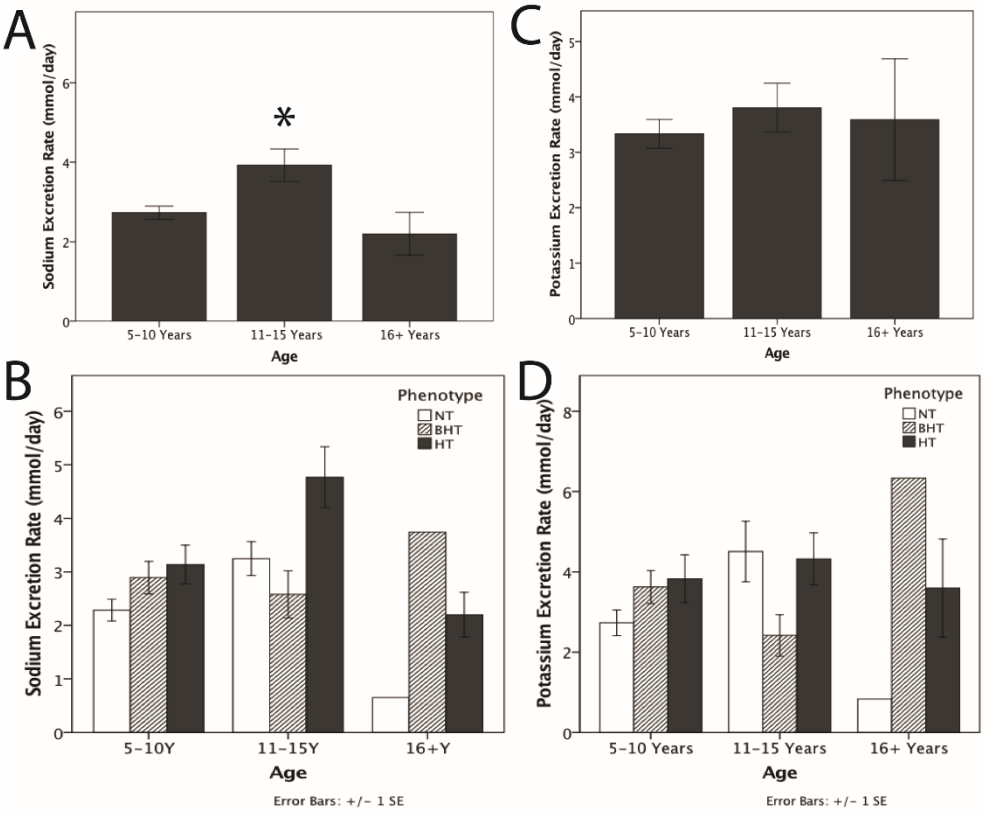

Figure 5.7: Urinary sodium excretion is elevated in adult animals compared to young animals.

Sodium excretion is elevated in adult (aged 11-15 years) animals compared to young adult (aged 5-10 years) animals ($p<0.05$ ANOVA with Tukey's post hoc comparison). Potassium excretion is similar in all age and age/phenotype groups.

CHAPTER SIX

DISCUSSION AND CONCLUSIONS

The African Green Monkey (AGM) is a highly translational large animal of spontaneous hypertension. It is a novel non-human primate model for a complex multifactorial disease that affects the health and life expectancy of one in three adults in the United States. While rodent models of disease have contributed largely to our understanding of hypertension and disease, 13% of hypertensive patients maintain elevated blood pressures while on three or more classes of anti-hypertensive therapies(Persell 2011). In 2015, over 78,000 deaths in the United States were directly attributed to hypertension (Benjamin, Virani et al. 2018), highlighting the detrimental health risks of this pathology that is expected to exceed $200 billion in direct spending by 2030.

Utilization of a non-human primate model of spontaneous hypertension offers specific advantages over traditional rodent models. AGMs are diurnal, like humans. Their upright posture allows for cyclical unloading of baroreceptors that resembles human physiology, unlike quadruped models. AGMs share over 90% of their gene sequence with humans and the two genomes have very high levels of synteny (Jasinska, Service et al. 2007). Unlike the closely-related rhesus monkey, the AGM is not endangered, easier to obtain, and typically less expensive to purchase (Jasinska, Schmitt et al. 2013). Compared to human studies, complex variables such as diet, reproduction, social interactions, and environmental factors can be controlled in this laboratory model. Large-scale genetic tools are becoming increasingly available for the analysis of complex diseases and traits (Huang, Ramensky et al. 2015), while their highly similar physiology to humans makes

them ideal for studies utilizing pharmacologic or physiological manipulations. The renal and cardiac pathologies reported in this dissertation strengthen the translational capacity of this model in the study of hypertension and associated cardiovascular diseases – as the hypertensive AGM exhibits several pathologies similar to essential hypertensive patients.

Like any model, the AGM has limitations. Due to their larger size compared to rodents, the associated costs of care and procurement are higher. As highly social creatures, they thrive in large group enclosures, which can be difficult for long-term maintenance and upkeep. Renal function studies, as previously described, require single housing, which may induce a stress response after removal from group housing. While any animal exhibiting signs of extreme distress is removed from single housing and all efforts are made to minimize stress for each animal, this induced stress is a confounding factor that should be considered in the interpretation of these results. Like any animal model, the AGM cannot recapitulate every clinical phenotype, however, the clinical relevance and importance of novel, unique, translational models in research cannot be understated. It is crucial that basic research scientists understand the mechanisms and factors that contribute to the development of pathologies as well as how disease processes are recapitulated in experimental animal models. The AGM represents an important opportunity for basic researchers to identify and study mechanisms of blood pressure control that may be unique to primates alone.

This dissertation details the first description of end-organ damage associated with hypertension in the AGM. In a cohort of 424 adult animals, 37% of the animals displayed systolic blood pressures over 140 mmHg while under light sedation. This rate of prevalence in the AGM is similar to the recent estimation from the American Heart

Association that 31.1% of adults in the United States have hypertension (reference?), however, there are important considerations to this interpretation. First, while genetic variability in the United States is quite heterogeneous, the AGM in the Caribbean likely has a relatively homogeneous level of genetic variability. Introduction of the AGM to the Caribbean likely occurred in the 1600s when the animals were brought to the islands as pets in the shipping trade. Long and stressful sea journeys likely had low survival rates that may have limited the genetic variability of the founding members of the population. Thus, the Caribbean AGM has likely undergone a significant bottleneck effect, which may influence the prevalence of hypertension described in the study. Analysis of the prevalence of hypertension in the Gambian population from which the Caribbean AGM (Svardal, Jasinska et al. 2017) is descended would provide data that clarify and provide context for the interpretation of this finding. Namely, it should be determined if the entire genus suffers from hypertension or if the introduction event of the AGM to the Caribbean selected for genes that promote hypertension. Rates of hypertension in humans are high in St. Kitts and Nevis (it was recently estimated that 53% of adults in the island country are hypertensive (Crews, Campbell et al. 2017)), so it is possible that the increased rates of hypertension in both human and nonhuman primates may be partially attributed to high levels of salt in the diet due to location. Additional experiments designed to test these hypotheses are worth exploring.

The prevalence of hypertension increases with age in the AGM. Aging is a major risk factor for the development of hypertension and cardiovascular disease in humans as vascular stiffness and sclerosis increase with age. The data in this dissertation do show an association of the prevalence of hypertension with age, but the mechanisms controlling

this relationship have yet to be identified. It is worth noting that other studies have shown development of atherosclerosis, insulin resistance, and obesity in the AGM, which are all often co-morbidities with hypertension in older patients, particularly in the context of obesity(references). Animals in the Caribbean research colony that we collaborate with are not obese, but other research colonies induce obesity in the species via a Western diet (Schmitt, Service et al. 2017). A comparative analysis of both the genetic and environmental factors contributing to the elevation of blood pressure in young and old, obese and non-obese AGMs would greatly enhance the translational capacity of the model species.

Changes in glomerular morphology and renal vascular hypertrophy are markers of renal damage associated with hypertension in the AGM. It is currently unknown if the proteinuria inducing renal damage is primarily located in the glomerulus, tubule, or both structures. Analysis of damage markers like vimentin or kidney injury molecule (Lopez-Giacoman and Madero 2015) would answer these questions and provide crucial data about the pathogenesis of hypertension in the AGM. Renal function studies found that HT AGMs have elevated urinary protein excretion compared to NT animals. Analysis of urinary protein excretion by age revealed that the elevation of blood pressure is associated with greater levels of proteinuria in young adult animals, which made up a larger majority of the renal function study cohort. Additional renal function studies focused on adult and mature adult animals will be an important contribution to the body of knowledge on the interaction of age and blood pressure elevation in the AGM.

Urinary sodium excretion is elevated in hypertensive animals, likely due to natriuresis in response to elevated blood pressure. Real-time blood pressure

measurements (obtained via wireless radio-telemetry) in association with shorter, more precise urine collection periods would determine how time of day, food intake, and activity would affect both blood pressure and natriuresis in the AGM. It is worth noting that, exact sodium intake is difficult to calculate with the current experimental equipment as primate chow can be thrown out of the cage or lost in the process of eating. New techniques and methods to accurately assess food intake will be needed to overcome this limitation.

Increased gene expression of adrenergic receptors in the renal medulla and left ventricular myocardium in the HT AGM suggests that sympathetic activation plays a role in the development and maintenance of hypertension in this model organism. Elevated heart rate, a possible consequence of increased sympathetic drive, and cardiac hypertrophy, associated with activation of the cardiac β-adrenergic receptors, were also associated with hypertension in the AGM. It is possible that sympathetic activation is elevated in the AGM increasing total peripheral resistance, and thus afterload, which could be assessed by measurement of sympathetic tone via ganglionic blockade or administration of selective α- and β-adrenergic receptor antagonists. It is also possible that decreased renal sympathetic nerve activation has increased renal adrenergic receptor transcription. Assessment of renal sympathetic activation via renal denervation or selective afferent nerve ablation would clarify the contribution of the sympathetic nervous system in this possibly neurogenic model of hypertension.

Additionally, prior studies demonstrated that hypertension in the AGM was ameliorated by administration of captopril, an ACE inhibitor, and not furosemide, a loop diuretic, suggesting activation of the renin-angiotensin-aldosterone system may be associated with hypertension in the AGM(Martin, Palmour et al. 1990). Our reported measurements of similar plasma renin activity, liver angiotensin expression, and renal cortical renin content suggest that the AGM is a 'normal-renin' model of hypertension. Assuming that the HT AGM in our hands responds to captopril with decreases in blood pressure, this suggests that other mechanisms, such as activation of RAAS in the central nervous system, may be contributing to the hypertension seen in this model. These model-defining experiments are crucially important to the determination of the mechanism causing hypertension in this unique animal model. However, downstream measurement of additional components of the RAAS, such as aldosterone, have not been published to date. It is also possible that alternate enzymes, such as chymase, that catalyze the formation of Ang II are increased in the HT AGM.

The possibility that a unique gene or gene variant may be causal in the development of hypertension in this model cannot be ignored. The unique history in the founding of the Caribbean AGMs may have resulted in high frequencies of detrimental genes in the population which result in an increased risk of hypertension. Future studies to further characterize the AGM as a model of spontaneous hypertension include, but are not limited to, long-term studies that measure the development of hypertension and the progression of end-organ damage throughout life, radio-telemetric measurement of blood pressure in conscious group-housed animals, further characterization of the renin-angiotensin-aldosterone system, the effect of renal denervation in the AGM, the

pharmacological effects of anti-hypertensive medications in the AGM and genetic and epidemiological analysis of the Caribbean and African populations of the AGM.

The complex social hierarchy in the AGM (Dunbar 1974) represents a unique opportunity to study the effects of psychosocial stress on severity and pathogenesis of hypertension in a large animal model similar to humans. Social stress, such as work stress, is increasingly being recognized as important in the pathogenesis of cardiovascular disease (Thayer, Yamamoto et al. 2010). As AGMs exist in multi-male and multi-female groups in the wild, social hierarchies are complex and have long-term effects on reproductive success in both males and females (Fairbanks and McGuire 1986, Isbell, Cheney et al. 2002, Seyfarth and Cheney 2003). Dominance in the male AGM is associated with increased paternity success (Weingrill, Willems et al. 2010), and decreased plasma norepinephrine levels (Dillon, Raleigh et al. 1992) but no studies have been published assessing the relationship between blood pressure and hierarchy status in the AGM. While complex social organization is not unique to primates, a prolonged period of dependence and learning is unique to primates. In addition, Old World monkeys and humans share similar patterns and rates of limbic and cortical brain development (Palmour, Mulligan et al. 1997). The AGM provides a unique opportunity to investigate the effects of these complex behaviors on health and disease in well-described social hierarchies in a laboratory setting.

Within this dissertation, I have presented evidence that supports the hypothesis that the African Green Monkey is a novel translational non-human primate model of spontaneous hypertension that recapitulates the pathologies observed in hypertensive human patients. The AGM is a novel tool in the investigation of hypertension that may

increase the translational success of anti-hypertensive therapies and interventions and the increased utilization of this model for basic science research may discover mechanisms of blood pressure control unique to primates. Our data (Rhoads, Goleva et al. 2017) combined with that of others (Martin, Palmour et al. 1990) suggests that hypertension in the AGM is non-renin dependent but that elevated blood pressure is still sensitive to captopril (an ACE inhibitor) administration, similar to many essential hypertensive patients. In addition, our data support the idea that hypertension in the AGM is sympathetically-driven.

The future of cardiovascular research in the AGM is bright and promising, as the AGM is a large-animal model organism that can be utilized in many unique ways. As a non-endangered, invasive pest species in the Caribbean, procurement of the animals serves two main purposes – preservation of the agricultural business on the island (to which the local AGM population is a large threat) and improved availability of the model organism for scientific research purposes. Future research should further characterize the response of the hypertensive AGM to anti-hypertensive pharmacological agents which would create a useful profile for researchers to appropriately match this model to the human drug responses seen in clinical practice and research.

Of course, as with any scientific research, the findings presented here have created additional questions that need to be answered. It is currently unknown what initiates the development of spontaneous hypertension in the AGM or what genetic factors may contribute to the pathology. The temporal nature of the end-organ damage is also unknown – does structural damage occur first, due to some mechanism other than elevated pressure, or is the causal primary factor the elevation of blood pressure? Aging

is associated with spontaneous hypertension in the AGM, but the role of sex has not yet been elucidated. As the use of the AGM in blood pressure and cardiovascular research increases, the answers to these questions and the immense translational potential of the AGM will hopefully be realized.

BIBLIOGRAPHY

Abdala, A. P., F. D. McBryde, N. Marina, E. B. Hendy, Z. J. Engelman, M. Fudim, P. A. Sobotka, A. V. Gourine and J. F. Paton (2012). "Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat." J Physiol **590**(17): 4269-4277.

Akita, S., F. M. Sacks, L. P. Svetkey, P. R. Conlin, G. Kimura and D. A.-S. T. C. R. Group (2003). "Effects of the Dietary Approaches to Stop Hypertension (DASH) diet on the pressure-natriuresis relationship." Hypertension **42**(1): 8-13.

Alt, J. M., H. Hackbarth, F. Deerberg and H. Stolte (1980). "Proteinuria in rats in relation to age-dependent renal changes." Lab Anim **14**(2): 95-101.

Andelman, S. J. (1987). "Evolution of concealed ovulation in the vervet monkeys (Cercopithecus aethiops)." The American Naturalist **129**(6): 785-799.

Animals, N. R. C. U. C. f. t. U. o. t. G. f. t. C. a. U. o. L. (2011). Guide for the Care and Use of Laboratory Animals. Washington, DC, National Academies Press (US).

Arnal, J.-F., L. Warin and J.-B. Michel (1992). "Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhbition of nitric oxide synthase." Journal of Clinical Investigation **90**: 647-652.

Aronow, W. S., C. Ahn, A. D. Mercando and S. Epstein (1996). "Association of average heart rate on 24-hour ambulatory electrocardiograms with incidence of new coronary events at 48-month follow-up in 1,311 patients (mean age 81 years) with heart disease and sinus rhythm." Am J Cardiol **78**(10): 1175-1176.

Astor, B. C., K. Matsushita, R. T. Gansevoort, M. van der Velde, M. Woodward, A. S. Levey, P. E. Jong, J. Coresh, C. Chronic Kidney Disease Prognosis, B. C. Astor, K. Matsushita, R. T. Gansevoort, M. van der Velde, M. Woodward, A. S. Levey, P. E. de Jong, J. Coresh, M. El-Nahas, K. U. Eckardt, B. L. Kasiske, J. Wright, L. Appel, T. Greene, A. Levin, O. Djurdjev, D. C. Wheeler, M. J. Landray, J. N. Townend, J. Emberson, L. E. Clark, A. Macleod, A. Marks, T. Ali, N. Fluck, G. Prescott, D. H. Smith, J. R. Weinstein, E. S. Johnson, M. L. Thorp, J. F. Wetzels, P. J. Blankestijn, A. D. van Zuilen, V. Menon, M. Sarnak, G. Beck, F. Kronenberg, B. Kollerits, M. Froissart, B. Stengel, M. Metzger, G. Remuzzi, P. Ruggenenti, A. Perna, H. J. Heerspink, B. Brenner, D. de Zeeuw, P. Rossing, H. H. Parving, P. Auguste, K. Veldhuis, Y. Wang, L. Camarata, B. Thomas and T. Manley (2011). "Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts." Kidney Int **79**(12): 1331-1340.

Atchison, D. K., P. Harding and W. H. Beierwaltes (2011). "Hypercalcemia reduces plasma renin via parathyroid hormone, renal interstitial calcium, and the calcium-sensing receptor." Hypertension **58**(4): 604-610.

Atchison, D. K., M. C. Ortiz-Capisano and W. H. Beierwaltes (2010). "Acute activation of the calcium-sensing receptor inhibits plasma renin activity in vivo." Am J Physiol Regul Integr Comp Physiol **299**(4): R1020-1026.

Atkins, H. M., C. J. Willson, M. Silverstein, M. Jorgensen, E. Floyd, J. R. Kaplan and S. E. Appt (2014). "Characterization of ovarian aging and reproductive senescence in vervet monkeys (Chlorocebus aethiops sabaeus)." Comp Med **64**(1): 55-62.

Bachmann, S., J. Peters, E. Engler, D. Ganten and J. Mullins (1992). "Transgenic rats carrying the mouse renin gene—Morphological characterization of a low-renin hypertension model." Kidney International **41**(1): 24-36.

Bakris, G. L., R. R. Townsend, M. Liu, S. A. Cohen, R. D'Agostino, J. M. Flack, D. E. Kandzari, B. T. Katzen, M. B. Leon, L. Mauri, M. Negoita, W. W. O'Neill, S. Oparil, K. Rocha-Singh, D. L. Bhatt and S. H.-. Investigators (2014). "Impact of Renal Denervation on 24-Hour Ambulatory Blood Pressure: Results From SYMPLICITY HTN-3." J Am Coll Cardiol **64**(11): 1071-1078.

Baldellou, M. and A. Adan (1998). "Diurnal and Seasonal Variations in Vervet Monkeys activity." Psychological Reports **83**(2): 675-685.

Barki-Harrington, L., C. Perrio and H. A. Rockman (2004). "Network intergration of the adrenergic system in cardiac hypertrophy." Cardiovascular Research **63**: 391-402.

Baylis, C. (1994). "Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor." J Clin Invest **94**(5): 1823-1829.

Belova, L. A. (2000). "Angiotensin II-Generating Enzymes." Biochemistry (Moscow) **65**(12): 1337-1345.

Benjamin, E. J., M. J. Blaha, S. E. Chiuve, M. Cushman, S. R. Das, R. Deo, S. D. de Ferranti, J. Floyd, M. Fornage, C. Gillespie, C. R. Isasi, M. C. Jimenez, L. C. Jordan, S. E. Judd, D. Lackland, J. H. Lichtman, L. Lisabeth, S. Liu, C. T. Longenecker, R. H. Mackey, K. Matsushita, D. Mozaffarian, M. E. Mussolino, K. Nasir, R. W. Neumar, L. Palaniappan, D. K. Pandey, R. R. Thiagarajan, M. J. Reeves, M. Ritchey, C. J. Rodriguez, G. A. Roth, W. D. Rosamond, C. Sasson, A. Towfighi, C. W. Tsao, M. B. Turner, S. S. Virani, J. H. Voeks, J. Z. Willey, J. T. Wilkins, J. H. Wu, H. M. Alger, S. S. Wong, P. Muntner, C. American Heart Association Statistics and S. Stroke Statistics (2017). "Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association." Circulation **135**(10): e146-e603.

Benjamin, E. J., S. S. Virani, C. W. Callaway, A. M. Chamberlain, A. R. Chang, S. Cheng, S. E. Chiuve, M. Cushman, F. N. Delling, R. Deo, S. D. de Ferranti, J. F. Ferguson, M. Fornage, C. Gillespie, C. R. Isasi, M. C. Jimenez, L. C. Jordan, S. E. Judd, D. Lackland, J. H. Lichtman, L. Lisabeth, S. Liu, C. T. Longenecker, P. L. Lutsey, J. S. Mackey, D. B. Matchar, K. Matsushita, M. E. Mussolino, K. Nasir, M. O'Flaherty, L. P. Palaniappan, A. Pandey, D. K. Pandey, M. J. Reeves, M. D. Ritchey, C. J. Rodriguez, G.

A. Roth, W. D. Rosamond, U. K. A. Sampson, G. M. Satou, S. H. Shah, N. L. Spartano, D. L. Tirschwell, C. W. Tsao, J. H. Voeks, J. Z. Willey, J. T. Wilkins, J. H. Wu, H. M. Alger, S. S. Wong, P. Muntner, E. American Heart Association Council on, C. Prevention Statistics and S. Stroke Statistics (2018). "Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association." Circulation **137**(12): e67-e492.

Bertel, O., F. R. Buhler, W. Kiowski and B. E. Lutold (1980). "Decreased Beta-adrenoreceptor responsiveness as related to age, blood pressure, and plasma catecholamines in patients with essential hypertension." Hypertension **2**: 130-138.

Bhatt, D. L., D. E. Kandzari, W. W. O'Neill, R. D'Agostino, J. M. Flack, B. T. Katzen, M. B. Leon, M. Liu, L. Mauri, M. Negoita, S. A. Cohen, S. Oparil, K. Rocha-Singh, R. R. Townsend, G. L. Bakris and S. H.-. Investigators (2014). "A controlled trial of renal denervation for resistant hypertension." N Engl J Med **370**(15): 1393-1401.

Bonnardeaux, A., E. Davies, X. Jeunemaitre, I. Fery, A. Charru, E. Clauser, L. Tiret, F. Cambien, P. Corvol and F. Soubrier (1994). "Angiotensin II Type 1 Receptor Gene Polymorphisms in Human Essential Hypertension." Hypertension **24**: 63-69.

Borch-Johnsen, K., P. K. Andersen and T. Deckert (1985). "The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus." Diabetologia **28**(8): 590-596.

Brenner, B. M., B. J. Ballerman, M. E. Gunning and M. L. Zeidel (1990). "Diverse biological actions of atrial natriuretic peptide." Physiological Reviews **70**(3): 665-687.

Brosnihan, K. B., P. Li, D. Ganten and C. M. Ferrario (1997). "Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of RAS." Am J Physiol **273**(6 Pt 2): R1908-1915.

Buford, T. W. (2016). "Hypertension and aging." Ageing Res Rev **26**: 96-111.

Chen, J. A., S. C. Fears, A. J. Jasinska, A. Huang, N. B. Al-Sharif, K. E. Scheibel, T. D. Dyer, A. M. Fagan, J. Blangero, R. Woods, M. J. Jorgensen, J. R. Kaplan, N. B. Freimer and G. Coppola (2018). "Neurodegenerative disease biomarkers Abeta1-40, Abeta1-42, tau, and p-tau181 in the vervet monkey cerebrospinal fluid: Relation to normal aging, genetic influences, and cerebral amyloid angiopathy." Brain Behav **8**(2): e00903.

Cheng, S., V. Xanthakis, L. M. Sullivan and R. S. Vasan (2012). "Blood pressure tracking over the adult life course: patterns and correlates in the Framingham heart study." Hypertension **60**(6): 1393-1399.

Chopra, S., D. Cherian, P. P. Verghese and J. J. Jacob (2013). "Physiology and clinical significance of natriuretic hormones." Indian J Endocrinol Metab **17**(1): 83-90.

Civantos Calzada, B. and A. Aleixandre de Artinano (2001). "Alpha-adrenoceptor subtypes." Pharmacol Res **44**(3): 195-208.

Cody, R. J., Jr., R. F. Rodger, L. H. Hartley, J. Burton and J. A. Herd (1982). "Acute hypertension in a nonhuman primate: humoral and hemodynamic mechanisms." Hypertension **4**(2): 219-225.

Collett, J. A., A. K. Hart, E. Patterson, J. Kretzer and J. L. Osborn (2013). "Renal angiotensin II type 1 receptor expression and associated hypertension in rats with minimal SHR nuclear genome." Physiol Rep **1**(5): e00104.

Cooper, S. J. (2008). "From Claude Bernard to Walter Cannon. Emergence of the concept of homeostasis." Appetite **51**(3): 419-427.

Coote, J. H. (2005). "A role for the paraventricular nucleus of the hypothalamus in the autonomic control of heart and kidney." Exp Physiol **90**(2): 169-173.

Cosenzi, A., E. Bernobich, M. Bonavita, G. Bertola, R. Trevisan and G. Bellini (2002). "Antihypertensive treatment with enrasentan (SB217242) in an animal model of hypertension and hyperinsulinemia." J Cardiovasc Pharmacol **39**(4): 488-495.

Cowley, A. W., Jr. (1992). "Long-term control of arterial blood pressure." Physiol Rev **72**(1): 231-300.

Crews, D. C., K. N. Campbell, Y. Liu, O. Bussue, I. Dawkins and B. A. Young (2017). "Chronic kidney disease and risk factor prevalence in Saint Kitts and Nevis: a cross-sectional study." BMC Nephrol **18**(1): 7.

Custodis, F., J. C. Reil, U. Laufs and M. Bohm (2013). "Heart rate: a global target for cardiovascular disease and therapy along the cardiovascular disease continuum." J Cardiol **62**(3): 183-187.

Dahl, L. K., K. D. Knudsen, M. Heine and G. Leitl (1967). "Effects of chronic excess salt ingestion. Genetic influence on the development of salt hypertension in parabiotic rats: evidence for a humoral factor." J Exp Med **126**(4): 687-699.

Dampney, R. A. L., M. J. Coleman, M. A. P. Fontes, Y. Hirooks, J. Horiuchi, J. W. Polson, P. D. Potts and T. Tagawa (2001). "Central Mechanism underlying short-term and long-term regulation of the cardiovascular system." Proceedings of the Australian Physiological and Pharmacological Society **32**(1): 1-12.

Davidson, M. B. and J. K. Hix (2006). "Association of Imparied Diurnal Blood Pressure Variation with a Subsequent Decline in Glomerular Filtration Rate." Arch Intern Med **166**: 846-852.

Davies, B. and T. Morris (1993). "Physiological Parameters in Laboratory Animals and Humans." Pharmaceutical Research **10**(7): 1093-1095.

Denic, A., R. J. Glassock and A. D. Rule (2016). "Structural and Functional Changes With the Aging Kidney." Adv Chronic Kidney Dis **23**(1): 19-28.

DiBona, G. F. (2004). "The sympathetic nervous system and hypertension: recent developments." Hypertension **43**(2): 147-150.

DiBona, G. F. and M. Esler (2010). "Translational medicine: the antihypertensive effect of renal denervation." Am J Physiol Regul Integr Comp Physiol **298**(2): R245-253.

DiBona, G. F. and U. C. Kopp (1997). "Neural control of renal function." Physiol Rev **77**(1): 75-197.

Dietz, J. R. (2005). "Mechanisms of atrial natriuretic peptide secretion from the atrium." Cardiovasc Res **68**(1): 8-17.

Dillon, J. E., M. J. Raleigh, M. T. McGuire, D. Bergin-Pollack and A. Yuwiler (1992). "Plasma catecholamines and social behavior in male vervet monkeys (Cercopithecus aethiops sabaeus)." Physiol Behav **51**(5): 973-977.

Donoghue, M., F. Hsieh, E. Baronas, K. Godbout, M. Gosselin, N. Stagliano, N. Donovan, B. Woolf, K. Robison, R. Jeyaseelan, R. E. Breitbart and S. Acton (2000). "A novel angiotensin-coverting enzyme-related carboxypeptidase (ACE2) converts Angiotensin I to Angiotensin 1-9." Circ Res **87**: e1-e9.

Dubey, R. K., S. Oparil, B. Imthurn and E. K. Jackson (2002). "Sex hormones and hypertension." Cardiovasc Res **53**(3): 688-708.

Dunbar, R. I. M. (1974). "Observations on the Ecology and Social Organization of the Green Monkey, Cercopithecus sabaeus, in Senegal." Primates **15**(4): 351-350.

Elhawary, A. M. and C. C. Y. Pang (1994). "Alpha1B-adrenoceptors mediate renal tubular sodium and water reabsorption in the rat." British Journal of Pharmacology **111**: 819-824.

Ervin, F. R. (1993). A History of the St. Kitts Vervet Monkey. Heritage Magazine. Bassaterre, St. Kitts & Nevis. **4**.

Esler, M. (2000). "The Sympathetic System and Hypertension." American Journal of Hypertension **13**: 99S-105S.

Esler, M. and D. Kaye (2000). "Sympathetic nervous system activation in essential hypertension, cardiac failure and psychosomatic heart disease." J Cardiovasc Pharmacol **35**(7 Suppl 4): S1-7.

Esler, M. D., M. Bohm, H. Sievert, C. L. Rump, R. E. Schmieder, H. Krum, F. Mahfoud and M. P. Schlaich (2014). "Catheter-based renal denervation for treatment of patients with treatment-resistant hypertension: 36 month results from the SYMPLICITY HTN-2 randomized clinical trial." Eur Heart J **35**(26): 1752-1759.

Esteban, V., M. Ruperez, E. Sanchez-Lopez, J. Rodriguez-Vita, O. Lorenzo, H. Demaegdt, P. Vanderheyden, J. Egido and M. Ruiz-Ortega (2005). "Angiotensin IV

activates the nuclear transcription factor-kappaB and related proinflammatory genes in vascular smooth muscle cells." Circ Res **96**(9): 965-973.

Evans, L. C., A. Dayton, C. Yang, P. Liu, T. Kurth, K. W. Ahn, S. Komas, F. C. Stingo, P. W. Laud, M. Vannucci, M. Liang and A. W. Cowley, Jr. (2018). "Transcriptomic analysis reveals inflammatory and metabolic pathways that are regulated by renal perfusion pressure in the outer medulla of Dahl-S rats." Physiol Genomics **50**(6): 440-447.

Fairbanks, L. A. (1990). "Reciprocal benefits of allomothering for female vervet monkeys." Animal Behavior **40**: 553-562.

Fairbanks, L. A. and M. T. McGuire (1986). "Age, reproductive value and dominance-related behavior in vervet monkey females: cross-generational influences on social relationships and reproduction." Animal Behavior **34**: 1710-1721.

Farah, B. Q., D. G. Christofaro, P. B. Balagopal, B. R. Cavalcante, M. V. de Barros and R. M. Ritti-Dias (2015). "Association between resting heart rate and cardiovascular risk factors in adolescents." Eur J Pediatr **174**(12): 1621-1628.

Feld, L. G., J. B. Van Liew, R. G. Galaske, J. W. Boylan, H. Chang, N. Manz and P. Muir (1977). "Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat." Kidney International **12**(5): 332-343.

Ferguson, S. S. and R. D. Feldman (2014). "beta-adrenoceptors as molecular targets in the treatment of hypertension." Can J Cardiol **30**(5 Suppl): S3-8.

Flicek, P., M. R. Amode, D. Barrell, K. Beal, K. Billis, S. Brent, D. Carvalho-Silva, P. Clapham, G. Coates, S. Fitzgerald, L. Gil, C. G. Giron, L. Gordon, T. Hourlier, S. Hunt, N. Johnson, T. Juettemann, A. K. Kahari, S. Keenan, E. Kulesha, F. J. Martin, T. Maurel, W. M. McLaren, D. N. Murphy, R. Nag, B. Overduin, M. Pignatelli, B. Pritchard, E. Pritchard, H. S. Riat, M. Ruffier, D. Sheppard, K. Taylor, A. Thormann, S. J. Trevanion, A. Vullo, S. P. Wilder, M. Wilson, A. Zadissa, B. L. Aken, E. Birney, F. Cunningham, J. Harrow, J. Herrero, T. J. Hubbard, R. Kinsella, M. Muffato, A. Parker, G. Spudich, A. Yates, D. R. Zerbino and S. M. Searle (2014). "Ensembl 2014." Nucleic Acids Res **42**(Database issue): D749-755.

Foss, J. D., G. D. Fink and J. W. Osborn (2013). "Reversal of genetic salt-sensitive hypertension by targeted sympathetic ablation." Hypertension **61**(4): 806-811.

Foss, J. D., R. D. Wainford, W. C. Engeland, G. D. Fink and J. W. Osborn (2015). "A novel method of selective ablation of afferent renal nerves by periaxonal application of capsaicin." Am J Physiol Regul Integr Comp Physiol **308**(2): R112-122.

Frame, A. A. and R. D. Wainford (2018). "Mechanisms of altered renal sodium handling in age-related hypertension." Am J Physiol Renal Physiol **315**: F1-F6.

Fu, C., H. Wang, S. Wang, Y. Shi, X. Zhou, K. Sun, J. Chen, X. Song, H. Xue and R. Hui (2008). "Association of beta 1-adrenergic receptor gene polymorphisms with left ventricular hypertrophy in human essential hypertension." Clin Biochem **41**(10-11): 773-778.

Fu, C., H. Wang, S. Wang, Y. Shi, X. Zhou, K. Sun, J. Chen, X. Song, H. Xue and R. Hui (2008). "Association of beta (1)-adrenergic receptor gene polymorphisms with left ventricular hypertrophy in human essential hypertension." Clinical Biochemistry **41**: 773-778.

Fuiano, G., S. Sund, G. Mazza, M. Rosa, A. Caglioti, G. Gallo, G. Natale, M. Andreucci, B. Memoli, L. De Nicola and G. Conte (2001). "Renal hemodynamic response to maximal vasodilating stimulus in healthy older subjects." Kidney Int **59**(3): 1052-1058.

Gentleman, R., V. Carey, D. Bates, B. Bolstad, M. Dettlings, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, J. Gentry, K. Hornik, T. Hothorn, W. Huber, S. Iacus, R. A. Irizarry, F. Leisch, C. Li, M. Maechlers, A. Rossini, G. Sawitzki, C. Smith, G. Smyth, L. Tierney, J. Yang and J. Zhang (2004). "Bioconductor: open software development for computational biology and bioinformatics." Genome Biology **5**(10): R80.

Gillman, M. W., W. B. Kannel and A. Belanger (1993). "Influence of heart rate on mortality among persons with hypertension: The Framingham Study." American Heart Journal **125**(4): 1148-1154.

Gillman, M. W., W. B. Kannel, A. Belanger and R. B. D'Agostino (1993). "Influence of heart rate on mortality among persons with hypertension: the Framingham Study." American Heart Journal **125**(4): 1148-1154.

Glassock, R. J. and C. Winearls (2009). "Ageing and the glomerular filtration rate: Truths and Consequences." Transactions of the American Clinical and Climatological Association **120**: 419-428.

Go, A. S., D. Mozaffarian, V. L. Roger, E. J. Benjamin, J. D. Berry, M. J. Blaha, S. Dai, E. S. Ford, C. S. Fox, S. Franco, H. J. Fullerton, C. Gillespie, S. M. Hailpern, J. A. Heit, V. J. Howard, M. D. Huffman, S. E. Judd, B. M. Kissela, S. J. Kittner, D. T. Lackland, J. H. Lichtman, L. D. Lisabeth, R. H. Mackey, D. J. Magid, G. M. Marcus, A. Marelli, D. B. Matchar, D. K. McGuire, E. R. Mohler, 3rd, C. S. Moy, M. E. Mussolino, R. W. Neumar, G. Nichol, D. K. Pandey, N. P. Paynter, M. J. Reeves, P. D. Sorlie, J. Stein, A. Towfighi, T. N. Turan, S. S. Virani, N. D. Wong, D. Woo, M. B. Turner, C. American Heart Association Statistics and S. Stroke Statistics (2014). "Heart disease and stroke statistics--2014 update: a report from the American Heart Association." Circulation **129**(3): e28-e292.

Guyenet, P. G. (2006). "The sympathetic control of blood pressure." Nat Rev Neurosci **7**(5): 335-346.

Guyton, A. C. (1991). "Blood Pressure Control-Special Role of the Kidneys and Body Fluids." Science **252**(5014): 1813-1816.

Guyton, A. C. and J. E. Hall (2006). Textbook of Medical Physiology Philadelphia, PA, Elsevier Inc.

Haber, E., T. Koerner, L. B. Page, B. Kliman and A. Purnode (1969). "Application of a Radioimmunoassay for Angiotensin I to the Physiologic Measurement of Plasma Renin Activity in Normal Human Subjects." The Journal of Clinical Endocrinology & Metabolism **29**(10): 1349-1355.

Haddy, F. J. (2006). "Role of dietary salt in hypertension." Life Sci **79**(17): 1585-1592.

Hall, J. E., L. Mizelle, D. A. Hildebrandt and M. W. Brands (1990). "Abnormal Pressure Natriuresis: A Cause or Consequence of Hypertension?" Hypertension **15**(6:1): 547-559.

Hamdani, N. and J. van der Velden (2009). "Lack of specificity of antibodies directed against human beta-adrenergic receptors." Naunyn Schmiedebergs Arch Pharmacol **379**(4): 403-407.

Hamet, P., E. Testaert, R. Palmour, P. Larochelle, M. Cantin, J. Gutkowska, Y. Langlois, F. Ervin and J. Tremblay (1989). "Effect of prolonged infusion of ANF in normotensive and hypertensive monkeys." Am J Hypertens **2**(9): 690-695.

Hamet, P., J. Tremblay, S. C. Pang, R. Skuherska, E. Schiffrin, R. Garcia, M. Cantin, J. Genest, R. M. Palmour, F. R. Ervin, S. Martin and R. Goldwater (1986). "Cyclic GMP as Mediator and Biological Marker of Atrial Natriuretic Factor " Journal of Hypertension **4**(supplement 2): S49-S56.

Harrison, M. J. S. (1983). "Age and sex difference in the diet and feeding strategies of the Green monkey, Cercopithecus sabaeus." Animal Behavior **31**: 969-977.

Healy, D. P., P. A. Munzel and P. A. Insel (1985). "Localization of beta1- and beta2-adrenergic receptors in rat kidney by autoradiography." Circ Res **57**: 278-284.

Heidenreich, P. A., J. G. Trogdon, O. A. Khavjou, J. Butler, K. Dracup, M. D. Ezekowitz, E. A. Finkelstein, Y. Hong, S. C. Johnston, A. Khera, D. M. Lloyd-Jones, S. A. Nelson, G. Nichol, D. Orenstein, P. W. Wilson, Y. J. Woo, C. American Heart Association Advocacy Coordinating, C. Stroke, R. Council on Cardiovascular, Intervention, C. Council on Clinical, E. Council on, Prevention, A. Council on, Thrombosis, B. Vascular, C. Council on, C. Critical, Perioperative, Resuscitation, N. Council on Cardiovascular, D. Council on the Kidney in Cardiovascular, S. Council on Cardiovascular, Anesthesia, C. Interdisciplinary Council on Quality of and R. Outcomes (2011). "Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association." Circulation **123**(8): 933-944.

Hendel, M. D. and J. P. Collister (2006). "Renal denervation attenuates long-term hypertensive effects of Angiotensin ii in the rat." Clin Exp Pharmacol Physiol **33**(12): 1225-1230.

Ho, J. E., M. G. Larson, A. Ghorbani, S. Cheng, E. E. Coglianese, R. S. Vasan and T. J. Wang (2014). "Long-term cardiovascular risks associated with an elevated heart rate: the Framingham Heart Study." J Am Heart Assoc **3**(3): e000668.

Hoek, F. J., F. A. Kemperman and R. T. Krediet (2003). "A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate." Nephrol Dial Transplant **18**(10): 2024-2031.

Holt, N. F. and K. L. Haspel (2010). "Vasopressin: a review of therapeutic applications." J Cardiothorac Vasc Anesth **24**(2): 330-347.

Hoy, W. E., R. N. Douglas-Denton, M. D. Hughson, A. Cass, K. Johnson and J. F. Bertram (2003). "A stereological study of glomerular number and volume: preliminary findings in a multiracial study of kidneys at autopsy." Kidney Int Suppl(83): S31-37.

Huang, Y. S., V. Ramensky, S. K. Service, A. J. Jasinska, Y. Jung, O. W. Choi, R. M. Cantor, N. Juretic, J. Wasserscheid, J. R. Kaplan, M. J. Jorgensen, T. D. Dyer, K. Dewar, J. Blangero, R. K. Wilson, W. Warren, G. M. Weinstock and N. B. Freimer (2015). "Sequencing strategies and characterization of 721 vervet monkey genomes for future genetic analyses of medically relevant traits." BMC Biol **13**: 41.

Husdan, H. and A. Rapoport (1967). "Estimation of Creatinine by the Jaffe Reaction." Clinical Chemistry **14**(3): 222-238.

Iaccarino, G., E. Barbato, E. Cipoletta, A. Fiorillo and B. Trimarco (2001). "Role of the sympathetic nervous system in cardiac remodeling in hypertension." Clin Exp Hypertens **23**(1-2): 35-43.

Iemitsu, M., T. Miyauchi, S. Maeda, S. Sakai, T. Kobayashi, N. Fujii, H. Miyazaki, M. Matsuda and I. Yamaguchi (2001). "Physiological and pathological cardiac hypertrophy induce different molecular phenotypes in the rat." Am J Physiol Regul Integr Comp Physiol **281**(6): R2029-2036.

Imai, E., M. Horio, K. Yamagata, K. Iseki, S. Hara, N. Ura, Y. Kiyohara, H. Makino, A. Hishida and S. Matsuo (2007). "Slow decline of glomerular filtration rate in the Japanese general population: a longitudinal 10 year follow up study " Hypertension Research **31**(3): 433-441.

Intengan, H. D. and E. L. Schiffrin (2001). "Vascular Remodeling in Hypertension: Roles of Apoptosis, Inflammation, and Fibrosis." Hypertension **38**(3): 581-587.

Irie, F., H. Iso, T. Sairenchi, N. Fukasawa, K. Yamagishi, S. Ikehara, M. Kanashiki, Y. Saito, H. Ota and T. Nose (2006). "The relationships of proteinuria, serum creatinine, glomerular filtration rate with cardiovascular disease mortality in Japanese general population." Kidney Int **69**(7): 1264-1271.

Isbell, L. A., D. L. Cheney and R. M. Seyfarth (2002). Why vervet monkeys (Cercopithecus aethiops) live in multimale groups. The Guenons: Diversity and Adaptation in African Monkeys. G. a. Cords.

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**(1): 103-124.

Izdebski, E., J. Izdebski, I. Cybulska, M. Makowiecka-Ciesla and A. Trzebski (2006). "Moderate exercise training reduces arterial chemoreceptor reflex drive in mild hypertension." Journal Physiological Pharmacology **57**(Suppl 11): 93-102.

Jacob, F., L. A. Clark, P. A. Guzman and J. W. Osborn (2005). "Role of renal nerves in development of hypertension in DOCA-salt model in rats: a telemetric approach." Am J Physiol Heart Circ Physiol **289**(4): H1519-1529.

Jafar, T. H., P. C. Stark, C. H. Schmid, M. Landa, G. Maschio, P. E. de Jong, D. de Zeeuw, S. Shanhinfar, R. Toto and A. S. Levey (2003). "Progression of Chronic Kidney Disease: The Role of Blood Pressure Control, Proteinuria, and Angiotensin-Converting Enzyme Inhibition A Patient-Level Meta-Analysis." Annals of Internal Medicine **139**: 244-252.

Jannetta, P. J., R. Segal, S. K. Wolfson, Jr., M. Dujovny, A. Semba and E. E. Cook (1985). "Neurogenic hypertension: etiology and surgical treatment. II. Observations in an experimental nonhuman primate model." Ann Surg **202**(2): 253-261.

Jasinska, A. J., M. K. Lin, S. Service, O. W. Choi, J. DeYoung, O. Grujic, S. Y. Kong, Y. Jung, M. J. Jorgensen, L. A. Fairbanks, T. Turner, R. M. Cantor, J. Wasserscheid, K. Dewar, W. Warren, R. K. Wilson, G. Weinstock, J. D. Jentsch and N. B. Freimer (2012). "A non-human primate system for large-scale genetic studies of complex traits." Hum Mol Genet **21**(15): 3307-3316.

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**(2): 122-143.

Jasinska, A. J., S. Service, M. Levinson, E. Slaten, O. Lee, E. Sobel, L. A. Fairbanks, J. N. Bailey, M. J. Jorgensen, S. E. Breidenthal, K. Dewar, T. J. Hudson, R. Palmour, N. B. Freimer and R. A. Ophoff (2007). "A genetic linkage map of the vervet monkey (Chlorocebus aethiops sabaeus)." Mamm Genome **18**(5): 347-360.

Jensen, B. C., P. M. Swigart and P. C. Simpson (2009). "Ten commercial antibodies for alpha-1-adrenergic receptor subtypes are nonspecific." Naunyn Schmiedebergs Arch Pharmacol **379**(4): 409-412.

Johns, E. J. and M. H. Abdulla (2013). "Renal nerves in blood pressure regulation." Curr Opin Nephrol Hypertens **22**(5): 504-510.

Kang, K. T. (2014). "Endothelium-derived Relaxing Factors of Small Resistance Arteries in Hypertension." Toxicol Res **30**(3): 141-148.

Karam, Z. and J. Tuazon (2013). "Anatomic and physiologic changes of the aging kidney." Clin Geriatr Med **29**(3): 555-564.

Kavanagh, K., L. A. Fairbanks, J. N. Bailey, M. J. Jorgensen, M. Wilson, L. Zhang, L. L. Rudel and J. D. Wagner (2007). "Characterization and heritability of obesity and associated risk factors in vervet monkeys." Obesity (Silver Spring) **15**(7): 1666-1674.

Knickman, J. R. and E. K. Snell (2002). "The 2030 problem: caring for aging baby boomers." Health Serv Res **37**(4): 849-884.

Kraft-Schreyer, N. and E. T. Angelakos (1985). "Noninvasive measurement of blood pressure in African Green Monkeys." American Journal of Primatology **9**(4): 285-294.

Kraft-Schreyer, N., H. Kushner and E. T. Angelakos (1987). "Development of a nonhuman primate model for spontaneous hypertension. Blood pressures in first-generation offspring." Hypertension **9**(6 Pt 2): III57-63.

Kraft-Schreyer, N., H. Kushner and E. T. Angelakos (1991). "Discriminant analysis of transmission of elevated blood pressure in first generation offspring of African green monkeys." Clin Exp Hypertens A **13**(1): 35-52.

Krum, H., M. Schlaich, R. Whitbourn, P. A. Sobotka, J. Sadowski, K. Bartus, B. Kapelak, A. Walton, H. Sievert, S. Thambar, W. T. Abraham and M. Esler (2009). "Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study." The Lancet **373**(9671): 1275-1281.

Kundu, M. C., M. C. May, J. Chosich, A. P. Bradford, B. Lasley, N. Gee, N. Santoro, S. E. Appt and A. J. Polotsky (2013). "Assessment of luteal function in the vervet monkey as a means to develop a model for obesity-related reproductive phenotype." Syst Biol Reprod Med **59**(2): 74-81.

Kurien, B. T., N. E. Everds and R. H. Scofield (2004). "Experimental animal urine collection: a review." Lab Anim **38**(4): 333-361.

Kurtz, A. (2011). "Renin release: sites, mechanisms, and control." Annu Rev Physiol **73**: 377-399.

Kushner, H., N. Kraft-Schreyer, E. T. Angelakos and E. M. Wudarski (1982). "Analysis of reproductive data in a breeding colony of African green monkeys." J Med Primatol **11**(2): 77-84.

Lamb, E. J., J. Wood, H. J. Stowe, S. E. O'Riordan, M. C. Webb and R. N. Dalton (2005). "Susceptibility of glomerular filtration rate estimations to variations in creatinine methodology: a study in older patients." Ann Clin Biochem **42**(Pt 1): 11-18.

Lemere, C. A., A. Beierschmitt, M. Iglesias, E. T. Spooner, J. K. Bloom, J. F. Leverone, J. B. Zheng, T. J. Seabrook, D. Louard, D. Li, D. J. Selkoe, R. M. Palmour and F. R. Ervin (2004). "Alzheimer's Disease Aβ Vaccine Reduces Central Nervous System Aβ Levels in a Non-Human Primate, the Caribbean Vervet." The American Journal of Pathology **165**(1): 283-297.

Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-408.

Lopez-Giacoman, S. and M. Madero (2015). "Biomarkers in chronic kidney disease, from kidney function to kidney damage." World J Nephrol **4**(1): 57-73.

Luscher, T. F. (1990). "Imbalance of endothelium-dervied relaxing and contracting factors: A new concept in hypertension?" American Journal of Hypertension **3**: 317-330.

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 to cold pressor stress, captopril administration, and acute bolus of atrial natriuretic factor." American Journal of Hypertension **3**: 27-32.

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." Am J Hypertens **3**(1): 27-32.

McGuire, M. T. (1974). " The History of the St Kitts Vervet " Caribbean Quarterly **20**(2): 37-52.

McKenna, O. C. and E. T. Angelakos (1968). "Adrenergic Innervation of the Canine Kidney." Circulation Research **22**.

Mezzano, S. M., M. Ruiz-Ortega and J. Egido (2001). "Angiotensin II and renal fibrosis." Hypertension **38**(part 2): 635-638.

Michel, M. C., O. E. Brodde and P. A. Insel (1990). "Peripheral adrenergic receptors in hypertension." Hypertension **16**(2): 107-120.

Michel, M. C., O. E. Brodde and P. A. Insel (1990). "Peripheral adrenergic receptors in hypertension." Hypertension **16**: 107-120.

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**(6): 441-450.

Molskness, T. A., D. L. Hess, G. M. Maginnis, J. W. Wright, J. W. Fanton and R. L. Stouffer (2007). "Characteristics and regulation of the ovarian cycle in vervet monkeys (Chlorocebus aethiops)." Am J Primatol **69**(8): 890-900.

Mori, T., A. Polichnowski, P. Glocka, M. Kaldunski, Y. Ohsaki, M. Liang and A. W. Cowley, Jr. (2008). "High perfusion pressure accelerates renal injury in salt-sensitive hypertension." J Am Soc Nephrol **19**(8): 1472-1482.

Muntner, P., J. He, L. Hamm, C. Loria and P. K. Whelton (2002). "Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States." J Am Soc Nephrol **13**(3): 745-753.

Murakami, S., M. Saitoh, T. Kubo, T. Koyama and M. Kobayashi (2000). "Renal disease in women with severe preeclampsia or gestational proteinuria." Obstet Gynecol **96**(6): 945-949.

Nagata, M. and W. Kriz (1992). "Glomerular damage after uninephrectomy in young rats. II. Mechanical stress on podocytes as a pathway to sclerosis." Kidney International **42**(1): 148-160.

Navar, L. G., L. Zou, A. Von Thun, C. Tarng Wang, J. D. Imig and K. D. Mitchell (1998). "Unraveling the Mystery of Goldblatt Hypertension." News Physiol Sci **13**: 170-176.

Norman Jr, R. A. and D. J. Dzielak (1982). "Role of renal nerves in onset and maintenance of spontaneous hypertension." Am J Physiol Heart and Circulatory Physiology **243**(2): H284-H288.

Ofstad, J. and B. M. Iversen (2005). "Glomerular and tubular damage in normotensive and hypertensive rats." Am J Physiol Renal Physiol **288**(4): F665-672.

Okamoto, K., R. Tabei, M. Fukushima, S. Nosaka, Y. Yamori, K. Ichijima, H. Haenara, M. Matsumoto, T. Maruyama, Y. Suzuki and M. Tamegai (1966). "Further Observations of the Development of a Strain of Spontaneously Hypertensive Rats." Japanese Circualtion Journal **30**.

Osborn, J. L., G. F. DiBona and M. D. Thames (1981). "Beta-1 receptor mediation of renin secretion elicited by low-frequency renal nerve stimulation." The Journal of Pharmacology and Experimental Therapeutics **216**(2): 265-269.

Palatini, P. and S. Julius (1996). "Heart rate and the cardiovascular risk." Journal of Hypertension **15**: 3-17.

Palmour, R. M., J. Mulligan, J. J. Howber and F. R. Ervin (1997). "Of monkeys and men: vervets and the genetics of human-like behaviors." American Journal of Human Genetics **61**: 481-488.

Palmour, R. M., J. Mulligan, J. J. Howbert and F. Ervin (1997). "Of monkeys and men: vervets and the genetics of human-like behaviors." Am J Hum Genet **61**(3): 481-488.

Pandrea, I., C. Apetrei, J. Dufour, N. Dillon, J. Barbercheck, M. Metzger, B. Jacquelin, R. Bohm, P. A. Marx, F. Barre-Sinoussi, V. M. Hirsch, M. C. Muller-Trutwin, A. A. Lackner and R. S. Veazey (2006). "Simian immunodeficiency virus SIVagm.sab infection of Caribbean African green monkeys: a new model for the study of SIV pathogenesis in natural hosts." J Virol **80**(10): 4858-4867.

Paton, J. F., P. A. Sobotka, M. Fudim, Z. J. Engelman, E. C. Hart, F. D. McBryde, A. P. Abdala, N. Marina, A. V. Gourine, M. Lobo, N. Patel, A. Burchell, L. Ratcliffe and A. Nightingale (2013). "The carotid body as a therapeutic target for the treatment of sympathetically mediated diseases." Hypertension **61**(1): 5-13.

Pechere-Bertschi, A. and M. Burnier (2004). "Female sex hormones, salt, and blood pressure regulation." Am J Hypertens **17**(10): 994-1001.

Persell, S. D. (2011). "Prevalence of resistant hypertension in the United States, 2003-2008." Hypertension **57**(6): 1076-1080.

Pfeifer, S. P. (2017). "The Demographic and Adaptive History of the African Green Monkey." Mol Biol Evol **34**(5): 1055-1065.

Pinto, V., J. Amaral, E. Silva, S. Simao, J. M. Cabral, J. Afonso, M. P. Serrao, P. Gomes, M. J. Pinho and P. Soares-da-Silva (2011). "Age-related changes in the renal dopaminergic system and expression of renal amino acid transporters in WKY and SHR rats." Mech Ageing Dev **132**(6-7): 298-304.

Poiseuille, J. (1846). "Experimental research on the movement of liquids in tubes of very small diameters." Memoires presentes par divers savants a l'Academie Royale des Sciences de I'Institut de France **IX**: 433-544.

Polderman, K. H., C. D. Stehouwer, G. J. van Kamp, G. A. Dekker, F. W. Verheugt and L. J. Gooren (1993). "Influence of sex hormones on plasma endothelin levels." Ann Intern Med **118**(6): 429-432.

Procino, G., M. Carmosino, S. Milano, M. Dal Monte, G. Schena, M. Mastrodonato, A. Gerbino, P. Bagnoli and M. Svelto (2016). "beta3 adrenergic receptor in the kidney may be a new player in sympathetic regulation of renal function." Kidney Int **90**(3): 555-567.

Rapp, J. P. and H. Dene (1985). "Development and characteristics of inbred strains of Dahl salt sensitive and salt resistant rats." Hypertension **7**(3 Part 1): 340-349.

Reckelhoff, J. F., H. Zhang and J. P. Granger (1998). "Testosterone Exacerbates Hypertension and Reduces Pressure-Natriuresis in Male Spontaneously Hypertensive Rats." Hypertension **31**(1): 435-439.

Reckelhoff, J. F., H. Zhang and K. Srivastava (2000). "Gender Differences in Development of Hypertension in Spontaneously Hypertensive Rats : Role of the Renin-Angiotensin System." Hypertension **35**(1): 480-483.

Remuzzi, G., P. Ruggenenti and A. Benigni (1997). "Understanding the nature of renal disease progression." Kidney International **51**(1): 2-15.

Renna, N. F., N. de Las Heras and R. M. Miatello (2013). "Pathophysiology of vascular remodeling in hypertension." Int J Hypertens **2013**: 808353.

Research, T. F. f. B. (2016). The Critical Role of Nonhuman Primates in Medical Research [white paper].

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**(3): R211-R218.

Rizzi, E., D. A. Guimaraes, C. S. Ceron, C. M. Prado, L. C. Pinheiro, A. Martins-Oliveira, R. F. Gerlach and J. E. Tanus-Santos (2014). "beta1-Adrenergic blockers exert antioxidant effects, reduce matrix metalloproteinase activity, and improve renovascular hypertension-induced cardiac hypertrophy." Free Radic Biol Med **73**: 308-317.

Rogers, J. L., A. R. Mitchell, C. Maric, K. Sandberg, A. Myers and S. E. Mulroney (2007). "Effect of sex hormones on renal estrogen and angiotensin type 1 receptors in female and male rats." Am J Physiol Regul Integr Comp Physiol **292**(2): R794-799.

Salomonsson, M., M. Oker, S. Kim, H. Zhang, J. E. Faber and W. J. Arendshorst (2001). "Alpha1-adrenoceptor subtypes on rat afferent arteriole assessed by radioligand binding and RT-PCR." Am J Physiol Renal Physiol **281**: F172-F178.

Santos, R. A. S., A. C. Simoes e Silva, C. Maric, D. M. R. Silva, R. P. Machado, I. de Buhr, S. Heringer-Walther, S. V. B. Pinheiro, M. T. Lopes, M. Bader, E. P. Mendes, V. S. Lemos, M. J. Campagnole-Santos, H. Schultheiss, R. Speth and T. Walther (2002). "Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas." Proc Natl Acad Sci U S A **100**(14): 8258-8263.

Sarganas, G., A. Schaffrath Rosario and H. K. Neuhauser (2017). "Resting Heart Rate Percentiles and Associated Factors in Children and Adolescents." J Pediatr **187**: 174-181 e173.

Scherzer, P., A. Gal-Moscovici, D. Sheikh-Hamad and M. M. Popovtzer (2015). "Sodium-pump gene-expression, protein abundance and enzyme activity in isolated nephron segments of the aging rat kidney." Physiol Rep **3**(6).

Schindelin, J., C. T. Rueden, M. C. Hiner and K. W. Eliceiri (2015). "The ImageJ ecosystem: An open platform for biomedical image analysis." Mol Reprod Dev **82**(7-8): 518-529.

Schlaich, M. P., D. M. Kaye, E. Lambert, M. Sommerville, F. Socratous and M. D. Esler (2003). "Relation between cardiac sympathetic activity and hypertensive left ventricular hypertrophy." Circulation **108**(5): 560-565.

Schlaich, M. P., F. Socratous, S. Hennebry, N. Eikelis, E. A. Lambert, N. Straznicky, M. D. Esler and G. W. Lambert (2008). "Sympathetic Activation in Chronic Renal Failure." Journal of the American Society of Nephrology **20**(5): 933-939.

Schmitt, C. A., S. K. Service, A. J. Jasinska, T. D. Dyer, M. J. Jorgensen, R. M. Cantor, G. M. Weinstock, J. Blangero, J. R. Kaplan and N. B. Freimer (2017). "Obesity and obesogenic growth are both highly heritable and modified by diet in a nonhuman primate model, the African green monkey (Chlorocebus aethiops sabaeus)." Int J Obes (Lond).

Schneider, C. A., W. S. Rasband and K. W. Eliceiri (2012). "NIH Image to ImageJ: 25 years of image analysis." Nature Methods **9**(7): 671-675.

Seccareccia, F., F. Pannozzo, F. Dima, A. Minoprio, A. Menditto, C. Lo Noce and S. Giampaoli (2001). "Heart Rate as a Predictor of Mortality: The MATISS Project." American Journal of Public Health **91**(8).

Seyfarth, R. M. and D. L. Cheney (1984). "Grooming, alliances and reciprocal altruism in vervet monkeys." Nature **308**(5959): 541-543.

Seyfarth, R. M. and D. L. Cheney (2003). The structure of social knowledge in monkeys. Animal Social Complexity: Intelligence, culture, and individualized societies. F. B. M. De Waal and P. L. Tyack, Harvard University Press.

Shemesh, O., H. Golbetz, J. P. Kriss and B. D. Myers (1985). "Limitations of creatinine as a filtration marker in glomerulopathic patients." Kidney International **28**(5): 830-838.

Shimokama, T., S. Haraoka, H. Horiguchi, F. Sugiyama, K. Murakami and T. Watanabe (1998). "The Tsukuba hypertensive mouse (transgenic mouse carrying human genes for both renin and angiotensinogen) as a model human malignant hypertension: development of lesions and morphometric analysis." Virchows Archive **432**(169).

Shively, C. A., S. L. Willard, T. C. Register, A. J. Bennett, P. J. Pierre, M. L. Laudenslager, D. W. Kitzman, M. K. Childers, R. W. Grange and S. B. Kritchevsky (2012). "Aging and physical mobility in group-housed Old World monkeys." Age (Dordr) **34**(5): 1123-1131.

Sillen-Tulberg, B. and A. P. Moller (1993). "The relationship between concealed ovulation and mating systems in anthropoid primates: a phylogenetic analysis." The American Naturalist **141**(1): 1-25.

Singh, I. and S. Linas (1996). "beta2-Adrenergic function in cultured rat proximal tubule epithelial cells." Am J Physiol Renal Physiol **271**(1): F71-F77.

Skov, K., P. Hamet, J. R. Nyengaard and M. Mulvany (2001). "Morphology of renal afferent arterioles and glomeruli, heart weight, and blood pressure in primates." American Journal of Hypertension **14**: 331-337.

Skov, K., P. Hamet, J. R. Nyengaard and M. J. Mulvany (2001). "Morphology of renal afferent arterioles and glomeruli, heart weight, and blood pressure in primates." Am J Hypertens **14**(4 Pt 1): 331-337.

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**: 792-796.

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 amoung African Green Monkeys." American Journal of Clinical Nutrition **39**: 792-796.

Stachenfeld, N. S. (2008). "Acute effects of sodium ingestion on thirst and cardiovascular function." Curr Sports Med Rep **7**(4 Suppl): S7-13.

Steklis, H. D., G. L. Brammer, M. J. Raleigh and M. T. McGuire (1985). "Serum testosterone, male dominance, and aggression in captive groups of vervet monkeys (Cercopithecus aethiops sabaeus)." Horm Behav **19**(2): 154-163.

Storsberg, A. D. (1993). "Structure, function, and regulation of adrenergic receptors." Protein Science **2**: 1198-1209.

Sullivan, J. C. (2008). "Sex and the renin-angiotensin system: inequality between the sexes in response to RAS stimulation and inhibition." Am J Physiol Regul Integr Comp Physiol **294**(4): R1220-1226.

Sun, Z. (2015). "Aging, arterial stiffness, and hypertension." Hypertension **65**(2): 252-256.

Svardal, H., A. J. Jasinska, C. Apetrei, G. Coppola, Y. Huang, C. A. Schmitt, B. Jacquelin, V. Ramensky, M. Muller-Trutwin, M. Antonio, G. Weinstock, J. P. Grobler, K. Dewar, R. K. Wilson, T. R. Turner, W. C. Warren, N. B. Freimer and M. Nordborg (2017). "Ancient hybridization and strong adaptation to viruses across African vervet monkey populations." Nat Genet **49**(12): 1705-1713.

Symplicity, H. T. N. I., M. D. Esler, H. Krum, P. A. Sobotka, M. P. Schlaich, R. E. Schmieder and M. Bohm (2010). "Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial." Lancet **376**(9756): 1903-1909.

Taddei, S., A. Virdis, P. Mattei, L. Ghiadoni, A. Gennari, C. B. Fasolo, I. Sudano and A. Salvetti (1995). "Aging and endothelial function in normotensive subjects and patients with essential hypertension." Circulation **91**: 1981-1987.

Tanaka, H., F. A. Dinenno, K. D. Monahan, C. M. Clevenger, C. A. DeSouza and D. R. Seals (2000). "Aging, Habitual Exercise, and Dynamic Arterial Compliance." Circulation **102**(11): 1270-1275.

Tanase, H., Y. Yamori, C. T. Hansen and W. Lovenberg (1982). "Heart size in inbred strains of rats." Hypertension **4**: 864-872.

Thayer, J. F., S. S. Yamamoto and J. F. Brosschot (2010). "The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors." Int J Cardiol **141**(2): 122-131.

Thrasher, T. N. (2002). "Unloading arterial baroreceptors causes neurogenic hypertension." American Journal of Physiology: Regulatory Intergrative Comparative Physiology(282): R1044-R1053.

Toeh, H., A. Quan, S. W. S. Leung and R. Y. K. Man (2000). "Differential effects of 17beta-estradiol and testosterone on the contractile responses of porcine coronary arteries." British Journal of Pharmacology **129**: 1301-1308.

Tostes, R. C., Z. B. Fortes, G. E. Callera, A. C. Montezano, R. M. Touyz, R. C. Webb and M. H. Carvalho (2008). "Endothelin, sex and hypertension." Clin Sci (Lond) **114**(2): 85-97.

Townsend, R. R., F. Mahfoud, D. E. Kandzari, K. Kario, S. Pocock, M. A. Weber, S. Ewen, K. Tsioufis, D. Tousoulis, A. S. P. Sharp, A. F. Watkinson, R. E. Schmieder, A. Schmid, J. W. Choi, C. East, A. Walton, I. Hopper, D. L. Cohen, R. Wilensky, D. P. Lee, A. Ma, C. M. Devireddy, J. P. Lea, P. C. Lurz, K. Fengler, J. Davies, N. Chapman, S. A. Cohen, V. DeBruin, M. Fahy, D. E. Jones, M. Rothman, M. Böhm, J. Aoki, B. Batson, M. Böhm, J. W. Choi, D. L. Cohen, G. Dangas, S. David, J. Davies, C. M. Devireddy, D. Kandzari, K. Kario, D. P. Lee, P. C. Lurz, M. Patel, K. Patel, R. E. Schmieder, A. S. P. Sharp, J. Singh, K. Tsioufis, A. Walton, T. Weber, J. Weil, T. Zeller, K. Ziada, K. Tanabe, R. Wilkins, F. Mahfoud, C. East, R. Wilensky, J. Contreras, S. Steigerwalt, N. Chapman, J. P. Lea, D. Reedus, S. Hoshide, A. Ma, K. Fengler, L. Svetkey, A. Rao, A. Schmid, A. F. Watkinson, A. Brown, D. Tousoulis, I. Hopper, M. Suppan, T. Agdirlioglu, E. Noory and C. Chasen (2017). "Catheter-based renal denervation in patients with uncontrolled hypertension in the absence of antihypertensive medications (SPYRAL HTN-OFF MED): a randomised, sham-controlled, proof-of-concept trial." The Lancet **390**(10108): 2160-2170.

Van Der Kuyl, A. C., J. T. Dekker and J. Goudsmit (1996). "St. Kitts Green Monkeys Originate From West Africa: Genetic Evidence From Feces." American Journal of Primatology **40**: 361-364.

Vanderheyden, P. M. (2009). "From angiotensin IV binding site to AT4 receptor." Mol Cell Endocrinol **302**(2): 159-166.

Verhaaren, H. A., R. M. Schieken, M. Mosteller, J. K. Hewitt, L. J. Eaves and W. E. Nance (1991). "Bivariate genetic analysis of left ventricular mass and weight in pubertal

twins (the Medical College of Virginia Twin Study)." American Journal of Cardiology **68**: 661-668.

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**(12): 1921-1933.

Weingrill, T., E. P. Willems, M. Krützen and R. Noë (2010). "Determinants of Paternity Success in a Group of Captive Vervet Monkeys (Chlorocebus aethiops sabaeus)." International Journal of Primatology **32**(2): 415-429.

Weinstein, J. R. and S. Anderson (2010). "The aging kidney: physiological changes." Adv Chronic Kidney Dis **17**(4): 302-307.

Whelton, P. K., R. M. Carey, W. S. Aronow, D. E. Casey, Jr., K. J. Collins, C. Dennison Himmelfarb, S. M. DePalma, S. Gidding, K. A. Jamerson, D. W. Jones, E. J. MacLaughlin, P. Muntner, B. Ovbiagele, S. C. Smith, Jr., C. C. Spencer, R. S. Stafford, S. J. Taler, R. J. Thomas, K. A. Williams, Sr., J. D. Williamson and J. T. Wright, Jr. (2018). "2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines." Hypertension **71**(6): e13-e115.

Worobey, M., P. Telfer, S. Souquiere, M. Hunter, C. A. Coleman, M. J. Metzger, P. Reed, M. Makuwa, G. Hearn, S. Honarvar, P. Roques, C. Apetrei, M. Kazanji and P. A. Marx (2010). "Island biogeography reveals the deep history of SIV." Science **329**(5998): 1487.

Yoon, S. S., C. D. Frayer and M. D. Carroll (2015). Hypertension prevalence and control among adults: United States, 2011-2014. . NCHS data brief. Hyattsville, MD, National Center for Health Statistics. **220**.

Yugandhar, V. G. and M. A. Clark (2013). "Angiotensin III: a physiological relevant peptide of the renin angiotensin system." Peptides **46**: 26-32.

Zaman, M. A., S. Oparil and D. A. Calhoun (2002). "Drugs targeting the renin-angiotensin-aldosterone system." Nat Rev Drug Discov **1**(8): 621-636.

Zimmerman, M. A. and J. C. Sullivan (2013). "Hypertension: what's sex got to do with it?" Physiology (Bethesda) **28**(4): 234-244.

Zimmerman, M. C., E. Lazartigues, R. V. Sharma and R. L. Davisson (2004). "Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system." Circ Res **95**(2): 210-216.

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PEER REVIEWED PUBLICATIONS

1. Franklin, B. M., Xiang, L., Collett, J. A., **Rhoads, M. K.**, & Osborn, J. L. (2015) Open Problem-based Instruction Impacts Understanding of Physiological Concepts Differently in Undergraduate Students. *Advances in Physiology Education, 39(4),* 327-334

2. **Rhoads, M. K.**, Goleva, S. B., Beierwaltes, W. H., & Osborn, J. L. (2017). Renal Vascular and Glomerular Pathologies Associated with Spontaneous Hypertension in the Non-human Primate Chlorocebus aethiops sabaeus. *American Journal of Physiology- Regulatory, Comparative, and Integrative Physiology, 313(3),* R211-R218

PUBLISHED ABSTRACTS

1. **Megan K Rhoads***, Caitlin Morrisey, Lexi Sunnenberg, and Jeffrey L Osborn. "Differential Impacts of Environmental Salinity on *Procambarus clarkii* and *Orconectes rusticus*" Experimental Biology, Boston, MA April 2013.

2. **Megan K Rhoads***, Amy Beierschmitt, Denise Huggins, Jason A Collett, and Jeffrey L Osborn. "Spontaneous essential hypertension in the non-human primate *Chlorocebus aethiops sabaeus*, the African Green Monkey" Experimental Biology, San Diego, CA April 2014

3. **Megan K Rhoads***, Slavina B Goleva, Amy Beierschmitt, and Jeffrey L Osborn. "Altered Renal and Cardiac Morphology in a Non-Human Primate of Spontaneous Essential Hypertension" High Blood Pressure Research Scientific Sessions, San Francisco, CA September 2014

4. **Megan K Rhoads***, Slavina B Goleva, Amy Beierschmitt, and Jeffrey L Osborn. "Spontaneous Hypertension and Cardiorenal Pathologies in the African Green Monkey" Experimental Biology, Boston, MA March 2015

5. **Megan K Rhoads*** and Jeffrey L Osborn. "Specific Renal Outer Medulla Upregulation of α- and β- adrenergic receptors in Spontaneously Hypertensive African Green Monkeys" Council on Hypertension Scientific Sessions, Washington D.C. September 2015

6. **Megan K Rhoads*** and Jeffrey L Osborn. "Increased Cardiac and Renal Adrenergic Receptor Expression in Spontaneously Hypertensive Caribbean Vervets." Council on Hypertension Scientific Sessions, Orlando, FL September 2016

7. **Megan K Rhoads***, Lin Xiang, Brandon M Franklin, and Jeffrey L Osborn. "Problem-based Learning Increases Motivation and Learning Strategy Use in Both Low- and High-achieving Students in an Upper-level Undergraduate Physiology Course." Experimental Biology, Chicago, IL April 2017

8. **Megan K Rhoads***, Chelsea C Weaver and Jeffrey L Osborn. “Sex and Hypertension in the African Green Monkey, a Translational Model of Human Cardiovascular Disease.” Experimental Biology, Chicago, IL April 2017

9. **Megan K Rhoads*** and Jeffrey L Osborn. “Prevalence of Spontaneous Hypertension in African Green Monkeys is Age-related.” Council on Hypertension Scientific Sessions, San Francisco, CA September 2017

10. **Megan K Rhoads*** and Jeffrey L Osborn. “Hypertensive and Pre-Hypertensive African Green Monkeys Display Increased Proteinuria.” Experimental Biology, San Diego, CA April 2018

11. **Megan K Rhoads***, Chelsea C Weaver, and Jeffrey L Osborn. “APOL1-like gene insertion associated with increased blood pressure in the African Green Monkey.” Experimental Biology, San Diego CA April 2018