

https://dspace.org/

Sexual dimorphism and renin-angiotensin-aldosterone system (RAAS) in hypertension and left ventricular hypertrophy in Spontaneously Hypertensive Rat (SHR)

Selebano, Kopano

2024

University of the Witwatersrand, Johannesburg

https://hdl.handle.net/10539/46948

Selebano, Kopano . (2024). Sexual dimorphism and renin-angiotensin-aldosterone system (RAAS) in hypertension and left ventricular hypertrophy in Spontaneously Hypertensive Rat (SHR) [Master`s dissertation, University of the Witwatersrand, Johannesburg]. WIReDSpace. https://hdl.handle.net/10539/46948

Downloaded from a DSpace repository. https://dspace.org/

Sexual dimorphism and renin-angiotensin-aldosterone system (RAAS) in hypertension and left ventricular hypertrophy in Spontaneously Hypertensive Rat (SHR)

Kopano Selebano

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine

Johannesburg, South Africa,

2024

Declaration

I, Kopano Selebano, declare that this dissertation is my own, except to the extent indicated in the contribution and acknowledgements sections. It is being submitted for the Degree of Master of Science in Medicine in the School of Physiology, Faculty of Health Sciences, at the University of the Witwatersrand, Johannesburg. The work contained in this dissertation has not been submitted before any degree or examination at any other university. I hereby certify that the studies contained in this dissertation have been approved by the Animal Ethics Research Committee, University of the Witwatersrand, Johannesburg.



Kopano Selebano

Signed on 27 day of March 2024.





Assoc Prof Frédéric Michel

Dr. Lebogang Palesa Mokotedi

Dedication

I dedicate this dissertation to my parents with profound gratitude and love. I thank them for their boundless support and unwavering love, this dissertation is a testament of their support and faith in me.

Abstract

Background: The renin-angiotensin-aldosterone system (RAAS) regulates blood pressure and is linked to hypertensive left ventricular hypertrophy. Sex differences in the involvement of the RAAS in the development of hypertension and left ventricular hypertrophy are unclear, specifically in spontaneously hypertensive rats (SHRs). This study aimed to investigate the sexual difference in the RAAS effects on hypertension and ventricular remodelling in spontaneously hypertensive rats (SHRs).

Methods: Thirty SHRs and Wistar Kyoto rats (WYKs) were assigned to four groups, namely; male SHR (n=8), female SHR (n=8), male WKY (n=7), and female WKY (n=7). Body weight, blood pressure and echocardiography parameters were measured before termination. Concentrations of RAAS parameters were measured using enzyme-linked immunosorbent assays (ELISA) at termination (after 7 months). The picrosirius red stain was used to determine collagen content in the left ventricle.

Results: SHRs, in comparison to WKYs, had significantly higher blood pressure, greater heart and left ventricular mass, greater heart wall thickness, greater area of collagen and impaired left ventricular relaxation (reduced lateral e'), and increased filling pressures (increased E/e') ($p<0.05$). SHRs also had significantly reduced end-diastolic volume, stroke volume and mid-wall fractional shortening ($p<0.05$). Females, in comparison to males, had reduced end-diastolic volume, stroke volume and mid-wall fractional shortening but had greater physiological growth ($p<0.05$). Female SHRs exhibited higher conscious and anaesthetised systolic and diastolic blood pressures, along with greater plasma concentrations of angiotensin II (ANG II) compared to other groups ($p<0.05$).

Conclusion: Compared to WKYs, SHRs developed concentric hypertrophy, impaired diastolic and systolic function. Compared to males, females developed greater physiological left ventricular growth with lower left ventricular relaxation and systolic function. The physiological cardiac differences may partly be influenced by factors such as body weight and blood volume. Additionally, female SHRs had elevated blood pressure, which may be due to increased plasma concentrations of ANG II.

Acknowledgements

To my supervisors, Associate Professor Frédéric Michel and Dr. Lebogang Palesa Mokotedi. I am immensely grateful for the opportunity I have been afforded to pursue the degree. Your patience, guidance, encouragement and support is what has truly carried me throughout this journey. I thank you for imparting your knowledge and research expertise for the betterment of my research skillset. I am grateful to be exposed to research at this level. I thank you for holding me accountable and inspiring me to unlock my potential and become a better version of myself.

I would like to further thank the Intergrated Molecular Physiology Research Initiative for knowledge and access to equipment. I wish to express my gratitude to the staff at the Wits Research Animal Facility for their support in the maintenence and welfare of the animals.

Lastly to my parents who have always reminded me of the importance of seeing things through, thank you for your great support. Your words of encouragement and affirmations kept me going. To my sister who has been an academic role model to me, thank you for the drive and showing me that it is possible to achieve the goals I set out for myself.

Table of Contents

Declaration ii

Dedication iii

Abstract iv

Acknowledgements vi

Table of Contents vii

List of Figures x

List of Tables xi

Abbreviations xii

Chapter 1: Introduction & Study Rationale 1

Chapter 2: Literature Review 4

2.1. Hypertension 5

2.1.1 Role of oestrogen in hypertension 6

2.1.2 Role of testosterone in hypertension 7

2.1.3 Role of renin-angiotensin-aldosterone in hypertension 9

2.1.4 Sex differences in the role of the renin-angiotensin-aldosterone system in hypertension 12

2.2. Left ventricular hypertrophy 14

2.2.1 Role oestrogen in left ventricular hypertrophy 16

2.2.2 Role of testosterone in left ventricular hypertrophy 18

2.2.3 Role of the renin-angiotensin-aldosterone system in left ventricular 19

2.2.4 Sex differences in role of the renin-angiotensin-aldosterone system in ... 21

2.3. Aim and objectives 39

Chapter 3: Methods 40

3.1. Experimental design 41

3.2. Body weight measurements 43

3.3. Non-invasive measurement of blood pressure (NIBP) 43

3.4. Urine sampling 43

3.5. Echocardiography 44

3.6. Blood and organ sampling 48

3.7. Enzyme-linked immunosorbent assays 48

3.8. Histology (quantification of fibrosis) 49

3.9. Statistical Analysis 49

Chapter 4: Results 51

4.1. Characterisation of experimental and control groups 52

4.2. Left ventricular geometry and systolic function 58

4.3. Left ventricular diastolic function 65

4.4. Systemic and urinary concentrations of RAAS components 67

4.5. Angiotensin II concentrations 69

Chapter 5: Discussion 71

5.1. Effects of hypertension on cardiac remodelling 72

5.2. Effects of hypertension on diastolic function 74

5.3. Effects of hypertension on systolic function 75

5.4. Effects of sex on cardiac remodelling 77

5.5. Effects of sex on diastolic function 79

5.6. Effects of sex on systolic function 81

5.7. Effects of strain and sex on blood pressure and the role of the ANG II..... 82

Chapter 6: Limitations and Conclusion..... 86

6.1. Limitations..... 87

6.2. Conclusion 88

Appendices 112

Appendix A: Animal ethics clearance certificate 113

List of Figures

Figure 3.1: Experimental design to address the role of the RAAS in the sexual dimorphism related to the development of hypertension and left ventricular hypertrophy in SHR.....42

Figure 3.2: Example of 2-D M-mode image illustrating measurements of the LV dimensions. 44

Figure 3.3: Example of the early diastolic filling velocity (E) and late diastolic filling (A) pulse wave trans mitral-valve Doppler image. 45

Figure 3.4: Example of the early diastolic mitral annulus velocity (e'), late diastolic annulus velocity (a') and peak systolic velocity (s') echocardiography image illustrating tissue Doppler image obtained from the mitral annulus.....46

Figure 4.1: Left ventricular weight (A) and normalised left ventricular weight (B) of 7-month-old male and female SHR and WKY. 56

Figure 4.2: Left ventricular collagen area fraction of 7-month-old male and female SHR and WKY groups. 57

Figure 4.3: Left ventricular internal diameter in diastole of 7-month-old male and female SHR and WKY. 62

Figure 4.4:Left ventricular posterior wall thickness in diastole of 7-month-old male and female SHR and WKY. 63

Figure 4. 5: Relative wall thickness of 7-month-old male and female SHR and WKY. ..... …64

Figure 4. 6: Concentration of angiotensin II in 7-month-old male and female SHR and WKY. 70

List of Tables

Table 2.1: Sex differences in the development of hypertension in SHR..... 25

Table 2.2: Sex differences in the development of left ventricular hypertrophy in SHR ..... …37

Table 4.1: Characteristics of 7-month-old male and female SHR and WKY..... 54

Table 4.2: Left ventricular geometry and systolic function of 7-month-old male and female SHR or WKY..... 60

Table 4.3: Left ventricular diastolic parameters in 7-month-old male and female SHR or WKY..... 66

Table 4.4: Systemic and urinary concentrations of RAAS components in 7-month-old male and female SHR or WKY..... 68

Abbreviations

A	Maximum late mitral inflow velocity
a'	Late peak tissue lengthening velocity
ACE	Angiotensin converting enzyme
ACE 1	Angiotensin converting enzyme 1
ACE 2	Angiotensin converting enzyme
AGT	Angiotensinogen
ANG I	Angiotensin I
ANG II	Angiotensin II
ANG (1-7)	Angiotensin (1-7)
ANG (1-12)	Angiotensin (1-12)
ANF	Atrial natriuretic factor
ANOVA	Analysis of variance
ATPase	Adenosine triphosphatase
AT_1R	Angiotensin II type 1 receptor
AT_2R	Angiotensin II type 2 receptor

BH_4	Tetrahydrobiopterin
Ca^{2+}	Calcium
cGMP-PKG	Cyclic phosphate-protein kinase G
cm/s	Centimetres per second
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DD	Diastolic dysfunction
E	Maximum early mitral inflow velocity
e'	Early peak tissue lengthening velocity
e'/a'	Early to late mitral annular diastolic tissue lengthening velocity ratio
E/A	Early to late diastolic filling velocity ratio
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
E/e'	Left ventricular filling pressure
EF	Ejection fraction

eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assays
ENaC	Epithelial sodium channel
endFS	Endocardial fractional shortening
ERα	Oestrogen receptor α
ERβ	Oestrogen receptor β
ERK	Extracellular regulated protein kinase
ET-1	Endothelin-1
HDAC	Histone deacetylase proteins
IGF-1	Insulin like growth factor 1
IL-6	Interleukin-6
IVSDd	Interventricular septum diameter in diastole
IVSDs	Interventricular septum diameter in systole
L-NAME	L-Nitroarginine methyl ester
LV	Left ventricle
LVH	Left ventricular hypertrophy

LVEDV	Left ventricular end-diastolic volume
LVESV	Left ventricular end-systolic volume
LVIDd	Left ventricular internal diameter in diastole
LVIDs	Left ventricular internal diameter in systole
LVM	Left ventricular mass
LVPWTd	Left ventricular posterior wall thickness in diastole
LVPWTs	Left ventricular posterior wall thickness in systole
MAP	Mean arterial pressure
MAPK	Mitogen-activated protein kinase
MasR	Mas receptor
midFS	Mid-wall fractional shortening
MMP	Matrix metalloproteinases
mRNA	Messenger ribonucleic acid
mTORC1	Rapamycin complex 1
Na^+	Sodium

NaCl	Sodium chloride
NE	Norepinephrine
NF-κB	Nuclear factor kappa B
ng/mL	Nanograms per millilitre
NIBP	Non-invasive blood pressure
NO	Nitric oxide
NOX	Nicotinamide adenine dinucleotide phosphate oxidases
pg/mL	Picograms per millilitre
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
RWT	Relative wall thickness
s'	Peak systolic velocity
SBP	Systolic blood pressure
SERCA	Sarcoplasmic reticulum adenosine triphosphatase
SHR	Spontaneously Hypertensive Rat
SNS	Sympathetic nervous system

SOD	Superoxide dismutase
STE	Speckle Tracking Echocardiography
SV	Stroke volume
TDI	Tissue Doppler Imaging
TGF-β	Transforming growth factor β
TNF-α	Tumour necrosis factor
VSMC	Vascular smooth muscle cells
WKY	Wistar-Kyoto Rat
WRAF	Wits Research Animal Facility

Chapter 1: Introduction & Study Rationale

Hypertension and left ventricular hypertrophy are significant risk factors for cardiovascular morbidity and mortality worldwide (Yildiz et al., 2020). While extensive research has been conducted to understand the pathophysiology of these conditions, there remains a notable gap in our understanding of the role of sexual dimorphism and the renin-angiotensin-aldosterone system (RAAS) in the development and progression of hypertension and left ventricular hypertrophy, particularly in the context of spontaneously hypertensive rats (SHRs). Previous studies have demonstrated differences in blood pressure regulation, cardiac structure, and function between male and female SHRs (Al-Gburi et al., 2017; Chan et al., 2011), suggesting the existence of sex-specific factors that may modulate the cardiovascular response to hypertension. Additionally, the RAAS has been implicated in the pathogenesis of hypertension and left ventricular hypertrophy (Radin et al., 2002), yet its precise involvement and potential sexual dimorphism remain incompletely understood.

Given the pronounced differences in blood pressure and cardiac parameters observed between male and female SHRs, as well as between SHRs and Wistar Kyoto rats (WKYs), there arises a need to elucidate the underlying mechanisms driving sexual dimorphism on the involvement of the RAAS in hypertension and left ventricular hypertrophy. Therefore, the study aims to explore the potential impact of sex-specific factors such as RAAS on the development and progression of hypertension and left ventricular hypertrophy, with a focus on explaining any differential effects on diastolic function and systolic performance between male and female SHRs and WKYs.

By addressing these research questions, the study aims to advance our understanding of the complex interplay between sex, the RAAS, and cardiovascular pathophysiology, ultimately informing the development of more targeted and effective

therapeutic strategies for the management of hypertension and left ventricular hypertrophy in function of sex.

This dissertation commences by detailing the existing literature in Chapter 2, providing a comprehensive overview of the research relating to sexual dimorphism and the RAAS in the context of hypertension and left ventricular hypertrophy. Chapter 3 outlines the methodological approaches utilised in the present study, while Chapter 4 outlines the results from these methodologies. Within Chapter 5, the findings will be contextualised and analysed in light of the existing literature, and Chapter 6 will underscore the limitations and draw conclusions from this study.

Chapter 2: Literature Review

2.1. Hypertension

Hypertension is a public health challenge in both developed and developing countries (Mohsen Ibrahim, 2018; Parati et al., 2021; Wierzejska et al., 2020). Hypertension is defined by the insistent chronic rise in blood pressure where the systolic pressure is greater or equal to 140 mmHg and/or where the diastolic pressure is greater or equal to 90 mmHg (Koracevic et al., 2023). Hypertension is the prime causative factor for cardiovascular diseases (CVD) such as heart failure (Boehme et al., 2018) and is recognised as one of the leading causes of mortality worldwide (McNamara et al., 2019). Globally, hypertension is considered to be the leading risk factor for death, with the death toll being 10.8 million in 2019 (Schutte et al., 2023). Hypertension is a major cause of early mortality in sub-Saharan Africa (Sharma et al., 2021). In particular, South Africa is the most affected of the sub-Saharan countries with a prevalence that has risen from 21% in 1998 to 77.3% in 2008 amongst individuals whose age fell under the 15 - 98 years range (Ntuli et al*.,* 2015 ; Sharma et al., 2021). In South Africa, hypertension-related mortality rates are estimated to contribute to 9% of all deaths, indicating the considerable burden of hypertension (Diallo et al., 2021). Therefore focusing on the fundamental causes and understanding the mechanisms that contribute to the development of hypertension is important to improve treatment strategies and the management of hypertension.

Hypertension can be classified as either primary (idiopathic) or secondary (with known etiology) hypertension (Brouwers et al., 2021). Primary hypertension is a multifactorial disease due to its numerous causative factors which are modifiable and non-modifiable. Modifiable risk factors include urbanisation, physical inactivity, unhealthy diets, psychosocial stress and alcohol consumption (Brouwers et al., 2021; Vijaykumar et al., 2023). Non-modifiable risk factors include ethnicity, sex and age, with sex having

an especially strong correlation to the control of hypertension (Vijaykumar et al., 2023). Furthermore, there are multiple molecular mechanisms associated with hypertension such as inflammation (Brouwers et al., 2021). These mechanisms contribute to the damage of vascular endothelial and smooth muscle cells which all result in hypertension. Males in both clinical and experimental studies (as shown in table 2.1) typically have higher blood pressures than females (Colafella & Denton, 2018; Maris et al., 2005; Sandberg & Ji, 2012 Sullivan et al., 2010). Adult males have a 27% risk of developing hypertension, whereas adult females have a 12% risk (Anna et al., 2016). In addition, premenopausal women are protected from cardiovascular comorbidities associated with the development of hypertension compared to age-matched males (Xue et al., 2013). This is due to gonadal hormones namely, oestrogen and testosterone.

2.1.1 Role of oestrogen in hypertension

Experimental studies show that the progression of hypertension is delayed in female rats. This is due to oestrogen intercepting with mechanisms that are involved in hypertension (Velásquez, 2018). The same phenomenon can be seen clinically as premenopausal women have lower blood pressures than age matched men and postmenopausal women (Maranon & Reckelhoff, 2013). However, postmenopausal women have higher adverse cardiovascular outcomes than age-matched males (Colafella & Denton, 2018).

Oestrogen is involved in hypertension directly by acutely inhibiting vasoconstriction and indirectly through modulation of mechanisms involved in the presentation of hypertension (Khalil, 2015). Oestrogen acts through two receptors namely, oestrogen receptor α (ERα) and β (ERβ) (Pabbidi et al., 2018). Binding of oestrogen to ERβ mediates vasodilation through NO production and endothelin-1 (ET-1) inhibition, while

binding to ERα is involved in protection against vascular damage (Pabbidi et al., 2018). Oestrogen counteracts the growth of vascular smooth muscle cells (VSMC) mediated by ANG II (Khalil, 2015). Increased growth of VSMC reduces vessel lumen which in turn increases pressure. Furthermore, oestrogen counteracts ANG II-induced hypertensive effects (Aryan et al., 2020). Oestrogen also acts as an antioxidant as it inhibits nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and production of ROS and free radicals (Khalil, 2015). Oestrogen increases β-adrenergic receptor expression in female rats (Riedel et al., 2019). An increase in β-adrenergic stimulation induces vasodilation in females (Riedel et al., 2019). It can control vessel response to β-adrenergic stimulation and therefore bring about dilation (Riedel et al., 2019). Administration of oestrogen to male and female gonadectomised rats increased baroreflex sensitivity and reduced sympathetic activation and lowered blood pressure (Boese et al., 2017). Additionally, no change in SNS activity or blood pressure is seen with an infusion of oestrogen in intact male rats (Boese et al., 2017).

Furthermore, oestrogen hyperpolarises VSMC and causes an efflux of calcium (Ca^{2+}) out of the cell and bringing about relaxation of VSMC (Khalil, 2015). Nuclear factor kappa B (NFκB) controls numerous inflammatory pathways that mediate hypertension such as activation of tumour necrosis factor α (TNFα) and interleukin-6 (IL-6) (Sabbatini & Kararigas, 2020). Oestrogen inhibits NFκB and its subunits which results in inflammatory pathways not being activated (Sabbatini & Kararigas, 2020).

2.1.2 Role of testosterone in hypertension

While oestrogen drives the cardiovascular system away from hypertension and protects the vasculature, testosterone does the opposite (Costa et al., 2015). Testosterone and androgen receptors are correlated to an increase in blood pressure as well as vessel collagen deposition (Seachrist et al., 2000). In experimental models

of hypertension, testosterone increases blood pressure in hypertensive male rats and increases arterial stiffens in females (Loh & Salleh, 2017). Testosterone is positively correlated with blood pressure, as levels of plasma testosterone increase so does blood pressure (Loh & Salleh, 2017). Administration of testosterone to ovariectomised female SHRs and WKYs increases blood pressure (Loh & Salleh, 2017). Ovariectomised female rats reflect postmenopausal women. Additionally, castration reduces blood pressure in SHR males and other models of hypertension (Kienitz & Quinkler, 2008). Furthermore, infusion of testosterone to castrated male SHR reverses effects (Kienitz & Quinkler, 2008).

Testosterone acts as a prooxidant and contributes to deleterious effects of the cardiovascular system (Tostes et al., 2016). Testosterone increases levels of oxidative stress through activation of NADPH oxidase by ANG II and exacerbates endothelial dysfunction (Cruz-Topete et al., 2020; Zhang et al., 2023). Infusion of testosterone to ovariectomised female SHR treated with oestrogen increases ROS (Costa et al., 2015). Furthermore, testosterone also contributes to hypertension though the induction of ET-1 release (Seachrist et al., 2000). This causes vasocontraction and chronically leads to hypertension.

Reckelhoff & Granger, 2015 showed that in intact and gonadectomised male and female SHR of the same age, testosterone administration increases blood pressure of ovariectomised female SHR to levels similar to intact male SHR Furthermore, pressure-natriuresis is reduced in intact male SHR and the same phenomenon is seen in female SHR receiving testosterone (Reckelhoff & Granger, 2015). Blunting of pressure-natriuresis increases blood volume and causes hypertension (Reckelhoff & Granger, 2015).

While the influence of sex hormones play a significant role in the development of hypertension, studies have shown that inappropriate activation of the RAAS is also an important contributor to the pathogenesis of hypertension (Velez, 2009).

2.1.3 Role of renin-angiotensin-aldosterone in hypertension

The RAAS is an important regulator of blood pressure and volume and has been implicated in the presentation of hypertension (Pugliese et al., 2020). The RAAS functions as a systemic endocrine and tissue paracrine/autocrine system and activation of both systemic and tissue RAAS are independent of each other (Duprez, 2006). The RAAS has two main pathways namely, classical and nonclassical. With decreased blood pressure or reduced sodium chloride (NaCl) delivery to macula densa, the juxtaglomerular apparatus in the kidney is stimulated to release renin (Goto et al., 2000). Renin is responsible for cleaving angiotensinogen (AGT) to angiotensin 1 (ANG I) (Martyniak & Tomasik, 2023). Furthermore, ANG II is produced from the cleaving of angiotensin ANG I, by angiotensin-converting enzyme one (ACE1), to ANG II (Ames et al., 2019). ANG II acts on two receptors namely, angiotensin type 1 and 2 receptors (AT_1R and AT_2R) (Goto et al., 2000).

The binding of ANG II to AT_1R activates the RAAS via the classical RAAS pathway (Liao & Wu, 2021). Binding initiates the hypertensive effects of ANG II such as vascular contraction, aldosterone release, renal sodium absorption which all are involved in blood volume and regulation (Goto et al., 2000). Under pathological conditions, ANG II increases the expression of chemokines and cytokines which increase leukocyte in vessel walls, thus causing vascular damage (Duprez, 2006). Furthermore, ANG II has been shown to facilitate sympathetic nerve activity which is important in the causation and maintenance of hypertension (Duprez, 2006). Additionally, ANG II-induced increased levels in aldosterone induce expression and

activity of epithelial sodium channel (ENaC), which is involved in increased sodium (Na^+) reabsorption in the distal nephron and collecting ducts thus causing an increase in blood pressure (Goto et al., 2000). Contrary to the classical RAAS pathway is the non-classical pathway which involves angiotensin (1-7) (ANG (1-7)) Martyniak & Tomasik, 2023). Conversion of ANG I into ANG (1-7) is mediated by angiotensin-converting enzyme two (ACE2) (Wang et al., 2020). The non-classical pathway is initiated when ANG (1-7) binds to angiotensin type 2 receptor (AT_2R) (Cristina Simões Silva et al., 2020). This brings about effects that are essentially opposite actions of classical the ANG II - AT_1R pathway (Cristina Simões Silva et al., 2020). ANG (1-7) attenuates oxidative stress, increases nitric oxide (NO) release, and amplifies bradykinin-induced vasodilation (Gouldsborough et al., 2003).

In hypertensive individuals, RAAS activity is often heightened, contributing to elevated blood pressure levels (Ferrari, 2013; Nwia et al.,2023). ANG (1-12), a substrate for the formation of ANG II, is greater in hypertensive individuals compared normotensive individuals (Ferrario et al., 2021). Furthermore, the activity of ACE is greater in hypertensive patients (Hristova et al., 2019). This alludes to the idea of increased formation of ANG II in hypertensive individuals. Furthermore as popularly known, renin secretion observed in hypertension leads to excessive ANG II production and ultimately its pressor effects, leading to vasoconstriction and sodium retention (Stoicescu et al., 2011). Additionally, genetic variations in RAAS components, such as ACE gene polymorphisms, have been associated with hypertension susceptibility (Zhang et al., 2019).

Studies have shown increased expression of all components of RAAS including increased expression of AT_1R in SHR (Sáinz et al., 2004; Williamson & Tai, 2017). Increased expression levels often allude to activation of RAAS, which in literature has

been strongly correlated to increased blood pressure (Williamson et al., 2017). It is widely known that SHRs have greater RAAS activation, this greater activation is in part the reason for higher blood pressures seen in SHRs compared to WKYs (Williamson et al., 2017). At 4 weeks (young SHRs) have higher renal levels of ANG II compared to WKY (Gouldsborough et al., 2003). Additionally, ANG II infusion at this age in SHR results in increased pressor response compared to WKY (Gouldsborough et al., 2003). This is indicative of the increased activity of AT_1R in SHR even before the onset of hypertension (Gouldsborough et al., 2003). Adult SHR at 16 weeks, when hypertension is already established, has greater renal upregulation of ACE1 and AT_1R comapred WKY (Williamson & Tai, 2017). Concomitant with this was a greater average systolic and diastolic blood pressure in SHR compared to WKY (Williamson & Tai, 2017). Additionally, a study showed a decrease in ACE2 accompanied by an increase in ACE1 in SHR than WKY (Yang et al., 2013). These studies mentioned above suggest greater RAAS activation in SHRs than age matched WKYs throughout their life spans.

The sympathetic nervous system (SNS) and its association with the RAAS are important in the development of hypertension (Valensi, 2021). SHRs have an increased SNS activation than WKY (Grisk & Rettig, 2004). This activation is implicated in the development of hypertension (Grisk & Rettig, 2004). Pro sympathetic neurotransmitter production induced by nerve stimulation is mediated by ANG II (Takeshi, 1977). This highlights a nexus between RAAS and the SNS. A study (Takeshi, 1977) investigating norepinephrine (NE) production by nerve stimulation showed that the SHR has greater NE than WKY, suggesting greater SNS activation in SHR. Furthermore, SNS response to ANG II infusion is greater in SHR (Nagase et al., 1996). Denervation of renal SNS in SHR decreases intrarenal NE, ANG II and

AT_1R all of which delay the onset of hypertension (Simko et al., 2022). The increased RAAS activation together with increased activation of other factors such as the SNS in SHR exacerbates and drives hypertension (Valensi, 2021). In addition to its well-established role in hypertension, the RAAS exhibits notable sex differences in its contribution to the pathogenesis of hypertension (Sabbatini & Kararigas, 2020).

2.1.4 Sex differences in the role of the renin-angiotensin-aldosterone system in hypertension

Sexual dimorphism has been investigated in the regulation of the RAAS and has been correlated to blood pressure (Reckelhoff, 2023).

Expression of RAAS components and activation is modulated by gonadal hormones (Nwia et al., 2023). Oestrogen upregulates and activates the non-classical vasodilatory pathway [ANG (1-7) - AT_2R] (Novella et al., 2019). Furthermore, oestrogen directly reduces the expression of classical RAAS components and essentially the activity of the classical vasoconstrictor pathway (ANG II – AT_1R) (Fardoun et al., 2020). An experimental study (Sandberg & Ji, 2003) showed chronic administration of oestrogen reduced angiotensin converting enzyme (ACE) activity, ANG II levels, kidney AT_1R expression, aldosterone secretion and ultimately vascular contraction. Furthermore, the lack of oestrogen has been implicated in the increased expression of RAAS components and activation of the vasoconstrictor pathway (Mkhize et al., 2023). Another experiment that reduced levels of oestrogen due to ovariectomy increases plasma renin activity (PRA), ANG II and aldosterone levels thus suggesting increased RAAS activity (Hidetomo et al., 2008). Furthermore, oestrogen administration to the very same ovariectomised female rats reduced effects (Hidetomo et al., 2008).

However, testosterone on the other hand favours the classical vasoconstrictor pathway (ANG II – AT_1R) (Sandberg & Ji, 2003). Testosterone upregulates the expression of prohypertensive RAAS components (Santos et al., 2023). Testosterone potentiates the pressor effects of ANG II (Pingili et al., 2020). This results in an increase in vasocontraction and blood volume and therefore results in higher blood pressure (Pingili et al., 2020). Plasma renin activity (PRA) is positively correlated with testosterone levels (Kienitz & Quinkler, 2008). This suggests an increase in RAAS activation in males due to testosterone. A study (Seachrist et al., 2000)shows that castration of male SHR reduces levels of ANG II and levels are restored with testosterone infusion.

Expression of these components have been alluded to increased RAAS activity as administration of testosterone to ovariectomised female SHR increased PRA levels, and messenger ribonucleic acid (mRNA) AGT. Additionally, castration of WKY male reduces mRNA levels of AGT while testosterone administration to female WKY increases the mRNA levels of AGT (Seachrist et al., 2000).

Regarding the renal pressure-natriuresis, male SHR excrete less sodium (Na^+) than female SHR at the same perfusion rate, indicating high testosterone-induced levels of aldosterone compared to female SHR (Reckelhoff et al., 2015). Sodium retention results in increased water reabsorption, leading to an increased blood volume and pressure (Hinrichs et al., 2020). Castration of male SHR restores the pressure-natriuresis and lowers blood pressure (Reckelhoff et al., 2015).

The interplay between hormones and the RAAS highlights the influence of oestrogen and testosterone on blood pressure regulation (Nwia et al., 2023). While oestrogen has been associated with reduced RAAS activity, through the non-classical RAAS pathway, testosterone appears to favour the classical vasoconstrictor pathway (Nwia

et al., 2023). These hormonal dynamics shed light on the gender differences observed in the RAAS.

While the influence of sex hormones and the RAAS system play a significant role in the development of hypertension, these factors can also influence the remodelling processes of the heart (Wu et al., 2019).

2.2. Left ventricular hypertrophy

Left ventricular hypertrophy (LVH) develops as an adaptive response to hemodynamic stress, aiming to normalise wall stress and maintain cardiac output (Ferreira et al., 2022). However, sustained pressure overload or volume overload can lead to maladaptive remodelling characterised by myocardial fibrosis, impaired contractility, and diastolic dysfunction (Schimmel et al., 2022). Depending on the type of overload, the myocardium in the left ventricle assumes different morphological patterns of arrangement (Nauta et al., 2020). Wall stress caused by pressure overload (like in hypertension) results in a type of cardiac remodelling known as concentric hypertrophy characterised by an increase in the left ventricular wall thickness due to parallel additions of sarcomeres into the left ventricular wall (Yildiz et al., 2020). In the case of volume overload, known to cause eccentric hypertrophy, sarcomeres are added in series and the left ventricular lumen begins to expand (Yildiz et al., 2020). Left ventricular hypertrophy is influenced by various factors, including genetic predisposition, neurohormonal activation and mechanical stress (Sayin & Oto, 2022; Gannon & Link, 2021; Takano et al., 2020).

Mechanical stress such as pressure (hypertension) and volume overload on the myocardium can induce second messenger signalling pathways that induce cardiomyocyte hypertrophy (Caturano et al., 2022). These pathways include protein kinase C, phosphatidylinositol, Raf-1 kinase and extracellular regulated protein kinase

(ERK) (Yamazaki & Yazaki, 1999). In response to hypertrophic growth, cardiomyocytes increase their cytosolic Ca^{2+} which in turn is able to stimulate calcineurin (Zou et al., 2001). Activation of pathways associated with calcineurin leads to gene expression of myocardial proteins (Grammer et al., 2006). Furthermore calcineurin is able to synergise with other signalling pathways such as mitogen-activated kinase (MAPK) pathways to cause left ventricular hypertrophy (Sugden, 1999). Together these pathways lead to increased myocardial protein synthesis which essentially defines cardiac hypertrophy.

Beyond hypertensive-induced cardiomyocyte hypertrophy, fibrosis is also an integral part of cardiac remodelling (Wang et al., 2022). Induced by pressure overload, stimulatory cytokines transforming growth factor β (TGF-β) is implicated in the process of fibrosis and is considered to be a prime stimulatory cytokine of fibrosis (Kuwahara et al., 2002). TGF-β induces activation of cardiac fibroblast into myofibroblasts (Kuwahara et al., 2002). Myofibroblasts are involved in mediating fibrosis by production of TGF-β, which further leads to activation of fibroblasts (Kuwahara et al., 2002). Increased deposition of collagen I (thick, tensile) fibres, decreased collagen III (thin, elastic) and the cross linking of collagen fibres is integral in cardiac remodelling (Yamamoto et al., 2002).

Together cardiomyocyte hypertrophy and fibrosis lead to cardiac remodelling, specifically LVH. (Ding et al., 2020). These processes are compensatory and are in response to increased pressure overload (Ding et al., 2020). Progression of fibrosis perturbs the structural integrity of the cardiac extracellular matrix (ECM) while hypertrophy increases cardiomyocyte size (Hanna et al., 2021; Nicks et al., 2020). Together cardiomyocyte hypertrophy and fibrosis impair the diastolic (relaxation) function and eventually systolic (contraction) function of the heart (Frangogiannis,

2020). In echocardiography, this improper relaxation of the myocardium can be indexed by reduced early peak tissue lengthening velocity at the mitral annulus (e') and late peak tissue lengthening velocity at the mitral annulus (a') (Kadappu & Thomas, 2015). Beyond the hemodynamic influence on cardiac remodelling and ventricular hypertrophy, there are multiple factors with numerous mechanisms and pathways that are involved as shown in table 2.2. These factors include RAAS and gonadal hormones. The role and mechanisms of these factors are described below.

2.2.1 Role oestrogen in left ventricular hypertrophy

Oestrogen plays an integral part in the inhibition of pressure overload induced cardiac hypertrophy through its receptors (Babiker et al., 2015). Both of its receptors are present in cardiomyocytes and cardiac fibroblasts (Da Silva et al., 2021). Oestrogen through its receptors inhibits activity of mitogen-activated kinase (MAPK) pathways in cardiac myocytes (Babiker et al., 2015). As already mentioned, there are numerous signalling pathways and kinases associated with MAPK such as p38-MAPKs, c-Jun N-terminal kinases (JNKs) and ERKs (Xiao et al., 2021). The activation and cascade of these kinases in the face of increased pressure overload on the heart initiate cardiac hypertrophy (Xiao et al., 2021). Oestrogen interferes with these pathways and drives the myocardium away from hypertrophy (Takano et al., 2020). Additionally, oestrogen interferes with the cascade of hypertrophic pathways induced by TGFβ (Pedram et al., 2013). Oestrogen also drives the myocardium from hypertrophy through inducing an increase in expression of atrial natriuretic factor (ANF) which has antihypertrophic effects (Babiker et al., 2015). Numerous studies (Bhuiyan & Fukunaga, 2010; Ramírez-Hernández et al., 2024; Voloshenyuk & Gardner, 2010; Westphal et al., 2012) have reported on oestrogen deficiency and development of hypertrophy in female rats. The same phenomenon occurs in clinical studies where post-menopausal

women are associated with increased adverse cardiovascular outcomes compared to males (Kamińska et al., 2023). However, female rats do not experience cessation of the oestrous cycle and therefore it would be required that they are ovareictomised in order to be compared to post-menopausal women in clinical trials (Garate-carrillo et al., 2020). A study showed that DOCA-salt female mice without ERβ led to maladaptive cardiac fibrosis through changes in cardiac collagen I and III distribution (Mice et al., 2011). This suggests the importance of oestrogen in driving the heart away from remodelling. Furthermore, another study showed that infusion of ERα agonist (16α-LE2) attenuates the level of cardiac fibrosis and slows development of myocardial hypertrophy in mice (Westphal et al., 2012).

Oestrogen influences tetrahydrobiopterin (BH_4), which is important in the production of NO through NOS modulation (Zhao et al., 2014). However, in cases where there is a low level of oestrogen, BH_4 in myocardium favours production of ROS rather than NO (Zhao et al., 2014). Increased oxidative stress in the myocardium leads ventricular remodelling (Martins et al., 2022). In vivo studies, prohypertrophic effects brought by ET-1 are abolished by ERβ agonists suggesting oestrogen blunts deleterious pressor effects that ET-1 causes in myocardium (Mice et al., 2011). TGFβ stimulates production of ANG II and ET-1 in fibroblasts (Pedram et al., 2015). ERβ agonists prevent the fibrosis induced by ANG II and ET-1 (Pedram et al., 2015). Furthemrore, oestrogen interferes with the cascade of hypertrophic pathways induced by TGFβ (Pedram et al., 2013).

Oestrogen is involved in ANG II mediated cardiac hypertrophy. A study showed that in SHR and normotensive rats, oestrogen deficiency leads to increase in the expression of AT_1R (Eickels et al., 2005). This increases the chances of pressor

effects of ANG II (Eickels et al., 2005). Furthermore, oestrogen can also lead to physiological cardiac hypertrophy (Dworatzek et al., 2014). Females may have an increased response to pressure overload which is exhibited through increased cardiac mass (Oláh et al., 2019). However, the remodelling is physiological as there is no fibrosis (Foryst-Ludwig et al., 2011). This physiological type of remodelling is associated with MAPK signaling pathways and protein kinase B (Oláh et al., 2019). Moreover ERβ is directly involved in the cascade of these processes (Oláh et al., 2019).

2.2.2 Role of testosterone in left ventricular hypertrophy

As shown in table 2.2, males typically exhibit a greater left ventricular hypertrophy compared to females. The binding of testosterone to androgen receptors and translocation to cardiomyocyte nucleus leads to enhanced cardiac gene expression (Thum and Borlak, 2002). This includes enhanced gene coding for myosin heavy chains (Thum and Borlak, 2002). Left ventricular metabolism of testosterone in male SHR leads to increased metabolites of testosterone such as dihydrotestosterone and androstenedione (Thum and Borlak, 2002). Which have a high affinity to androgen receptors and are able to induce remodelling through binding to these androgen receptors (Thum and Borlak, 2002). Additionally, in cardiomyocytes testosterone induces growth through promotion of protein synthesis through activation of signalling pathways such as rapamycin complex 1 pathway (mTORC1) (Thum and Borlak, 2002). Activation of the pathway involve activation of extracellular signal-regulated kinases 1/2 (ERK 1/2) (Silva et al., 2006). This kinase is a component of MAPKs, which are all involved cell proliferation (Silva et al., 2006). Furthermore, blockage of mTORC1 attenuates testosterone induced cardiomyocyte hypertrophy (Silva et al., 2006). Furthermore, testosterone is able to increase levels of growth factors such as

insulin like growth factor 1 (IGF-1) in the cardiac tissue which is responsible for cardiac hypertrophic growth (Żebrowska et al., 2017). Either than the mechanisms mentioned above, testosterone is also able to influence other factors such as the SNS which is independently implicated in hypertrophy. Testosterone induces SNS activation, this activation increases the contractility of the heart which increases mechanical stress and thus causes hypertrophy (Ely et al., 1997; Meyer et al., 2010). An experiment (Cavasin et al., 2003) showed that testosterone administration to mice increased myocyte cardiomyocyte size and left ventricular dimensions. Although the exact mechanisms in which testosterone induces hypertrophy weren't elucidated in the experiment, the already above mentioned mechanisms could be an explanation.

2.2.3 Role of the renin-angiotensin-aldosterone system in left ventricular hypertrophy

The increased cardiac expression and activity of cardiac RAAS components are associated with cardiac remodelling, marked by an increased LV mass in hypertensive rats (Li et al., 2008). Unlike cardiac myocyte hypertrophy that happens due to pressure overload, fibrosis occurs through the activation of RAAS (Weber et al., 1992). Experimental studies show that components of RAAS are present in the rat myocardium (Lijnen & Petrov, 1999). A study showed ANG II infusion in SHR increases left ventricular mass (LVM) (Reddy et al., 1996). Expression, protein production and activity of the classical cardiac RAAS components are increased in rat myocardial disease such as LVH (Schunkert et al., 1993). Additionally, the non-classic pathway components (which are cardioprotective) are also upregulated in cardiac hypertrophy in the SHR compared to WKY however, upregulation is compensatory (Li et al., 2008). Fibrotic events are mainly through the effects of ANG II via AT_1R (Kai, 2002).

ANG II stimulates tissue growth factor β-1 (TGF-β1) in cardiac fibroblasts and through binding to AT_1R (Kai, 2002). TGF-β1 induces activation of cardiac fibroblast into myofibroblasts (Kuwahara et al., 2002). Myofibroblasts are involved in mediating fibrosis by production of TGF-β1, which in a feedforward fashion further perpetuates activation of fibroblasts into myofibroblasts (Kuwahara et al., 2002).

Cardiac fibroblasts are involved in the deposition of thick tensile type 1 collagen fibres in the cardiac ECM (Nikolov & Popovski, 2022). ANG II suppresses and downregulates MMPs (Brilla, 2000). MMPs are involved in the degradation of collagen (Nikolov & Popovski, 2022). The increased deposition and reduced degradation of collagen in the cardiac ECM results in fibrosis (Varo et al., 2015). This renders the heart stiff and impairs its diastolic (relaxation) function.

Cardiac stretch such as pressure overload releases ANG II from cardiomyocyte and upregulates its receptors, however, the upregulation of AT_1R (which mediate hypertrophy) is greater than that of AT_2R (Lijnen & Petrov, 1999). A study showed that in SHR due to pressure overload there is an increase in expression and activity of ACE and inhibition of ACE reduces not only blood pressure but progression LVH as well (Reddy et al., 1996). Furthermore, another study reported that expression and activity of cardiac RAAS components were increased in hypertensive rats with cardiac remodelling (Lijnen & Petrov, 1999).

Aldosterone is also implicated in cardiac fibrosis. Activation of mineralocorticoids receptor in epithelial cells are involved in initiation of cardiac fibrosis (Brilla, 2000). Chronic administration of aldosterone induces cardiac fibrosis in experimental models of hypertension (rats) (Weber et al., 1992). In SHR, activation of RAAS is enhanced, hence a greater degree of fibrosis compared to normotensives (Dworatzek et al., 2014).

Together, ANG II and aldosterone work synergistically to induce cardiac fibrosis which changes the cardiac matrix through increased collagen deposition and causes LVH (Buffolo et al., 2022). In echocardiography, LVH is indexed by increased left ventricular posterior wall thickness in diastole (LVPWTd), interventricular septum diameter in diastole (IVSDd) and relative wall thickness (RWT) which studies have shown to be greater in SHR than WKYs (Slama et al., 2005; Haas et al., 1995). LVH renders the ventricle stiff and impacts the myocardial relaxation function and causes an impaired LV filling ability.

2.2.4 Sex differences in role of the renin-angiotensin-aldosterone system in left ventricular hypertrophy

As previously mentioned, experimental studies in tables 2.1 and 2.2 show that classical RAAS pathway is more activated in males compared to females. This in part supports the idea that males exhibit adverse cardiovascular events sooner compared to age-matched males. Increased RAAS activation in males results in increased pressure overload which ultimately results in left ventricular hypertrophy (Dalpiaz et al., 2015; Radin et al., 2002; Reckelhoff et al., 2015). A study showed (Radin et al., 2002) that compared to female Spontaneously Hypertensive Heart Failure rats (SHHF), male SHHF had an increased RAAS activation at 5 - 8 month (marked by increased PRA, serum AGT and ACE). Furthermore, the study showed increased LVH in male rats compared to females (Radin et al., 2002). Lesser RAAS activation in the study was suspected to be due to oestrogen as oestrogen in known to downregulate the RAAS. The sexual dimorphism seen in RAAS activation is primarily due to sexual hormones. Typically, testosterone upregulates RAAS while oestrogen down regulates it (Sun et al., 2011). A study (Dalpiaz et al., 2015) showed that castration reduced cardiac ACE levels and cardiac hypertrophy in male SHR, suggesting a role of

testosterone in ANG II mediated hypertrophy. On the other hand, the same study showed that ovariectomy increased cardiac hypertrophy in female SHRs.

Increased RAAS activation is suggestive of high ANG II levels. As already mentioned, males have greater activation thus suggesting increased ANG II levels in males (Pendergrass et al., 2008). A study (Li et al., 2015) showed greater cardiac expression of AGT and ACE in ventricles of male mice compared to female mice. In hypertensive mice, ANG II increases NADPH oxidases in the myocardium of male and ovariectomised female rats while having no effect on intact female rats (Komukai et al., 2010). Furthermore, ANG II in hypertensive conditions induces proliferation and differentiation of cardiac fibroblasts, activation of NF_KB and MAPK signalling pathways (Komukai et al., 2010). However, oestrogen inhibits fibrosis and these signalling pathways (Komukai et al., 2010). Beyond cell proliferation, binding of cardiac ANG II to AT_1R brings induces inflammation, oxidative stress and apoptosis which are all implicated in hypertrophy and fibrosis (Xu et al., 2010). Although oestrogen interferes with the cascade of hypertrophic pathways (Pedram et al., 2013). Oestrogen may also contribute the development of cardiac remodelling. Regarding oestrogen's involvement with RAAS in development of hypertrophy, oestrogen is said to play a permissive role as a study showed that it augments effects of ACE inhibitors (Eickels et al., 2005). Furthermore, hypertrophy is modulated by histone deacetylase proteins (HDACs) class I and II (Pedram et al., 2013). HDAC I is prohypertrophic while II is antihypertrophic (Pedram et al., 2013). Oestrogen and ERβ agonists attenuate ANG II induced HDAC I production, thus promoting hypertrophy (Pedram et al., 2013). Additionally, ANG II inhibits HDAC class II and results in hypertrophy (Pedram et al., 2013). However, effects were reversed with oestrogen infusion and ERβ agonism (Pedram et al., 2013). However, testosterone indirectly causes cardiac hypertrophy

through the RAAS. A study (Mishra et al., 2015) showed that infusion of ANG II to testosterone administered and intact male rats increase left ventricle-to-body weight. However, this effect was not seen in castrated rats that did not receive testosterone (Mishra et al., 2015). The increase in left ventricular mass in the above-mentioned study was defined by increased cardiomyocyte size and fibrosis (Mishra et al., 2015). Furthermore, indirectly through elevation of blood pressure testosterone is able to induce cardiac fibrosis. Fibrosis together with testosterone signalling pathways lead to left ventricular hypertrophy (Diaconu et al., 2021).

Table 2.1: Sex differences in the development of hypertension in SHR

Author	Age of rat	Methods	MAP measurements	RAAS measurements	Other measurements
(Yanes et al., 2006)	8 MO SHR Male & female	Catheterization (consciously 4 days post implantation) ELISA for RAAS Western blot for renal AGT	Similar MAP between sexes at 16 MO for catheterization & NIBP.	Systemic levels: no sex differences Renal levels: AGT & renin: male SHR > female SHR ANG II: male SHR < female SHR	
(Maris et al., 2005)	6 MO SHR & WKY Male & female	MAP by Telemetry (conscious)	SHR SBP: male > female DBP: male > female WKY: SBP: male > female (10 mm Hg) DBP: male = female		
Sullivan et al., 2010)	12 WK SHR Male & female	MAP by Telemetry (conscious) ELISA for ANG(1-7) Western blot for renal AT_1R, AT_2R and MasR RT-PCR for AT_1R, AT_2R and MasR expression	Baseline: MAP: male > female ANG II infusion: MAP: male > female Significance in male in 4 days. Significance in female in 8 days	Baseline: Renal ANG(1-7): female > male ANG II infusion: Renal ANG(1-7): female > male AT_1R: decreased in male : no change in female AT_2R: increased in male : no change in female	

				MasR: no change in male : increased in female	
(Reckelhoff et al., 2015)	4 WK SHR Male and female	MAP by Tail plethysmography (weekly BP from 5wk – 7wk weeks) (conscious) Gonadectomy	<u>Intact:</u> MAP: male > females <u>Castrated:</u> MAP: males = females <u>Ovariectomised + testosterone:</u> MAP: males < females <u>Ovariectomised + intact female:</u> MAP: ovx female = intact female		
(Iliescu et al., 2007)	9 WK SHR Male	Catheterization (MAP measured consciously 8 days post implantation) Western blot for markers of oxidative stress.	<u>Castration:</u> MAP: castrated males > intact males <u>NADPH oxidase inhibition:</u> Castrated: no effect on MAP. Intact: reduced BP by 15 mm Hg.		**Oxidative stress** <u>Castration:</u> Reduced NADPH oxidase & NADPH-stimulated superoxide anion generation. <u>Apocynin:</u> Reduced NADPH oxidase & NADPH-stimulated superoxide anion generation in intact but not castrated.

					The above led to a decrease in MAP.
(Sartori et al., 2007)	15 & 18 WK SHR Male & female	Catheterisation (MAP measured consciously 8 days post implantation) Western blot for markers of oxidative stress	<u>MAP:</u> Males > females		**Oxidative stress** <u>Systemic:</u> male = female <u>Urinary:</u> F2-isoprostane: male<female. <u>Whole kidney:</u> F2-isoprostane: male>female.
(Sullivan et al., 2007)	12-14 WK SHR Male & female	Tail cuff (consciously) Gonadectomy at 10 WK Na^+ -selective electrode Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit.	<u>Castration:</u> Decreased MAP <u>Ovariectomy:</u> No effect on MAP		**Salt intake** <u>Na^+ -selective electrode</u> Na^+ intake: male > female decreased by gonadectomy. **Oxidative stress**

		(measured H_2O_2 in urine) Western blot for markers of anti-oxidants			Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit : H_2O_2: male > female Castration: Reduced H_2O_2 excretion in males compared to intact males Ovariectomy: increased H_2O_2 excretion compared to intact females. **Antioxidants** Renal SOD level and activity (superoxide dismutase) males > females Gonadectomy reduced antioxidants in males and had no effect in females
(Fortepiani & Reckelhoff, 2005)	8 WK SHR & WKY Male & female	Catheterization (no mention of when BP was recorded post implantation) Gas chromatography-negative ion chemical ionisation mass spectrometric assay for	MAP:SHR > WKY. MAP: Male > female.		**Oxidative stress** Renal F_2-isoprostanes: male SHR > female SHR. male SHR (300%)> male WKY. female SHR (70%) > female WKY.

		renal oxidative stress markers.			Inhibition of F2-isoprostane by "tempol" reduced blood pressure in adult male SHR but not adult female (administration of tempol at onset of HTN 12 weeks). Inhibition of F2-isoprostane by tempol from birth reduced blood pressures in both sexes of SHR. Inhibition of F2-isoprostane had no effect on WKY BP No direct correlation between renal F2-isoprostane levels and BP.
(Outrobe & Le, 2018)	2-6 WK SHR & WKY Male and female	Tail cuff (conscious) Cannulation (anaesthetised and done with dose of ketamine that didn't affect MAP) BP was observed at 2,4,3 & 6 WK	<u>Tail cuff:</u> MAP: SHR>WKY (evident at 4 weeks of age). <u>Canulation:</u> MAP: SHR = WKY		

(Loh & Salleh, 2017)	**8 WK SHR & WKY** **Male & female**	Gonadectomy & Sham Cannulation (anaesthetised) ELISA for testosterone concentrations	Sham: MAP: Males > females Castration: Reduction of MAP in male SHR & WKY Ovariectomy: Reduction of MAP in male SHR & WKY		**Testosterone infusion** Increased MAP in ovariectomised female SHR & WKY. **Gonadectomy & Sham** Sham: Male SHRs had highest levels of testosterone compared to every other group. Castration: Reduced plasma testosterone in male SHR & WKY. Ovariectomy: Reduced plasma testosterone in female SHR & WKY. study showed a positive correlation between BP and testosterone.
(Fortepiani et al., 2015)	**4-18 MO SHR** **Male & female**	Catheterization (anaesthetised) Radioimmunoassay for plasma renin levels.	MAP at 4 WK: Males > females MAP at 18 MO in females:	Plasma renin activity: postmenopausal rat (18 MO) > young premenopausal rat (4&8 MO)	

			Intact females > ovariectomised females No difference between genders for MAP at 18 MO. Postmenopausal rats had similar MAP to adult males. <u>Ovariectomy:</u> No effect on MAP		
(Sullivan et al., 2007)	**8-16 WK SHR** **Male & female**	Telemetry (conscious) Gonadectomy	<u>MAP for 9-16 WK:</u> Male > female <u>Castration at 16 WK:</u> Reduced MAP in male <u>Ovariectomy at 16 weeks:</u> No effect on MAP	<u>AT_1R expression:</u> Male > female Castration nor ovariectomy had effect on AT_1R expression. <u>Plasma ANG II:</u> Females > males <u>Renal ANG II</u> Male & female were comparable	**Oxidative stress:** <u>Renal superoxide levels:</u> Males > females. <u>Castration:</u> No effect on superoxide levels. <u>Ovariectomy:</u> Increased superoxide levels in ovx females than in intact females.
(Bhatia et al., 2012)	**SHR** **Male & female**	**T**elemetry (conscious)	<u>Baseline MAP:</u> Males > females <u>ANG II infusion:</u> MAP: Males > females Males reached significance after 5 days		**Systemic oxidative stress:** <u>Baseline</u> 8-isoprostane: comparable in male & female H_2O_2 excretion: males > female

			of infusion and females at 8.		NADPH oxidase comparable in male and female <u>ANG II infusion:</u> increased 8-isoprostane in males and not females Increased H_2O_2 excretion in males (290%) and in females (189%) Increased NADPH oxidase in males (43%) and female (154%). **Renal oxidative stress:** <u>Baseline:</u> O_2^- : male > female <u>ANG II infusion:</u> Increased O_2^- in male (30%) and female (40%). **Systemic/plasma antioxidant levels:** <u>Baseline:</u> SOD: male < female <u>ANG II infusion:</u>

					SOD: increased only in male. **Renal antioxidant levels:** <u>Baseline:</u> Urinary AOP: male < female. <u>ANG II infusion</u>: Urinary AOP: decreased in male (41%) and females (39%). male < females
(Xue et al., 2005)	**12-16 WK mice Male & female**	Telemetry (conscious)	<u>Baseline MAP:</u> Similar across groups <u>ANG II infusion:</u> Increased MAP in both male and female but more significantly in males. <u>Castration:</u> Attenuated hypertension. <u>Ovariectomy</u>: Augments hypertension.		
(Elmarakby et al., 2016)	**12-13 WK SHR Male & female**	Catheterisation (anaesthetised)	<u>Baseline MAP:</u> Male > female <u>Acute ANG II infusion (1hr) MAP:</u>		

			Male > female. Significant increase in males and no effect in females.		
(Sáinz et al., 2004)	**13-14 WK SHR & WKY.** **Male & female**	Tail cuff (conscious) Cannulation (anaesthetised)	<u>Tail cuff:</u> MAP: male > female <u>Cannulation:</u> MAP: male > female Males had greater MAP in both modes of MAP measurement <u>Castration:</u> Reduced MAP but more delayed compared to females. <u>Ovariectomy:</u> Reduced MAP quicker than in males	<u>Plasma renin activity:</u> Male SHR > male WKY. No difference in females <u>Gonadectomy:</u> No change in PRA levels between males and females and across strains.	**Oxidative stress** <u>L-NAME administration</u>**:** Increased MAP in both sexes but more significantly in male. Male > female **Hormone infusion** <u>Oestrogen:</u> Oestrogen infusion to males reduced PRA levels <u>Androgens:</u> Androgenisation to females increased PRA levels.
(Silva-antonialli et al., 2004)	**SHR** **Male & female**	BP by tail cuff (conscious) RT-PCR for receptor expression. Vessel reactivity by F-60 microdisplacement transducer Gonadectomy	MAP: male > female	<u>Vascular AT_1R/AT_2R ratio:</u> Male > female <u>Vascular AT_1R activity & response to ANG II:</u> Male > females <u>Vascular AT_2R activity & response to ANG II:</u> Males < females <u>Renal AT_1R:</u>	

				Male > female Renal AT_2R: Male < female <u>Ovariectomy:</u> Increased AT_1R similar to male Oestrogen infusion reversed effects.	
(Melo et al., 2020)	**12 WK SHR** **Male & female**	BP by Tail cuff (conscious) RAAS activity by Fluorimetry oxidative stress by flow cytometry Gonadectomy	<u>Sham & gonadectomised:</u> MAP: male > female	<u>ACE & ACE2 activity:</u> Male < females <u>Castration:</u> no effect on ACE increased ACE2 <u>Ovariectomy:</u> Reduced ACE2	**Oxidative stress** <u>Castration:</u> O_2^- & H_2O_2: male > female
(Venegas et al., 2009)	**20 WK mice** **Male & female**	Catheterisation	<u>ACE inhibition:</u> MAP: male = females <u>ANG II infusion:</u> MAP: male > female		
(Pendergrass et al., 2008a)	**15 WK HTN Lewis & normotensive Lewis rat** **Male & female**	Narco biosystem device for MAP	<u>MAP:</u> Hypertensive male > hypertensive female and normotensive male and female.	<u>AGT levels & activity:</u> Hypertensive male > hypertensive females <u>ACE levels & activity:</u> Hypertensive male > hypertensive females <u>ANG II levels:</u> Hypertensive male > hypertensive female	

				<u>ANG(1-7) levels:</u> Hypertensive male < hypertensive female <u>Renal ANG II levels:</u> Hypertensive male had greater levels compared to all groups.	

Table 2.2: Sex differences in the development of left ventricular hypertrophy in SHR

Author	Age of rat	Methods	Cardiac measurements	RAAS measurements	Other measurements
(Chan et al., 2011)	**3-30 MO SHR & WKY** **Male & female**	Tail cuff Echocardiography	MAP: SHR > WKY Male SHR > Female SHR LV weights: SHR > WKY LVPWTd: Male SHR > Male & female WKY until 15 MO (wall thinning). Female SHR was delayed in wall thickening LVIDd: Decrease in male SHR at 9-12 MO. Increase in diameter after 15 MO. FS: Decreased in male SHR at 15 MO & remained constant in female SHR. E/A:		

			Increased in male at 18 MO & constant in female SHR		
(Wallen, Cserti, Belanger, & Wittnich, 2000b)	**Age matched male and female SHR & WKY**	Cannulation	Heart weight: Male SHR > female SHR. Male WKY > female WKY. Heart weight - body weight: Female SHR > male SHR. Female WKY > male WKY.		
(Radin et al., 2002)	**5-13 MO SHHF Male & female**	Tail cuff Echocardiography Radioimmunoassay	MAP: Before CHF, SBP in male and female were equal Heart weight: Male SHHF > female SHHF Heart weight – body weight: Female SHHF > male SHHF IVS: Male SHHF > female SHHF IVS – body weight: Female SHHF > male SHHF	ACE activity: Male SHHF > female SHHF PRA: Male SHHF > female SHHF	Renal endothelin excretion: Female SHHF > male SHHF

			FS: Male SHHF > female SHHF (13 MO+)		
(Al-Gburi et al., 2017)	**5-36 WK SHR Male & female**	Tail cuff Picrosirius red staining Gelatin zymography	MAP: Males > female Lumen/wall ratio: Males < females Heart mass – body weight: Males > females Myocardial collagen: Males > females (29 WK)	Cardiac AT2R: Males < females	Matrix metalloproteinases activity: Males > females
(Romero et al., 2013)	**10 WK SHR Male & female**	Picrosirius red staining L-arginine substrate for NOS activity Immunohistochemistry for TGF-β	MAP: Males > females Myocardial collagen: Males > females		NO system activation: Males < females Oxidative stress: Males > females Cardiac NOS activity: Males < females TGF-β: Males > females

2.1. Aim and objectives

The study aimed to determine the sexual dimorphism of the role of the RAAS on hypertension and left ventricular hypertrophy in SHRs.

The specific objectives of this study were to determine:

a) the effects of the RAAS on blood pressure in male and female SHRs and WKYs after 7 months.

b) the effects of the RAAS on left ventricular remodelling, diastolic function and systolic function in male and female SHRs and WKYs after 7 months.

c) the effects of hypertension and sex on left ventricular remodelling, diastolic and systolic function in SHRs and WKYs after 7 months.

Chapter 3: Methods

3.1. Experimental design

All experiments were approved by the Animal Ethics Research Committee of the University of the Witwatersrand under the AREC clearance number 2021/03/03/C. Sixteen (16) 6-week-old male (n = 8) and female (n = 8) SHR and Fourteen (14) 6-week-old male (n = 7) and female (n = 7) WKY were used in this study. The rats were obtained from the Wits Research Animal Facility (WRAF). Rats were housed individually, in temperature-controlled rooms (25 ± 2^{o}C) and were allowed free access to food and drinking water. All rats were offered the same diet throughout the experiment.

Figure 3.1 illustrates the experimental design. Following the habituation period of two weeks, body weights were measured weekly. Blood pressure was measured non-invasively once a week over the last 5 weeks prior to termination. A day prior to termination, urine was collected and stored for further measurement. At 7-month-old, on the day of termination, rats were anaesthetised, anaesthetised blood pressure was measured and echocardiography was performed. Blood samples were collected by thoracotomy and stored for further measurement. Hearts were dissected, weighted and stored for further measurement. Termination was at 7 months since we wanted to examine the long-term effects on hypertensive levels responsible for marked compensated hypertrophy in male and female SHR

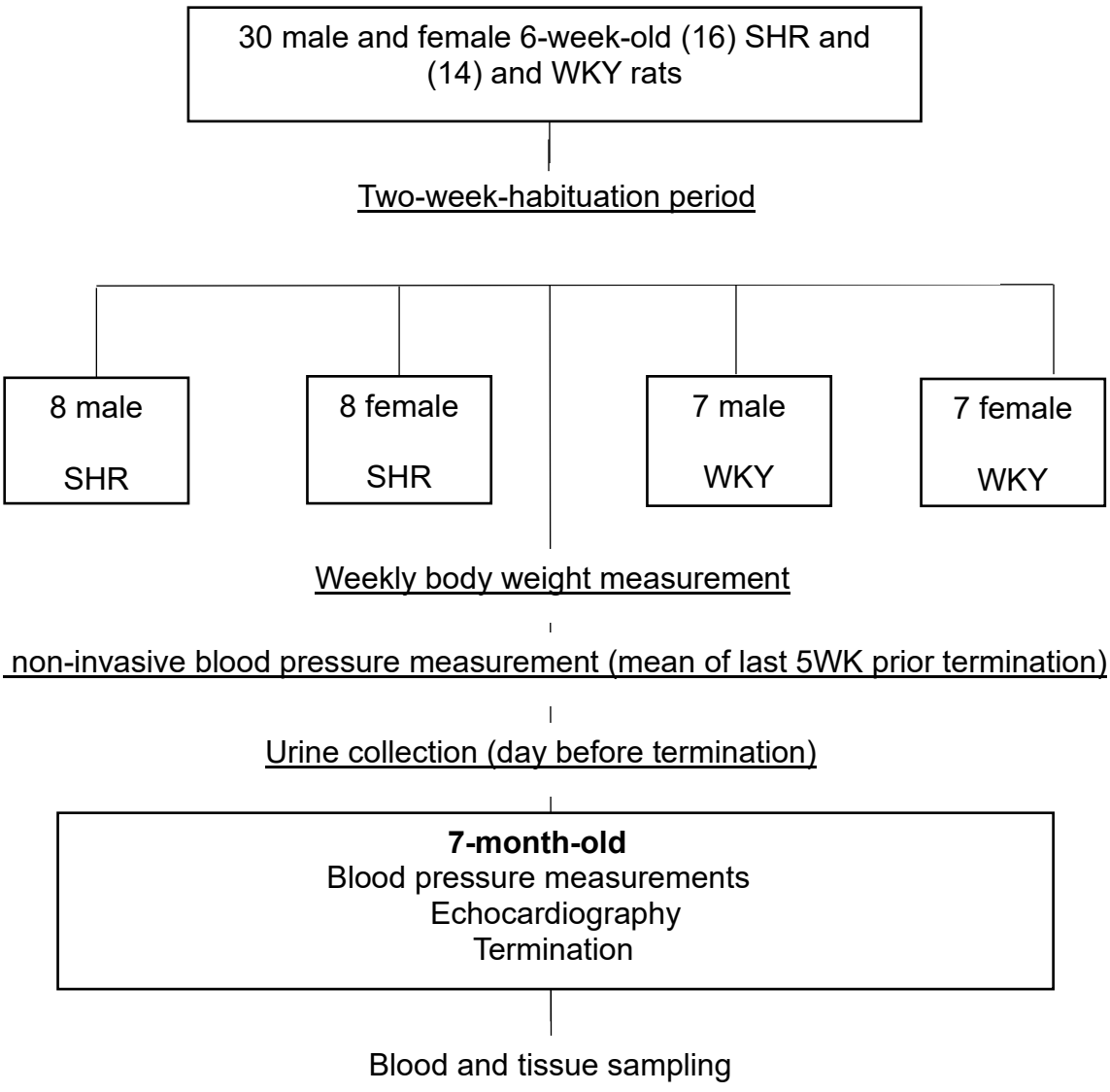


Figure 3.1: Experimental design to address the role of the RAAS in the sexual dimorphism related to with the development of hypertension and left ventricular hypertrophy in SHR

3.2. Body weight measurements

Using the Snowrex Electronic Scale (Clover Scales, Johannesburg, South Africa), the mean of the final six body weights before termination (measured weekly) including the final body weight at termination were considered and used. Animals were habituated to the measurements in the first two weeks of arrival.

3.3. Non-invasive measurement of blood pressure (NIBP)

For each animal, blood pressure readings were performed the last five weeks prior to termination once a week using the Biopac Non-Invasive Blood Pressure Measurement 250 System (NIBP). The rats were habituated to the procedure. While conscious, the rat was placed into the restrainer and attached to its tail was a cuff sensor. The tail was preheated on a heating pad. In a single event, blood pressure readings were measured twice using the tail-cuff sensor that is built into the NIBP 250 system. The cuff occluded vessels and once the inflation threshold was reached, the cuff gradually deflated allowing blood flow and thus resulting in a linear fall in pressure recorded by the blood pressure system. The mean of the two consecutive readings for each rat was calculated. The mean for the five weeks was then calculated and reported.

On the day of termination, blood pressure was measured using the same procedure (excluding the restrainer) while rats were anaesthetised and immobile.

3.4. Urine sampling

The day before termination, rats were placed individually into metabolic cages (equipped with urine containers) overnight for 8 hours without access to food and water. In the morning, the urine was collected and the samples were centrifuged for 5 minutes at 1500 rpm. The residues that sedimented were eliminated and the volume of urine was measured using a measuring cylinder. Urine sampling was done to measure urinary and angiotensinogen concentrations.

3.5. Echocardiography

After 7 months, rats were anaesthetised using isoflurane (2%, inhaled). Rats were individually placed in a gas chamber (connected to the isoflurane tank) until they were anaesthetised and immobile. Rats were removed from the chamber and placed in a left lateral decubitus position with their thoracic area shaven. Measurements were carried out by an experienced scientist using a non-invasive, high-resolution (paediatric-10Mhz) ultrasound probe that is connected to an echocardiogram (Siemens, Acuson SC2000, Diagnostic ultrasound system; Siemens Medical Solutions, USA, Inc.).

Left ventricular dimensions were assessed using M-mode echocardiography (Figure 3.2). Measurements of the internal diameter of the left ventricle in systole (LVIDs) and diastole (LVIDd), the interventricular septum diameter in systole (IVSDs) and diastole (IVSDd), and the left ventricular posterior wall thickness in systole (LVPWTs) and diastole (LVPWTd) were measured from the parasternal long axis view (Figure 3.2). Using the Teichholz formulas, these dimensions were used to derive indices that reflect systolic performance namely: (left ventricular end-diastolic volume (LVEDV) = [7/ (2.4) + LVEDD)] x $LVEDD^3$, the left ventricular end-systolic volume (LVESV) = [7/ (2.4 + LVESD)] x $LVESD^3$ and the stroke volume (SV) = LVEDV – LVESV (Teichholz & Herman, 1976).

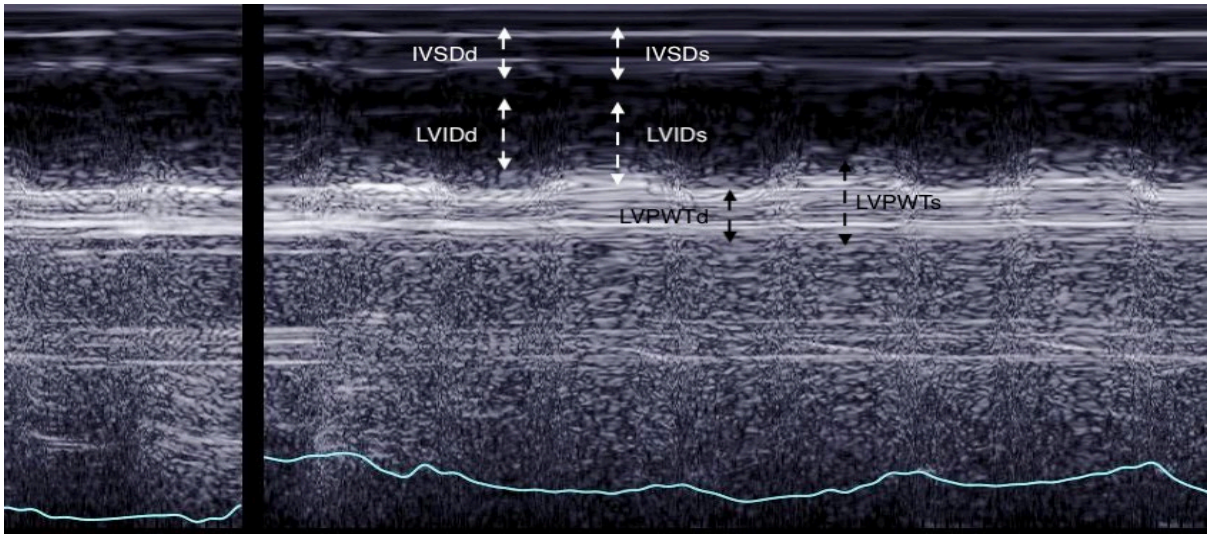


Figure 3.2: Example of 2-D M-mode image illustrating measurements of the LV dimensions.

Interventricular septum diameter in systole (IVSDs), intraventricular septum diameter in diastole (IVSDd), left ventricular internal diameter in end-systole (LVIDs), left ventricular internal diameter in end-diastole (LVIDd), left ventricular posterior wall thickness in systole (LVPWTs) and left ventricular posterior wall thickness in diastole (LVPWTd).

Left ventricular diastolic performance was assessed using pulsed Doppler and tissue Doppler imaging (TDI). Using pulsed Doppler imaging (Figure 3.3), the early (E) and late (A) diastolic inflow velocities were obtained in the apical four-chamber view with the sample volume placed at the mitral valve leaflet tip. The E/A ratio was used as an index of diastolic function. Velocities of the myocardial lengthening were measured by placing the cursor at the lateral corner of the mitral annulus.

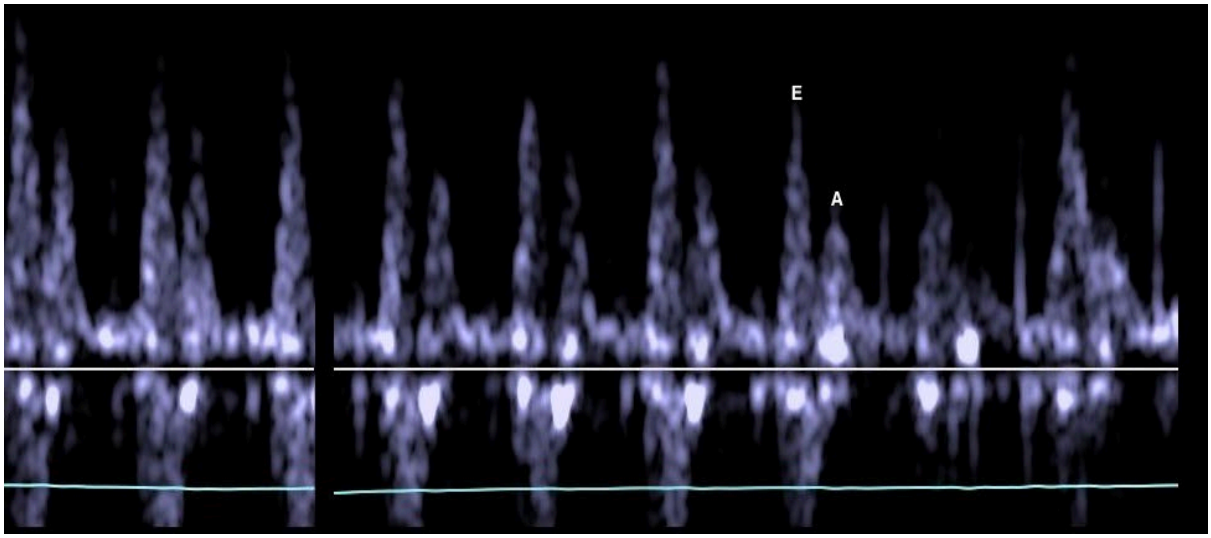


Figure 3.3: Example of the early diastolic filling velocity (E) and late diastolic filling (A) pulse wave trans mitral-valve Doppler image.

To determine diastolic function using tissue Doppler imaging (TDI) (figure 3.4), peak myocardial tissue lengthening velocities during early (e') and late (a') diastole were recorded at the lateral mitral annulus in the apical four-chamber view. The E/A ratio was used as an index of diastolic function, e' as an index of myocardial relaxation, the e'/a' ratio as an index of myocardial stiffness and the E/e' ratio as an index of the left ventricular filling pressures. E/A ratios between 1.5 and 2.5 were considered normal and ratios below 1.5 indicated possible diastolic function. E/e' ratios between 8 and 12 were considered to be normal and ratios beyond 12 were considered a pathology.

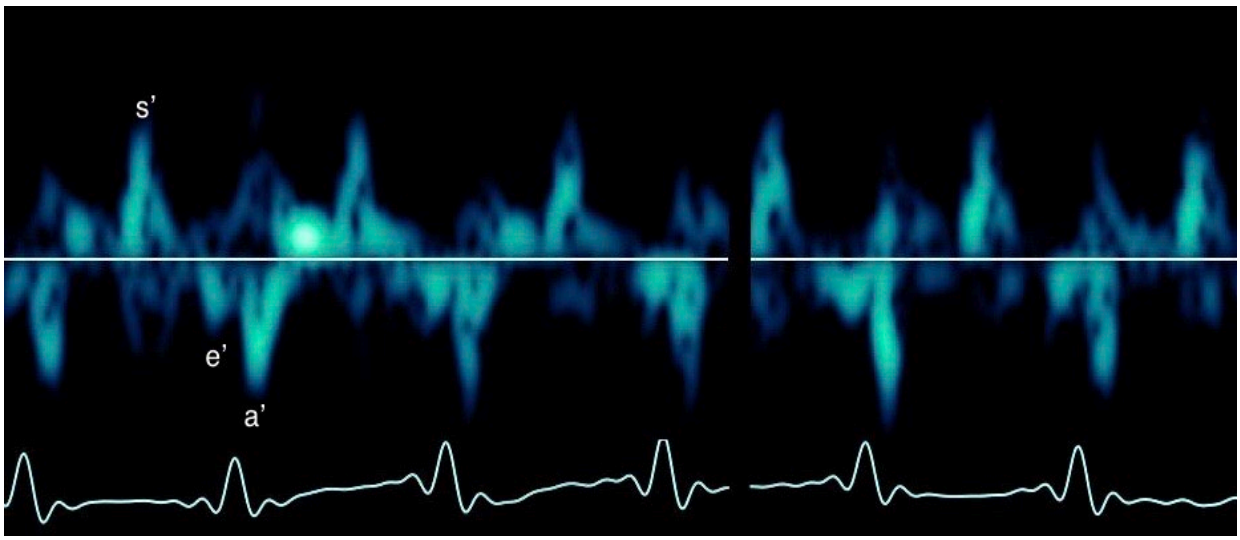


Figure 3.4: Example of the early diastolic mitral annulus velocity (e'), late diastolic annulus velocity (a') and peak systolic velocity (s') echocardiography image illustrating tissue Doppler image obtained from the mitral annulus.

3.6. Blood and organ sampling

After echocardiography, blood was collected by thoracotomy in clot activator or Ethylenediaminetetraacetic acid (EDTA) tubes. Tubes were centrifuged at 1500 rpm. Plasma and serum were collected and stored at -80°C until assayed. The estimated glomerular filtration rate was calculated by multiplying the urine volume with the urinary creatinine concentration and dividing the product by the serum creatinine.

The hearts were harvested and weighed. Further dissections on the heart were performed, the right ventricular wall was separated from the interventricular septum and the left ventricle was weighed.

3.7. Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assays (ELISA) were used to measure the concentration of systemic and urinary RAAS as well as urinary creatinine concentrations (used to measure estimated glomerular filtration rate). Urinary creatinine concentrations were measured using a Cr (Creatinine) ELISA Kit (catalogue no: E-EL-0058; Elabscience, Inc., Wuhan, China; sensitivity = 0.75 ug/mL, detection range = 1.25-80 ug/mL, coefficients of variation <10%). Renin concentrations in serum were measured using a Rat REN (Renin) ELISA Kit (catalogue no: E-EL-R0030; Elabscience, Inc., Wuhan, China; sensitivity = 9.38 pg/mL, detection range = 15.63-1000 pg/mL, coefficient of variation <10%). Serum Aldosterone concentrations were measured using an ALD (Aldosterone) ELISA Kit (catalogue no: E-EL-0070; Elabscience, Inc., Wuhan, China; sensitivity = 18.75 pg/mL, detection range = 31.25-2000 pg/mL, coefficient of variation < 10%). Serum and urinary angiotensinogen concentration measurements were done using a Rat AGT ELISA Kit (catalogue no: E-

EL-R0199; Elabscience, Inc., Wuhan, China; sensitivity = 37.5 ng/mL, detection range = 62.5-4000 ng/mL, coefficient of variation < 10%)

3.8. Histology (quantification of fibrosis)

Heart tissue was harvested, and the left ventricle was then cut out and fixed in ten percent (10%) buffered formalin. The samples were dehydrated and embedded in paraffin wax using standard techniques. Embedded tissue was thinly sliced at four μm using the Leica RM2125RT microtome and sections were prepared on microscope slides. The tissue sections were deparaffinised, rehydrated and stained with picrosirius red stain for an hour. Post staining, sections were washed in acidified water, removed and shaken vigorously to remove excess water. Sections were further dehydrated in 100% ethanol, cleared in xylene and finally mounted to a coverslip using the acrytol mounting medium.

To observe the collagen distribution across the LV extracellular matrix, sections were viewed and analysed using the Zeiss Axioskop 2 plus microscope equipped with a 208 colour Axiocam. Sections were viewed at 10x objective lens under both bright-field and polarized light. Neat and untorn areas with a high concentration of red staining were used for the quantification of the area of fibrosis. Three areas, using ImageJ Software (version 1.53m), were measured and the mean was calculated.

3.9. Statistical Analysis

Analyses were performed using the GraphPad Prism 9.2.0 Software. All parameters were tested for normality across all groups using the Shapiro-Wilk test. Parameters that did not pass the normality test were logged or square-rooted and retested for normality. The selection between the logged and square-rooted data to perform the statistical test was based on the lowest p-value obtained from the normality results of both the logged and square-rooted data sets. The following parameters were log-

transformed: body weight, conscious systolic blood pressure, mean of aldosterone concentrations and the following were square root transformed: heart weight, left ventricular weight, left ventricular internal diameter in diastole (LVIDs, left ventricular end-systolic volume (LVESV), early peak tissue lengthening velocity at the lateral mitral annulus (e'), late peak tissue lengthening velocity at the lateral mitral annulus (a'), filling pressure (E/e'), collagen fractional area of fibrosis, concentrations of renin, serum and urinary angiotensinogen, estimated glomerular filtration rate (eGFR), ANG II. Normally distributed parameters were expressed as mean ± standard deviation and those that were not normally distributed were expressed as the median and interquartile range. A two-way analysis of variances (ANOVAs) was performed on non-transformed or transformed parameters. In case of a significant interaction, the ANOVA was followed by a multiple comparison Tukey Kramer post hoc test. Differences across all groups were considered to be statistically significant at $P \leq 0.05$.

Chapter 4: Results

4.1. Characterisation of experimental and control groups

Table 4.1 shows the characteristics of the 7-month-old male and female SHR and WKY. No significant interaction was noted for the body weight ($p=0.1914$). WKYs had significantly heavier body weights compared to the SHRs ($p<0.0001$). Similarly, for the normalised heart weight, no significant interaction was noted ($p=0.6811$). SHR normalised hearts were significantly heavier than the WKYs ($p<0.0001$). No significant interaction was noted regarding the conscious systolic blood pressure ($p=0.5204$) however, SHRs had significantly higher conscious systolic blood pressures compared to the WKYs ($p<0.0001$). SHRs had significantly lower estimated glomerular filtration rates than the WKYs ($p=0.0453$) and no significant interaction was noted ($p=0.4582$). Body weights of the males were significantly heavier compared to females ($p<0.0001$). Female normalised hearts were significantly heavier than the male ($p<0.0001$). No significant difference was seen between the sexes regarding the conscious systolic blood pressure ($p=0.2175$) and the estimated glomerular filtration rate ($p=0.1077$).

A significant interaction was noted for the heart weights ($p=0.0493$). Male SHR hearts were significantly heavier than the other three groups ($p<0.0001$ vs female SHR, $p=0.0149$ vs male WKY and $p<0.0001$ vs female WKY). Male WKY hearts were significantly heavier than the female WKY and SHR (both $p<0.0001$). Heart weights were similar between the two female groups ($p=0.9761$). A significant interaction was noted for the anaesthetised systolic blood pressure ($p=0.0003$). Female SHRs had significantly higher anaesthetised systolic blood pressures than the other three groups ($p=0.04$ vs male SHR, $p<0.0001$ vs male WKY and $p<0.001$ vs female WKY). Male SHRs had significantly higher anaesthetised systolic pressures compared to the male and female WKYs (both $p<0.0001$). The anaesthetised systolic blood pressure was similar between the two WKY groups ($p=0.1805$). A significant interaction was noted

for the anaesthetised diastolic blood pressure (p=0.0051). Female SHRs had significantly higher diastolic blood pressures than the other three groups (p=0.0006 vs female SHR, p<0.0001 vs male WKY and p<0.0001 vs female WKY). Male SHRs had significantly higher anaesthetised diastolic blood pressures than the male and female WKYs (p=0.0112 and p=0.0083, respectively). The anaesthetised diastolic blood pressure was similar between the two WKY groups (p=0.4613) and no significant difference was seen between the two male groups (p=0.1667).

A significant interaction was noted for the conscious diastolic blood pressure (p=0.0025). Female SHRs had significantly higher conscious diastolic blood pressures than the other three groups (p=0.006 vs male SHR, p<0.0001 vs male WKY and p<0.0001 vs female WKY). Male SHRs had significantly higher conscious diastolic blood pressures than male and female WKYs (p=0.0112 and p=0.0083, respectively). The conscious diastolic blood pressure was similar between the two WKY groups (p=0.9992).

Table 4.1: Characteristics of 7-month-old male and female SHR and WKY

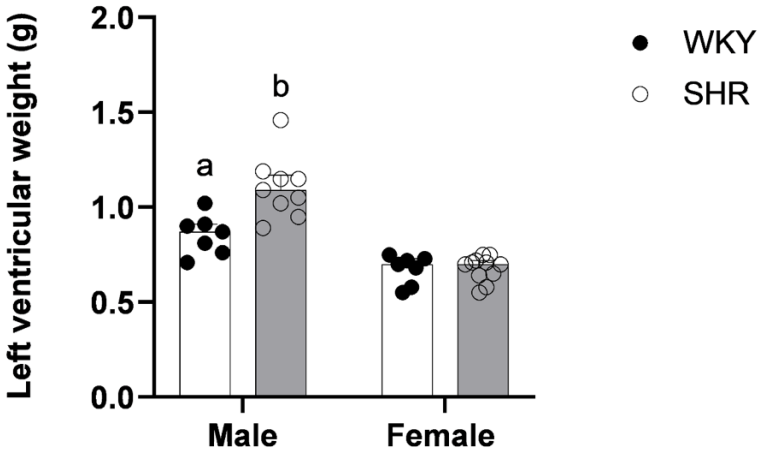
Parameters	**Male WKY(n=7)**	**Male SHR (n=8)**	**Female WKY (n=7)**	**Female SHR (n=8)**
Body weight, g	390 ± (372-411)	364 ± (296-392)***	226 ± (221-276)†††	206 ± (194-220.50)†††**
Heart weight, g	1.31 ± (1.18-1.42)[b]	1.49 ± (1.26-1.83)[a]	0.93 ± (0.81-1.10)†††	0.95 ± (0.83-1.10)†††*
Normalised heart weight, g/10^3	0.33 ± 0.02	0.42 ± 0.03***	0.39 ± 0.03†††	0.47 ± 0.05†††***
Anaesthetised SBP, mmHg	118 ± 9	147 ± 3[a]	108 ± 6	160 ± 11[c]
Anaesthetised DBP, mmHg	89 ± 12	105 ± 18[a]	79 ± 8	124 ± 14[c]
Conscious SBP, mmHg	128 ± (126-136)	176 ± (154-189)[a]	126 ± (117-133)	171 ± 7(164-179)[c]
Conscious DBP, mmHg	88 ± (86-93)	97 ± (94-100)[a]	90 ± (83-93)	107 ± (98-116)[c]
Estimated GFR, mL/min	0.36 ± (0.33-0.83)	0.34 ± (0.12-0.83)*	0.33 ± (0.40-1.02)	0.45 ± (0.10-0.68)*

Data expressed as mean ± SD (normalised heart weight, anaesthetised systolic and diastolic blood pressure) and median - IQR (body weight, heart weight, conscious systolic, diastolic blood pressure and estimated GFR. SBP: systolic blood pressure, DBP: diastolic blood pressure, GFR: glomerular filtration rate. *p<0.05 and ***p<0.001 vs WKY, †††P <0.001 vs male (Two-way ANOVA); *a* male SHR vs all other 3 groups, *b* male WKY vs all other group, *c* female SHR vs all other 3 groups (Two-way ANOVA followed by a Tukey Kramer post hoc test)

Figure 4.1A shows the left ventricular weights where a significant interaction was noted ($p=0.0032$). Male SHR left ventricles were significantly heavier than the other three groups ($p<0.0001$ vs female SHR, $p=0.0005$ vs male WKY and $p<0.0001$ vs female WKY). Male WKY left ventricles were significantly heavier than the female WKY ($p=0.0211$) and female SHR ($p=0.0121$). The left ventricular weight was similar between the two female groups ($p=0.9996$). Figure 4.1B shows the normalised left ventricular weight and no significant interaction was noted ($p=0.0658$). SHR normalised left ventricles were significantly heavier than that of the WKY ($p<0.0001$). Females had normalised left ventricles that were significantly heavier than that of the males ($p=0.0005$).

Figure 4.2A shows significant collagen accumulation in the SHR compared to the WKY rats as shown by the increased red staining of cardiac tissue sections visualised under bright-field microscopy. Figure 4.2B shows cardiac tissue sections under polarised light, where thin fibres show more cross linking appear green and thick collagen fibres appear red or orange. Both figures show significantly greater cardiac remodelling through greater deposition and cross-linking of collagen I and III fibres in SHR compared to WKY. Figure 4.2C shows the left ventricular collagen area fraction (marker of collagen accumulation and fibrosis) of the groups (mean ± SD), of which no significant interaction was noted ($p=0.6767$). The left ventricular collagen area fraction was significantly greater in SHRs compared to WKYs ($p=0.0223$).No significant differences were observed between the sexes ($p=0.5826$).

A



B

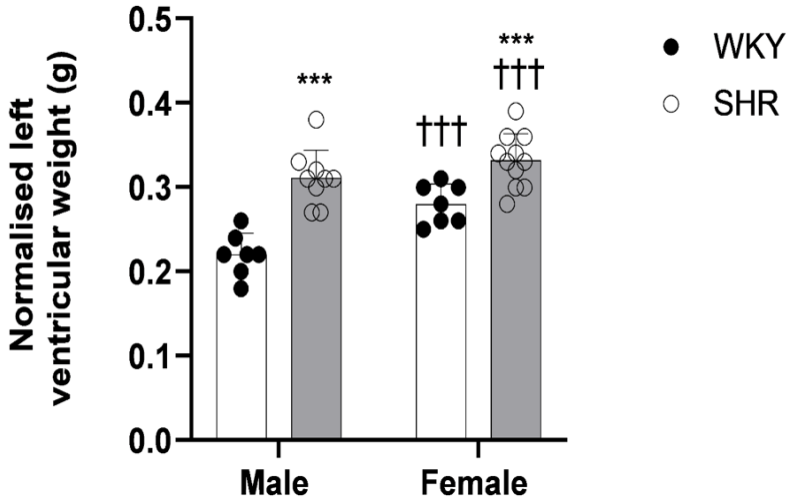


Figure 4.1: Left ventricular weight (A) and normalised left ventricular weight (B) of 7-month-old male and female SHR and WKY.

Data expressed as mean ± SD (B) and Median - IQR (A). ***p<0.001 vs WKY, †††P <0.001 vs male, *a* male WKY vs all other groups, *b* male SHR vs all other groups (Two-way ANOVA followed by a Tukey Kramer post hoc test).

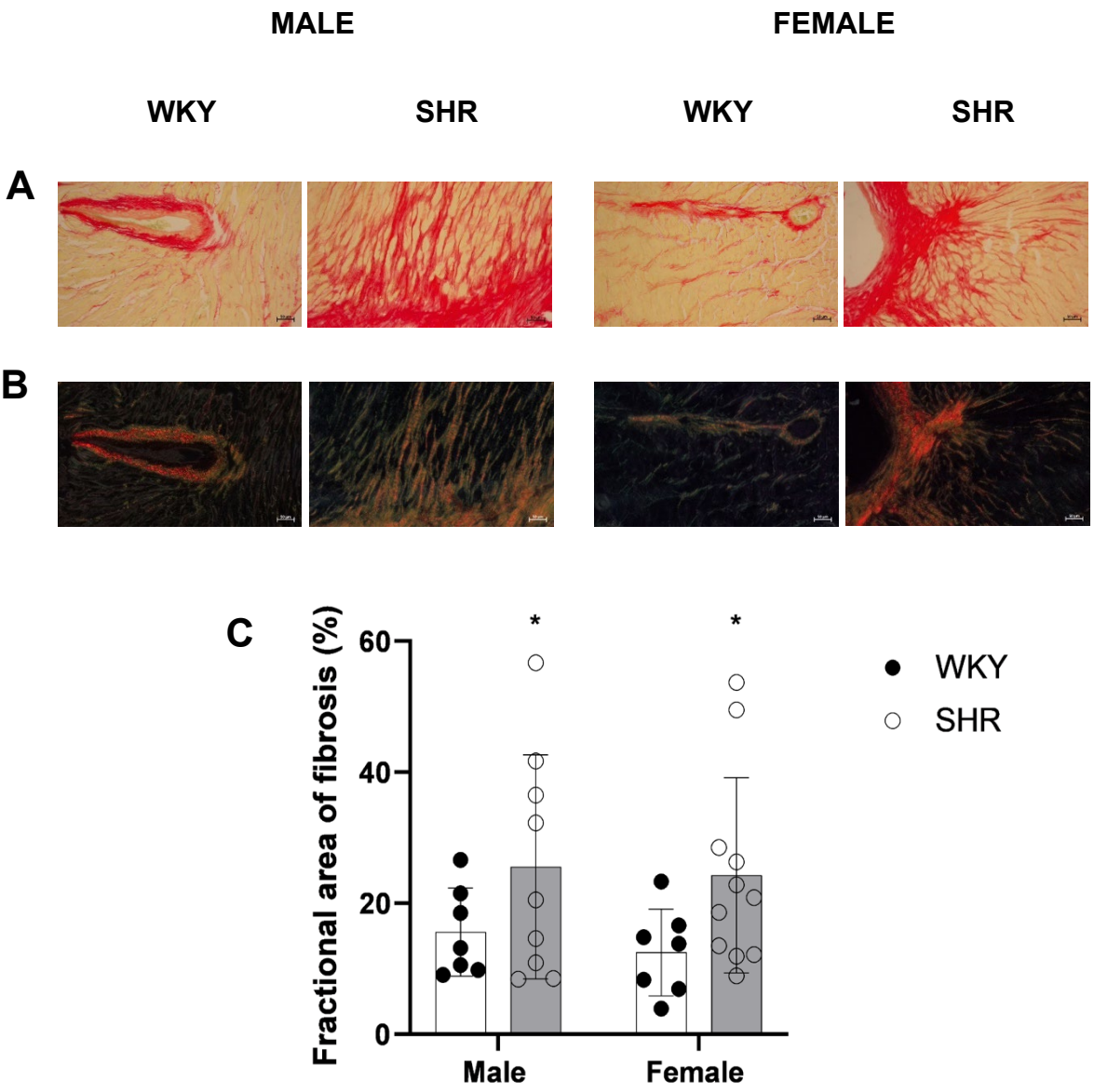


Figure 4.2: Left ventricular collagen area fraction 7-month-old male and female SHR and WKY groups.

Representative picrosirius red stained micrographs imaged at x100 magnification viewed in (A) bright-field and under (B) polarised light. (C) % collagen area fraction calculated from picrosirius red stained LV sections (SHR: n=8, WKY: n=7). Data expressed as mean ± SD. *p<0.05 vs WKY (Two-way ANOVA followed by a Tukey Kramer post hoc test).

4.2. Left ventricular geometry and systolic function

Table 4.2 shows the M-mode-derived echocardiographic parameters. There was no significant interaction noted for the left ventricular interventricular septum diameter in systole (LVIDs) (p=0.7138). Moreover, no significant difference was seen for LVIDs across sex (p=0.4122) and strain (p=0.7025). No interaction was noted for the IVSDs (p=0.8066). No significant difference was noted for the IVSDs when strains were compared (p=0.8783). Males had significantly greater IVSDs compared to the females (p=0.0089). Similarly, no interaction was noted for the IVSDd (p=0.6713). The difference in IVSDd between the SHR and WKY was not significant (p=0.3881). Males had a larger IVSDd compared to the females (p=0.0253). Concerning the LVPWTs, no significant interaction was noted. Furthermore, no significant difference was seen for sex (p=0.2764) and strain (p= 0.2505). Statistical analyses showed no significant interaction (p=0.8742) and no significant differences for sex (p=0.3172) and strain (p=0.631) for the LVESV. No significant interaction was noted for the left ventricular end-diastolic volume (LVEDV) (p=0.6271). SHRs had significantly lower LVEDV compared to the WKYs (p=0.0395). The males had a significantly greater LVEDV compared to females (p=0.0359).

No significant interaction was noted for the stroke volume (SV) (p=0.6295). SHRs had significantly lower SV than the WKYs (p=0.0036). Males had a significantly greater stroke volume than females (p= 0.0384).

Regarding the ejection fraction (EF), no interaction was noted (p=0.9456) and no significant differences were observed between sexes (p=0.8780) and strains (p=0.2326). No significant interaction was noted for the endocardial fractional shortening (endFS) (p=0.1786) and no differences were seen across both sex (p=0.143) and strain (p=0.3572).

The mid-wall fractional shortening (midFS) of the SHRs was significantly lower than the WKYs ($p=0.0075$) and no significant interaction was seen ($p=0.9917$). MidFS of the males was significantly greater compared to females ($p=0.0032$).

Table 4.2: Left ventricular geometry and systolic function of 7-month-old male and female SHR or WKY

Parameters	Male WKY (n=7)	Male SHR (n=8)	Female WKY (n=7)	Female SHR (n=8)
Cardiac geometry				
LVIDs, cm	0.33 ± (0.26-0.48)	0.28 ± (0.22-0.66)	0.31 ± (0.19-0.35)	0.30 ± (0.21-0.60)
IVSDs, cm	0.38 ± 0.09	0.37 ± 0.07	0.30 ± 0.04††	0.34 ± 0.07††
IVSDd, cm	0.27 ± 0.08	0.31 ± 0.11	0.21 ± 0.01†	0.22 ± 0.09†
LVPWTs, cm	0.28 ± 0.09	0.34 ± 0.06	0.34 ± 0.07	0.34 ± 0.06
Systolic function				
LVESV, ml	0.14 ± (0.05-0.27)	0.10 ± (0.06-0.84)	0.11 ± (0.04-0.17)	0.09 ± (0.04-0.50)
LVEDV, ml	0.73 ± 0.19	0.54 ± 0.19*	0.53 ± 0.13†	0.41 ± 0.23†*
SV, ml	0.57 ± 0.19	0.36 ± 0.15**	0.42 ± 0.12†	0.26 ± 0.12†**
EF	77.60 ± 8.71	82.20 ± 27.15	75.37 ± 7.59	71.9 ± 0.99
endFS, %	43.30 ± 12.20	44.90 ± 4.10	42.80 ± 8.90	34.40 ± 9.30
midFS, %	29.50 ± 5.20	22.70 ± 3.10**	21.90 ± 8.20††	15.20 ± 4.70††**

Data expressed as mean ± SD (IVSDs, IVSDd, LVPWTs, LVEDV, SV, endFS, midFS) and median - IQR (LVIDs, LVESV, EF). LVIDs: left ventricular internal diameter end systole, IVSDs: interventricular septal diameter end systole, IVSDd: interventricular septal diameter end diastole, LVPWTs: left ventricular posterior wall thickness end systole, LVESV: left ventricular end systolic volume, LVEDV: left ventricular end diastolic volume, SV: stroke volume, EF: ejection fraction, endFS: endocardial fractional shortening, midFS: midwall fractional shortening. *p<0.05 and **p<0.01 vs WKY, †p <0.05 and ††p<0.01 vs male (Two-way ANOVA followed by a Tukey *Kramer* post hoc test)

Figure 4.3 shows the differences in LVIDd across the four groups. No significant interaction was noted for the left ventricular interventricular septum diameter in diastole (LVIDd) (p=0.911). The LVIDd was significantly lower in SHRs compared to WKYs (p=0.0283). Males had a significantly larger LVIDd than females (p=0.0356).

Figure 4.4 shows the Left ventricular posterior wall thickness in diastole (LVPWTd) of the four groups. No significant interaction was noted (p=0.3202). The SHR had a significantly greater LVPWTd than the WKY (p=0.0035). There was no significant difference observed across the sexes regarding the LVPWTd (p=0.0576).

Figure 4.5 illustrates the relative wall thickness (RWT) of which no significant interaction was noted (p=0.0939). RWT was significantly larger in SHR compared to the WKYs (p=0.0018). Males had a significantly lower relative wall thickness compared to females (p= 0.0486).

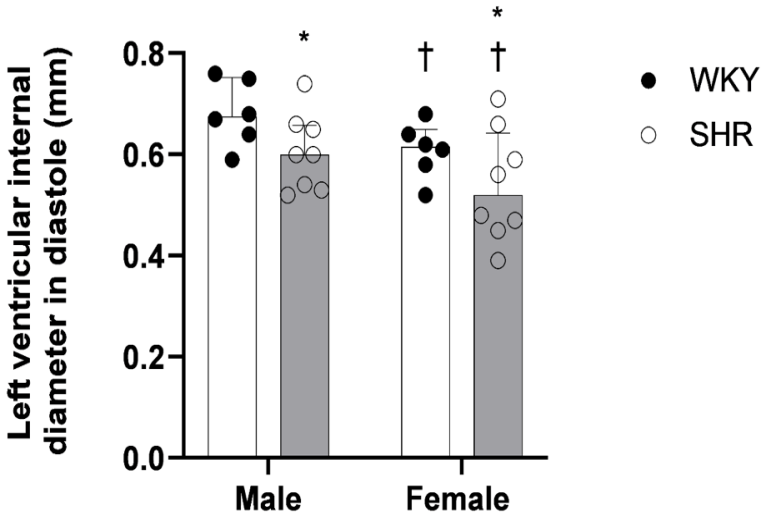


Figure 4.3: Left ventricular internal diameter in diastole of 7-month-old male and female SHR and WKY.

Data expressed as mean ± SD. *p<0.05 vs WKY, †p<0.05 vs male (Two-way ANOVA followed by a Tukey Kramer post hoc test).

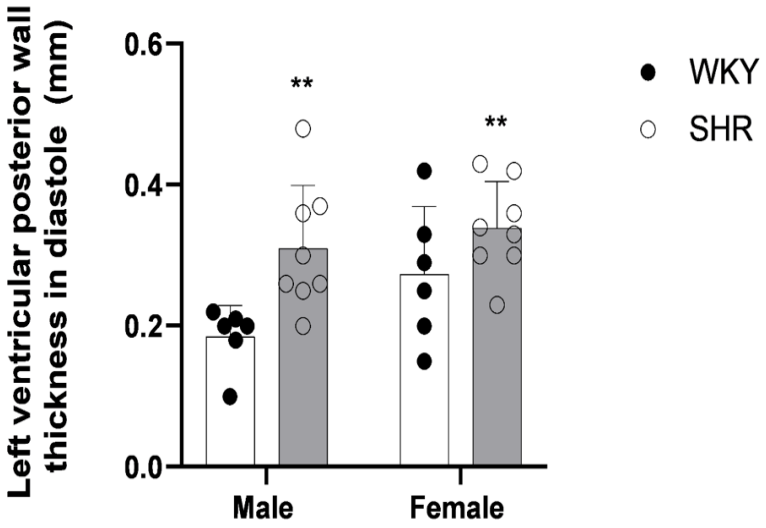


Figure 4.4:Left ventricular posterior wall thickness in diastole of 7-month-old male and female SHR and WKY.

Left ventricular posterior wall thickness in diastole of 7-month-old male and female SHR and WKY. Data expressed as mean ± SD. **p<0.01 vs WKY (Two-way ANOVA followed by a Tukey Kramer post hoc test).

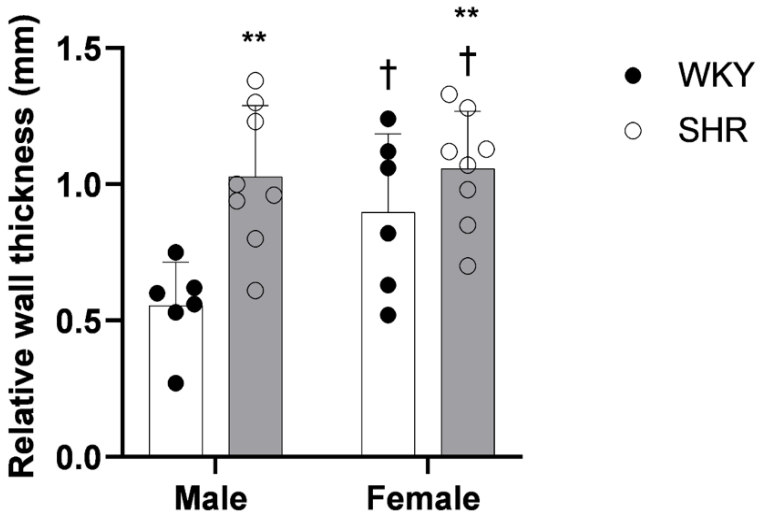


Figure 4. 5: Relative wall thickness of 7-month-old male and female SHR and WKY. Data expressed as mean ± SD. **p<0.01 vs WKY, †P <0.05 vs male (Two-way ANOVA followed by a Tukey Kramer post hoc test).

4.3. Left ventricular diastolic function

Table 4.3 shows the pulsed Doppler and tissue Doppler velocities. For the E, no significant interaction was noted (p=0.7233) and no significant difference was observed for both sex (p=0.4861) and strain (p=0.1067). No significant interaction was noted (p=0.3848) and no significant difference was seen when comparing sexes (p=0.9709) and strains (p=0.4595) for the early to late diastolic filling ratio (E/A). Regarding e', no significant interaction was noted (p=0.6113). The e' was significantly lower in the SHRs compared to the WKYs (p=0.004). No interaction was seen for a' (p=0.1625). Regarding e' and a', no significant difference was observed across the sexes and p-values were p=0.7314 and p=0.1697 respectively. For the early to late peak tissue lengthening velocity ratio e'/a', of which no significant interaction was noted (p=0.1149), no significant differences were observed for both the sex (p=0.24790 and strain (p=0.2905). No significant interaction was noted for the E/e' (p=0.6687). SHRs had significantly greater filling pressures compared to the WKY (p=0.0095). Comparing sexes for E/e', no significant difference was seen (p=0.529).

Table 4.3: Left ventricular diastolic function parameters in 7-month-old male and female SHR or WKY

Parameters	Male WKY (n=7)	Male SHR (n=8)	Female WK (n=7)	Female SHR (n=8)
Pulsed Doppler				
E, cm/s	55.50 ± (39-92)	91.00 ± (37-414)	50.00 ± (31-106)	80.50 ± (22-271)
E/A	2.43 ± 0.80	2.48 ± 0.98	2.80 ± 1.16	2.15 ± 1.07
Tissue Doppler				
e', cm/s	4.00 ± (3.0-6.0)	3.00 ± (2.0-4.0)**	4.50 ± (2.0-7.0)	3.00 ± (2.0-4.0)**
E/e'	13.90 ± (9.4-23.67)	45.50 ± (92.5-103.50)**	12.50 ± (6.2-21.5)	25.60 ± (12.50-90.33)**
e'/a'	0.50 ± (0.44-0.75)	1.00 ± (0.60-1.33)	0.70 ± (0.33-2.5)	0.80 ± (0.60-1.50)

Data expressed as mean ± SD (E/A) and median - IQR (E, A, e', a', s', E/e', e'/a'). e': early peak tissue lengthening velocity at the lateral mitral annulus, a': late peak tissue lengthening velocity at the lateral mitral annulus, s': peak systolic velocity, E: maximum early mitral inflow velocity, A: maximum late mitral inflow velocity, E/A: early to late diastolic filling velocity ratio, e'/a': early to late peak tissue lengthening velocity ratio. *p<0.05 and ***p<0.001 vs WKY, †P <0.05 vs male (Two-way ANOVA followed by a *Tukey* Kramer post hoc test)

4.4. Systemic and urinary concentrations of RAAS components

Table 4.4 shows the plasma, serum and urinary concentrations of components of the renin-angiotensin-aldosterone system. For the interaction, sex and strain for all parameters shown in the table, no significant differences were noted. Serum renin: p=0.3411 (interaction), p=0.7168 (sex) and p=0.7522 (strain). The mean of serum aldosterone: p=0.1821 (interaction), p=0.6615 (sex) and p=0.2679 (strain). Serum angiotensinogen: p=0.497 (interaction), p=0.1175 (sex) and p=0.6687 (strain). Finally, urinary angiotensinogen: p=0.0677 (interaction), p=0.1577 (sex) and p=0.7602 (strain).

Table 4.4: Systemic and urinary concentrations of RAAS components in 7-month-old male and female SHR or WKY

Parameters	**Male WKY (n=7)**	**Male SHR (n=8)**	**Female WKY (n=7)**	**Female SHR (n=8)**
Serum renin, pg/mL	55.0 ± (49.7-105.5)	60.2 ± (53.5-98.9)	61.1 ± (49.2-167.8)	56.6 ± (48.7-1249)
Serum aldosterone, pg/mL	268.0 ± (94.9-331.6)	187.6 ± (82.6-353.4)	164.1 ± (62.5-308.2)	280.7 ± (1.8-6.6)
Serum angiotensinogen, ng/mL	3.00 ± (1.3-18.6)	3.80 ± (1.66-24.3)	8.30 ± (2.1-23.0)	6.00 ± (1.3-25.7)
Urinary angiotensinogen, ng/mL	99.7 ± 13.4	109.1 ± 15.9	119.4 ± 10.2	106.4 ± 14.0

Data expressed as mean ± SD (urinary angiotensinogen) and median - IQR (plasma renin, mean of aldosterone, aldosterone to renin, angiotensin II, serum angiotensinogen). (Two-way ANOVA followed by a *Tukey* Kramer post hoc test)

4.5. Angiotensin II concentrations

Figure 4.6 illustrates the varying concentrations of ANG II across the four groups. A significant interaction was noted for the concentration of ANG II ($p=0.0213$). The female SHR had a significantly higher concentration of ANG II compared to the three other groups ($p=0.0007$ vs male SHR, $p=0.0012$ vs male WKY and $p=0.0057$ vs female WKY). Comparing the two male groups (SHR and WKY), no significant difference was observed ($p=0.9992$). No significant difference was seen between the male and female WKY ($p=0.8699$). Lastly, no significant difference was observed between male SHR and female WKY ($p=0.9010$).

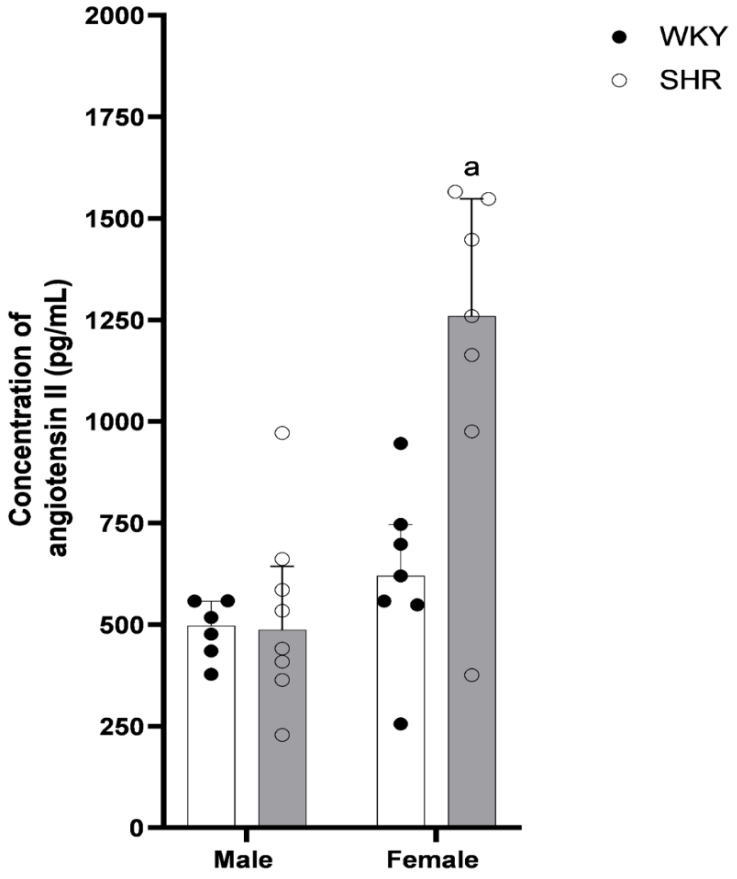


Figure 4. 6: Concentration of angiotensin II in 7-month-old male and female SHR and WKY.

Data expressed as mean ± SD. *a* female SHR vs all other groups (Two-way ANOVA followed by a Tukey Kramer post hoc test).

Chapter 5: Discussion

The main findings of this study are as follows:

Compared to WKYs, SHRs had concentric hypertrophy (increased thickness of the heart wall and overall muscle mass), impaired left ventricular relaxation (reduced lateral e') and increased filling pressures (increased E/e'). In addition, SHRs had impaired systolic function characterised by increased end-diastolic volume, stroke volume and mid-wall fractional shortening. Compared to males, females had greater physiological left ventricular myocardial growth (increased normalised heart and left ventricular weights and relative wall thickness). No differences between the sexes were observed for indices of diastolic function. Additionally, females had a reduced systolic performance (decreased end-diastolic volume, stroke volume, mid-wall fractional shortening and peak systolic velocity). Finally female SHRs had greater anaesthetised systolic and diastolic pressure as well as conscious diastolic blood pressure compared to all other groups. In addition, female SHRs had greater plasma concentrations of ANG II compared to other groups which may be responsible of the higher blood pressure in 7-month-old female SHR.

These findings highlight the sexual dimorphism in blood pressure and cardiac remodelling in hypertension and the development of LVH.

5.1. Effects of hypertension on cardiac remodelling

The results of the present study show that chronic hypertension resulted in notable changes in cardiac geometry. SHRs had increased heart and left ventricular mass compared to WKYs. In addition, SHRs had increased left ventricular posterior wall thickness and relative wall thickness compared to WKYs. Indeed, the type of cardiac remodelling that takes place as a result of chronic untreated hypertension in SHRs has been shown to be concentric left ventricular hypertrophy (Legrice et al., 2012). In SHRs, hypertension triggers an escalation in systemic vascular resistance,

necessitating the heart to exert more force to circulate blood against the heightened resistance across the body (Messerli et al., 2017). This augmented afterload places an added burden on the left ventricle, promoting it to undergo structural adaptations. In response to the heightened afterload, the left ventricle undergoes hypertrophy, characterised by the thickening of its muscular wall (Davies et al., 2018). This adaptive change allows the heart to generate increased force to overcome the elevated arterial resistance and maintain sufficient blood flow. In the context of hypertension, the left ventricle may undergo concentric remodelling, which signifies an adaptation where the chamber's size may decrease, and its walls become thicker (Cramariuc & Gerdts., 2016). Consequently, there is an increase in the posterior wall thickness and relative wall thickness. The increase in the thickness of the left ventricular wall and the overall muscle mass of the chamber contributes to the greater left ventricular mass in SHRs compared to WKYs.

The molecular mechanisms that cause concentric hypertrophy in SHRs remain incompletely understood. In the current study, SHRs had significant collagen accumulation in their left ventricles compared to WKYs. Our findings align with previous studies that have also documented myocardial fibrosis in SHRs (Xin et al., 2020). The mechanical strain imposed by hypertension on the heart chamber walls sets in motion a cascade of molecular signalling pathways, including the activation of TGF-β pathway (Wu et al., 2016). This activation, in turn, prompts the stimulation of fibroblasts, specialised cells responsible for collagen synthesis. The resulting fibrotic response leads to an accumulation of collagen within the heart tissue, making it stiffer and less compliant (Pagan et al., 2015). This increased stiffness subsequently drives the development of concentric hypertrophy as the left ventricle adapts to the elevated afterload. Therefore, taken together, our findings suggest that hypertension likely

induces heightened myocardial fibrosis, which, in turn, may impact the myocardial remodelling processes, ultimately leading to the development of concentric hypertrophy.

5.2. Effects of hypertension on diastolic function

In the present dissertation, pulse and tissue Doppler imaging echocardiographic techniques were used to determine the diastolic function. No significant differences were observed between SHRs and WKYs regarding E and E/A (markers of left ventricular relaxation). Although the E and E/A ratio were similar between WKYs and SHRs, lateral e' was significantly reduced and filling pressures (E/e') were increased in SHRs compared to WKYs which suggests a pseudonormal filling pattern. The increased filling pressures (E/e') in SHRs may have resulted in a compensatory increase in early diastolic filling (E) resulting in moderate to severe diastolic dysfunction. This further suggests that tissue Doppler imaging techniques are more suitable and sensitive in the investigation of diastolic dysfunction and need to be measured in addition to pulse Doppler measurements. Similar to our results, animal studies have shown impaired diastolic filling characterised by an increase in filling pressures and impaired left ventricular relaxation in SHRs (J. Wang et al., 2017).

There are several mechanisms by which hypertension can cause impaired left ventricular relaxation and increased filling pressures. In SHRs, hypertension may bring about a systemic proinflammatory state which is important in the presentation of diastolic dysfunction (Kuwahara et al., 2004). This proinflammatory state is marked by an increase in cytokines such as tumour necrosis factor (TNF)-α and IL-6 (Plitt et al., 2018). In cardiomyocytes, these cytokines decrease the bioavailability of endothelial nitric oxide (NO) and attenuate the activity of cyclic phosphate-protein kinase G (cGMP-PKG) (Lakhani et al., 2019). This flow of events together with collagen

deposition synergistically leads to the stiffening of cardiomyocytes by way of hypertrophy and ultimately diastolic dysfunction in the left ventricle. In addition to proinflammatory markers, animal studies have shown hypertension in SHRs may result in abnormal intracellular calcium handling and reduced expression of sarcoplasmic reticulum ATPase (SERCA) pumps which in turn leads to compromised relaxation (Williams et al., 2014; Dupont et al., 2012).

Pro-fibrotic pathways are important in left ventricular hypertrophy and diastolic dysfunction and may also be affected in SHRs. It is widely known that chronic elevated pressures induce pro-fibrotic pathways (Nwabuo, 2020). Persistent hypertension brings about a fibrogenic response in the myocardium, where there is activation of resident cardiac fibroblasts into myofibroblasts. These myofibroblasts upregulate depositions of proteins such as Platelet Derived Growth Factors in the cardiac interstitium, which are important in the fibrotic response (Frangogiannis, 2018). These events may destabilise the structural integrity of the cardiac ECM(9. Fibroblast activation and collagen deposition in the cardiac ECM, specifically type 1 collagen fibres, is associated with stiffening of the left ventricle because of their size and strength as compared to the more thin and elastic type 3 collagen fibres (Schimmel et al., 2022). Taken together, our results suggest that hypertension may have resulted in fibrosis, which rendered the heart stiff. This stiffness may have led to impaired left ventricular relaxation and increased filling pressures.

5.3. Effects of hypertension on systolic function

In the present study, the end-diastolic volume, stroke volume and mid-wall fractional shortening were reduced in SHRs compared to WKYs. Similar to our results, previous studies have reported impaired systolic function characterised by reduced end-

diastolic volumes, stroke volume and mid-wall fractional shortening in SHRs (Cingolani et al., 2015; Kokubo et al., 2007; Potnuri et al., 2022).

Reduced left ventricular end-diastolic volume in the SHRs is suspected to be due to left ventricular remodelling caused by chronic hypertension. Indeed, left ventricular myocardial remodelling in this context negatively impacted the distensibility and contractility of the heart. Left ventricles in the SHRs were not able to reasonably distend to accommodate inflow from the left atrium, hence reducing left ventricular end-diastolic volume in SHRs. Consequently, this may have been the reason for a reduction in stroke volume, which alluded to possible systolic dysfunction.

Although ejection fraction and endocardial fractional shortening were similar between SHRs and WKYs, mid-wall fractional shortening was reduced in SHRs. Indeed, mid-wall fractional shortening is a more valid index of systolic function especially in cases where concentric hypertrophy has manifested (Ono et al., 2002). This rationale is due to ejection fraction being sensitive to both pressure overload and left ventricular remodelling as these factors influence the percentage of ejection. Ejection fraction is therefore a better indicator of ventricular-arterial coupling rather than contractility (Guihaire et al., 2013). Hence the preservation of ejection fraction may have been due to the compensatory hypertrophic response, which maintained the overall ejection fraction even though the ventricular function was impaired.

Studies in SHRs have reported on the reduction of the mid-wall fractional shortening in cases where left ventricular hypertrophy has manifested (Kokubo et al., 2007). In cases of concentric hypertrophy, the increased thickness of the heart wall and the altered cardiac geometry can result in compromised systolic function (Haas et al., 1995). Consequently, mid-wall fractional shortening may be reduced since the heart

encounters greater difficulty in contracting efficiently and shortening the muscle fibres situated within the central region of the ventricular wall (Lin et al., 2020).

Wachtell et al., 2010 showed an independent negative association between the mid-wall fractional shortening and the left ventricular mass in the case of hypertension. This validated previous studies that reported the improvement of systolic performance marked by a less depressed mid-wall fractional shortening when treatment was administered and regression of left ventricular hypertrophy was apparent. A statistically significant depressed mid-wall fractional shortening in SHRs as seen in the present dissertation, together with a reduced left ventricular end diastolic volume and stroke volume strongly suggests systolic dysfunction.

5.4. Effects of sex on cardiac remodelling

The differences in heart and left ventricular weights between males and females together with the opposing results when normalised to body weight, can be due to several physiological factors characteristic of the sexes. Typically, males have larger body sizes and more lean muscle mass compared to females, which leads to larger organ sizes (Wells, 2007). Moreover, sex hormone effects, particularly testosterone, has been shown to contribute to the development of larger heart and left ventricles in males (Silva et al., 2008). Indeed, testosterone has been associated with increased muscle mass and cardiac hypertrophy, leading to larger heart sizes in males (Rubio-Gayosso et al., 2013).

Interestingly, when normalised to body weight, females had heavier heart and left ventricular weights compared to males. In addition, females had increased relative wall thickness compared to males. These results suggest that females may have relatively larger hearts for their body size compared to males. One possible explanation may be the variations in cardiac remodelling and adaptation to

physiological demands. Women tend to have smaller bodies and less muscle mass compared to males (Sanada et al., 2012), which may cause a higher workload on the heart. As a compensatory mechanism, the female heart may undergo hypertrophic remodelling to meet the demands of cardiac output, resulting in relatively larger heart and left ventricular weights when adjusted for body weight and increased relative wall thickness (Chen, 2012).

Oestrogen in a pathological context provides cardioprotective effects (Dworatzek et al., 2014). However, oestrogen may function physiologically as a growth factor (Gürgen et al., 2011). Indeed, the effect of oestrogen in favouring cardiac remodelling has been demonstrated and it is connected to a relevant modification of the expression of genes involved in myocardial hypertrophy and fibrosis (Kararigas et al., 2014; Dworatzek et al., 2014). In females, oestrogen may promote a more compact myocardial structure, leading to increased relative wall thickness compared to males. This structural adaptation could contribute to the observed differences in left ventricular weights when normalised to body weight. Furthermore, higher eGFR indicated normal kidney function. Suggesting blood pressures should be lower in SHR female. However, this is not the case in fact SHR females have the highest blood pressure and greater LVH mass when normalised. However, in this context, the greater LVH in females is physiological and is suspected to be due to oestrogen.

In support of this, a study showed that the administration of oestrogen to female rats induced an increase in the heart-to-body weight ratio through activation of an intracellular signalling pathway (Wittnich, 2015). Experimental studies have reported the role of oestrogen in cardiac physiology, demonstrating that its binding to ERβ receptors activate signalling pathways such as the MAPKs pathways, ultimately leading to cardiac hypertrophy (Dworatzek et al., 2014). Furthermore, another study

showed that ERα mediates physiological cardiac remodelling (Kararigas et al., 2014). This study revealed that without ERα, physiological remodelling does not occur, suggesting that ERα, rather than ERβ, plays a pivotal role in this process (Kararigas et al., 2014). This understanding highlights the importance of oestrogen-induced cardiac growth in maintaining cardiac function and adapting to physiological demands. In further validating the normal physiological growth in females, no differences were observed in fibrosis between males and females. Fibrosis is typically associated with pathological conditions (Kong et al., 2014). This confirms that the increased normalised heart and left ventricular weights in females were indeed attributed to normal physiological growth.Taken together, results of our study suggest that the greater physiological left ventricular myocardial growth in this case was possibly due to the growth effects of oestrogen.

5.5. Effects of sex on diastolic function

In the present study no significant differences were seen between males and females regarding diastolic function indices. In contrast to our results, several experimental studies (Douglas et al., 1998; Kitpipatkun, & Sukwan, 2022; Shah et al., 2018) have reported increased filling pressures and impaired left ventricular relaxation in males compared to females. The progression of diastolic dysfunction is accelerated in male rats compared to female rats (Chan et al., 2011). However, female rats typically have a higher degree of cardiac compensatory remodelling than males, which may play a role in modulating diastolic dysfunction (Beaumont et al., 2017; Friberg et al., 1985; Wallen et al., 2000).

In the present study, males of both species may have developed diastolic dysfunction earlier than females. Male rats having a worsened diastolic function compared to female rats could be due to increased activation and stimulation of factors that

increase cardiac remodelling, which consequently then result in diastolic dysfunction. These factors may include testosterone. Testosterone can directly and indirectly lead to diastolic dysfunction. Testosterone activates rapamycin complex 1 (mTORC1) in cardiomyocytes and this induces protein synthesis and growth of cardiomyocytes (Silva et al., 2008). Testosterone has been positively correlated to blood pressure and shown to increase blood pressure (Loh & Salleh, 2017). This may result in an increased afterload which may induce cardiac remodelling and cause diastolic dysfunction. Males are further associated with increased sympathetic nervous system activation, which promotes cardiac remodelling independently of blood pressure (B. Wang, 1999). However no differences were seen for diastolic indices at 7-month-old. SHRs are used in investigating sex differences in human hypertension and hypertension induced cardiac remodelling. However, due to the complexity of sex differences in mechanisms, differences might not always be seen across all parameters of diastolic dysfunction. Males might have developed diastolic dysfunction earlier than females. However, females may have developed diastolic dysfunction to a similar degree as males of the present study due to increased compensatory physiological concentric LVH. The fact that no differences between sexes were seen for diastolic function implies that maybe the rats were still progressing into diastolic dysfunction. Male rats typically progress through stages of heart failure earlier than female rats. So, if males in this context have less cardiac growth, this might imply that rats still in the early stages of disease progression. So, the increased growth seen in females was compensatory and due to protective effects of estrogen.

5.6. Effects of sex on systolic function

Differences in the end diastolic volume, stroke volume, and mid-wall fractional shortening in female as compared to male rats may be associated with several physiological factors such as the differences in body weight and the impact of Frank-Starling mechanisms. First, the weight of the body is the primary factor that should be discussed in association with heart function. As was pointed out, the male rats are larger and heavier usually compared to the female rats. The difference in body weight may result in different heart sizes and functions. Studies have shown that larger animals tend to have greater cardiac dimensions and volumes to accommodate the increased metabolic demands of a larger body mass (Forman et al., 1997; Schaible & Scheuer, 1984). Therefore, the end diastolic volume and stroke volume may be higher in male rats, as they are larger in size, than in female rats.

Secondly, an important physiological mechanism in the heart, namely the Frank-Starling law, describes the relationship between end diastolic volume and stroke volume as a major effect on cardiac output control. Based on this law, a rise in end diastolic volume may lead to a similar rise in the stroke volume due to stretch-induced enhancement of myocardial contractility (Shiels & White, 2008). With the observations of the diversity between male and female rats, the decline observed in the female's end diastolic volume suggests a decreased stroke volume via the Frank-Starling mechanism. It implies that the left ventricle of female rats may contract at a reduced preload in comparison to males, thus affecting the cardiac performance.

Moreover, mid-wall fractional shortening is an index of myocardial contractility and function. The lower values in female rats may indicate that females have less contractile function than males. This could be influenced by factors such as hormonal differences, as oestrogen has been shown to have cardioprotective effects, whereas

testosterone may exert positive inotropic effects on the heart (Sun et al., 2011). Indirectly, oestrogen through it vasodilatory effects can reduce blood pressures and therefore work load imposed on the heart (Jazbutyte et al., 2008). Therefore, protecting the female hearts against increased force of contraction.

Testosterone through binding to its cardiac receptors can directly induce stronger cardiac contractions (Marsh et al., 1998). Testosterone may also lead to increased cardiac cytosolic Ca^{2+} through modulation of calcium channels, which can cause an increase in strength of contraction (Er et al., 2007). Moreover, testosterone can activate the SNS and cause an increase in cardiac contraction (Ely et al., 1997; Kumai et al., 1994; Meyer et al., 2010). Taken together, our results suggest that females had reduced systolic performance compared to males, which was attributed to the effect of body weight, hormonal influences, and variations in blood volume.

5.7. Effects of strain and sex on blood pressure and the role of the ANG II

Numerous studies have shown higher blood pressures in male SHRs compared to female SHRs at 3, 6 and 16 months (Reckelhoff et al., 2015; Maris et al., 2005; Yanes, et al., 2006). Higher blood pressures in males are associated with increased classical RAAS activation and effects of testosterone as factors contributing to hypertension (Loh & Salleh, 2017). Testosterone increases vessel collagen deposition, which affects vessel's ability to regulate blood flow and pressure (Seachrist et al., 2000). Testosterone can increase oxidative stress through production of ROS and free radicals which directly damage vessels (Costa et al., 2015). Moreover, testosterone is able to induce ET-1 release which causes vasoconstriction and chronically this can lead to hypertension (Seachrist et al., 2000). Furthermore, testosterone is able enhance pressor effects of ANG II, which are vasoconstriction and stimulation of aldosterone release. Aldosterone is responsible for salt and water retention, which

increases blood volume (Goto et al., 2000). Both vasoconstriction and water retention cause hypertension.

Additionally, lower systolic and diastolic blood pressure in females is believed to be due to the cardioprotective effects of oestrogen (Sullivan et al., 2007). Oestrogen induces NO (vasodilator) production and release while inhibiting ET-1 (vasoconstrictor) (Pabbidi et al., 2018). Oestrogen inhibits growth of vessel walls and acts as an antioxidant to prevent damage of vessels from ROS and free radicals (Khalil, 2015). Furthermore, oestrogen is able to attenuate sympathetic activation (Boese et al., 2017). Oestrogen also brings about vasodilation through stimulation of Ca^{2+} efflux (Mazzuca et al., 2015). Moreover, oestrogen inhibits NF_kB and its inflammatory processes its associated with (Sabbatini & Kararigas, 2020). Lastly, oestrogen reduces RAAS induced hypertensive effects through favouring the vasodilatory non-classical RAAS pathway (Novella et al., 2019). These different effects that testosterone and oestrogen have on systems and factors implicated in the development of hypertension are important in understanding sex differences in hypertension. In contrast, hypertension is exacerbated in adult female rats in the present study. One of the explanations may be an activation of the RAAS.

In the present study, the higher blood pressures in female SHRs may be attributed to elevated ANG II concentration. Other RAAS parameters in the study were similar between the groups. ANG II may mediate increased blood pressure (Nwia et al., 2023). ANG II causes constriction of blood vessels, which increases the peripheral resistance and in turn makes it harder for blood to flow through the vessels thus raising blood pressure (Goto et al., 2000; Muñoz-durango et al., 2016). Additionally, ANG II may act indirectly to increase blood pressure. ANG II stimulates the activation of the sympathetic nervous system (SNS) which increases heart rate and contractile

strength, leading to an elevation in blood pressure including diastolic blood pressure (Dong et al., 2015).

Moreover, ANG II increases oxidative stress which induces hypertension. Nicotinamide adenine dinucleotide phosphate oxidases (NOXs) are responsible for the production of reactive oxygen species (ROS) and free radicals (Zhang et al., 2023). These reactive oxygen species and free radicals increase vascular oxidative stress and damage vessels and contribute to hypertension (Zhang et al., 2023). In addition, an increase in oxidative stress directly reduces bioavailability of nitric oxide (vasodilator). Reactive oxygen species and free radicals can react with nitric oxide and produce perioxinitrite (a vasoconstrictor), thus mediating increased blood pressure. Unfortunately, we did not measure levels of reactive oxygen species. To confirm greater vascular remodelling in female SHRs, techniques such as vascular imaging techniques, morphometric analysis or vascular reactivity could have been employed to assess the effects of ANG II on vascular remodelling

Furthermore, ANG II stimulates aldosterone release, which consequently results in increased salt and water retention (Goto et al., 2000). This increases blood volume and exacerbates hypertension. However, in the present study, the similar aldosterone concentration between the groups suggest that aldosterone is responsible for the higher blood pressure in female SHRs.

Sexual dimorphism in RAAS activation has also been widely reported in experimental studies. A study showed that at 1, 7 and 9 months female SHRs have increased AT_2R and reduced ANG II mediated vasoconstriction (Al-Gburi et al., 2017). At 3 months, a study showed that female SHRs have higher AT_2R density compared to male SHRs (Sullivan et al., 2010). On the other hand, a study showed that at 4 months male SHRs have greater AT_1R density compared to female SHRs (Sullivan et al., 2007). Another

study showed increased renal renin and angiotensinogen concentrations in male SHRs than female SHRs at 16 months (Yanes, Romero, Iles, Iliescu, Gomez-Sanchez, et al., 2006). Lastly a study showed increased ANG II levels in male hypertensive Lewis rats compared to female Lewis rats (Pendergrass et al., 2008).

All these studies concluded that males generally have a greater RAAS activation compared to females. Another important question arising from the present results is the cause of the higher levels of ANG II observed only in 7-month-old female SHRs.

SHRs are an inbred genetic model of experimental hypertension, which is the origin of hypertension in the strain. As mentioned above there are sex difference in the RAAS activation in SHRs. Oestrogen receptor is a transcription factor that regulates gene expression (Lu et al., 2016). Oestrogen has been shown to increase plasma and tissue levels of renin and angiotensinogen (Brosnihan et al., 1999; Chen et al., 1992; Farrington et al., 1993) which may induce the production of ANG II.

Lastly, anaesthetised blood pressures were lower than conscious blood pressures. This may have been due to the anaesthetic effects of isoflourine, which may have reduced blood pressures through suppression of the SNS and baroreceptor reflex, increased vasodilation and a decrease in the heart's contraction. Together all these effects may have caused a reduction in blood pressure.

Chapter 6: Limitations and Conclusion

6.1. Limitations

There are several limitations to the present study. Catheterisation instead of the tail-cuff method could have been used to achieve more accurate blood pressure readings. Procedures that rats went through in the evaluation of blood pressure could have stressed the animals and therefore could have possibly further influenced readings. Oestrogen was suspected to have caused increased ANG II concentrations in the female SHRs. However, concentrations of oestrogen were not assayed. Measurement of oestrogen concentrations between female SHRs and WKYs could have strengthened the analysis and elucidation of the current study's results. However, studies have shown that SHRs have greater RAAS activation. Therefore, even in the event of measuring oestrogen, female SHR would still have had greater ANG II concentration compared to female WKYs. Furthermore, no statistically significant differences were observed for most of the RAAS parameters. Therefore, the use of other techniques such as immunohistochemistry, radioimmunoassay or western blot rather than ELISA could've been more useful in evaluating significant differences for RAAS parameters. Although stroke volume, ejection fraction and mid-wall fractional shortening were employed in the analysis of systolic function, the use of Speckle Tracking Echocardiography (STE) could have been advantageous. STE is highly sensitive and can detect subtle changes in myocardial deformation and preclinical systolic impairments. Therefore, providing early detection of cardiomyopathy. Additionally, increasing the duration of the study beyond 7 months may have allowed us to see statistically significant sex differences across most echocardiography parameters.

6.2. Conclusion

In conclusion, the results of this study show significant differences in blood pressure and cardiac parameters among female SHRs compared to other groups, including male SHRs, and male and female WKYs. Notably, female SHRs showed elevated anaesthetised and conscious blood pressure which can be explained by the heightened plasma concentrations of ANG II. Furthermore, compared to WKYs, SHRs demonstrated concentric hypertrophy, impaired left ventricular relaxation, and increased filling pressures, indicating distinct cardiac adaptations in hypertension. Moreover, sex-specific differences were evident, with females displaying greater physiological left ventricular myocardial growth and reduced systolic performance compared to males. These findings highlight the critical role of sex-specific factors in the pathogenesis of left ventricular hypertrophy, emphasising the importance of tailored approaches in managing cardiovascular diseases. Furthermore, they contribute to the growing body of evidence on sexual dimorphism and the involvement of the RAAS in hypertension and left ventricular hypertrophy in spontaneously hypertensive rats.

References

A. Boehme, C. Esenwa, M. E. (2018). Stroke: Risk factors and prevention. *Journal of the Pakistan Medical Association*, *60*(5), 412. https://doi.org/10.1161/CIRCRESAHA.116.308398.Stroke

Access, O. (2017). Cardiac hypertrophy and IGF-1 response to testosterone propionate treatment in trained male rats, 120–127. https://doi.org/10.1515/biol-2017-0014

Angiotensin, R., System, A., Simko, F., Baka, T., Stanko, P., Repova, K., … Paulis, L. (2022). Sacubitril / Valsartan and Ivabradine Attenuate Left Ventricular Remodelling and Dysfunction in Spontaneously Hypertensive Rats : Different Interactions with the, (Lv).

Babiker, F. A., Lips, D., Meyer, R., Delvaux, E., Zandberg, P., Janssen, B., … Doevendans, P. A. (2015). Estrogen Receptor ␣ Protects the Murine Heart Against Left Ventricular Hypertrophy. https://doi.org/10.1161/01.ATV.0000223344.11128.23

Beaumont, C., Walsh-Wilkinson, É., Drolet, M. C., Roussel, É., Arsenault, M., & Couet, J. (2017). Female rats with severe left ventricle volume overload exhibit more cardiac hypertrophy but fewer myocardial transcriptional changes than males. *Scientific Reports*, *7*(1), 1–12. https://doi.org/10.1038/s41598-017-00855-9

Bethany, E., & Zajacova Anna. (2016). Gender differences in hypertension among young adults. *HHS Public Access*, *61*(1), 1–17. https://doi.org/10.1080/19485565.2014.929488.Gender

Bhatia, K., Elmarakby, A. A., El-Remessey, A., & Sullivan, J. C. (2012). Oxidative stress contributes to sex differences in angiotensin II-mediated hypertension in

spontaneously hypertensive rats. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *302*(2), 274–282. https://doi.org/10.1152/ajpregu.00546.2011

Biotechnology, M. (n.d.). Testosterone, cytochrome P450, and cardiac hypertrophy ¨, 1537–1549.

Boese, A. C., Kim, S. C., Yin, K., Lee, J., & Hamblin, M. H. (2017). Sex Differences in Vascular Physiology and Pathophysiology : Estrogen and Androgen Signaling in Health and Disease. https://doi.org/10.1152/ajpheart.00217.2016

Brilla, C. G. (2000). Renin – angiotensin – aldosterone system and myocardial fibrosis, *47*, 1–3.

Brosnihan, K. B., Senanayake, P. S., Li, P., Ferrario, C. M., & Foundation, C. C. (1999). Bi-directional actions of estrogen on the renin-angiotensin system, *32*, 373–381.

Brouwers, S., Sudano, I., Kokubo, Y., & Sulaica, E. M. (2021). Seminar Arterial hypertension. *Lancet*, *6736*(21), 249–261.

Cavasin, M. A., Sankey, S. S., Yu, A., Menon, S., & Yang, X. (2003). Estrogen and testosterone have opposing effects on chronic cardiac remodeling and function in mice with myocardial infarction, *48202*, 1560–1569.

Chan, V., Fenning, A., Levick, S. P., Loch, D., Chunduri, P., Iyer, A., … Brown, L. (2011). Cardiovascular Changes During Maturation and Ageing in Male and Female Spontaneously Hypertensive Rats, *57*(4), 469–478.

Chen, H. I. (2012). Hemodynamic mechanism of ventricular hypertrophy in hypertension. *Chinese Journal of Physiology*, *55*(6), 369–379. https://doi.org/10.4077/CJP.2012.BAA088

Chen, Y. F., Naftilan, A. J., & Oparil, S. (1992). Androgen-dependent

angiotensinogen and renin messenger RNA expression in hypertensive rats. *Hypertension*, *19*(5), 456–463. https://doi.org/10.1161/01.HYP.19.5.456

Cingolani, O. H., Yang, X., Cavasin, M. A., & Carretero, O. A. (2015). in Adult Spontaneously Hypertensive Rats. https://doi.org/10.1161/01.HYP.0000052832.96564.0B

Colafella, K. M. M., & Denton, K. M. (2018). and associated cardiovascular disease. *Nature Publishing Group*, 1–17. https://doi.org/10.1038/nrneph.2017.189

Costa, T. J., Ceravolo, G. S., Santos, R. A., Oliveira, M. A. De, Araújo, P. X., Giaquinto, L. R., … Carvalho, M. H. C. (2015). Association of testosterone with estrogen abolishes the beneficial effects of estrogen treatment by increasing ROS generation in aorta endothelial cells, (39), 723–732. https://doi.org/10.1152/ajpheart.00681.2014

Cramariuc, D., & Gerdts, E. (2016). Epidemiology of left ventricular hypertrophy in hypertension : Implications for the clinic, *9072*(May). https://doi.org/10.1080/14779072.2016.1186542

Dalpiaz, P. L. M., Lamas, A. Z., Caliman, I. F., Ribeiro, R. F., & Abreu, G. R. (2015). Sex Hormones Promote Opposite Effects on ACE and ACE2 Activity , Hypertrophy and Cardiac Contractility in Spontaneously Hypertensive Rats, 1–15. https://doi.org/10.1371/journal.pone.0127515

Davies, C., Zerebiec, K., Rozanowska, A., Czestkowska, E., Długosz, D., Chyrchel, B., & Surdacki, A. (2018). Is left ventricular hypertrophy a friend or foe of patients with aortic stenosis? *Postepy w Kardiologii Interwencyjnej*, *14*(4), 328–337. https://doi.org/10.5114/aic.2018.78734

Dong, T., Chen, J., Tian, L., Wang, L., Jiang, R., Zhang, Z., & Xu, J. (2015). Role of the Renin-Angiotensin System , Renal Sympathetic Nerve System , and

Oxidative Stress in Chronic Foot Shock-Induced Hypertension in Rats, *11*. https://doi.org/10.7150/ijbs.10250

Dupont, S., Maizel, J., Mentaverri, R., Chillon, J. M., Six, I., Giummelly, P., … Slama, M. (2012). The onset of left ventricular diastolic dysfunction in SHR rats is not related to hypertrophy or hypertension. *American Journal of Physiology - Heart and Circulatory Physiology*, *302*(7), 1524–1533. https://doi.org/10.1152/ajpheart.00955.2010

Duprez, D. A. (2006). Role of the renin – angiotensin – aldosterone system in vascular remodeling and inflammation : a clinical review, 983–991.

Dworatzek, E., Mahmoodzadeh, S., Schubert, C., Westphal, C., Leber, J., Kusch, A., … Regitz-zagrosek, V. (2014). Sex differences in exercise-induced physiological myocardial hypertrophy are modulated by oestrogen receptor beta. https://doi.org/10.1093/cvr/cvu065

Eickels, M. Van, Schreckenberg, R., Doevendans, P. A., Meyer, R., & Schlu, K. (2005). The influence of oestrogen-deficiency and ACE inhibition on the progression of myocardial hypertrophy in spontaneously hypertensive rats, *7*, 1079–1084. https://doi.org/10.1016/j.ejheart.2005.03.004

Elmarakby, A. A., Bhatia, K., Crislip, R., & Sullivan, J. C. (2016). Hemodynamic responses to acute angiotensin II infusion are exacerbated in male versus female spontaneously hypertensive rats. *Physiological Reports*, *4*(1), 1–8. https://doi.org/10.14814/phy2.12677

ely-et-al-1997-spontaneously-hypertensive-rat-y-chromosome-increases-indexes-of-sympathetic-nervous-system-activity. (n.d.).

Er, F., Michels, G., Brandt, M. C., Khan, I., Haase, H., Eicks, M., … Hoppe, U. C. (2007). Impact of testosterone on cardiac L-type calcium channels and Ca2+

sparks: Acute actions antagonize chronic effects. *Cell Calcium*, *41*(5), 467–477. https://doi.org/10.1016/j.ceca.2006.09.003

Fardoun, M., Dehaini, H., Shaito, A., Mesmar, J., El-Yazbi, A., Badran, A., … Eid, A. H. (2020). *The hypertensive potential of estrogen: An untold story*. *Vascular Pharmacology* (Vol. 124). Elsevier Inc. https://doi.org/10.1016/j.vph.2019.106600

Farrington, P. F., Ogasawara, M., Suzumori, K., Tomoda, S., Berrebi, S., & Sasaki, M. (1993). A molecular variant of preeclampsia, *4*(may), 59–61.

Ferrari, R. (2013). RAAS inhibition and mortality in hypertension. *Global Cardiology Science and Practice*, *2013*(3), 34. https://doi.org/10.5339/gcsp.2013.34

Ferrario, C. M., Iyer, S. R., Burnett, J. C., Ahmad, S., Wright, K. N., Voncannon, J. L., … Groban, L. (2021). Angiotensin (1-12) in Humans With Normal Blood Pressure and Primary Hypertension. *Hypertension*, *77*(3), 882–890. https://doi.org/10.1161/HYPERTENSIONAHA.120.16514

Forman, D. E., Cittadini, A., Azhar, G., Douglas, P. S., & Wei, J. Y. (1997). Cardiac morphology and function in senescent rats: Gender-related differences. *Journal of the American College of Cardiology*, *30*(7), 1872–1877. https://doi.org/10.1016/S0735-1097(97)00411-7

Fortepiani, L. A., & Reckelhoff, J. F. (2005). Role of oxidative stress in the sex differences in blood pressure in spontaneously hypertensive rats. *Journal of Hypertension*, *23*(4), 801–805. https://doi.org/10.1097/01.hjh.0000163149.05083.13

Fortepiani, L. A., Zhang, H., Racusen, L., Roberts, L. J., & Reckelhoff, J. F. (2003). Characterization of an animal model of postmenopausal hypertension in spontaneously hypertensive rats. *Hypertension*, *41*(3 II), 640–645.

https://doi.org/10.1161/01.HYP.0000046924.94886.EF

Frangogiannis, N. G. (2018). Molecular Aspects of Medicine Cardiac fi brosis : Cell biological mechanisms , molecular pathways and therapeutic opportunities. *Molecular Aspects of Medicine*, 0–1. https://doi.org/10.1016/j.mam.2018.07.001

friberg1985.pdf. (n.d.).

Garate-carrillo, A., Gonzalez, J., Ceballos, G., Ramirez-sanchez, I., & Villarreal, F. (2020). Sex Related Differences in the Pathogenesis of Organ Fibrosis.

Goto, K., Fujii, K., Onaka, U., Abe, I., & Fujishima, M. (2000). Renin-Angiotensin System Blockade Improves Endothelial Dysfunction in Hypertension.

Gouldsborough, I., Lindop, G. B. M., & Ashton, N. (2003). Renal Renin-Angiotensin System Activity in Naturally Reared and Cross-Fostered Spontaneously Hypertensive Rats, 864–869. https://doi.org/10.1016/S0895-7061(03)00999-3

Grammer, J. B., Bleiziffer, S., Monticelli, F., Lange, R., & Bauernschmitt, R. (2006). Calcineurin and matrix protein expression in cardiac hypertrophy: Evidence for calcineurin B to control excessive hypertrophic signaling. *Basic Research in Cardiology*, *101*(4), 292–300. https://doi.org/10.1007/s00395-006-0598-z

Grisk, O., & Rettig, R. (2004). Interactions between the sympathetic nervous system and the kidneys in arterial hypertension. *Cardiovascular Research*, *61*(2), 238–246. https://doi.org/10.1016/j.cardiores.2003.11.024

Guihaire, J., Haddad, F., Boulate, D., Decante, B., Denault, A. Y., Wu, J., … Fadel, E. (2013). Non-invasive indices of right ventricular function are markers of ventricular-arterial coupling rather than ventricular contractility: insights from a porcine model of chronic pressure overload. *European Heart Journal Cardiovascular Imaging*, *14*(12), 1140–1149. https://doi.org/10.1093/ehjci/jet092

Haas, G. J., Mccune, S. A., Brown, D. M., & Cody, R. J. (n.d.). Echocardiographic

characterization of left ventricular adaptation in a genetically determined heart failure rat model, 806–811.

Haas, G. J., McCune, S. A., Brown, D. M., & Cody, R. J. (1995). Echocardiographic characterization of left ventricular adaptation in a genetically determined heart failure rat model. *American Heart Journal*, *130*(4), 806–811. https://doi.org/10.1016/0002-8703(95)90081-0

Hidetomo, R. H., Hiroe, N., Yoshihiko, I., Yuji, K., Yamanouchi, Y., Suzuki, H., & Words, K. (2008). Nephrology Estrogen and Angiotensin II Interactions Determine Cardio-Renal Damage in Dahl Salt-Sensitive Rats with Heart Failure, *0451*, 413–423. https://doi.org/10.1159/000112806

Hristova, M., Stanilova, S., & Miteva, L. (2019). Serum concentration of renin-angiotensin system components in association with ACE I/D polymorphism among hypertensive subjects in response to ACE inhibitor therapy. *Clinical and Experimental Hypertension*, *41*(7), 662–669. https://doi.org/10.1080/10641963.2018.1529782

Iliescu, R., Cucchiarelli, V. E., Yanes, L. L., Iles, J. W., & Reckelhoff, J. F. (2007). Sex Differences in Renal and Cardiovascular Function : Physiology and Pathophysiology Impact of androgen-induced oxidative stress on hypertension in male SHR, *4505*, 731–736. https://doi.org/10.1152/ajpregu.00353.2006.

Jazbutyte, V., Arias-Loza, P. A., Hu, K., Widder, J., Govindaraj, V., Von Poser-Klein, C., … Pelzer, T. (2008). Ligand-dependent activation of ERβ lowers blood pressure and attenuates cardiac hypertrophy in ovariectomized spontaneously hypertensive rats. *Cardiovascular Research*, *77*(4), 774–781. https://doi.org/10.1093/cvr/cvm081

Kadappu, K. K., & Thomas, L. (2015). Tissue doppler imaging in echocardiography:

Value and limitations. *Heart Lung and Circulation*, *24*(3), 224–233. https://doi.org/10.1016/j.hlc.2014.10.003

Kai, H. (2002). The Effect and Mechanism of Forsinopril on Ventricular Hypertrophy of SHR and Left Ventricular Pressure overloading Rat, *22*(1), 17–20.

Kararigas, G., Tiep, B., & Jarry, H. (2014). Molecular and Cellular Endocrinology Estrogen modulates cardiac growth through an estrogen receptor a -dependent mechanism in healthy ovariectomized mice. *Molecular and Cellular Endocrinology*, *382*(2), 909–914. https://doi.org/10.1016/j.mce.2013.11.011

Khalil, R. A. (2015). Sex Hormones as Potential Modulators of Vascular Function in Hypertension. https://doi.org/10.1161/01.HYP.0000172945.06681.a4

Kienitz, T., & Quinkler, M. (2008). Testosterone and Blood Pressure Regulation, 71–79. https://doi.org/10.1159/000119417

Kokubo, M., Uemura, A., & Matsubara, T. (2007). Noninvasive Evaluation of the Time Course of Change in Cardiac Function in Spontaneously Hypertensive Rats by Echocardiography, *28*(7), 601–609.

Komukai, K., Mochizuki, S., & Yoshimura, M. (2010). Gender and the renin–angiotensin– aldosterone system, *24*, 687–698. https://doi.org/10.1111/j.1472-8206.2010.00854.x

Kong, P., Christia, P., & Frangogiannis, N. G. (2014). The pathogenesis of cardiac fibrosis. *Cellular and Molecular Life Sciences*, *71*(4), 549–574. https://doi.org/10.1007/s00018-013-1349-6

Koracevic, G., Micic, S., Stojanovic, M., & Zdravkovic, M. (2023). A Need for Improvement in the Definition of Resistant Arterial Hypertension. *Medicina (Lithuania)*, *59*(4), 1–11. https://doi.org/10.3390/medicina59040803

Kumai, T., Tanaka, M., Watanabe, M., Matsumoto, C., & Kobayashi, S. (1994).

Possible Involvement of Androgen in Increased Norepinephrine Synthesis in Blood Vessels of Spontaneously Hypertensive Rats. *The Japanese Journal of Pharmacology*, *66*(4), 439–444. https://doi.org/10.1254/jjp.66.439

Kuwahara, F., Kai, H., Tokuda, K., Kai, M., Takeshita, A., Egashira, K., & Imaizumi, T. (2002). Transforming growth factor-β function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. *Circulation*, *106*(1), 130–135. https://doi.org/10.1161/01.CIR.0000020689.12472.E0

Kuwahara, F., Kai, H., Tokuda, K., Takeya, M., Takeshita, A., Egashira, K., & Imaizumi, T. (2004). Hypertensive Myocardial Fibrosis and Diastolic Dysfunction: Another Model of Inflammation? *Hypertension*, *43*(4), 739–745. https://doi.org/10.1161/01.HYP.0000118584.33350.7d

Lakhani, I., Sai, K., Leung, K., Tse, G., Pui, A., & Lee, W. (2019). Novel Mechanisms in Heart Failure With Preserved , Midrange , and Reduced Ejection Fraction, *10*(July), 1–6. https://doi.org/10.3389/fphys.2019.00874

Legrice, I. J., Pope, A. J., Sands, G. B., Whalley, G., Doughty, R. N., & Smaill, B. H. (2012). Progression of myocardial remodeling and mechanical dysfunction in the spontaneously hypertensive rat. https://doi.org/10.1152/ajpheart.00748.2011

Li, L., Yi-ming, W., Li, Z., Zhao, L., Yu, Y., Li, D., … Su, D. (2008). Local RAS and inflammatory factors are involved in cardiovascular hypertrophy in spontaneously hypertensive rats, *58*, 196–201. https://doi.org/10.1016/j.phrs.2008.06.009

Li, Y., Kishimoto, I., Saito, Y., Harada, M., Kuwahara, K., Izumi, T., … Nakao, K. (2015). Androgen Contributes to Gender-Related Cardiac Hypertrophy and Fibrosis in Mice Lacking the Gene, *145*(June), 951–958. https://doi.org/10.1210/en.2003-0816

Lijnen, P., & Petrov, V. (1999). Renin-Angiotensin System , Hypertrophy and Gene Expression in Cardiac Myocytes, *970*, 949–970.

Lin, W. T., Nithiyanantham, S., Hsieh, D. J., Day, R. C. C., Ying, J., & Kuo, L. C. (2020). Bioactive peptides attenuate cardiac apoptosis in spontaneously hypertensive rat hearts through activation of autophagy and mitochondrial biogenesis pathway, (October 2019), 1–7. https://doi.org/10.1002/tox.22916

Loh, S. Y., & Salleh, N. (2017). In fl uence of testosterone on mean arterial pressure : A physiological study in male and female normotensive WKY and hypertensive SHR rats, *104*(1), 25–34. https://doi.org/10.1556/2060.104.2017.1.3

Lu, K., Keen, H. L., Weatherford, E. T., Sequeira-lopez, M. L. S., Gomez, R. A., & Sigmund, C. D. (2016). Estrogen Receptor α Is Required for Maintaining Baseline Renin Expression. https://doi.org/10.1161/HYPERTENSIONAHA.115.07082

Maranon, R., & Reckelhoff, J. F. (2013). Sex and gender differences in control of blood pressure. *Clinical Science*, *125*(7), 311–318. https://doi.org/10.1042/CS20130140

Maris, M. E., Melchert, R. B., Joseph, J., & Kennedy, R. H. (2005). GENDER DIFFERENCES IN BLOOD PRESSURE AND HEART RATE IN SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO RATS, (October 2004), 35–39.

Marsh, J. D., Lehmann, M. H., Ritchie, R. H., Gwathmey, J. K., Green, G. E., & Schiebinger, R. J. (1998). Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation*, *98*(3), 256–261. https://doi.org/10.1161/01.CIR.98.3.256

Mazzuca, M. Q., Mata, K. M., Li, W., Rangan, S. S., & Khalil, R. A. (2015). Estrogen

Receptor Subtypes Mediate Distinct Microvascular Dilation and Reduction in [Ca 2 1] i in Mesenteric Microvessels of Female Rat, (February), 291–304.

Mc Namara, K., Alzubaidi, H., & Jackson, J. K. (2019). Cardiovascular disease as a leading cause of death: how are pharmacists getting involved? . *Integrated Pharmacy Research and Practice*, *Volume 8*, 1–11. https://doi.org/10.2147/iprp.s133088

Melo, A. F., Lima, P., Dalpiaz, M., Escouto, S., Januário, G., Aires, R., & Damacena, N. (2020). Involvement of sex hormones , oxidative stress , ACE and ACE2 activity in the impairment of renal function and remodelling in SHR. *Life Sciences*, *257*(May), 118138. https://doi.org/10.1016/j.lfs.2020.118138

Messerli, F. H., Rimoldi, S. F., & Bangalore, S. (2017). The Transition From Hypertension to Heart Failure: Contemporary Update. *JACC: Heart Failure*, *5*(8), 543–551. https://doi.org/10.1016/j.jchf.2017.04.012

Meyer, C., Rana, O. R., Saygili, E., Gemein, C., Becker, M., Nolte, K. W., … Schauerte, P. (2010). Augmentation of left ventricular contractility by cardiac sympathetic neural stimulation. *Circulation*, *121*(11), 1286–1294. https://doi.org/10.1161/CIRCULATIONAHA.109.874263

Mice, A., Gu, D., Kusch, A., Catar, R., Chaykovska, L., Hoff, U., … Dragun, D. (2011). Estrogen Receptor- ᴺʟ Signals Left Ventricular Hypertrophy Sex Differences in Normotensive Deoxycorticosterone, 648–654. https://doi.org/10.1161/HYPERTENSIONAHA.110.166157

Mishra, J. S., More, A. S., Gopalakrishnan, K., & Kumar, S. (2015). Testosterone plays a permissive role in angiotensin II-induced hypertension and cardiac hypertrophy in male rats, (August 2018). https://doi.org/10.1093/biolre/ioy179

Muñoz-durango, N., Fuentes, C. A., Castillo, A. E., & Kalergis, A. M. (n.d.). Role of

the Renin-Angiotensin-Aldosterone System beyond Blood Pressure Regulation : Molecular and Cellular Mechanisms Involved in End-Organ Damage during Arterial Hypertension, 1–17. https://doi.org/10.3390/ijms17070797

Nagase, M., Shimosawa, T., Ando, K., & Fujita, T. (1996). Local Renin-Angiotensin System in Sympathetic Overactivity of Spontaneously Hypertensive Rats.

No Title. (2018).

Novella, S., Pérez-Cremades, D., Mompeón, A., & Hermenegildo, C. (2019). Mechanisms underlying the influence of oestrogen on cardiovascular physiology in women. *Journal of Physiology*, *597*(19), 4873–4886. https://doi.org/10.1113/JP278063

Nwabuo, C. C. (2020). Pathophysiology of Hypertensive Heart Disease : Beyond Left Ventricular Hypertrophy.

Nwia, S. M., Leite, A. P. O., Li, X. C., & Zhuo, J. L. (2023a). Sex differences in the renin-angiotensin-aldosterone system and its roles in hypertension, cardiovascular, and kidney diseases. *Frontiers in Cardiovascular Medicine*, *10*(June). https://doi.org/10.3389/fcvm.2023.1198090

Nwia, S. M., Leite, A. P. O., Li, X. C., & Zhuo, J. L. (2023b). Sex differences in the renin-angiotensin-aldosterone system and its roles in hypertension, cardiovascular, and kidney diseases, (June). https://doi.org/10.3389/fcvm.2023.1198090

Ono, K., Masuyama, T., & Yamamoto, K. (n.d.). Echo Doppler Assessment of Left Ventricular Function in Rats with Hypertensive Hypertrophy, 109–117. https://doi.org/10.1067/mje.2002.115034

Outrobe, D., & Le, R. T. M. K. W. (2018). Blood pr essu r e a n d h ea r t r a t e developm en t in you n g spon t a n eou sly h yper t en sive r a t s, 794–800.

Pagan, L. U., Damatto, R. L., Cezar, M. D. M., Lima, A. R. R., Bonomo, C., Campos, D. H. S., … Okoshi, K. (2015). Long-term low intensity physical exercise attenuates heart failure development in aging spontaneously hypertensive rats. *Cellular Physiology and Biochemistry*, *36*(1), 61–74. https://doi.org/10.1159/000374053

Pedram, A., Razandi, M., Mahony, F. O., Lubahn, D., & Levin, E. R. (2015). Estrogen Receptor- ℓ Prevents Cardiac Fibrosis, *24*(November 2010), 2152–2165. https://doi.org/10.1210/me.2010-0154

Pedram, A., Razandi, M., Narayanan, R., Dalton, J. T., Mckinsey, T. A., & Margolis, B. (2013). Estrogen regulates histone deacetylases to prevent cardiac hypertrophy, *24*, 3805–3818. https://doi.org/10.1091/mbc.E13-08-0444

Pendergrass, K. D., Pirro, N. T., Westwood, B. M., Ferrario, C. M., Brosnihan, K. B., & Chappell, M. C. (2008a). Sex differences in circulating and renal angiotensins of hypertensive mRen (2). Lewis but not normotensive Lewis rats, *1095*, 10–21. https://doi.org/10.1152/ajpheart.01277.2007.

Pendergrass, K. D., Pirro, N. T., Westwood, B. M., Ferrario, C. M., Brosnihan, K. B., & Chappell, M. C. (2008b). Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology Sex differences in circulating and renal angiotensins of hypertensive mRen (2). Lewis but not normotensive Lewis rats, *1095*, 10–21. https://doi.org/10.1152/ajpheart.01277.2007.

Plitt, G. D., Spring, J. T., Moulton, M. J., & Agrawal, D. K. (2018). diastolic dysfunction Ac ce pt us cr t. *Expert Review of Cardiovascular Therapy*, *0*(0), 1. https://doi.org/10.1080/14779072.2018.1497485

Radin, M. J., Holycross, B. J., Sharkey, L. C., Shiry, L., Cune, S. A. M. C., Judith, M., … Gender, S. A. M. (2002). Gender modulates activation of renin-angiotensin

and endothelin systems in hypertension and heart failure, *43210*, 935–940.

Reckelhoff, J. F., & Granger, J. P. (2015). t t, 7–12.

Reckelhoff, J. F., Zhang, H., & Srivastava, K. (2015). Gender Differences in Development of Hypertension in Spontaneously Hypertensive Rats Role of the Renin-Angiotensin System.

Reddy, D. S., Singh, M., & Ghosh, S. (1996). Role of cardiac renin-angiotensin system in the development of pressure-overload left ventricular hypertrophy in rats with abdominal aortic constriction.

Riedel, K., Deussen, A. J., Tolkmitt, J., Weber, S., Schlinkert, P., Zatschler, B., … Kopaliani, I. (2019). Sex Differences in Cardiovascular and Cerebrovascular Physiology , Disease , and Signaling Mechanisms Estrogen determines sex differences in adrenergic vessel tone by regulation of endothelial ␣ -adrenoceptor expression, (16), 243–254. https://doi.org/10.1152/ajpheart.00456.2018

Romero, M., Caniffi, C., Bouchet, G., Elesgaray, R., Laughlin, M. Mac, Arranz, C., & Costa, M. A. (2013). Sex Differences in the Beneficial Cardiac Effects of Chronic Treatment with Atrial Natriuretic Peptide In Spontaneously Hypertensive Rats, *8*(8). https://doi.org/10.1371/journal.pone.0071992

Rubio-Gayosso, I., Ramirez-Sanchez, I., Ita-Islas, I., Ortiz-Vilchis, P., Gutierrez-Salmean, G., Meaney, A., … Ceballos, G. (2013). Testosterone metabolites mediate its effects on myocardial damage induced by ischemia/reperfusion in male Wistar rats. *Steroids*, *78*(3), 362–369. https://doi.org/10.1016/j.steroids.2012.12.004

Sabbatini, A. R., & Kararigas, G. (2020). Estrogen-related mechanisms in sex differences of hypertension and target organ damage, *7*, 1–17.

Sáinz, J., Osuna, A., Wangensteen, R., de Dios Luna, J., Rodríguez-Gómez, I., Duarte, J., … Vargas, F. (2004). Role of sex, gonadectomy and sex hormones in the development of nitric oxide inhibition-induced hypertension. *Experimental Physiology*, *89*(2), 155–162. https://doi.org/10.1113/expphysiol.2003.002652

Sanada, L. S., da Rocha Kalil, A. L., Tavares, M. R., Neubern, M. C. M., Salgado, H. C., & Fazan, V. P. S. (2012). Sural nerve involvement in experimental hypertension: Morphology and morphometry in male and female normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). *BMC Neuroscience*, *13*(1). https://doi.org/10.1186/1471-2202-13-24

Sandberg, K., & Ji, H. (2003). Sex and the Renin Angiotensin System : Progression of Kidney Disease. *Gender Issues in End-Stage Renal Disease*, *10*(1), 15–23. https://doi.org/10.1053/jarr.2003.50006

Sandberg, K., & Ji, H. (2012). Sex differences in primary hypertension, 1–21.

Sartori-valinotti, J. C., Iliescu, R., Fortepiani, L. A., Yanes, L. L., & Reckelhoff, J. F. (2007). Frontiers in Research Review : Gaseous Molecules and Radicals in Health and Disease SEX DIFFERENCES IN OXIDATIVE STRESS AND THE IMPACT ON BLOOD PRESSURE CONTROL AND CARDIOVASCULAR DISEASE, (November 2006), 938–945. https://doi.org/10.1111/j.1440-1681.2007.04643.x

Schaible, T. F., & Scheuer, J. (1984). Comparison of heart function in male and female rats. *Basic Research in Cardiology*, *79*(4), 402–412. https://doi.org/10.1007/BF01908140

Schimmel, K., Ichimura, K., Reddy, S., Haddad, F., & Humeres, C. D. (2022). Cardiac Fibrosis in the Pressure Overloaded Left and Right Ventricle as a Therapeutic Target, *9*(May), 1–23. https://doi.org/10.3389/fcvm.2022.886553

Schunkert, H., Jackson, B., Tang, S. S., Schoen, F. J., Smits, J. F. M., Apstein, C. S., & Lorell, B. H. (1993). Distribution and Functional Significance of Cardiac Angiotensin Converting Enzyme in Hypertrophied Rat Hearts, 1328–1339. https://doi.org/10.1161/01.CIR.87.4.1328

Schutte, A. E., Jafar, T. H., Poulter, N. R., Damasceno, A., Khan, N. A., Nilsson, P. M., … Tomaszewski, M. (2023). Addressing global disparities in blood pressure control: perspectives of the International Society of Hypertension. *Cardiovascular Research*, *119*(2), 381–409. https://doi.org/10.1093/cvr/cvac130

Sciences, V. I., Kitpipatkun, P., & Sukwan, C. (2022). Vet Integr Sci Vet Integr Sci Vet Integr Sci, *20*(1), 117–131.

Seachrist, D., Dunphy, G., Daneshvar, H., Caplea, A. N. N., Milsted, A. M. Y., Seachrist, D., … Testosterone, E. D. (2000). Testosterone Increases Blood Pressure and Cardiovascular and Renal Pathology in Spontaneously Hypertensive Rats, (1996).

Sensoring, C., & Carus, C. G. (2017). No Title. https://doi.org/10.1152/ajpregu.00231.2016

Sharma, J. R., Mabhida, S. E., Myers, B., Apalata, T., Nicol, E., Benjeddou, M., … Johnson, R. (2021). Prevalence of hypertension and its associated risk factors in a rural black population of mthatha town, south africa. *International Journal of Environmental Research and Public Health*, *18*(3), 1–17. https://doi.org/10.3390/ijerph18031215

Shiels, H. A., & White, E. (2008). The Frank-Starling mechanism in vertebrate cardiac myocytes. *Journal of Experimental Biology*, *211*(13), 2005–2013. https://doi.org/10.1242/jeb.003145

Silva-antonialli, M. M., Tostes, R. C. A., Fior-chadi, R., Hiromi, E., Helena, M., &

Carvalho, C. (2004). A lower ratio of AT 1 / AT 2 receptors of angiotensin II is found in female than in male spontaneously hypertensive rats, *62*, 587–593. https://doi.org/10.1016/j.cardiores.2004.01.020

Silva, P., Toyos, M., Wilson, C., Altamirano, F., Uhle, P., & Lavandero, S. (2006). Testosterone induces cardiomyocyte hypertrophy through mammalian target of rapamycin complex 1 pathway, 299–307. https://doi.org/10.1677/JOE-09-0044

Silva, P., Toyos, M., Wilson, C., Altamirano, F., Uhle, P., & Lavandero, S. (2008). Testosterone induces cardiomyocyte hypertrophy through mammalian target of rapamycin complex 1 pathway, 299–307.

Slama, M., Ahn, J., Peltier, M., Maizel, J., Chemla, D., Varagic, J., … Frohlich, E. D. (2005). Validation of echocardiographic and Doppler indexes of left ventricular relaxation in adult hypertensive and normotensive rats, 1131–1136. https://doi.org/10.1152/ajpheart.00345.2004.

Stoicescu, M., Csepento, C., Muţiu, G., & Bungău, S. (2011). The role of increased plasmatic renin level in the pathogenesis of arterial hypertension in young adults. *Romanian Journal of Morphology and Embryology*, *52*(SUPPL. 1), 419–423.

Sugden, P. H. (1999). Signaling in myocardial hypertrophy: Life after calcineurin? *Circulation Research*, *84*(6), 633–646. https://doi.org/10.1161/01.RES.84.6.633

Sullivan, J. C., Sasser, J. M., & Pollock, J. S. (2007a). Sex Differences in Renal and Cardiovascular Function : Physiology and Pathophysiology Sexual dimorphism in oxidant status in spontaneously hypertensive rats, *30912*, 764–769. https://doi.org/10.1152/ajpregu.00322.2006.

Sullivan, J. C., Sasser, J. M., & Pollock, J. S. (2007b). Sexual dimorphism in oxidant status in spontaneously hypertensive rats. *American Journal of Physiology -*

Regulatory Integrative and Comparative Physiology, *292*(2), 764–769. https://doi.org/10.1152/ajpregu.00322.2006

Sullivan, J. C., Semprun-Prieto, L., Boesen, E. I., Pollock, D. M., & Pollock, J. S. (2007). Sex and sex hormones influence the development of albuminuria and renal macrophage infiltration in spontaneously hypertensive rats. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *293*(4). https://doi.org/10.1152/ajpregu.00429.2007

Sun, J., Fu, L., Tang, X., Han, Y., Ma, D., Cao, J., … Ji, H. (2011). Testosterone modulation of cardiac β-adrenergic signals in a rat model of heart failure. *General and Comparative Endocrinology*, *172*(3), 518–525. https://doi.org/10.1016/j.ygcen.2011.04.019

Takeshi, N. (1977). NII-Electronic Library Service. *Chemical Pharmaceutical Bulletin*, *57*(534), 364–370. Retrieved from http://www.mendeley.com/research/geology-volcanic-history-eruptive-style-yakedake-volcano-group-central-japan/

Teichholz, L. E., & Herman, V. (1976). Problems in Echocardiographic Presence or Absence Volume Determinations : Correlations in the of Asynergy, *D*(January).

Tostes, R. C., Carneiro, F. S., Carvalho, M. H. C., & Reckelhoff, J. F. (2016). Reactive oxygen species : players in the cardiovascular effects of testosterone. https://doi.org/10.1152/ajpregu.00392.2014

Varo, N., Iraburu, J., Varela, M., Etayo, J. C., & Dı, J. (2015). Chronic AT 1 Blockade Stimulates Extracellular Collagen Type I Degradation and Reverses Myocardial Fibrosis in Spontaneously Hypertensive Rats.

Velásquez, D. (2018). No 主観的健康感を中心とした在宅高齢者における 健康関連指標に関する共分散構造分析Title. *New England Journal of Medicine*, *372*(2), 2499–2508. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/7556065%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC394507%0Ahttp://dx.doi.org/10.1016/j.humpath.2017.05.005%0Ahttps://doi.org/10.1007/s00401-018-1825-z%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/27157931

Velez, J. C. Q. (2009). The importance of the intrarenal renin-angiotensin system. *Nature Clinical Practice Nephrology*, *5*(2), 89–100. https://doi.org/10.1038/ncpneph1015

Venegas-pont, M., Sartori-valinotti, J. C., Glover, P. H., Reckelhoff, J. F., & Ryan, M. J. (2009). Sexual Dimorphism in the Blood Pressure Response to Angiotensin II in Mice After Angiotensin-converting Enzyme Blockade. *American Journal of Hypertension*, *23*(1), 92–96. https://doi.org/10.1038/ajh.2009.203

Vijaykumar, N., K, H., G, S., Badiger, S., & Hegde, H. (2023). Effect of modifiable and non-modifiable risk factors on hypertension among diabetics: An observational study. *National Journal of Physiology, Pharmacy and Pharmacology*, *13*(0), 1. https://doi.org/10.5455/njppp.2023.13.01002202307032023

Wachtell, K., Gerdts, E., Palmieri, V., Olsen, M. H., Nieminen, M. S., Papademetriou, V., … Devereux, R. B. (n.d.). In-treatment midwall and endocardial fractional shortening predict cardiovascular outcome in hypertensive patients with preserved baseline systolic ventricular function : the Losartan Intervention For Endpoint reduction study ¨ f g , 1541–1546. https://doi.org/10.1097/HJH.0b013e328339f943

Wallen, W. J., Cserti, C., Belanger, M. P., & Wittnich, C. (2000a). Gender-differences in myocardial adaptation to afterload in normotensive and hypertensive rats. *Hypertension*, *36*(5), 774–779. https://doi.org/10.1161/01.HYP.36.5.774

Wallen, W. J., Cserti, C., Belanger, M. P., & Wittnich, C. (2000b). Gender-Differences in Myocardial Adaptation to Afterload in Normotensive and Hypertensive Rats, 774–780.

Wang, B. (1999). The Role of the Renin-Angiotensin and Cardiac Sympathetic Nervous Systems in the Development of Hypertension and Left Ventricular Hypertrophy in Spontaneously Hypertensive Rats.

Wang, J., Li, Z., Wang, Y., Zhang, J., Zhao, W., Fu, M., … Ge, J. (2017). Qiliqiangxin Enhances Cardiac Glucose Metabolism and Improves Diastolic Function in Spontaneously Hypertensive Rats. *Evidence-Based Complementary and Alternative Medicine*, *2017*. https://doi.org/10.1155/2017/3197320

Weber, K. T., Brilla, C. G., Campbell, S. E., Zhou, G., Matsubara, L., & Guarda, E. (1992). Pathologic Hypertrophy With Fibrosis : The Structural Basis for Myocardial Failure, 75–85.

Wells, J. C. K. (2007). Sexual dimorphism of body composition. *Best Practice and Research: Clinical Endocrinology and Metabolism*, *21*(3), 415–430. https://doi.org/10.1016/j.beem.2007.04.007

Westphal, C., Schubert, C., Prelle, K., Penkalla, A., Fliegner, D., Petrov, G., & Regitz-zagrosek, V. (2012). Effects of Estrogen , an ER a Agonist and Raloxifene on Pressure Overload Induced Cardiac Hypertrophy, *7*(12), 1–8. https://doi.org/10.1371/journal.pone.0050802

Williams, S., Pourrier, M., McAfee, D., Lin, S., & Fedida, D. (2014). Ranolazine improves diastolic function in spontaneously hypertensive rats. *American Journal of Physiology - Heart and Circulatory Physiology*, *306*(6), 867–881. https://doi.org/10.1152/ajpheart.00704.2013

Williamson, C. R., & Tai, T. C. (2017). Comparative Analysis of Renin-Angiotensin

System (RAS) -Related Gene Expression Between Hypertensive and Normotensive Rats, 20–24. https://doi.org/10.12659/MSMBR.901964

Wittnich, C. (2015). The Role of 17 β -Estradiol in Myocardial Hypertrophy in Females in the Presence and Absence of Hypertension, 347–353. https://doi.org/10.1007/s10557-015-6603-8

Wu, M., Peng, Z., Zu, C., Ma, J., Lu, S., Zhong, J., & Zhang, S. (2016). Losartan attenuates myocardial endothelial-to-mesenchymal transition in spontaneous hypertensive rats via inhibiting TGF-β/Smad Signaling. *PLoS ONE*, *11*(5), 1–13. https://doi.org/10.1371/journal.pone.0155730

Xin, J. J., Dai, Q. F., Lu, F. Y., Zhao, Y. X., Liu, Q., Cui, J. J., … Yu, X. C. (2020). Antihypertensive and Antifibrosis Effects of Acupuncture at PC6 Acupoints in Spontaneously Hypertensive Rats and the Underlying Mechanisms. *Frontiers in Physiology*, *11*(August), 1–12. https://doi.org/10.3389/fphys.2020.00734

Xu, J., Carretero, O. A., Liao, T., Peng, H., Shesely, E. G., Xu, J., … Yang, X. (2010). Local angiotensin II aggravates cardiac remodeling in hypertension, *2689*(37), 1328–1339. https://doi.org/10.1152/ajpheart.00538.2010.

Xue, B., Johnson, A. K., & Hay, M. (2013). Sex differences in angiotensin II- and aldosterone-induced hypertension: The central protective effects of estrogen. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *305*(5), 459–463. https://doi.org/10.1152/ajpregu.00222.2013

Xue, B., Pamidimukkala, J., & Hay, M. (2005). Sex differences in the development of angiotensin II-induced hypertension in conscious mice. *American Journal of Physiology - Heart and Circulatory Physiology*, *288*(5 57-5). https://doi.org/10.1152/ajpheart.00969.2004

Yamamoto, K., Masuyama, T., Sakata, Y., & Nishikawa, N. (2002). M yocardial

stiffness is determined by ventricular fibrosis , but not by compensatory or excessive hypertrophy in hypertensive heart, *55*, 76–82.

Yamazaki, T., & Yazaki, Y. (1999). Role of tissue angiotensin II in myocardial remodelling induced by mechanical stress. *Journal of Human Hypertension*, *13*, S43–S47. https://doi.org/10.1038/sj.jhh.1000747

Yanes, L. L., Romero, D. G., Iles, J. W., Iliescu, R., Gomez-Sanchez, C., & Reckelhoff, J. F. (2006). Sexual dimorphism in the renin-angiotensin system in aging spontaneously hypertensive rats. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *291*(2), 383–391. https://doi.org/10.1152/ajpregu.00510.2005

Yanes, L. L., Romero, D. G., Iles, J. W., Iliescu, R., Gomez-sanchez, C., Reckelhoff, J. F., … Reckelhoff, J. F. (2006). Sexual dimorphism in the renin-angiotensin system in aging spontaneously hypertensive rats, 383–391. https://doi.org/10.1152/ajpregu.00510.2005.

Yang, Z., Yu, X., Cheng, L., Miao, L., Li, H., Han, L., & Jiang, W. (2013). Effects of enalapril on the expression of cardiac angiotensin-converting enzyme and angiotensin-converting enzyme 2 in spontaneously hypertensive rats. *Archives of Cardiovascular Diseases*, *106*(4), 196–201. https://doi.org/10.1016/j.acvd.2013.01.004

Yildiz, M., Oktay, A. A., Stewart, M. H., Milani, R. V., Ventura, H. O., & Lavie, C. J. (2020). Left ventricular hypertrophy and hypertension. *Progress in Cardiovascular Diseases*, *63*(1), 10–21. https://doi.org/10.1016/j.pcad.2019.11.009

Zhang, Y., Shi, F., Yu, Z., Yang, A., Zeng, M., Wang, J., … Ma, X. (2019). A cross-sectional study on factors associated with hypertension and genetic

polymorphisms of renin-angiotensin-aldosterone system in Chinese hui pilgrims to hajj. *BMC Public Health*, *19*(1), 1–11. https://doi.org/10.1186/s12889-019-7357-1

Zhang, Z., Zhao, L., Zhou, X., Meng, X., & Zhou, X. (2023). Role of inflammation, immunity, and oxidative stress in hypertension: New insights and potential therapeutic targets. *Frontiers in Immunology*, *13*(January), 1–18. https://doi.org/10.3389/fimmu.2022.1098725

Zhao, Z., Wang, H., Jessup, J. A., Lindsey, S. H., & Chappell, M. C. (2014). Sex and Gender Differences in Cardiovascular Physiology – Back to the Basics Role of estrogen in diastolic dysfunction, 628–640. https://doi.org/10.1152/ajpheart.00859.2013

Zou, Y., Hiroi, Y., Uozumi, H., & Takimoto, E. (2001). Calcineurin Plays a Critical Role in the Development of Pressure Overload – Induced Cardiac Hypertrophy.

Appendices

Appendix A: Animal ethics clearance certificate

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

CLEARANCE CERTIFICATE NUMBER: 2021/03/03/C

APPLICANT: Prof F Michel
School: School of Physiology; Department: N/A; Location: WRAF

PROJECT TITLE: The involvement of intrarenal renin angiotensin system (RAS) in the sexual dimorphism associated with the development of hypertension in Spontaneously Hypertensive Rat (SHR)
Category: C; Species and Numbers involved: *28X each of male and female, 1 month old Spontaneously Hypertensive Rats (SHR) and 7X each of male and female, 1 month old Wistar Kyoto Rats (WKY)*

Approval is hereby given for the use of animals for the research project named above and described in the application reviewed by a quorate meeting of the AREC held on 30 Mar 2021. This approval remains valid until 27 Apr 2023 and is conditional to the following (if blank there are no special conditions):

Condition 1	Condition 2	Condition 3	Condition 4

All material changes to the approved research must be reported to the AREC before they are implemented. Failure to do so will invalidate this clearance certificate.

An annual progress report must be provided to the AREC.

The use of these animals is subject to AREC guidelines on the use and care of laboratory animals, is limited to the procedures described in the application and is subject to additional conditions listed below:

I, the Chair of the AREC (or my designated representative) am satisfied that the proposed research is ethical as judged by local law, international standards and University policy.

Signed: _____ Date: 28 April 2021

(Deputy Chairperson of the AREC)

I am satisfied that the persons listed in this application are competent to perform the procedures described in the application, in the context of Section 23 (1) (c) of the veterinary and Para-veterinary Professions Act (19 of 1982).

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Wits Research Animal Facility (WRAF): Dr Kim Jardine