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Impact of Gender on Adrenergic-Induced Cardiac Dilatation and Systolic Dysfunction in Spontaneously Hypertensive Rats

Mhlengi Mthokozisi Magubane

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

Johannesburg, 2014

Abstract

Left ventricular hypertrophy (LVH) is more frequently associated with LV dilatation and systolic chamber dysfunction in males than in females. The mechanisms of this effect are uncertain. As excessive adrenergic stimulation may be responsible for LV dilatation and systolic chamber dysfunction in hypertension, in my dissertation I aimed to assess whether gender determines the adverse effects on LV chamber remodeling following 6 months of daily β-adrenergic receptor (AR) stimulation (isoproterenol [ISO] at 0.04 $mg.kg^{-1}day^{-1}$) in spontaneously hypertensive rats (SHR). LV dilatation was assessed in vivo from LV end diastolic diameter (EDD) (echocardiography) and *ex vivo* from the volume intercept at 0 mm Hg pressure (V_0) of the LV diastolic pressure-volume relationship (isolated, perfused heart technique). LV systolic function was determined *in vivo* from LV endocardial fractional shortening (FS_{end}) and *ex vivo* from the slope (LV end systolic elastance [LV E_{es}]) of the LV end systolic pressure-volume relationship (isolated, perfused heart technique). As compared to saline-treated male SHR (n=13), male SHR receiving ISO for 6 months (n=13) developed an increased LV EDD (Male Saline: 6.56±0.20 mm; Male ISO: 7.78±0.29 mm; $p<0.05$) and LV V_0 (Male Saline: 0.22±0.01 ml; Male ISO: 0.31±0.02 ml; $p<0.05$). In contrast, ISO administration failed to modify LV EDD (Female Saline, n=13: 6.06±0.15 mm; Female ISO, n=12: 6.33±0.15 mm) or LV V_0 (Female Saline: 0.17±0.01ml; Female ISO: 0.17±0.01 ml) in female SHR. In addition, there was a gender-ISO interactive effect on LV E_{es} ($p<0.05$; Male Saline: 2268±336 $mmHg.ml^{-1}$; Male ISO: 1623±164 $mmHg.ml^{-1}$; Female Saline: 1910±219 $mmHg.ml^{-1}$; Female ISO: 2302±230 $mmHg.ml^{-1}$). In conclusion, as compared to female SHR, male SHR are more susceptible to the adverse effects of chronic β-AR activation on LV cavity dimensions and systolic chamber function. These results suggest that the higher prevalence of LV dilatation and systolic chamber dysfunction in males than in females with

LVH may be attributed to an increased susceptibility to the adverse effects of adrenergic stimulation.

Declaration

I, Mhlengi Mthokozisi Magubane, declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science of Medicine, in the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa. The work contained in this thesis, has not been submitted for any degree or examination in this university, or any other university.

.....

Mhlengi Mthokozisi Magubane

Signed on the.....day of……2014

I certify that the studies contained in this dissertation have the approval by the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics approval number is 2010/21/04

……

Mhlengi Mthokozisi Magubane

Signed on the.....day of…… 2014



....

Frederic Michel (supervisor) Angela J. Woodiwiss (supervisor)

Date..... Date.....

Dedication

Firstly, I would like to dedicate this research to my mother Nokuphula Euginia Khumalo who made sure that I am provided for from the first grade up until the postgraduate level. Secondly, I dedicate it to my grandmother Nokuthula Khumalo for lovingly and patiently giving me direction in my life; she has played a significant role in my life. Lastly, I dedicate this work to my supervisors, Dr Frederic Michel, and Professors Angela J. Woodiwiss and Gavin Norton for their endless guidance and support.

Publications and presentations

Data presented in this dissertation have been presented in the form of a poster presentation at the 41st Annual Conference of the Physiology Society of Southern Africa held at the University of Medunsa, September 2013. The title of a poster presentation was "Sex comparison of cardiomyocyte apoptosis in Spontaneously Hypertensive Rats that received short-term β adrenergic agonist"

Although during the cause of my MSc I learnt the techniques of echocardiography and isolated perfused hearts, these are specialized techniques which require a high degree of skill which only comes with experience. Hence in the interests of collecting accurate and hence meaningful data an expert with 20 years of experience collected these data. Importantly, I was responsible for all the daily administrations of isoproterenol and saline vehicles in these animals, assisted with echocardiography and the collection of all the rest of the data including assessment of cardiomyocyte apoptosis and necrosis.

Acknowledgements

I would like to thank my supervisors, Frederic Michel, Angela Woodiwiss and Gavin Norton for their endless patience, commitment and invaluable teaching, I will always be grateful. In addition, I would like to thank Mr Zwelibi Majola and Ms Margaret Badenhorst for their Technical support.

I would also like to thank the Central Animal Services and their professional staff members for their guidance and help during the study and the husbandry that they provided for the rats used in the present dissertation. I would like to thank my fellow members of our laboratory for their assistance and support received during my degree. Finally I would like to thank my family and friends for their support during the many hard hours and challenging times.

List of Abbreviations

AC	adenylate cyclase
AESC	animal ethics screening committee
ANOVA	analysis of variance
AV	arteriovenous
$beats.min^{-1}$	beats per min
BP	blood pressure
Ca^{2+}	calcium
$CaCl_2$	calcium chloride
cAMP	adenosine 3′,5′-cyclic monophosphate
CHF	congestive heart failure
DNA	deoxyribonucleic acid
E_n	slope of the systolic (σ)-strain relation
FS_{end}	endocardial fractional shortening
FS_{mid}	midwall fractional shortening
g	grams
$g.kg^{-1}$	grams per kilogram
HF	heart failure
HR	heart rate
HRV	heart rate variability
ISO	isoproterenol
LV EDD	left ventricular end diastolic diameter
LV ESD	left ventricular end systolic diameter
LVED PWT	left ventricular end diastolic posterior wall thickness

LVES PWT	left ventricular end systolic posterior wall thickness
LVH	left ventricular hypertrophy
MAPK	mitogen-activated protein kinases
MI	myocardial infarction
mm Hg	millimeters of mercury
$mmol.kg^{-1}$	millimoles per kilogram
NE	norepinephrine
NET	norepinephrine transporter
p value	probability value
PLB	phospholamban
RAAS	renin-angiotensin-aldosterone system
SD	Sprague-Dawley
SEM	standard error of the mean
SHR	spontaneous hypertensive rat
SNS	sympathetic nervous system
TAC	transverse aortic constriction
TDT	terminal deoxynucleotidyl transferase
TG	transgenic
TUNEL	terminal deoxynucleotidyltransferasedUTP nick end labeling
WKY	wistar-kyoto
WT	wild-type
α	alpha
β	beta

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Preface

Heart failure is a disease caused by cardiac dysfunction and is characterized by left ventricular hypertrophy or dilatation due to, among other things pressure overload (hypertension). Hypertension is the number one regulating risk factor for the development of heart failure in developing countries. Although, heart failure accounts for a substantial proportion of morbidity and mortality in both men and women, studies have shown that men are at a higher risk of developing heart failure than women. However, the efforts to understand gender influences on the risks of cardiovascular disease in the context of hypertension have not been well established. A leading cause of heart failure in South Africa is hypertension. It is crucial to understand the impact of gender on the development of heart failure in the settings of hypertension.

The progression to heart failure in systemic hypertension is associated with the initial development of left ventricular hypertrophy, said to be a compensatory mechanism. It is suggested that the compensated hypertensive hypertrophy could be as a consequence of sympathetic nervous system (SNS) activation. Indeed, previous studies from our laboratory have studied the impacts of excessive SNS activation on cardiovascular changes, using the beta adrenergic receptor agonist, daily (isoproterenol, ISO). In this regard, these studies revealed that SNS activation causes compensatory ventricular hypertrophy to cardiac dilatation. In addition, previous studies have reported gender-related differences in SNS activation (with reduced activation in females). Hence it is possible that gender difference in progression to heart failure may be explained by differential responses to beta-adrenergic activation in the context of hypertension.

My study therefore aimed to investigate the impact of gender on beta-adrenergic receptor stimulation in the setting of hypertensive animal model. The aims, objectives and justification for this study are further included in chapter one of the introduction.

Chapter One - Introduction

1. Introduction

Heart failure (HF) is a common cause of cardiovascular disease worldwide (Regitz-Zagrosek & Seeland, 2011) including in South Africa (Stewart et al. 2008). Similar to what has been reported on in other communities of African descent (Akinkugbe et al. 1991), the major risk factor for congestive heart failure is hypertension in urban, economically developing communities of black African ancestry in South Africa (Stewart et al. 2008). There is no question that the main challenge in this regard is the lack of access to adequate health care in these communities where a large proportion of individuals with hypertension go undetected or untreated (Steyn et al. 2001). Furthermore, when treated for hypertension only few hypertensives from these communities achieve target blood pressures (Steyn et al. 2001), highlighting the importance of specific anti-hypertensive treatment for black African ancestry.

In the progression from hypertension to heart failure it is now acknowledged that there are factors other than blood pressure which predict this transition process. Identifying and targeting these changes in patients with hypertension is a critical issue. Animal and human studies have provided significant evidence to suggest that males and females respond differently to cardiovascular diseases (Adams et al. 1999, Halm et al. 2000, Hayward et al. 2000, Dubey et al. 2002, & Rossouw et al. 2002). In epidemiologic studies, women with congestive heart failure appear to have better survival rates than men with congestive heart failure (Schocken et al. 1992, Ho et al. 1993, & Adams et al. 1999). In the Framingham Heart Study, once heart failure is present, the median survival time was noted to be 3.2 years for women and only 1.7 years for men (Ho et al. 1993). In the NHANES-I study; the 1-year mortality rate was 23.8% for women with heart failure and 54.4% for men with heart failure (Schocken et al. 1992). Data from the FIRST study showed that higher survival rates for women were evident in the case of non-ischaemic as opposed to ischaemic causes of heart

failure (Adams et al. 1999). In South Africa, men with heart failure from urban, developing communities are more likely to have impaired systolic function than women with heart failure, although women present with more overt symptoms of heart failure (Stewart et al. 2008).

Despite numerous studies implicating gender as a possible factor in mediating the progression to heart failure, studies on the effects of gender on the progression to heart failure in the presence of hypertension (pressure overload) are limited. Moreover, as neurohumoral activation, particularly the sympathetic nervous system (SNS) plays a key role in hypertension and the progression to heart failure, the impact of gender on SNS-induced heart failure remains to be elucidated. Hence the aim of my study was to determine the impact of gender on β-adrenergic-induced cardiac dilatation and dysfunction. As a background to this aim, in chapter one, I will first discuss cardiac hypertrophy, cardiac dilatation and cardiac dysfunction. I will then discuss the effects of gender on cardiac hypertrophy and the progression to heart failure; the role of the sympathetic nervous system in the progression to heart failure; the impact of gender on sympathetic nervous system activation; and then finally, the impact of gender on sympathetic nervous system-induced progression to heart failure.

1.1 Cardiac hypertrophy

The progression to heart failure in pressure overload states such as systemic hypertension is characterized by the initial development of concentric left ventricular hypertrophy, which is an increase in the ratio of wall thickness to chamber dimension (Grossman et al. 1975, Swynghedauw et al. 1999, Hein et al. 2003, & Opie et al. 2006). Left ventricular hypertrophy is a physiological compensatory response to adapt to increased stress or tension on the walls of the heart. This physiological adaptation is according to La Place's law [wall tension = (systolic

pressure x chamber radius)/ 2 (wall thickness)] (Grossman et al. 1975, & Laskey et al. 1984). This adaptive response is characterized by the parallel replication of contractile units (sarcomere) in cardiomyocytes, which increases the cardiomyocyte width (Grossman et al. 1975). The increase in the left ventricular wall thickness normalizes systolic left ventricular wall stress in order to maintain pump function (ejection fraction and cardiac output) (Grossman et al. 1975). The development of concentric ventricular hypertrophy is an early milestone during the clinical development of heart failure and as such it is an important independent risk factor for cardiac mortality and morbidity (Levy et al 1990, & Hunter and Chien 1999).

There are two types of cardiac hypertrophy, namely concentric hypertrophy – caused primarily by chronic left ventricular (LV) pressure overload; and also eccentric hypertrophy – caused primarily by chronic LV volume overload (Grossman et al. 1975, & Ganau et al. 1992). As discussed above concentric hypertrophy is characterized by wall thickening in order to resist increased systolic wall stress due to pressure overload and thus acting as feedback inhibition. As the wall thickens by parallel replication of sarcomeres (figure 1.1), concentric hypertrophy may reduce cardiac filling because of a reduced radius (Grossman et al. 1975, & Norton et al. 1993). Hence the diastolic pressure-volume curve would be left shifted compared to normal, and the volume at a pressure of 0 mm Hg (V_0) would be decreased (figure 1.1). Concentric hypertrophy is therefore characterized by an increased wall thickness to radius ratio.

With respect to eccentric hypertrophy, in response to volume overload induced increases in end diastolic wall stress the chamber enlarges due to a series replication of sarcomeres (figure 1.1).

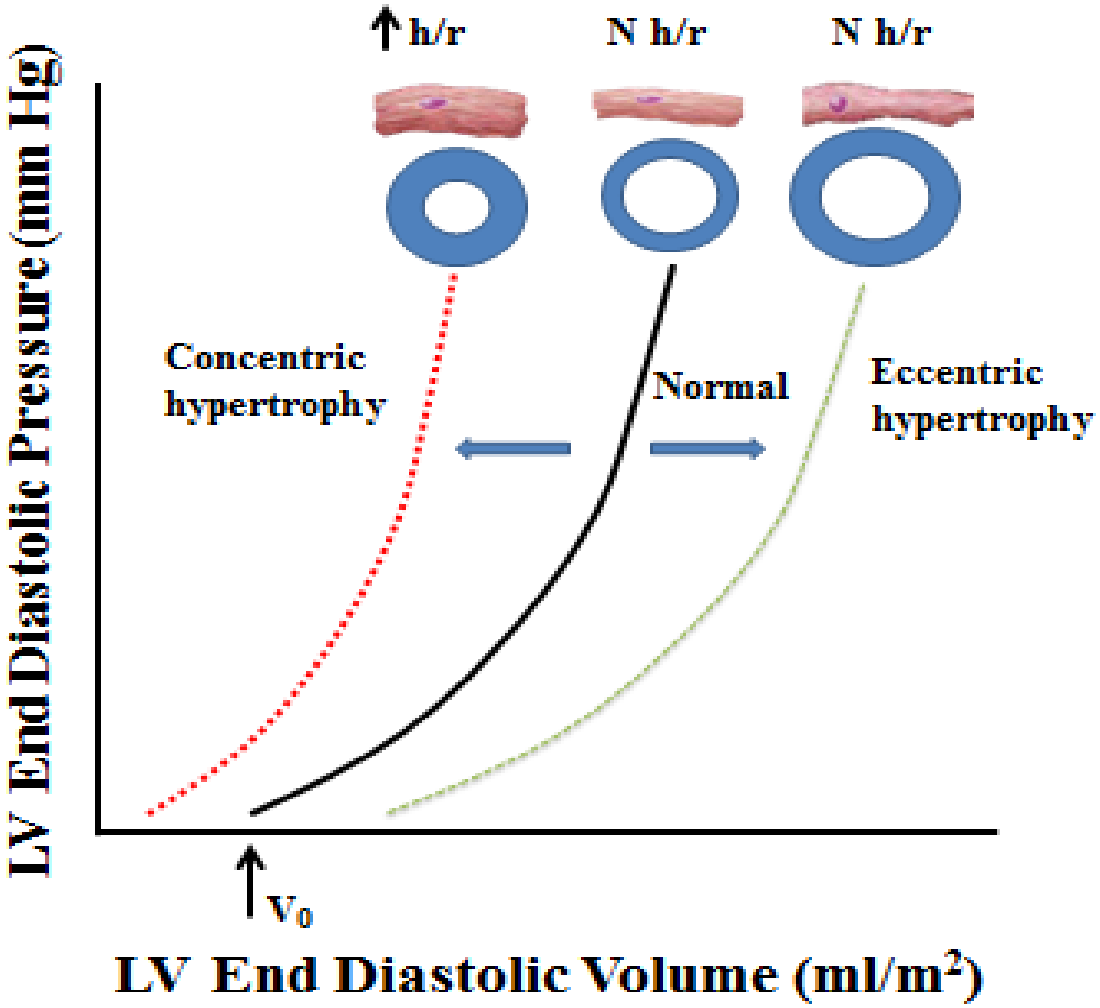


Figure 1.1: Diagrammatic representation of changes in the left ventricular end diastolic pressure-volume relationship in cardiac hypertrophy. The normal diastolic pressure-volume relationship is indicated by the solid line; a left shift in the pressure-volume relationship with decreased volume at which left ventricular (LV) end diastolic pressure equals zero (V_0) and increased wall thickness to radius ratio as occurs in concentric hypertrophy is indicated by the dotted line; and a right shift in the pressure-volume relationship with an increased V_0 but normal wall thickness to radius ratio as occurs in eccentric hypertrophy is indicated by the dashed line

According to the law of La Place, the enlarged chamber leads acutely to an increased peak systolic wall stress, which then causes wall thickening of a sufficient magnitude to resist systolic stress (Grossman et al. 1975, & Laskey et al. 1984). It should then be noted that chamber enlargement together with wall thickening results in comparable ratios of wall thickness to chamber dimensions as that of a normal ventricle (figure 1.1). However, ventricular eccentric hypertrophy shifts the diastolic pressure-volume curve to the right and V_0 is increased.

Although both concentric and eccentric hypertrophy are adaptive processes that occur in response to increased loading conditions (pressure or volume respectively), in order to reduce wall stress back to normal levels, these processes are not limitless. Moreover they are associated with the activation of neurohumoral pathways which have detrimental effects on the heart. Hence cardiac hypertrophy although initially a compensatory response may become maladaptive. Maladaptive hypertrophy occurs when the ventricle enlarges; the ventricular wall becomes thinner and consequently the wall thickness to radius ratio decreases (Swynghedauw et al. 1999, Janicki et al. 2004, & Opie et al. 2006). In the next section I will therefore discuss the processes by which compensatory hypertrophy progresses to maladaptive (LV dilatation).

1.2 Cardiac dilatation

As described above, cardiac dilatation is a maladaptive process characterised by chamber enlargement and wall thinning. The presence of ventricular dilatation can be detected *in vivo* using echocardiography. Alternatively, *ex vivo* assessments can be made using an isolated perfused heart system. The details of these two methods (*in vivo* and *ex vivo*) will be discussed thoroughly under the methods section (see chapter 2). However, it is important to discuss the relative accuracy of these two methods in determining the presence of LV dilatation.

In this regard *in vivo* assessments are confounded by the impact of heart rate and loading conditions. Indeed, increases in volume load would result in increased filling volumes and therefore enlarged chamber dimensions. Hence, an increased left ventricular end diastolic diameter (LV EDD) would be measured on echocardiography. Alternatively, increases in pressure load could result in decreases in stroke volume and hence enlarged chamber dimensions. Furthermore, increases in heart rate could similarly result in enlarged chamber dimensions as a consequence of reductions in stroke volume. In comparison the isolated perfused heart system allows for load and heart rate independent measures. Firstly, the hearts are paced at a fixed rate and secondly pressures are recorded at stepwise increments in volume. As the heart is isolated from the body, there are also no external neurohumoral influences. Therefore LV dilatation is defined as a right shift in the diastolic pressure-volume curve obtained from an isolated perfused heart system (Gibbs et al. 2004, & Gilbert and Glantz 1989) (figure 1.2).

Our group have studied factors which contribute to the transition from cardiac hypertrophy to cardiac dilatation, including neurohumoral activation (Badenhorst et al. 2003a, Gibbs et al. 2004, Veliotes et al. 2005, & Osadchii et al. 2007). These studies showed that sympathetic nervous system activation plays a key role (Woodwiss et al. 2001, Badenhorst et al. 2003a, Gibbs et al. 2004, & Veliotes et al. 2005). In addition, increased activity of the renin-angiotensin-aldosterone system has been shown to mediate cardiac dilatation (Veliotes et al. 2005). Indeed, aldosterone receptor blockade prevents the progression from compensatory cardiac hypertrophy to cardiac dilatation. Increased sympathetic nervous system activity is associated with alterations in the characteristics of cardiac collagen (Woodiwiss et al. 2001, & Badenhorst et al. 2003a).

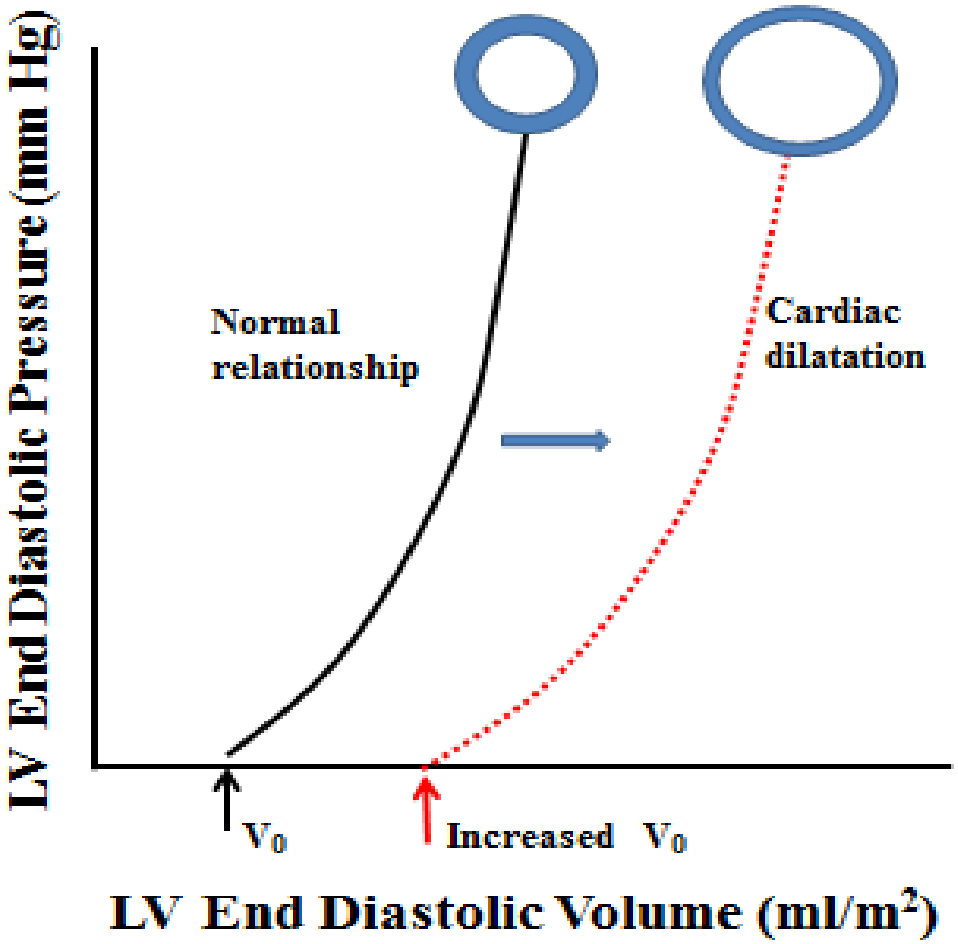


Figure 1.2: Example of the left ventricular end diastolic pressure-volume relationship

The normal diastolic pressure-volume relationship is indicated by the solid line, and a right shift in the pressure-volume relationship, indicative of cardiac dilatation, is indicated by the dashed line. The volume intercept [volume at which left ventricular (LV) end diastolic pressure equals zero (V_0)] is increased in the right shifted relationship.

Although synthesis of cardiac collagen is increased with sympathetic nervous system activation, the new collagen has reduced cross-links (Woodiwiss et al. 2001, & Badenhorst et al. 2003a). Consequently the tethering of cardiomyocytes is reduced. This results in side-to-side slippage of the cardiomyocytes and ultimately dilatation. In addition, cardiomyocyte death by either apoptosis or necrosis is also believed to play a role (Yussman et al. 2002). Subsequent to cardiomyocyte death, side-to-side slippage has also been shown to occur and hence dilatation (Yussman et al. 2002).

The presence of LV dilatation is not benign; it is indeed a maladaptive process which impacts on cardiac function (Janicki et al. 2004). In particular, pump (chamber) function, either in the presence or the absence of myocardial dysfunction, is impaired. In the next section I will therefore discuss measurements of cardiac function and the impacts of LV dilatation on cardiac function.

1.3 Cardiac function and the impacts of cardiac dilatation

Cardiac function includes both diastolic function (capacity of the heart to fill with blood during diastole) and systolic function (capacity of the heart to eject blood during systole). I will focus my discussion on systolic cardiac function as this is most relevant to the impacts of cardiac dilatation. Although cardiac function can be assessed *in vivo* using echocardiography, as discussed in section 1.2 above, these measurements are influenced by heart rate and loading conditions. Hence, *ex vivo* assessments are required to assess cardiac function in the absence of the influences of heart rate, loading conditions and neurohumoral activity. Using isolated perfused heart systems, pressure and volume are recorded. Systolic cardiac chamber function is assessed from the slope of the linear portion of the developed (systolic minus diastolic)

pressure-volume relationship (E_{es}) (figure 1.3). Intrinsic systolic myocardial function is assessed from the slope of the systolic stress-strain relationship (E_n), where stress is calculated from left ventricular systolic stress = 1.36 x LV developed pressure x $(LVV)^{2/3}$ / [LVV + (0943 x LV mass)]$^{2/3}$ – $LVV^{2/3}$ (Norton et al. 2002, Badenhorst et al. 2003b, & Veliotes et al. 2005) (figure 1.3).

As cardiac dilatation is known to be a precursor of pump dysfunction and clinical heart failure (Pfeffer et al. 1992, Gaudron et al. 1993, Vasan et al. 1997), the question arises as to how cardiac dilatation results in reduced pump function? As cardiac dilatation is associated with a reduced relative wall thickness, according to La Place's Law, wall tension or stress will be increased. An increase in wall stress will result in an increase in myocardial oxygen consumption; hence the myocardial oxygen demand-to-supply ratio may be increased in cardiac dilatation. An increase in myocardial oxygen demand-to-supply ratio may subsequently lead to a reduction in myocardial contractility. Alternatively, cardiac chamber function may be reduced in the absence of alterations in myocardial contractility (Norton et al. 2002). It is possible that in a dilated ventricle, inappropriate force transduction occurs during the contraction of cardiomyocytes, which would result in pump dysfunction (Sallin 1969). In addition, in a dilated ventricle larger chamber volumes may be required to produce cardiomyocyte stretch in order to recruit the Frank-Starling effect. Indeed, when the structure of the ventricle changes the mechanics of systolic function are affected which results in a low cardiac output (Laskey et al. 1984, & Cohn et al. 2000).

Having discussed left ventricular hypertrophy, cardiac dilatation and the progression to systolic dysfunction, I will now discuss the literature on the effects of gender on left ventricular hypertrophy and the progression to heart failure.

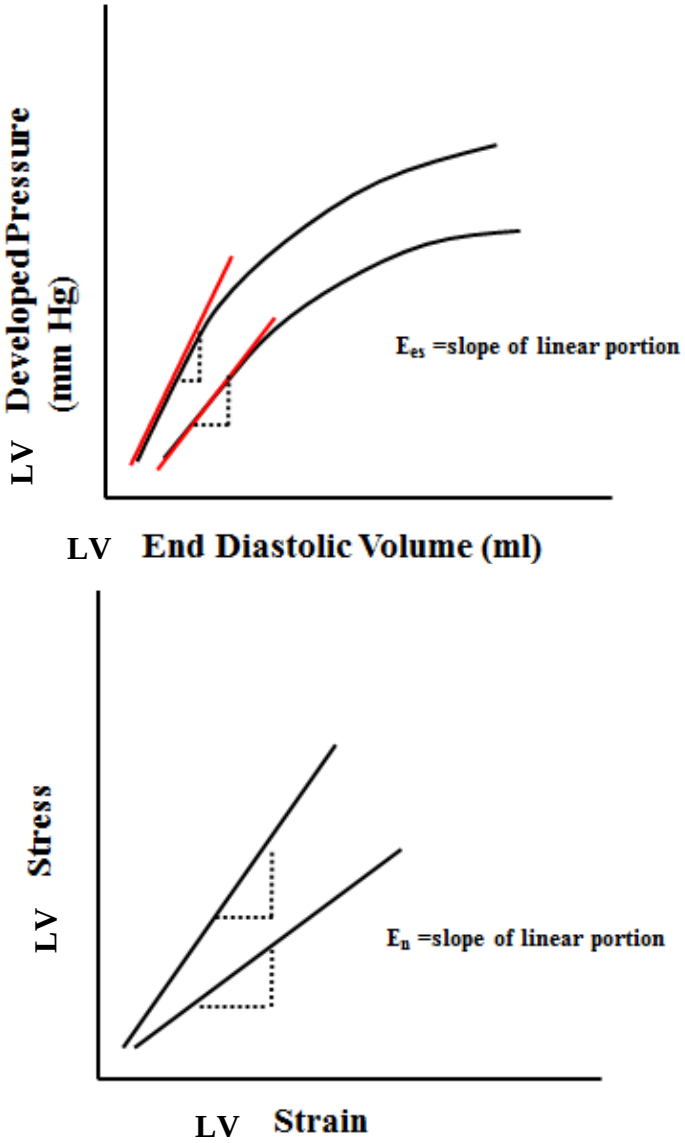


Figure 1.3: Diagrammatic representation of changes in the slope of the developed pressure-volume relationship (upper panel) and the stress-strain relationship (lower panel). A decreased slope of the developed pressure-volume relationship (E_{es}) indicates decreased systolic chamber function, and a decreased slope of the stress-strain relationship (E_n) indicates decreased systolic myocardial function.

1.4 Gender effects on left ventricular hypertrophy and the progression to heart failure

Gender has been shown to quantitatively and qualitatively influence the characteristics of left ventricular hypertrophy (LVH), as well as progressive heart failure (HF) (Topol et al. 1985, Carrol et al. 1992, & Aurigemma et al. 1994). The observational Studies of Left Ventricular Dysfunction (SOLVD) patient registry reported a higher annual risk for heart failure-related mortality in women (Bourassa et al. 1993); however several studies have suggested that women with HF have better survival rates than men (McKee et al. 1971, Schocken et al. 1992, Ho et al. 1993, & Adams et al. 1999). The apparent controversial data in the SOLVD study may be due to higher age or other confounding factors in women. In 652 members of the Framingham Study the median survival after the onset of heart failure was 1.7 years in men and 3.2 years in women (Ho et al. 1993). Possible explanations for the gender differences in HF survival include myocardial adaptation to pressure overload (Schaible et al. 1984, Buttrick et al. 1993, Witt et al. 2008, & Fliegner et al. 2010). In this regard, human studies have reported that hypertensive women develop a more marked concentric hypertrophy and higher indices of systolic function than men (Carrol et al. 1992, Aurigemma et al. 1994). Indeed, the preponderance of extreme hypertensive hypertrophy with a subnormal wall stress resulting in a supernormal pump or ejection performance is higher in elderly black women than men (Topol et al. 1985, & Douglas et al. 1995). However, these findings may be because men with extensive hypertrophy have succumbed to the complications of cardiovascular disease at an earlier age. Nevertheless, in autopsies, apoptosis and necrosis of cardiomyocytes are less evident in the failed hearts of women than in those belonging to men (Guerra et al. 1999, & Olivetti et al. 1997). These findings suggest that the female gender may be protected against cardiac damage and dysfunction.

Animal studies have provided similar evidence to human studies. Indeed, animal studies have shown the effects of gender on cardiac changes, not only with load and heart rate dependent echocardiographic measurements obtained *in vivo*; but importantly with load and heart rate-independent measures of cardiac dilatation and pump dysfunction *ex vivo* under controlled conditions. A summary of the most pertinent animal studies assessing the effects of gender on LVH and the progression to heart failure is provided in table 1.1. Most of these studies used *in vivo* assessments of cardiac remodelling in response to either pressure (Pfeffer et al. 1982, Douglas et al. 1998, & Tamura et al. 1999) or volume (Brower et al. 2003, Skavdahl et al. 2005, Dent et al. 2010, & Dent et al. 2012) overload. In addition, two of the studies assessed cardiac dimensions *in vivo* in a genetically hypertensive model; namely the Spontaneous Hypertensive Rat (SHR) (Pfeffer et al. 1982, & Tamura et al.1999). Hence the data from these studies need to be interpreted with caution as the results may be confounded by differences in loading conditions or heart rate. However, other studies used both *in vivo* and *ex vivo* (Gardener et al. 2002, Weinberg et al. 1999, & Chan et al. 20110 or *ex vivo* (Jain et al. 2002, & Monasky et al. 2008) assessments of cardiac dimensions and function in either pressure (Weinberg et al. 1999, & Chan et al. 2011) or volume (Gardner et al. 2002) overload conditions. Compared to *in vivo* assessments *ex vivo* assessments of cardiac dimensions and function are a more reliable technique, because they allow for the exclusion of the influences of loading conditions and heart rate as discussed in section 1.2 above.

Table 1.1: Summary of animal studies reporting on the effects of gender on the progression from left ventricular hypertrophy to heart failure.

Author	**Model**	**Animal strain**	***In vivo/ Ex vivo***	**Findings & Conclusion**
Pfeffer et al. 1982	Pressure overload (hypertension)	Spontaneously Hypertensive Rats (SHRs)	*In vivo*	Female SHR had normal heart dimensions and function; male SHR had LV dilatation & LV dysfunction and HF.
Douglas et al.1998	Pressure overload induced by aortic banding	Wistar rats	*In vivo*	Female rats were protected from chamber dilatation and sustained concentric hypertrophy compared to male rats.
Tamura et al. 1999	Pressure overload (hypertension)	Spontaneously Hypertensive Rats (SHRs)	*In vivo*	Hypertrophy was present from 2, 4 and 6 months in both genders. At 18 months, males had a cardiac dilatation, whereas females only had it at 24 months. Therefore, reduced adaptive hypertrophic reserve was observed in males.
Weinberg et al. 1999	Pressure overload induced by aortic banding (stenosis)	Wistar rats	*In vivo* & *Ex vivo*	In contrast to females, males showed a reduced contractility after 6 weeks of pressure overload, but the patterns of LVH & systolic wall stress were similar in both genders. Hence males adapt differently to pressure overload despite a similar LVH and systolic wall stress to females.
Gardner et al. 2002	Volume overload, Arteriovenous (AV) fistula	Sprague-Dawley (SD) rats	*In vivo* & *Ex vivo*	Male rats had LV dilatation and increased compliance compared to female rats. Hence female rats adapt more favourably to volume overload than male rats.
Jain et al. 2002	Pressure overload after myocardial	Dahl salt-sensitive (DS)	*Ex vivo*	Females developed LV concentric hypertrophy with no dilatation, whereas males had LV eccentric hypertrophy and dilatation. Females responded more

	infarction (MI)	rats		favourably to the hemodynamic overload after large MI.
Gao et al. 2003	Over-expression of β-adrenergic receptors	Transgenic (TG) mice	*In vivo*	Male mice had LV dilatation & pump dysfunction compared to female mice, Therefore, there was greater survival in female transgenic mice compared to male mice. The LV dilatation and pump dysfunction in the male mice was improved with castration.
Brower et al. 2003	Volume overload, Arteriovenous (AV) fistula	Sprague-Dawley (SD) rats	*In vivo*	Compared to male rats there was reduced heart failure in female rats which was associated with preserved LV function and chamber size.
Skavdahl et al. 2005	Pressure overload, aortic banding	β-estrogen receptor knockout (β-ERKO) & wild-type (WT) mice	*In vivo*	WT male mice developed hypertrophy when subjected to TAC, also sham females had hypertrophy after TAC. Therefore, oestrogen receptor-β plays an important role in attenuating the hypertrophic response to pressure overload.
Monasky et al. 2008	Pressure overload, Isoproterenol (ISO)	Lewis-Brown Norway F1 (LBN F1) rats	*Ex vivo*	Basic mechanical performance of healthy isolated myocardium under physiological conditions is not different between males and females, and a different response to stress must underlie gender-based differences in cardiac performance.
Dent et al. 2010	Volume overload, Arteriovenous (AV) shunt.	Sprague-Dawley (SD) rats	*In vivo*	Male rats in contrast to female rats showed increased LV dimensions and reduced pump function. Gender differences in cardiac function may be due to differences in the type of cardiac remodelling as a consequence of AV shunt.

Chan et al. 2011	Pressure overload (hypertension)	Spontaneously Hypertensive Rats (SHRs)	*In vivo* & *Ex vivo*	At 15 months of age, SHR males develop LV dilatation, systolic & diastolic dysfunction, but not females. Therefore, ageing male SHRs in contrast to female SHRs are more susceptible to heart failure.
Dent et al. 2012	Volume overload, Arteriovenous (AV) shunt.	Sprague-Dawley (SD) rats	*In vivo*	β-AR mechanism is downregulated in males and upregulated in females. Therefore, oestrogen is responsible for the upregulation of β-AR mechanism and maintenance of cardiac function in females exposed to volume overload.

With respect to LVH, most authors reported that females develop concentric hypertrophy and males develop eccentric hypertrophy in response to either pressure (Douglas et al. 1998, Tamura et al. 1999, & Jain et al. 2002) or volume overloads (Gardner et al. 2002). Hypertensive female rats were noted to have a greater capacity for hypertrophy compared to hypertensive male rats (Tamura et al. 1999). Indeed, Pfeffer et al. (1982) and Chan et al. (2011) reported that LV dilatation occurred in male spontaneously hypertensive rats (SHR) but not in female SHR. These findings are in line with the fact that healthy male rats have larger cardiomyocytes than female rats presumably because of the increased volume load from a larger body mass (Bai et al. 1999). Moreover, in transgenic mice with over-expression of β-adrenergic receptors, Gao et al. (2003) reported from *in vivo* assessment that LV dilatation occured in male but not female mice. Furthermore, in a review by Du (2004) it was suggested that male mice are more sensitive than their female counterparts to genetic interventions leading to pathologic hypertrophy and HF.

In addition to gender effects on LV dimensions, previous studies have reported on gender differences in LV function. In response to pressure (Pfeffer et al. 1982, Weinberg et al. 1999, & Chan et al. 2011) or volume (Gardner et al. 2002, Brower et al 2003, & Dent et al. 2010) overload, male rats developed systolic dysfunction and were more susceptible to HF in comparison to female rats. Monasky et al. (2008) demonstrated that both male and female rats have the same LV function under normal physiological conditions, but LV function was reduced in males under pressure overload. In addition, in response to β-adrenergic receptor over-expression male mice developed pump dysfunction and had reduced survival compared to female mice (Gao et al. 2003). Importantly in the latter study (Gao et al. 2003), the LV dilatation and pump dysfunction in the male mice was improved with castration. In addition, Dent et al. (2012) reported that β-adrenergic receptor upregulation occurred in female rats

which maintained cardiac function when exposed to volume overload. These authors claimed that oestrogen was responsible for the β-adrenergic receptor upregulation. Therefore, the observed sex differences in table 1.1 support the hypothesis that sex hormones are involved in protecting females against either pressure or volume overload induced progression to HF. However, the mechanisms by which this occurs are currently unclear.

Both oestradiol and testosterone have been proposed as candidates that may explain the gender-related differences in left ventricular remodelling and heart failure outcomes (Marsh et al. 1998, Mendelsohn et al. 1999, Gao et al 2003, Hodson et al. 2014). With respect to the potential role of testosterone in heart failure, testosterone has been shown to elicit hypertrophy of cardiomyocytes both *in vitro* and *in vivo* (Marsh et al. 1998, & Cabral et al. 1988). However, in contrast to what would be predicted, low circulating testosterone concentrations are a marker of a poor prognosis for the survival of men suffering from cardiac failure (Jankowska et al. 2006). Moreover, in men with chronic heart failure, testosterone-replacement therapy improves cardiac output as well as the symptoms of heart failure (Pugh et al. 2003), suggesting a protective effect of normal levels of testosterone. Recent findings from our laboratory nevertheless show that castration does not influence the extent of LV dilatation induced by chronic adrenergic activation (Hodson et al. 2014). Clearly the role of testosterone in the process of progression to heart failure is controversial.

With respect to the potential role of oestrogen in heart failure, oestrogen-replacement therapy given to postmenopausal women is associated with a better prognosis in hypertrophic cardiomyopathy, as well as a lower incidence of heart failure after a myocardial infarction (Reis et al. 2000, Shlipak et al. 2001, & Lindelfeld et al. 2003). In rodent models of cardiac pathology, oestrogen reduces myocardial hypertrophy induced by pressure or volume overload (Cabral et al. 1988, Malhotral et al. 1990, Weinberg et al. 1999, Sharkey et al. 1999, Gardner et

al. 2010). Skavdahl et al. (2005) have shown that β-oestrogen receptors are responsible for reducing the hypertrophic response to pressure overload in female rats. Oestrogen administration in ovariectomized female rats under volume overload prevents the degradation of collagen associated with matrix metalloproteinase (MMP) activation (Brower et al. 2002, Chancey et al. 2005 & Janicki et al. 2006). In addition, gender-related differences have also been observed in the activation of the mitogen-activated protein kinase (MAPK) pathway in compensated hypertrophy (Dash et al. 2003). As oestrogen decreases the activation of p38 MAPK (Angele et al. 2003, & Dash et al. 2003), the gender-specific regulation of p38 MAPK may be in part, responsible for the gender differences in the hypertrophic response following pathological conditions. In addition, oestrogen administration may prevent cardiomyocyte apoptosis and the development of pump dysfunction in pressure overload states (Dubey et al. 1998, Satoh et al. 2007, & Mahmoodzadeh et al. 2010). *In vitro* and *in vivo* studies have identified oestrogen as a potential inhibitor of cardiomyocyte apoptosis (Pattern et al. 2004, Satoh et al. 2007, & Fliegner et al. 2010), fibrosis and MMP activity (Dubey et al. 1998, & Mahmoodzadeh et al. 2010), as well as RAAS activity (Holycross et al. 1997, Schunkert et al. 1997, & Malkin et al. 2006).

The ability of gender and sex hormones to modify a number of the processes that may explain the progression from compensated hypertensive hypertrophy to HF raises the question as to whether there is a role for gender in the transition to HF. In this regard, activation of the sympathetic nervous system may be a key process in the transition to heart failure in hypertension. Hence before discussing the potential impact of gender on sympathetic nervous system-induced adverse effects on the heart, the role of the sympathetic nervous system in LVH and the progression to HF will be discussed.

1.5 Role of sympathetic nervous system activation in the progression to heart failure

A strong association has been demonstrated between sympathetic nervous activation and the failing heart (Kaye et al. 1996, Pepper et al. 1999, & Brunner-La Rocca et al. 2001). Activation of the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) is known to occur in response to increases in cardiac wall stress. Furthermore, continual cardiac wall stress would favour more sympathetic overactivity which has major adverse effects on cardiac remodelling (Brunner-La Rocca et al. 2001, Badenhorst et al. 2003a, & Veliotes et al. 2005). Indeed, a potential role of sympathetic nervous system activation in the progression to heart failure from hypertrophy is well documented (Anderson et al. 1989, Kaye et al. 1996, Pepper et al. 1999, Brunner-La Rocca et al. 2001, Badenhorst et al. 2003, Veliotes et al. 2005, Floras 2009, Triposkiadis et al. 2009, & DiBona et al. 2013). Studies have shown that sympathetic nervous system overactivation may contribute toward the transition to heart failure in LVH (Brunner-La Rocca et al. 2001, Badenhorst et al. 2003, Schlaich et al. 2003, & DiBona et al. 2013). In addition, in pressure overload induced ventricular hypertrophy an increased myocardial norepinephrine (NE) concentration has been measured in the coronary sinus (Agabiti-Rosei et al. 1987, Kelm et al. 1996, & Brunner-La Rocca et al. 2001). Similarly, in our laboratory, we have demonstrated that hypertensive rats with compensatory hypertrophy, when exposed to pressure overload conditions, produced increased myocardial NE concentrations in the coronary effluent (Veliotes et al. 2005). Moreover, transgenic animals with decreased adrenergic activation are protected against the development of HF when exposed to pressure overload conditions (Esposito et al 2002). In this regard, it has been shown that excessive sympathetic nervous system (SNS) activation down-regulates beta-adrenergic receptor (β-AR) mediated signal transduction which may ultimately result in depressed cardiac function (Böhm et al. 1995, Dhalla et al. 1997, & Osadchii et al. 2007). Finally, in our

laboratory we have further demonstrated that hypertensive animals with compensated hypertrophy are susceptible to chronic β-AR activation and consequently develop cardiac dilatation and pump dysfunction (Woodiwiss et al. 2001, Tsotetsi et al. 2001, Badenhorst et al. 2003, & Veliotes et al. 2005).

Importantly, the pathophysiological mechanism for this transition to HF from LVH in sustained (chronic) β-AR activation is explained by the increased NE concentrations that ultimately result in an increased inotropic response of the cardiomyocytes through post receptor activation of adenylate cyclase (AC), an increase in the intracellular concentrations of the second messenger cyclic adenosine monophosphate (cAMP) and subsequently myocardial contractility (Dhalla et al. 1997, & Dent et al. 2010). This signalling cascade leads to compensatory hypertrophy and subsequently dilatation. Among other pathways, *in vitro* and *in vivo* studies have shown that the mitogen-activated protein kinases (MAPK) are important switches in pathways that promote the transition to LV dilatation from hypertrophy (Sheng et al. 1997, Wang et al. 1998, Hunter et al. 1999, & Dash et al. 2003). For instance, in mice p38 mitogen-activated protein kinases are strongly activated by pressure overload which is usually accompanied by an increase in the rate of cardiomyocyte death (apoptosis) (Wang et al. 1998). Indeed, adrenergic activation promotes cardiomyocyte apoptosis through activation of β_1-adrenergic receptor cAMP-dependent protein kinase A and mitogen-activated protein kinase (MAPK) pathways (Singh et al. 2001) and indirectly through RAAS stimulation (De Angelis et al. 2002). Apoptosis or necrosis may reduce the capacity to tether cardiomyocytes and hence promote side-to-side slippage and consequently cardiac dilatation (Beltrami et al. 1995). Importantly, Chan et al. 2004 reported that the transition from cardiac hypertrophy to heart failure in hypertension may be prevented by the blockade of β-AR independent of blood pressure effects.

These data clearly show that excessive adrenergic activation is a crucial process in the transition from LV dilatation to HF in hypertension. However, it is unclear whether there are alternative blood pressure independent effects that may explain the transition to heart failure in hypertension. Importantly, the effects of gender on LVH and progression to HF (as discussed in section 1.4 above) suggest that gender may be one such alternative blood pressure independent effect. As SNS activation plays a key role in the transition from LVH to HF as discussed what evidence is there of gender effects on SNS activation?

1.6 Gender effects on sympathetic nervous system activation

It is known that gender differences exist in the regulation of the SNS. In healthy men and women at rest, the blood pressure (BP) is similar but heart rate (HR) tends to be higher in women (Hinojosa-Laborde et al. 1999). However, human studies have shown that measurements of SNS activity are reduced in healthy women compared to healthy men (Ng et al. 1993, & Jones et al. 1996). This presents a question: what is the mechanism underlying this increase in HR, in the presence of a decreased SNS activity in healthy women?

The differences in the development of hypertension in males and females are strongly associated with gender differences in the regulation of SNS activity (Hogarth et al. 2007, Lambert et al. 2007, & Coulson and Cockcroft 2011). Hypertension in women is associated with a lower level of sympathetic hyperactivity than in men (Hogarth et al. 2007). Gender differences have been demonstrated in the regulation of the SNS through the involvement of the beta-adrenergic system (Kneal et al. 2000, Hart et al. 2009, & Hogarth et al. 2011). Increased β_2-AR sensitivity offsets α-adrenergic mediated vasoconstriction in pre-menopausal women (Kneal et al. 2000). Whereas men have an increased SNS activity due to β-adrenergic

activation which ultimately results in reductions in coronary blood flow velocity in comparison to women (Momen et al. 2010). Furthermore, several human studies have evaluated gender differences in baroreflex regulation of cardiac SNS activity (Abdel-Rahman et al. 1994, & Desai et al. 1997). These studies have shown that the reflex bradycardia in response to increases in BP is diminished in women compared to in men. There is increasing evidence to show the existence of gender-related differences in sympathetic activation and baroreflex control of autonomic function (Yamasaki et al. 1996, Umetani et al. 1998, & Aroson et al. 2000). Disturbances in baroreflex activation are characterized by profound abnormalities in autonomic control; whereby there is sympathetic overactivity (Cohn et al. 1984, & Floras and Pitt et al. 1993) and parasympathetic withdrawal (Binkley et al. 1991, & Floras et al. 1993).

To my knowledge, there are no animal data available to show that bradycardia is different in males and females. However, with respect to gender differences in reflex tachycardia in response to a decrease in BP, there is conflicting evidence. One study has shown that female rats respond with increases in HR in response to low BP (Buñag et al. 1975). Whereas, another study reported that there are no gender differences in reflex-mediated tachycardia (Crofton et al. 1988). Several animal studies have used the mediators of the β-AR signalling pathway to show gender differences in SNS activation; including catecholamines, β-AR density and affinity, Ca^{2+} current density, cAMP, AC activity, and MAPK activation (Schwertz et al. 1999, Vizgirda et al. 2002, Dash et al. 2003, Dent et al. 2010, McIntosh et al. 2011, Dent et al. 2012). In these studies it has been shown that male rats have more β-AR and greater cAMP production, which indicates that males have an enhanced response to β-adrenergic stimulation (Schwertz et al. 1999, & Vizgirda et al. 2002). Transgenic male mice with phospholamban (PLB) over-expression exhibit contractile dysfunction and hypertrophy due to early p38 MAPK activation which promotes compensatory hypertrophy. Whereas

transgenic female mice show a temporal delay in p38 MAPK activation despite contractile dysfunction (Dash et al. 2003). This may be attributed to oestrogen which modulates MAP kinase phosphatase-1 (MKP-1) expression and thus inhibits MAPK activation (Nuedling et al. 1999).

Having summarized the data indicating that there are indeed gender differences in SNS activation and that SNS activation plays a key role in the transition from LVH to HF, the question then arises as to whether gender impacts on SNS-induced progression to HF.

1.7 Gender effects on sympathetic nervous system induced progression to heart failure

As discussed in the previous section, disturbances in baroreflex activation may result in abnormalities in autonomic control and subsequently sympathetic overactivity (Cohn et al. 1984, & Floras et al. 1993) as well as parasympathetic withdrawal (Binkley et al. 1991, & Floras et al. 1993). A method that measures noninvasive heart rate variability (HRV) has been largely used to explain the relationship between sympathetic and parasympathetic nervous system function in modulating heart rate (Mortara et al. 1994, Butler et al. 1997, Poniskowski et al. 1997, & Jiang et al. 1997). Using an analysis of heart rate variability (HRV) it is possible to study the sympathetic over-activation (Cohn et al. 1984, & Floras et al. 1993) as well as parasympathetic withdrawal (Binkley et al. 1991, & Floras et al. 1993). Among other indexes of HRV, high frequency heart rate fluctuations are associated with healthy cardiac function (Hyndman et al. 1971, Billman et al. 1990, Ryan et al. 1994); whereas, low beat to beat HRV is associated with heart failure (Saul et al. 1988, & Casolo et al. 1991). Indeed, gender-related differences of the HRV as an index of autonomic system function for normal subjects have been reported in several studies (Cowan et al. 1994, Ryan

et al. 1994, Huikuri et al. 1996, Ramaekers et al. 1998, & Umetani et al. 1998). A few of these studies reported an augmented parasympathetic activity as determined by a higher HRV in healthy women compared with healthy men (Ryan et al. 1994, & Huikuri et al. 1996). In contrast, other studies have shown a significantly lower HRV in healthy female subjects compared to healthy male subjects (Cowan et al. 1994, Ramaekers et al. 1998, & Umetani et al. 1998). Moreover, some studies reported similar HRV between men and women (Bigger et al. 1995, & Liao et al. 1995). Importantly, there is only one study that reported gender-related differences in HRV in patients with heart failure (Aroson et al. 2000) (table 1.2). This study reported that women with heart failure have a higher HRV compared to men with heart failure. Therefore, suggesting that women with heart failure have an attenuated sympathetic activation and augmented parasympathetic activity compared with men.

Other techniques used to assess sympathetic over-activity in human studies include increases in plasma norepinephrine (NE), central sympathetic outflow, and NE spillover from activated sympathetic nerve fibers (Pepper et al. 1999, Triposkiadis et al. 2009, & Mitoff et al. 2011). These techniques are reliable and are considered to best quantify cardiac SNS activity, particular in CHF patients (Hasking et al. 1986, Kaye et al. 1995, Newton et al. 1996, Rundqvist et al. 1997, & Mitoff et al. 2011). Most commonly, plasma NE is measured to assess SNS activity because it depends on the rate of immediate NE reuptake and clearance from the circulation (Esler et al. 1990). In CHF however, there is defective neuronal catecholamine reuptake that may result in accumulation of large amounts of NE in the cardiac sympathetic neuroeffector junctions (Liang et al. 2007). In normal conditions NE transporters (NET) recapture and remove more than 90% of these large amounts of NE into the sympathetic terminals (Eisenhofer et al. 1996, & Cingolani et al. 2011). However, in CHF the NET loses its function which results in decreased NE stores, NE extraneuronal clearance, and contractile impairments (Cingolani et al. 2011).

Table 1.2: Gender effects on sympathetic nervous system induced progression to heart failure

Human studies			
Author	**Technique**	**Markers of SNS activation**	**Findings & Conclusion**
Mitoff et al. 2011	Radiotracer, NE spillover	NE in coronary sinus plasma	Higher NE concentrations in women than in men with HF. Therefore, women have increased cardiac-specific sympathetic activation.
Aroson et al. 2000	Heart rate variability (HRV) analysis	Heart rate activation	HRV was higher in females with HF compared with males with HF. Therefore, females in HF have increased parasympathetic activation, whereas males in HF have increased sympathetic activation.
Animal studies			
Author	**Technique**	**Markers of SNS activation**	**Findings & Conclusion**
Gao et al. 2003	Over-expression of β-adrenergic receptors	β_2-adrenergic receptors	Male mice showed LV dilatation & pump dysfunction, but improved with castration compare to female mice. Therefore, greater survival in female transgenic mice vs. male mice.
Dash et al. 2003	Phospholamban, (PLB) overexpression= increase NE	NE-mediated p38 MAPK activation	Early p38 MAPK activation in TG Males & delay in TG female. Therefore, females exhibit a temporal delay for contractile dysfunction & hypertrophy. Oestrogen modulates MKP-1

			expression which inhibits MAPK activation.
Dent et al. 2010	Volume overload, AV shunt	Plasma catecholamines, β-AR affinity, AC activity	Increased catecholamines in males from AV shunt may account for the greater increase in cardiac output and higher degree of cardiac hypertrophy.
Dent et al. 2012	Volume overload, AV shunt	Plasma catecholamines, β-AR content, AC content	Decreased β_1-ARs & β_2-ARs in AV shunt males and ovariectomized females, but increased in sham females and those that underwent oestrogen therapy. AV shunt female rats maintain cardiac function because of sex-hormones.
Hodson et al. 2014	ISO	β-AR activation	ISO caused cardiac dilatation, but not pump dysfunction in both castrated and non-castrated male rats. Therefore, castration does not influence the extent of LV dilatation.

Importantly, gender appears to play a role in NET function. Indeed, Buonanno et al. 1982 demonstrated that blockade of NET with reboxetine in 12 healthy men may result in a greater increase in cardiac stroke volume and cardiac output than in 12 age-matched women. Therefore, suggesting that the male heart depends on NET more than the female heart. Gender-related studies that assess cardiac-specific sympathetic activity in patients with heart failure are limited. There is only one study (Mitoff et al. 2011) that incorporated patients with CHF (Table 1.2). Mitoff et al. 2011 studied 166 subjects: 48 with normal heart function and 118 with CHF but stable symptoms and left ventricle (LV) ejection fraction <35%. This study (Mitoff et al. 2011) demonstrated that patients with CHF have NET dysfunction. Importantly, this study confirmed that women have reduced NET function compared to men with or without HF. Therefore, it is clear in this regard that females have attenuated SNS activity compared to males.

Moreover, animal studies have documented gender-related differences in HF induced by SNS activation (Gao et al. 2003, Dash et al. 2003, Dent et al. 2010, & Dent et al. 2012) (Table 1.2). In this regard, several studies have shown the effects of gender on HF associated with increased SNS activity in response to volume overload (Dent et al. 2010, & Dent et al. 2012). Recently, in our laboratory we have demonstrated that castration in male Sprague Dawley rats does not prevent systolic chamber dysfunction after chronic β-adrenergic activation (Hodson et al. 2014). In contrast, another study suggests that castration offers protective effects against increases in LV diameters in transgenic mice with cardiac β_2-AR overexpression (Gao et al. 2003). In a study using transgenic mice with fourfold phospholamban (PLB) expression, gender differences in cardiomyopathy or hypertrophy induced by SNS activation were noted (Dash et al. 2003). This study (Dash et al. 2003) demonstrated that transgenic male mice exhibited earlier signs of progressive HF than their counterpart transgenic female mice. Moreover, ovariectomy did not offer cardioprotective

effects to β-adrenergic responsiveness in the hearts of ovariectomised female mice compared to the hearts of sham female mice and male mice (McIntosh et al. 2011). Similar evidence was demonstrated in Sprague Dawley rats after volume overload induced by arteriovenous shunt that the cardiac function was maintained in the sham female rats and the female rats which underwent oestrogen-replacement after ovariectomy compared with the male rats and ovariectomized female rats (Dent et al. 2012). These data suggest that oestrogen is responsible for maintaining cardiac function.

To my knowledge, the above mentioned studies are the only existing evidence of gender-related differences in HF induced by SNS activation. A summary of these studies is therefore provided in table 1.2**.** Importantly, from the existing animal studies in table 1.2 it is evident that none of these studies investigated the influence of gender on SNS activation and the progression to HF in a hypertensive animal model (Spontaneously Hypertensive Rats, SHRs).

Therefore, the aim of my study was to investigate the influence of gender on SNS-induced alterations in cardiac remodeling and function in Spontaneously Hypertensive Rats as discussed below.

1.8 Aim

To investigate the role of gender in the transition from compensated cardiac hypertrophy to heart failure using a model of adrenergic-induced cardiac dilatation and pump dysfunction in hypertensive rats.

1.9 Objectives

The primary objective of the current study was therefore to determine whether hypertensive female rats are less susceptible than hypertensive male rats to cardiac dilatation and pump dysfunction produced by long-term β-adrenergic receptor (AR) activation.

The secondary objective of the current study was to determine whether apoptosis or necrosis are possible mechanisms of gender differences in cardiac dilatation and pump dysfunction produced by β-adrenergic receptor (AR) activation.

Chapter Two - Materials and Methods

2. Study designs

For purposes of addressing the objectives of this dissertation, two studies were subsequently conducted; namely the long term and short term beta adrenergic receptor stimulation studies (see sections 2.1 & 2.3, respectively). The long term beta adrenergic receptor stimulation focused on the primary objective. Subsequently, the short term beta adrenergic receptor stimulation was to assess a possible mechanism responsible for beta adrenergic receptor stimulation induced gender-related differences on cardiac remodeling and function in Spontaneously Hypertensive rats. Each study design is thoroughly described in the subsequent subheadings.

2.1 Experimental protocol for the long-term β-adrenergic receptor stimulation study

The animal model of hypertensive heart disease studied was the Spontaneously Hypertensive Rat (SHR). SHRs were obtained at the age of nine months from the Central Animal Services of the University of the Witwatersrand. Premature cardiac dilatation and pump dysfunction was induced in SHR by chronic β-adrenergic receptor activation (β-AR), using a method established in our laboratory and previously described (Tsotetsi et al. 2001, Badenhorst et al. 2003, & Veliotes et al. 2005). A total of 30 male rats and 27 female rats were randomly assigned to four groups (Figure 2.1). Rats from two groups, 1 female group and 1 male group, received daily subcutaneous injections (0.1ml) of isoproterenol (ISO), a β-AR agonist, at the dose of 0.04 $mg.kg^{-1}$ for 6 months, as previously described (Veliotes et al. 2005). Rats from the other two groups, 1 female group and 1 male group, received daily subcutaneous injections of the saline vehicle (0.1 ml of 0.9 % saline solution) for 6 months. For the duration of the study the rats were housed in the Central Animal Services of the University of the Witwatersrand (2 rats per cage, minimum). To reduce the number of potential deaths from arrhythmias, rats were habituated to gradually increasing doses of ISO (1^{st} week 0.01 $mg.kg^{-1}.day^{-1}$, 2^{nd} week 0.02 $mg.kg^{-1}.day^{-1}$, 3^{rd} week 0.03 $mg.kg^{-1}.day^{-1}$, 4^{th}week 0.04 $mg.kg^{-1}.day^{-1}$). Rats in the saline group received daily injections of (0.1ml) of the saline vehicle for 2 weeks. Nevertheless, six rats, 2 females and 4 males, died due to arrhythmias towards the end of the experiments; all received chronic β-adrenergic receptor activation.

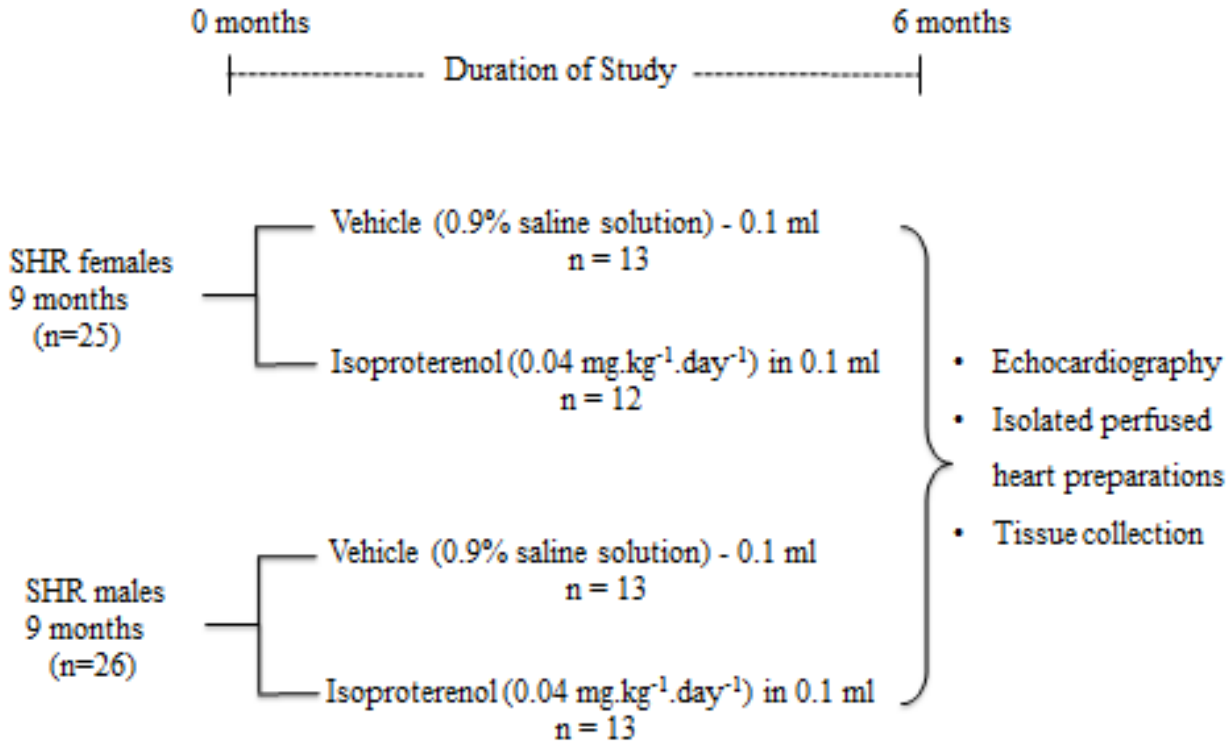


Figure 2.1: Summary of rat groups in the long-term β-adrenergic receptor stimulation study.

2.2 Cardiac structure and function:

2.2.1 Echocardiography:

Cardiac structure and function were assessed *in vivo* using two-dimensional directed M-mode echocardiography as previously described (Tsotetsi et al. 2001, & Veliotes et al. 2005). Left ventricular chamber dimensions and wall thickness were determined in systole and diastole by an experienced investigator blinded to the experimental groups, 24 hours after the last dose of isoproterenol or saline solution. Rats were anaesthetized with ketamine (75 $mg.kg^{-1}$) and xylazine (15 $mg.kg^{-1}$) 15 minutes before the procedure. The chest of the rats was shaved and an ultrasonic probe was placed on the chest of the animal to obtain M-mode images in systole and diastole. For the duration of three consecutive beats, the internal dimensions and wall thickness of the left ventricle were measured in systole and diastole (Figure 2.2). Relative wall thickness (RWT), Left ventricular endocardial (FS_{end}) and midwall (FS_{mid}) fractional shortening were then calculated (see equations below). FS_{end} and FS_{mid} were employed as measures of chamber and myocardial systolic function respectively.

- FS_{end} = (LV EDD-LV ESD)/LV EDD x 100

Where LV EDD = left ventricular end diastolic internal diameter and LV ESD = left ventricular end systolic internal diameter.

- FS_{mid} = [(LV EDD+LVED PWT)-(LV ESD+LVES PWT)]/(LV EDD +LVED PWT) x 100

Where LVED PWT = left ventricular end diastolic posterior wall thickness and LVES PWT = left ventricular end systolic posterior wall thickness.

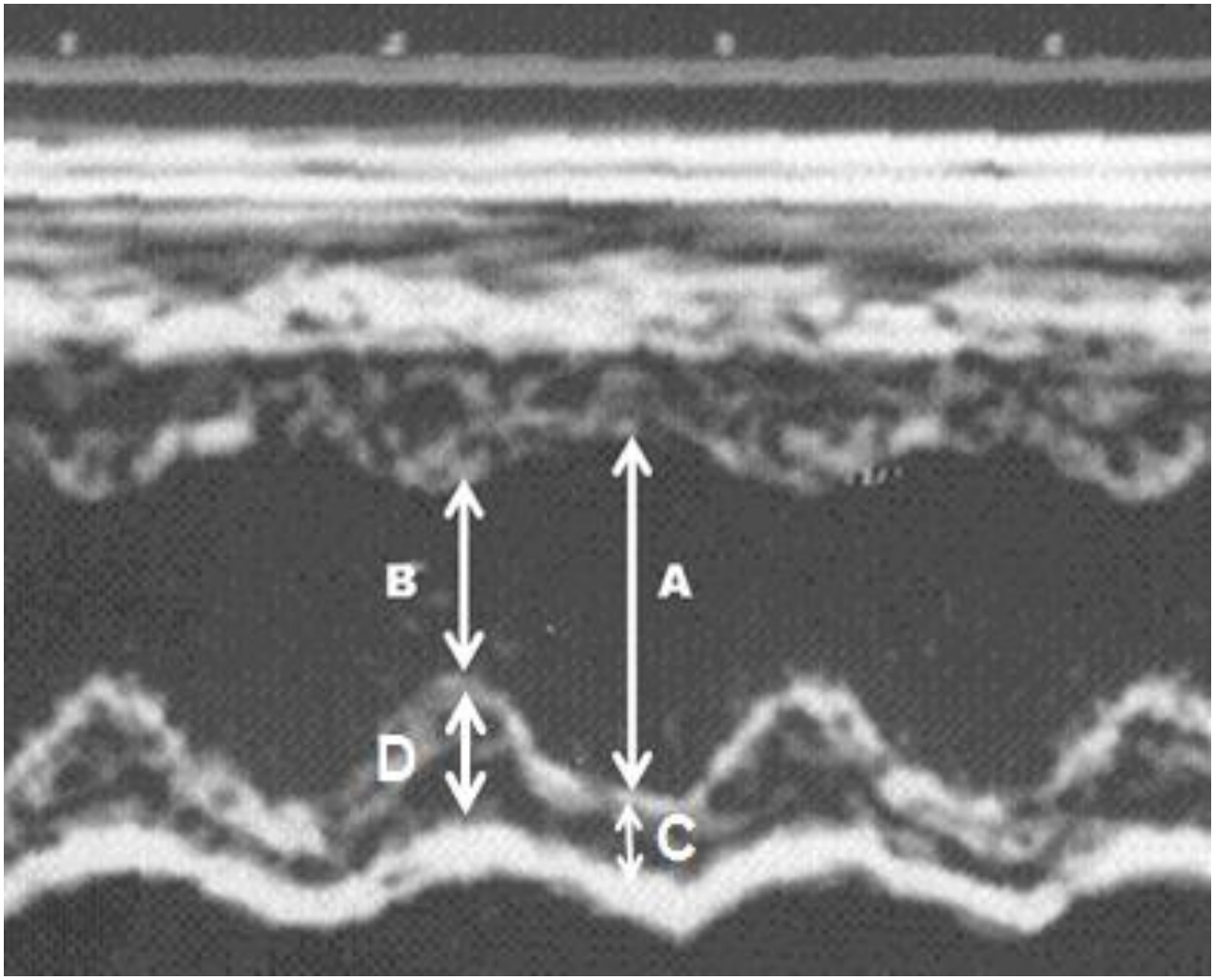


Figure 2.2: Representative example of an M-mode echocardiographic image of the left ventricle in a Spontaneously Hypertensive Rat. A, left ventricular end diastolic diameter (LV EDD); B, left ventricular end systolic diameter (LV ESD); C, left ventricular end diastolic posterior wall thickness (LVED PWT); D, left ventricular end systolic posterior wall thickness in systole (LVES PWT).

- Relative wall thickness = LVED PWT/(EDD/2)

Where LVED PWT = left ventricular end diastolic posterior wall thickness.

2.2.2 Blood pressure measurement:

After echocardiography, but still under anaesthesia an incision was made on the left side of the rat's neck. The left carotid artery was clamped and an incision in the left corotid artery was made for insertion of a catheter connected to a fluid filled Statham P23 pressure transducer. The clamp was removed and systolic and diastolic blood pressures were recorded under anaesthesia.

2.2.3 Isolated perfused heart preparations:

15-20 minutes after the administration of ketamine and xylazine (after echocardiography and blood pressure measurement), rats were euthanized by thoracotomy and the heart removed from the thoracic cavity. Excised hearts were placed in an ice-cold physiological saline solution, then weighed and mounted on an isolated perfused heart apparatus and load-independent measures of cardiac function and dimensions were then determined by an experienced investigator as previously described by our group (Tsotetsi et al. 2001, & Veliotes et al. 2005). Hearts were retrogradely perfused at a constant flow with a 37°C filtered physiological saline solution consisting of (in mM) 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$ and 10.0 glucose with a pH of 7.4. The solution was saturated with 95% O_2 and 5% CO_2 gas and filtered through a size 0.45µm Millipore Durapore membrane filter. Hearts were paced at 330-360 beats.min^{-1} with platinum electrodes attached to the left atrium and the apex of the heart. An empty latex balloon with a known wall volume (empty volume of 0.11 ml), coupled to a Statham P23 pressure transducer and a micromanipulator, was inserted through the mitral valve into the left

ventricular lumen (Figure 2.3 upper panel). The coronary flow rate was determined volumetrically and adjusted to achieve a flow of 12 $mL.min^{-1}.g$ of the wet heart weight. Left ventricular pressure, developed at incremental volumes, was recorded by a water-filled balloon-tipped cannula attached to a pressure transducer (Figure 2.3 lower panel). The volume of the balloon wall was measured with a water-displacement technique, and the same balloon was used throughout each study. A micromanipulator was used to gradually increase LV volumes to values that resulted in no further change in LV developed pressure (LV systolic minus diastolic pressure). LV pressures were determined at as many multiple small increments in volume as were practically possible to improve on the accuracy of curve fitting during analysis (Figure 2.4 left panel). From these data, left ventricular diastolic and systolic (developed) pressure-volume relations were derived to assess the extent of left ventricular dilatation and systolic chamber function respectively (Figure 2.4 right panel).

2.2.3.1 Intrinsic systolic myocardial function:

Intrinsic systolic myocardial function was determined from the slope of the systolic developed stress-strain relationship (myocardial systolic elastance-En) (Norton et al. 2002, Badenhorst et al. 2003b, & Veliotes et al. 2005). By calculating (from pressures and volumes) systolic stress and strain data, the impact of alterations in left ventricular chamber geometry on systolic function were eliminated (Weber et al. 1988). Left ventricular systolic stress and strain were calculated from previously described formulae (Weber et al. 1988a, & Norton et al. 2002) assuming a thick-walled spherical geometry of the left ventricle as follows:

$$\text{Left ventricular systolic stress} = 1.36 \times \frac{\text{LV developed pressure} \times (\text{LVV})^{2/3}}{[\text{LVV} + (0943 \times \text{LV mass})]^{2/3} - \text{LVV}^{2/3}}$$

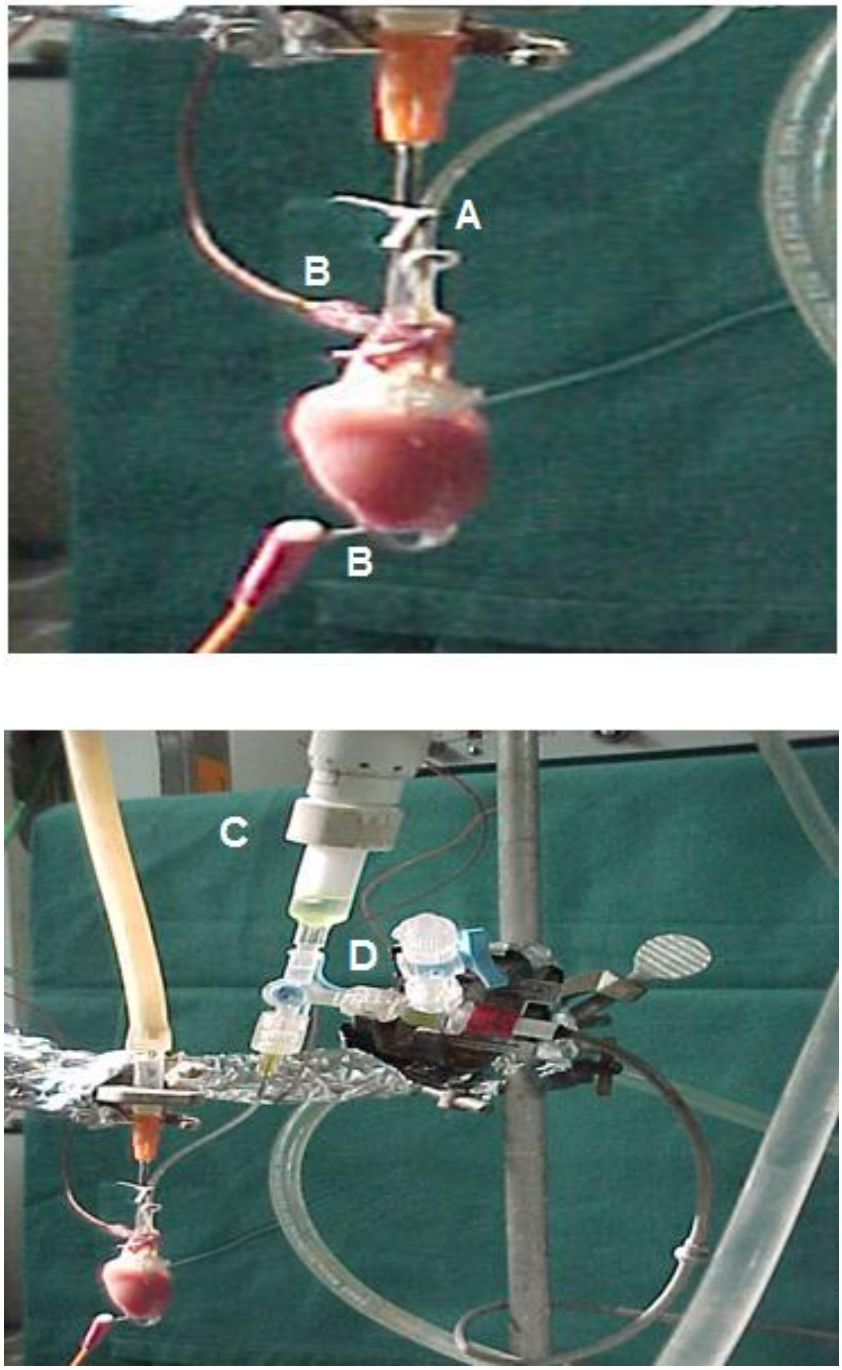


Figure 2.3: Representative example of the experimental setup for the isolated perfused heart apparatus. A, the fluid filled catheter connected to the balloon in the left ventricle lumen; B, platinum electrodes attached to the isolated heart; C, the micromanipulator; D, the pressure transducer

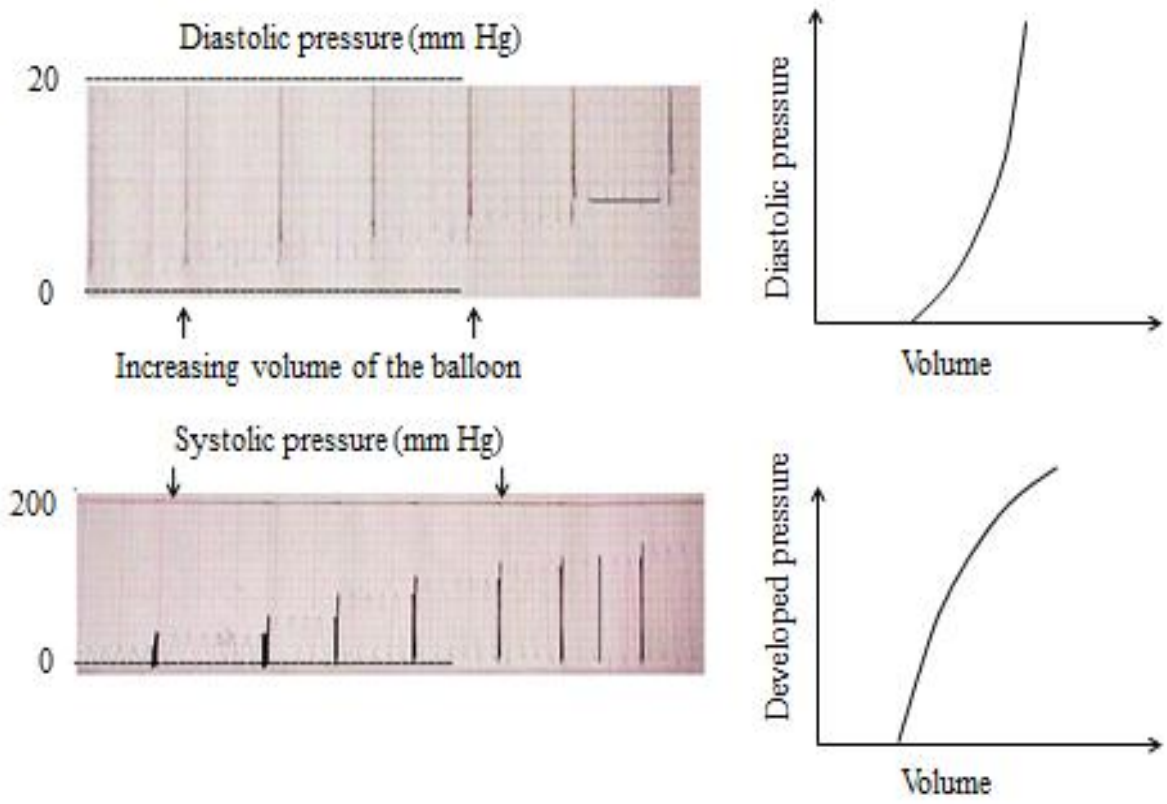


Figure 2.4: Diagrammatic example of derived diastolic and systolic (developed) pressure-volume curves (right panel). Representative example of typical recordings obtained for diastolic and systolic pressures in isolated, perfused heart preparations (left panel).

$$\text{Left ventricular systolic strain} = \frac{LVV^{1/3} + [LVV + (0.943 \times LV\ mass)]^{1/3}] - 1}{[LV\ V_0^{1/3} + [LV\ V_0 + (0.943 \times LV\ mass)]^{1/3}}$$

Where LVV is left ventricular volume and LV V_0 is the volume intercept of the LV developed pressure- volume relationship, i.e. LV volume when LV developed pressure = 0 mm Hg.

2.3 Experimental protocol for the short-term β-adrenergic receptor stimulation study:

As both cardiomyocyte apoptosis and necrosis occur with short-term β-adrenergic receptor stimulation (Goldspink et al. 2004), in the present study the impact of gender on cardiomyocyte apoptosis and necrosis induced by short-term β-adrenergic receptor stimulation was assessed. The SHRs were obtained at the age of nine months. A total of 14 male rats and 14 female rats (1 female rat died due to injection of ISO) were randomly assigned to four groups (figure 2.5). Habituation was done in one week by gradually increasing isoproterenol (ISO) doses to 0.04 $mg.kg^{-1}$ every 2 days. Following habituation, rats from two groups received daily injection of isoproterenol (ISO) for a week. Rats from the two other groups received daily injections of the saline vehicle for a week (figure 2.5).

2.4 Myocardial cellular changes responsible for HF:

Whole hearts, left ventricle and right ventricle were weighed. The free wall of the right ventricle was trimmed off the septum of the heart and weighed; subsequently, the left ventricle with the septum was then weighed. The body weight and tibial length were measured to account for variations in the growth of rats. All the tissue samples were stored at -70°C for histology and cell damage (TUNEL staining and pathological scores) assessments.

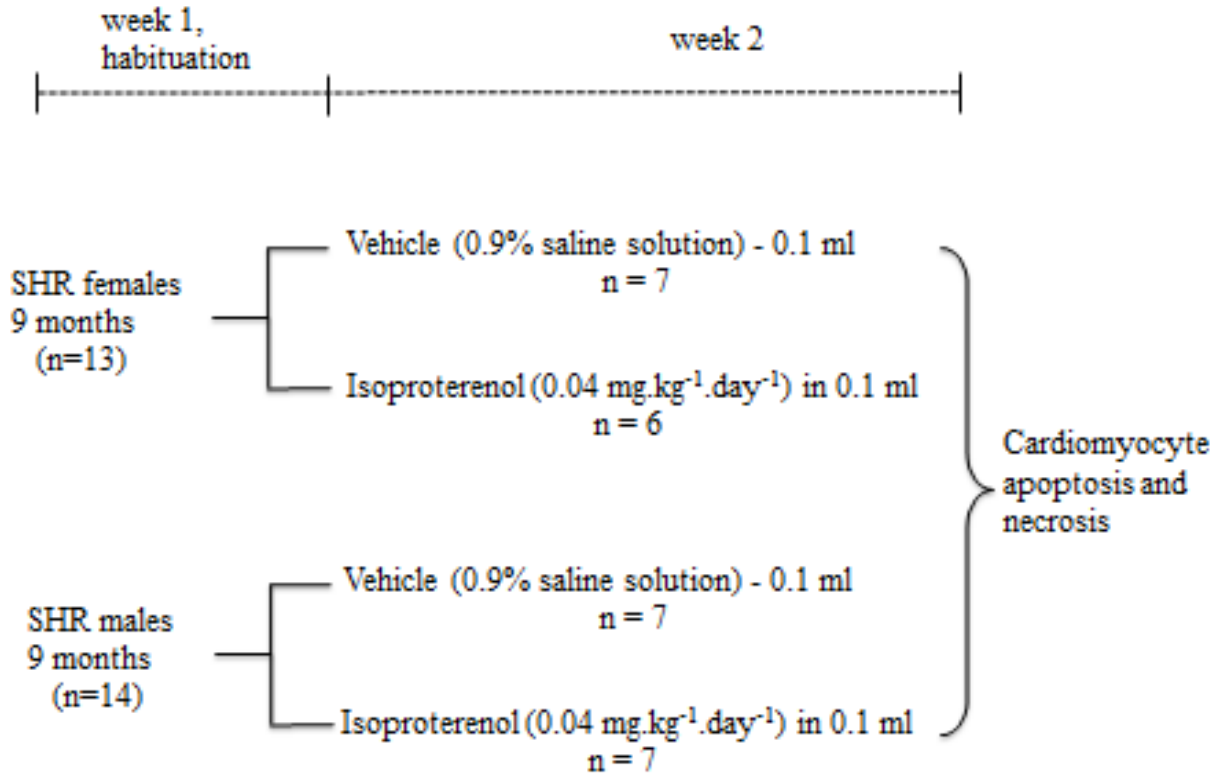


Figure 2.5: Summary of rat groups in the short-term β-adrenergic receptor stimulation study.

2.4.1 Cardiomyocyte necrosis:

As previously described by our group (Veliotes et al. 2005, Woodiwiss et al. 2001, & Badenhorst 2003), cardiomyocyte necrosis was assessed. A longitudinal slice of the left ventricle from the apex to the base through both the anterior and posterior left ventricular walls was obtained from all hearts and stored in formalin for subsequent histology. The left ventricular slice was processed for light microscopy and 5 μm-thick sections were cut through the wall thickness of the left ventricle. The sections were stained with Van Gieson which stains for fibrosis tissue a pink colour. The degree of myocyte necrosis was determined on each slice, where grade 0 indicates no histological evidence of fibrosis, grade 1 and 2 respectively indicate patchy fibrosis in less or more than 20% of the field, grade 3 and 4 respectively indicate diffuse contiguous subendocardial fibrosis in less or more than 50% of the field, and grade 5 and 6 respectively indicate full thickness (transmural) fibrosis in less or more than 50% of the field (figure 2.6).

2.4.2 Cardiomyocyte apoptosis:

As previously described by our group (Veliotes et al. 2005, & Badenhorst 2003), cardiomyocyte apoptosis was also assessed. The degree of apoptosis was quantified on myocardial tissue sections obtained from the same tissue blocks as used to assess the pathological score (Veliotes et al. 2005). Five μm thick sections were stained with a non-radioactive *in situ* apoptotic cell death detection kit (DeadEndTM Colorimetric TUNEL system, Promega, Madison, WI, USA). Nuclear deoxyribonucleic acid (DNA) fragments in the tissue sections were detected using terminal deoxynucleotidyltransferase (TdT) to incorporate biotinylated nucleotide at the 3'-OH DNA ends. Horseradish-peroxidase-labeled streptavidin binds to biotinylated nucleotides and subsequently stains dark brown in response to hydrogen peroxide and diaminobenzidine. Both positive (DNase treated) and negative (no

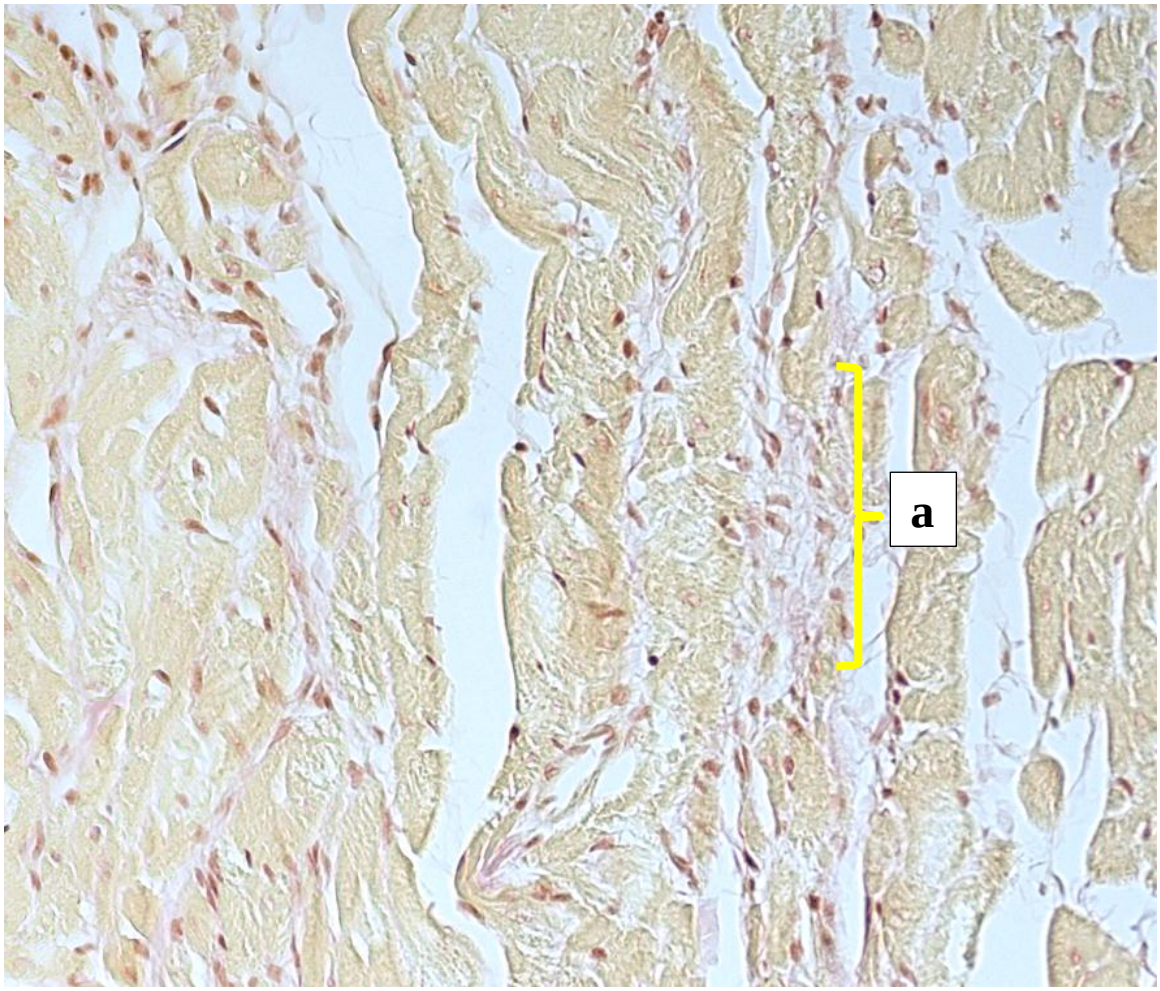


Figure 2.6: Representative myocardial tissue sections stained with Van Gieson's stain for necrosis. a, pink indicates necrotic section.

addition of TdT) control tissue sections were incorporated into each assay. The number of apoptotic cardiomyocyte nuclei and the total number of cardiomyocyte nuclei (haematoxylin and eosin stain) in each slide were counted on ten evenly spaced fields from the apex to the base, using a computer-based image acquisition and analysis system at 200 times magnification (Axiovision 3, Carl Zeiss, Gottingen, Germany) (refer to figure 2.7). Apoptotic total cardiomyocyte nuclei were expressed as a percentage of the total number cardiomyocyte nuclei, refer to formula below.

$$\text{Apoptosis (\%)} = \frac{\text{Apoptotic cardiomyocyte nuclei}}{\text{Total number of cardiomyocyte nuclei}} \text{ X } 100$$

2.5 Data analysis

A two-way analysis of variance (ANOVA) followed by Tukey-Kramer *post hoc* analysis was performed to determine differences in cardiac weights, left ventricular diameters and dimensions and systolic and diastolic function between the groups. A two-way ANOVA was also employed to determine differences in cardiomycyte apoptosis and pathological score. The level of significance was set at $P<0.05$. All statistical analysis was performed using GraphPad Prism 5 (Graph-pad Software Inc., San Diego, USA). All data are expressed as means±SEM.

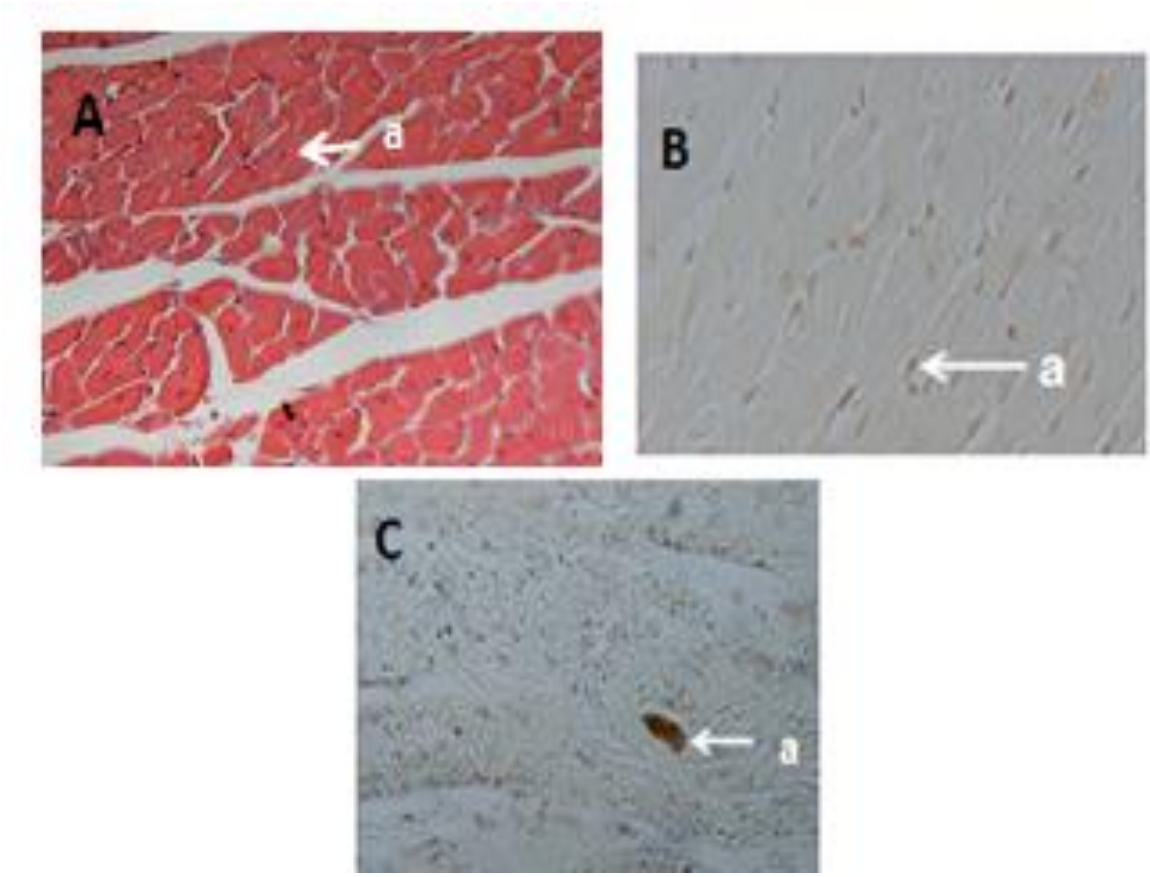


Figure 2.7: Representative examples of cardiac tissues sections stained with haemotoxylin and eosin (A) and TUNEL (B & C) after 1 week of injection with isoproterenol (200X magnification microscope). (A) H & E stained section, (a) indicates cardiomyocyte nuclei (200X magnification microscope); (B) TUNEL positive tissue section indicating numerous cardiomyocyte apoptotic nuclei (200X magnification microscope); (C) TUNEL tissue section indicating only one nucleus, (a) indicates apoptotic cardiomyocyte nucleus (400x magnification microscope).

Chapter Three - Results

3.1 Effects of long-term β-adrenergic receptor stimulation and gender on body weights, heart weights and blood pressures.

Table 3.1 shows the effects of gender and long-term β-adrenergic receptor stimulation on body and heart weights. At 15 months, male rats were heavier than female rats ($p<0.0001$) and tibial lengths were longer in the male rats compared to the female rats ($p<0.0001$). Heart, left ventricle and right ventricle weights were also greater in the male rats compared to the female rats ($p<0.0001$, $p<0.0001$ and $p=0.0002$, respectively). As a consequence of the relatively larger body weights in the male rats, the ratios of heart, left ventricle and right ventricle weights to body weight were lower than in the female rats. Isoproterenol administration had no effects on body weight in either male or female rats. However, isoproterenol administration resulted in increased heart, left ventricular and right ventricular weights in male rats but not in female rats. The systolic blood pressure values were similar in all the groups (mean±SEM, mm Hg; Male Sal: 126.0±10.6; Male Iso: 134.0±8.8; Female Sal: 136.6±6.6; Female Iso: 119.3±4.7; $p>0.05$). The diastolic blood pressure values were also similar in all the groups (mean±SEM, mm Hg; Male Sal: 87.1±6.4; Male Iso: 88.8±5.9; Female Sal: 89.0±5.4; Female Iso: 79.7±4.1; $p>0.05$).

3.2 Effects of long-term β-adrenergic receptor stimulation and gender on left ventricular dimensions *in vivo*

Table 3.2 and figure 3.1 show the effects of long-term β-adrenergic receptor stimulation and gender on left ventricle dimensions, *in vivo*. Left ventricular end diastolic diameter (figure 3.1), left ventricular end systolic diameter and posterior wall thickness in systole (table 3.2) were greater in the male rats compared to the female rats ($p<0.0001$, $p=0.0004$ and

Table 3.1: Effects of long-term β-adrenergic receptor stimulation on body, heart, left ventricular, right ventricular weights in male and female SHRs.

	Male		Female	
	Sal n=13	**Iso** n=13	**Sal** n=13	**Iso** n=12
Body weight (g)	379 ± 5.55*	384 ± 4.71*	221 ± 3.05	221 ± 4.33
Tibia length (mm)	42.0 ± 0.47*	42.1 ± 0.64*	36.5 ± 0.36	37.4 ± 0.46
Heart weight (g)	1.46 ± 0.04*	1.64 ± 0.04*†	1.15 ± 0.07	1.06 ± 0.03
Left ventricular weight (g)	1.16 ± 0.02*	1.26 ± 0.03*†	0.89 ± 0.04	0.81 ± 0.02
Right ventricular weight (g)	0.27 ± 0.01*	0.30 ± 0.02*†	0.22 ± 0.02	0.21 ± 0.01
HW/BWx100	0.39 ± 0.04*	0.43 ± 0.04*	0.52 ± 0.13	0.48 ± 0.03
LVW/BWx100	0.30 ± 0.02 *	0.34 ± 0.02 *	0.37 ± 0.02	0.41 ± 0.09
RVW/BWx100	0.07 ± 0.01*	0.08 ± 0.02*	0.10 ± 0.03	0.09 ± 0.02

BW, body weight; TL, tibial length; HW, heart weight; LVW, left ventricular weight; RVW, right ventricular weight; Sal, saline; Iso, isoproterenol. Data are expressed as means ± SEM. * $p<0.05$ vs female rats. † $p<0.05$ vs other groups (two-way ANOVA).

Table 3.2: Effects of long-term β-adrenergic receptor stimulation on left ventricular dimensions as assessed *in vivo* in male and female SHRs.

	Male		Female	
	Sal n=13	**Iso** n=13	**Sal** n=13	**Iso** n=12
LV ESD (mm)	3.26 ± 0.17*	4.33 ± 0.24*†	3.01 ± 0.23	3.01 ± 0.14
PWED (mm)	2.31 ± 0.11	2.13 ± 0.06	2.10 ± 0.06	2.12 ± 0.05
PWES (mm)	3.08 ± 0.08*	2.93 ± 0.06*†	2.68 ± 0.06	2.78 ± 0.06
Relative wall thickness	0.72 ± 0.05	0.56 ± 0.01†	0.70 ± 0.02	0.68 ± 0.02

LV, left ventricle; ESD, end systolic diameter; PWED, posterior wall thickness at end diastole; PWES: posterior wall thickness at end systole; Sal, saline; Iso, isoproterenol. Data are expressed as means ± SEM.* $p<0.05$ vs female rats. †$p<0.05$ vs other groups (two-way ANOVA).

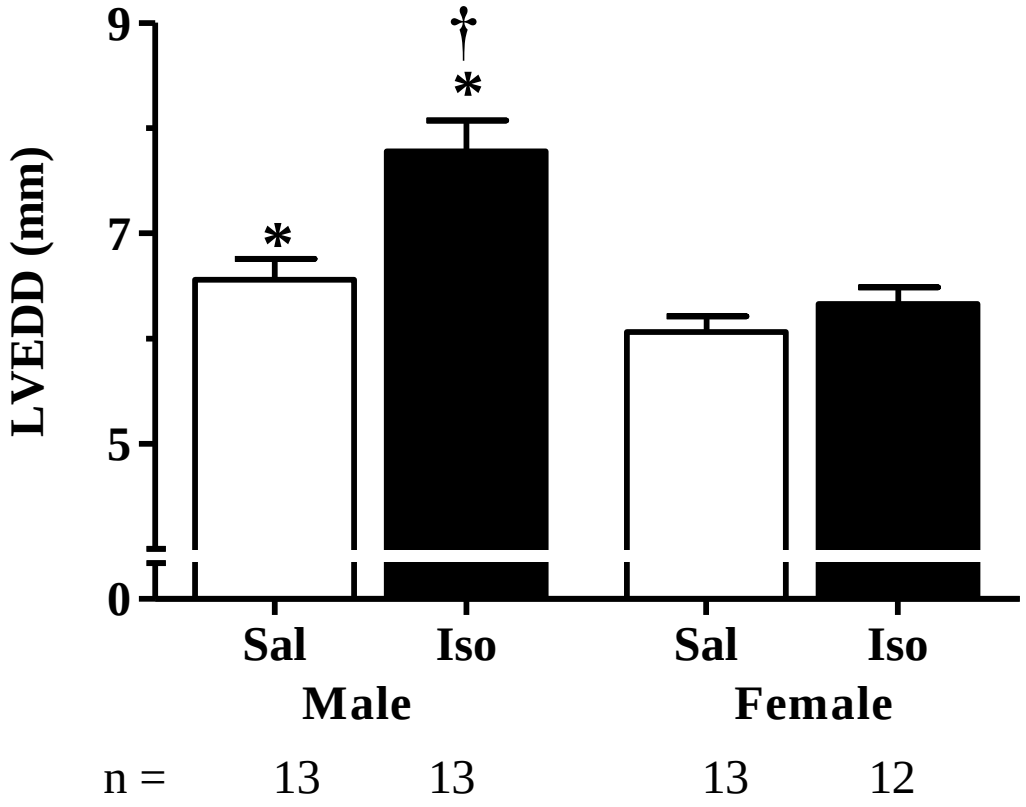


Figure 3.1: Effects of long-term β-adrenergic receptor stimulation on left ventricular end diastolic diameter (LVEDD) in male and female SHRs. Data are expressed as means±SEM. LVEDD, left ventricular end diastolic diameter; Sal, saline; Iso, isoproterenol.* $p<0.05$ vs female rats. † $p<0.05$ vs other groups (two-way ANOVA).

p=0.0002, respectively). Six months of isoproterenol administration increased left ventricular end diastolic (p=0.0242), and end systolic (p=0.0123) diameters in male rats, but not in female rats. In addition, isoproterenol resulted in a decrease in posterior wall thickness at end systole and in relative wall thickness (p=0.0364) (table 3.2) in the male rats but not in the female rats.

3.3 Effects of long-term β-adrenergic receptor stimulation and gender on left ventricular chamber dimensions *ex vivo*

Figure 3.2 shows left ventricular diastolic pressure-volume relations assessed using an isolated perfused heart system (upper panel) and the volume intercept at 0 mm Hg (lower panel) calculated from the left ventricular diastolic pressure-volume relations (left ventricular chamber volume). The left ventricular chamber volumes were greater in the male compared to the female rats (p<0.0001). Six months of isoproterenol administration resulted in a right shift in the left ventricular end diastolic pressure-volume relations in the male rats but not in the female rats. Consequently, the left ventricular chamber volume was increased in the male rats receiving isoproterenol compared to the other groups (p=0.004).

3.4 Effects of long-term β-adrenergic receptor stimulation and gender on left ventricular systolic chamber function

Figure 3.3 shows left ventricular systolic chamber function as indexed by endocardial fractional shortening (FS_{end}) determined using *in vivo* measurements (echocardiography). At

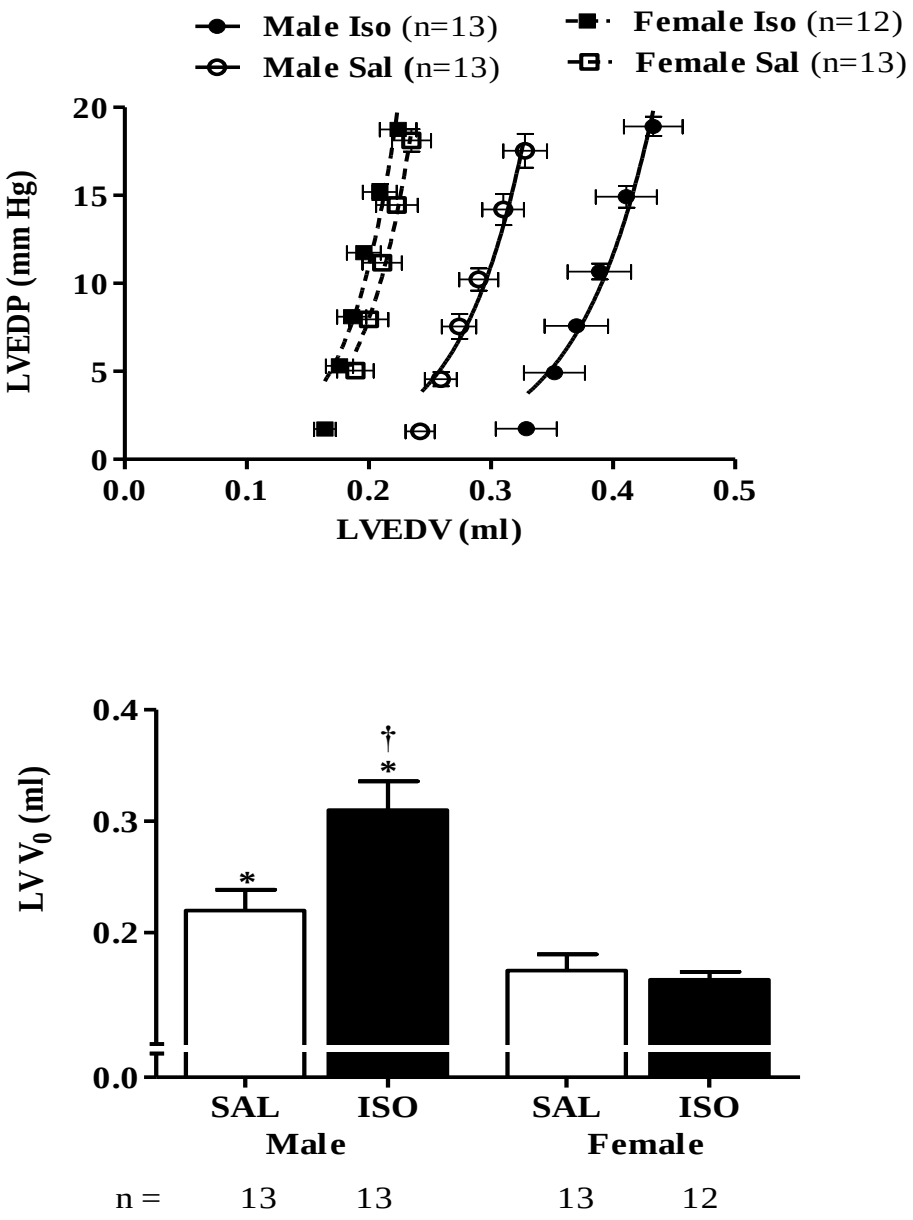


Figure 3.2: Effects of long-term β-adrenergic receptor stimulation on left ventricular chamber volumes as indexed by diastolic pressure-volume relations (upper panel) and the volume intercept at 0 mm Hg [V_0] (lower panel) in male and female SHRs. Iso, isoproterenol; Sal, saline. Data are expressed as means ± SEM. LV V_0, left ventricular volume intercept at 0 mm Hg; * $p<0.05$ vs female rats.† $p<0.05$ vs other groups (two-way ANOVA).

15 months, no difference (p=0.0819) in FS_{end} was observed between the male and female rats. Also, isoproterenol administration had no effect on FS_{end} in either male or female rats (p=0.4891). Figure 3.4 shows left ventricular systolic chamber function as indexed by load-independent systolic pressure-volume relations (upper panel) and the slope of these relations [left ventricular end systolic elastance, LV E_{es}] (lower panel) determined using *ex vivo* isolated perfused heart preparations. Gender had no effect on LV E_{es}. Isoproterenol decreased LV E_{es} in the male rats (p=0.0474) but not in the female rats (p>0.05).

3.5 Effects of long-term β-adrenergic receptor stimulation and gender on left ventricular intrinsic myocardial systolic function

Figure 3.5 shows left ventricular intrinsic midwall fractional shortening (FS_{mid}) determined *in vivo* using echocardiography. At 15 months, FS_{mid} was greater in the female compared to the male rats (p=0.03777). No difference in FS_{mid} was observed between the different groups of male and female rats that received isoproterenol or saline (p=0.8532). Figure 3.6 shows left ventricular intrinsic myocardial systolic function as indexed by left ventricular systolic stress-strain relations (upper panel) *ex vivo*. The lower panel shows the slope (left ventricular end systolic myocardial elastance - LV E_n) of the left ventricular systolic stress-strain relations. LV E_n was decreased in male compared to female rats (p=0.0190), but no ISO effect noted.

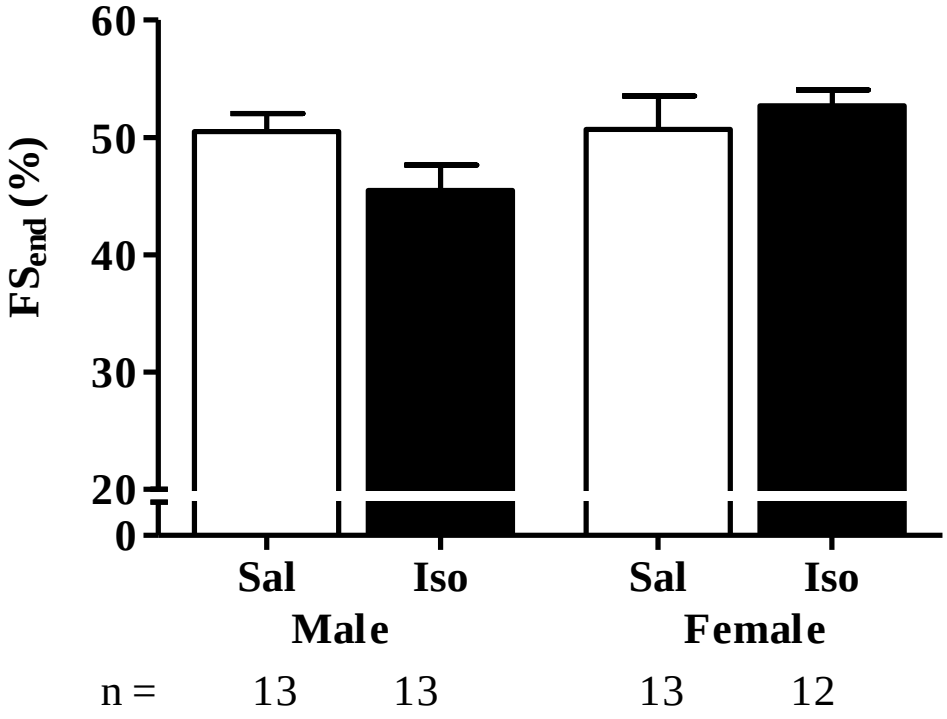


Figure 3.3: Effects of long-term β-adrenergic receptor stimulation on left ventricular systolic chamber function as indexed by endocardial fractional shortening (FSend) in male and female SHRs. Iso, isoproterenol; Sal, saline. Data are expressed as means ± SEM.

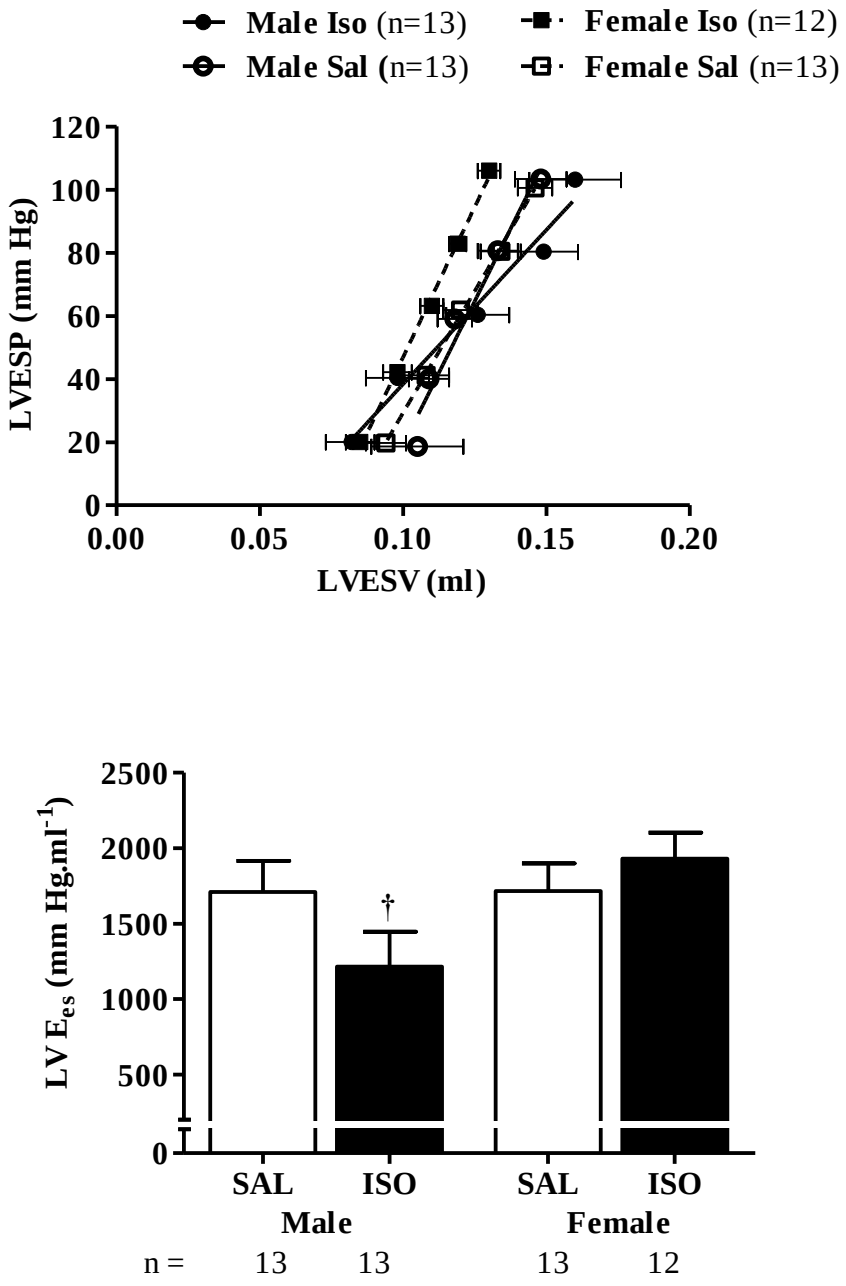


Figure 3.4: Effects of long-term β-adrenergic receptor stimulation on left ventricular systolic chamber function as indexed by pressure-volume relations. Iso, isoproterenol; Sal, saline. Data are expressed as means ± SEM. †$p<0.05$ vs other groups (two-way ANOVA).

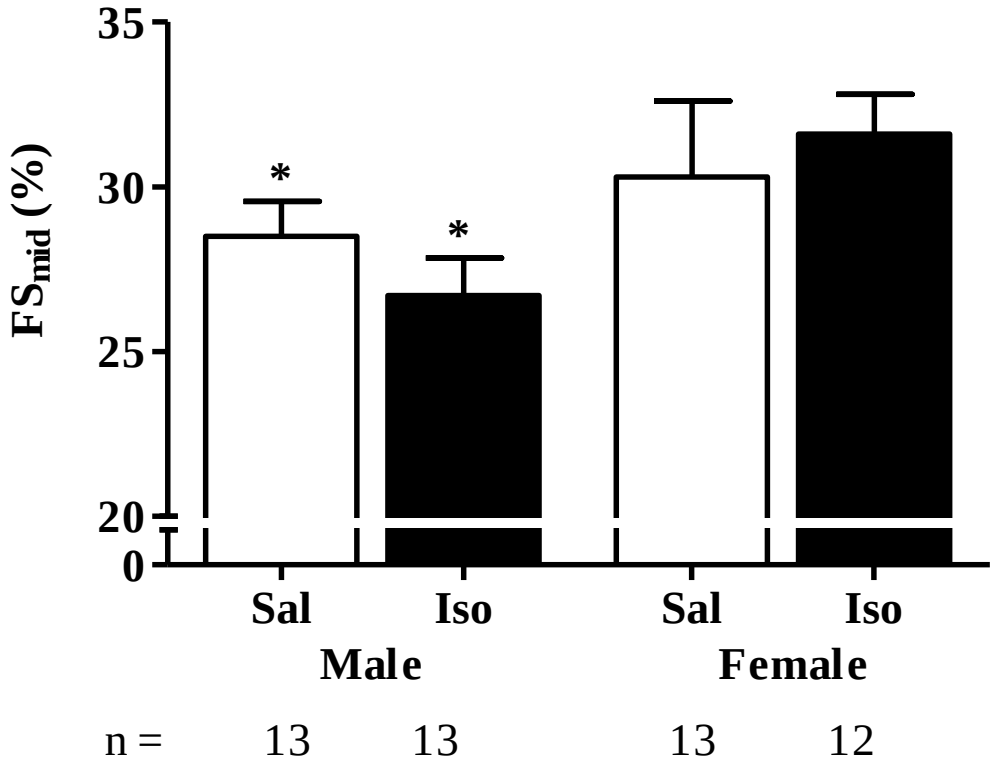


Figure 3.5: Effects of long-term β-adrenergic receptor stimulation on left ventricular intrinsic myocardial systolic function as indexed by midwall fractional shortening (FS_{mid}) in male and female Spontaneously Hypertensive Rats. Iso, isoproterenol; Sal, saline. Data are expressed as means ± SEM. *$p<0.05$ vs female rats.

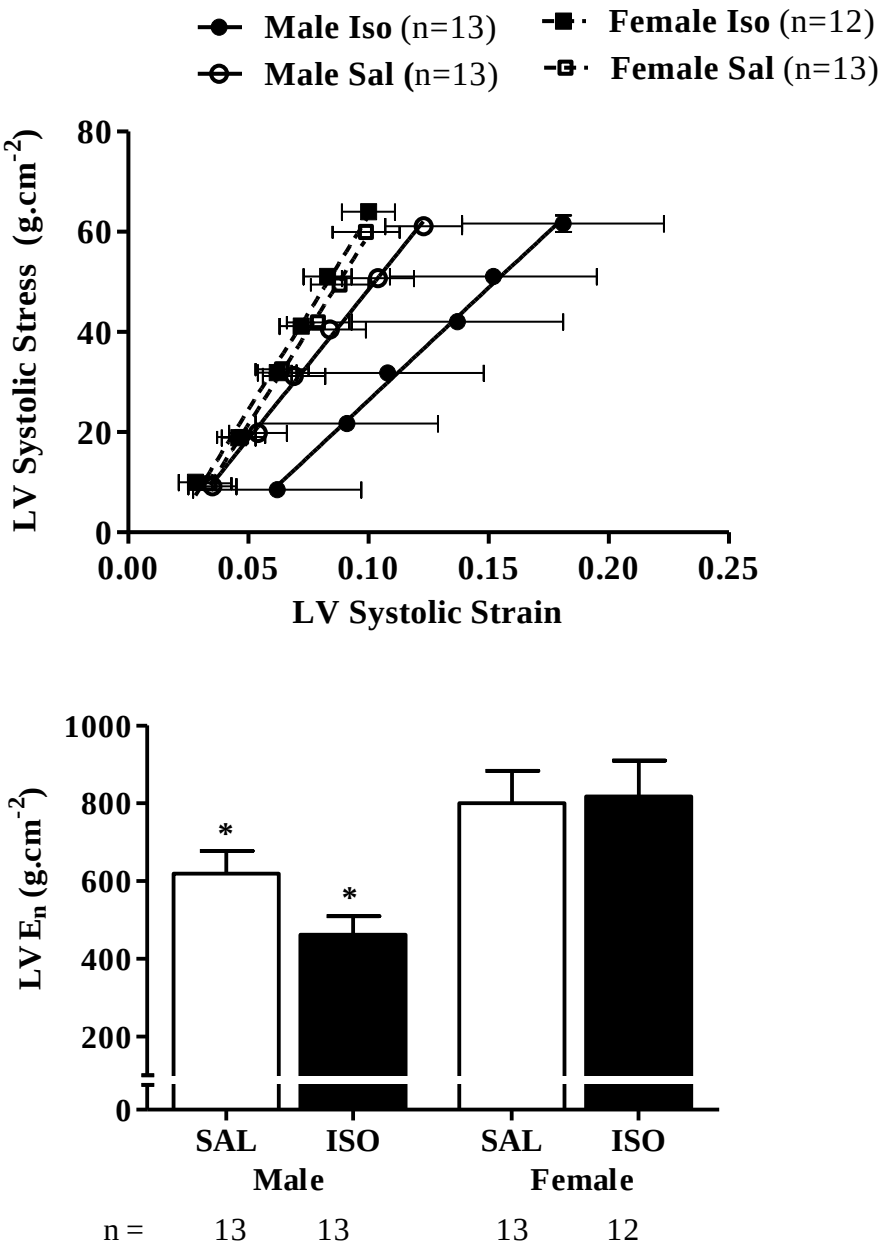


Figure 3.6 : Effects of long-term β-adrenergic receptor stimulation and gender on left ventricular intrinsic myocardial systolic function as indexed by LV systolic stress-strain relations (upper panel). The lower panel shows the slope (LV end systolic myocardial elastance-LV En) of the left ventricular systolic stress-strain relations in male and female Spontaneously Hypertensive Rats. Iso, isoproterenol; Sal, saline. Data are expressed as means ± SEM. * $p<0.05$ vs female rats.

3.6 Effects of short-term β-adrenergic receptor stimulation and gender on body, heart, left and right ventricular weights

Table 3.3 shows the effects of short-term β-adrenergic receptor stimulation and gender on body, heart, left and right ventricular weights. At 15 months, the body and heart weights were heavier in the male rats compared to the female rats ($p<0.0001$). Heart weights were heavier in both the male and female rats that received isoproterenol for a week ($p<0.05$). The ratios of heart, left ventricle and right ventricle weights to body weight were lower in the male than female rats.

3.7 Effects of short-term β-adrenergic receptor stimulation and gender on necrosis and apoptosis

Figure 3.7 shows the effects of short-term β-adrenergic receptor stimulation on pathological score (necrosis) shown in the upper panel and apoptotic cardiomyocytes (lower panel). After one week of daily isoproterenol (ISO) administration, there was no difference in pathological score and apoptosis of cardiomyocytes between the different experimental groups. However, there was a noticeable trend for ISO effect on apoptosis ($p=0.08$).

Table 3.3: Effects of short-term β-adrenergic receptor stimulation on body, heart, left and right ventricular weights in male and female SHRs.

	Male		**Female**	
	Sal	**Iso**	**Sal**	**Iso**
Body weight(g)	370.0 ± 2.27*****	352.9 ± 3.63*****	223.7 ± 1.59	215 ± 1.88
Heart weight (g)	1.38 ± 0.02*****	1.52 ± 0.02***†**	0.96 ± 0.02	1.02 ± 0.01†
Left ventricular weight (g)	1.02 ± 0.01*****	1.12 ± 0.02*****	0.72 ± 0.02	0.75 ± 0.004
Right ventricular weight (g)	0.29 ± 0.01*****	0.29 ± 0.01*****	0.19 ± 0.004	0.18 ± 0.03
HW/BW*100	0.37 ± 0.01*	0.43 ± 0.01*	0.46 ± 0.02	0.48 ± 0.01
LVW/BW*100	0.28 ± 0.01*	0.32 ± 0.01*	0.34 ± 0.01	0.35 ± 0.01
RVW/BW*100	0.08 ± 0.01*	0.08 ± 0.01*	0.082 ± 0.005	0.083 ± 0.004

BW, body weight; HW, heart weight; LVW, left ventricular weight; Sal, saline; Iso, isoproterenol. Data expressed as means ± SEM.*$p<0.05$ vs female rats. † $p<0.05$ vs other groups (two-way ANOVA).

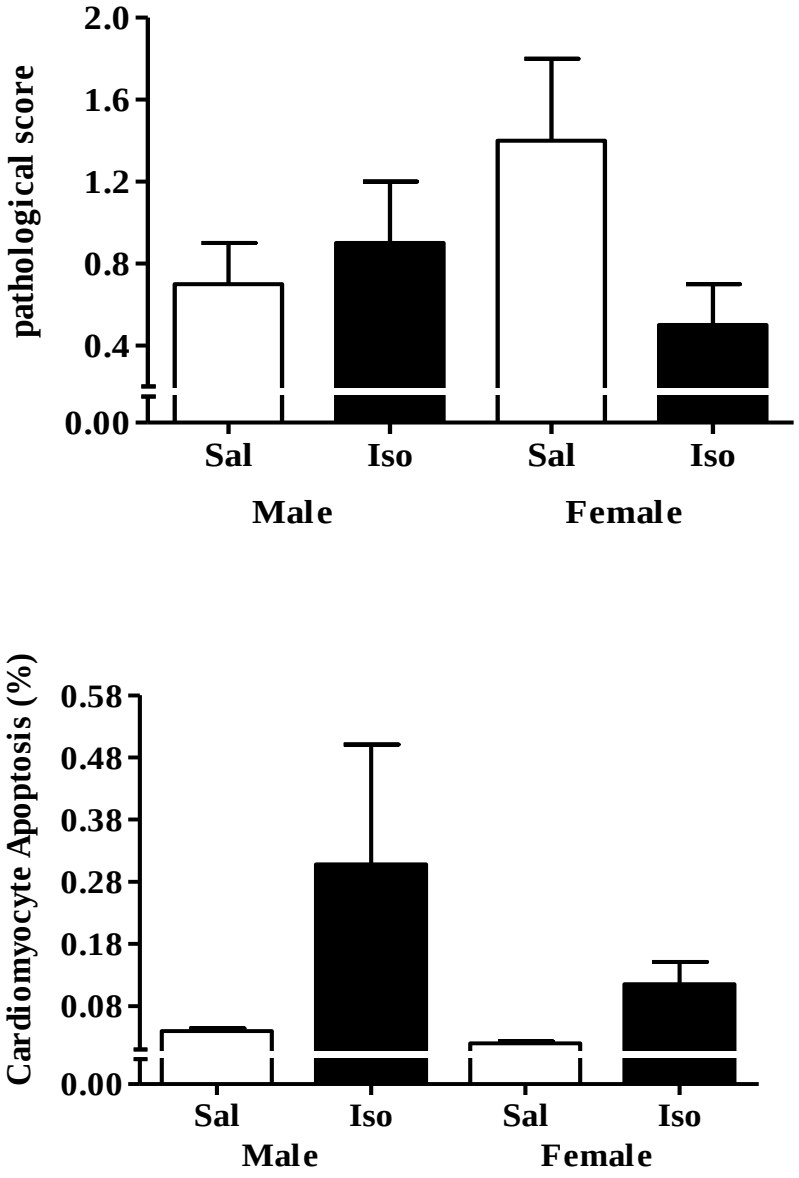


Figure 3.7: Effects of short-term β-adrenergic receptor stimulation on pathological score (necrosis) shown in the upper panel and apoptotic cardiomyocyte (lower panel) in male and female Spontaneously Hypertensive Rats. Iso, isoproterenol; Sal, saline. Male Sal (n=7); Male Iso (n=7); Female Sal (n=7); Female Iso (n=6). Data expressed as means ± SEM.

Chapter Four - Discussion

4.1 Main findings of the present study

The main findings of the present study are as follows: Chronic (six months) β-adrenergic receptor (β-AR) stimulation produced left ventricular hypertrophy (LVH) associated with cardiac dilatation in the male Spontaneously Hypertensive Rats (SHRs), but not in the female SHRs as determined *in vivo* by an increase in the left ventricular dimension, a decrease in the relative wall thickness, and *ex vivo* by a right shift in the volume intercept at 0 mm Hg pressure (V_0) of the left ventricular diastolic pressure-volume relationship. Moreover, chronic β-AR stimulation produced pump dysfunction in the male SHRs, but not in the female SHRs as determined *ex vivo* by a decrease in the left ventricular systolic chamber function (end systolic elastance, LV E_{es}). However, chronic β-AR stimulation did not impact on the myocardial systolic function when assessed both *in vivo* (systolic midwall fractional shortening, FS_{mid}) and *ex vivo* (systolic myocardial elastance, LV E_n) in both male and female SHRs.

4.2 Uniqueness of current study

This is the first study to assess *in vitro* the effects of gender on β-adrenergic induced cardiac remodeling and systolic pump dysfunction in SHRs. Previously, in our laboratory cardiac remodeling and function in SHRs exposed to the β-adrenergic stimulation has been extensively studied (Badenhorst et al. 2003b, Gibbs et al. 2004, & Veliotes et al. 2005). In these studies the SHRs were compared to Wistar Kyoto (WKY) rats, the original genetic normotensive strain. These studies have shown that the SHRs exposed to β-adrenergic stimulation developed progressive LVH associated with adverse ventricular remodeling (cardiac remodeling and pump dysfunction) earlier than SHRs not exposed to β-AR stimulation (Badenhorst et al. 2003b, & Gibbs et al. 2004). Therefore, these findings suggested that β-AR stimulation has a synergetic effect with pressure overload (hypertension)

to produce progressive LVH and adverse ventricular remodeling. There are limited studies that have assessed the effect of gender on cardiac remodeling and function in ageing SHRs (Pfeffer et al. 1982, Tamura et al. 1999, & Chan et al. 2011). Moreover, to my knowledge, there are no studies that have assessed the effect of gender on cardiac remodeling and function induced by chronic β-AR stimulation in a pressure overload animal model (SHRs).

4.3 Comparisons with previous studies

4.3.1 Gender influence on the progression from concentric left ventricular hypertrophy to dilatation

The present study has assessed the impact of gender on the LVH associated with cardiac dilatation in hypertensive rats after chronic β-adrenergic activation. The results from the current study show that the heart, left ventricle and right ventricle weights are heavier in the male SHRs compared with the female SHRs after the administration of either isoproterenol or saline vehicle. However, after the heart, left and right ventricular weights had been corrected for 100g body weight to eliminate gender differences, the female rats showed increases in the heart, left ventricular and right ventricular weights compared to male rats that received either isoproterenol or saline vehicle. Therefore the gender differences in heart and ventricular weights are due to differences in body weight.

In response to chronic β-adrenergic activation, the male rats but not the female rats developed heavier hearts than the saline-administered male rats. Hence, only the male rats developed hypertrophy in response to β-adrenergic activation. Moreover, the reduced relative wall thickness and the increased left ventricular dimensions in the isoproterenol-administered male rats compared to the female rats indicate that the male, but not the female rats, developed cardiac dilatation. In support of the current study, a previous study demonstrated

that male mice after two weeks of pressure overload induced by aortic banding developed concentric hypertrophy as indicated by a higher heart weight to body weight ratio than the female mice (Skavdahl et al. 2005). Moreover, following 20 weeks of aortic banding in Wistar rats it was demonstrated that the male rats developed cardiac dilatation whereas the female rats maintained concentric hypertrophy (Douglas et al. 1998). These data support the present study which has shown that six-months of isoproterenol administration produced cardiac dilatation in the male SHRs, but not in the female SHRs.

The influence of gender on left ventricular remodeling in healthy human conditions has previously been shown (Dannenberg et al. 1989, Lauer et al. 1991, & Levy et al. 1996). Importantly, the influence of gender on the characteristics of LVH and its progression to heart failure under pathological loading conditions has been reported in human and animal studies (Pfeffer et al. 1982, Topol et al. 1985, Carrol et al. 1992, Villari et al. 1992, Douglas et al. 1995, Douglas et al.1998, Tamura et al. 1999, Weinberg et al. 1999, Gardner et al. 2002, Brower et al. 2003, Monasky et al. 2008, Petrov et al. 2010, & Chan et al. 2011). The findings of the current study are in agreement with other human and animal studies. The human studies have shown gender-related differences in the left ventricles of women patients exposed to chronic pressure overload by aortic stenosis and indeed, have shown that women have smaller, more concentric hypertrophied left ventricles with better systolic function performance, whereas men have larger, more eccentric hypertrophied ventricles with depressed systolic function (Topol et al. 1985, Carrol et al. 1992, Villari et al. 1992, & Douglas et al. 1995). It is suggested that the existence of concentric hypertrophy in the female subjects during hemodynamic overload normalizes left ventricular systolic function (Devereux et al. 1987, Carrol et al. 1992, Villari et al. 1992, Liebson et al. 1993, & Aurigemma et al. 1994). Although these studies are in agreement with the current study in that they report a preserved systolic function with concentric hypertrophy in the female hearts

compared to the male hearts, their data were only assessed *in vivo*. Hence, the data from previous studies may be influenced by heart rate and loading conditions. Moreover, Carrol et al. (1992) demonstrated that elderly women (>60 years old) under chronic pressure overload induced by aortic stenosis had higher relative wall thickness and better preservation of systolic left ventricular function compared to men. However, this study failed to consider the hormonal influences with ageing on cardiac hypertrophy as aging is associated with menopause in females (Pfeffer et al. 1979, & Scheuer et al. 1987). It should therefore be acknowledged that ageing and gender play key roles in the differences of left ventricular adaptation to systolic pressure overload. Nevertheless, a study by Villari et al. (1995) reported similar findings in young female patients exposed to chronic aortic stenosis. Indeed as in the elderly women, young women had concentric hypertrophy and better ventricular systolic function; compared to young male patients who presented with characteristics of eccentric hypertrophy with reduced myocardial contractility and a depressed systolic left ventricular function. Hence, irrespective of whether pre- or post-menopausal women were assessed, female gender appears to protect against pressure overload induced cardiac remodelling and systolic dysfunction. Nevertheless, these studies were all conducted *in vivo* and hence the results may be influenced by differences in heart rate and loading conditions.

With respect to animal studies, studies in rodents have shown the effects of gender on the left ventricular adaptation to chronic pathological loading; including pressure (Pfeffer et al. 1982, Douglas et al. 1998, Tamura et al. 1999, & Jain et al. 2002) or volume (Gardner et al. 2002, & Dent et al. 2010) overload. Firstly, *in vivo* cardiac dimension assessments demonstrated that female SHRs (aged 6–18 months) had normal ventricular dimensions compared to the male SHRs which had ventricular dilatation at the age of 12 months (Pfeffer et al. 1982, & Tamura et al. 1999). In addition, Chan et al. (2011) demonstrated that male SHRs exhibited cardiac dilatation at the age of 15 months; whereas female SHRs showed

signs of the ventricular dilatation only at the age of 18 months. In this regard, it is clear that the male SHRs have an early onset for ventricular dilatation compared to the female SHRs. Secondly, in Wistar rats and Dahl Salt-Sensitive (DSS) rats at 20 weeks after pressure overload induced by aortic banding or genetic hypertension respectively, the female rats were protected from ventricular dilatation and had maintained concentric hypertrophy compared to the male rats that showed ventricular eccentric hypertrophy (Douglas et al. 1998, & Jain et al. 2002). Lastly, in Sprague-Dawley rats after volume overload induced by arteriovenous (AV) shunt, the male rats demonstrated LV dilatation and increased compliance compared to the female rats; whereas the female rats presented with left ventricular concentric hypertrophy (Gardner et al. 2002, Brower et al. 2003, Dent et al. 2010, & Dent et al. 2012). However, a study in isolated hearts from Wistar rats after 6 weeks of aortic banding (pressure overload) demonstrated that both the male and female hearts had a similar degree of hypertrophy (Weinberg et al. 1999). Clearly, it can be suggested that the transition to ventricular dilatation from ventricular hypertrophy depends largely on the duration of the pathologic loading. Furthermore, it should be noted that males exhibited an early onset for the transition from concentric LVH to dilatation.

4.3.2 Gender influence on the progression to systolic dysfunction in response to pressure overload

The present study has demonstrated that six months of β-adrenergic receptor stimulation to SHRs produced systolic dysfunction in the male rats, but not in the female rats as determined by a decrease in the left ventricular systolic chamber function *ex vivo* (end systolic elastance, LV E_{es}). Although, systolic chamber function *in vivo* (endocardial fractional shortening, FS_{end}) failed to show differences in all the groups, however the decreased posterior wall thickening in systole in the male ISO group would suggest a

decreased systolic chamber function. Importantly, the *in vivo* systolic function assessment is load and heart rate dependent. In contrast to the current study, other studies have shown a change in *in vivo* systolic function with dilatation in either volume or pressure overload (Pfeffer et al. 1982, Douglas et al. 1998, Tamura et al. 1999, & Brower et al. 2003, Skavdahl et al. 2005, Dent et al. 2010, & Dent et al. 2012). Whereas, the *ex vivo* systolic cardiac function assessment (LV E_{es}) is independent of load and heart rate. Therefore, compared to *in vivo* assessments, *ex vivo* assessments of systolic cardiac function are a more reliable technique, because these assessments allow for the exclusion of the influences of load and heart rate. The current study also demonstrated that six months of β-adrenergic receptor stimulation did not impact on myocardial systolic function when assssed both *in vivo* (systolic midwall fractional shortening, FS_{mid}) and *ex vivo* (systolic myocardial elastance, LV E_n) in the male and female SHRs.

In addition, to the differential impact of β-adrenergic activation in male versus female rats a gender effect was noticed in the current study, whereby both *in vivo* and *ex vivo* assessments demonstrated that the female rats had a higher myocardial systolic cardiac function compared to the male rats. These data therefore suggest that β-adrenergic activation initiates the progression from concentric hypertrophy to cardiac dysfunction primarily through mechanism other than reductions in myocardial systolic cardiac function in male SHRs. Indeed, this notion was clarified be other researchers, whereby it was proposed by Cohn (1995), and subsequently substantiated by data obtained in human (Vasan et al. 1997) and animal (Norton et al. 2002, Badenhorst et al. 2003, & Osadchii et al. 2007) studies that pump dysfunction can occur mainly as a consequence of cardiac dilatation rather than myocardial contractile disturbances. Moreover, in Sprague Dawley rats three months of β-adrenergic activation resulted in pump dysfunction as determined by decreased LV E_{es} but with no differences in myocardial systolic function as determined by LV E_n (Osadchii et al

2007). In addition, ageing SHRs demonstrated no changes in myocardial contractility despite cardiac dilatation and subsequently cardiac dysfunction in either male or female rats (Tamura et al. 1999, & Chan et al. 2011). In contrast to this concept, data from Sabbah (1998) suggested that cardiomyote apoptosis or necrosis may contribute to pump dysfunction and subsequently produce cardiac dilatation. We have shown that despite reductions in the myocardial contractility and cardiomyocyte apoptosis after three months of β-adrenergic activation, rats are still able to maintain myocardial contractility, possibly through the enhanced myocardial α-adrenergic receptor mediated contractile responses and as well as an increased presynaptic myocardial norepinephrine release (Osadchii et al 2007). In this regard, alterations in myocardial contractility can only be a consequence rather than a cause of pump dysfunction.

Similar to the findings of the present study, human studies have shown *in vivo* that women have a higher systolic function compared to men in healthy individuals (Buonanno et al. 1982, & de Simone et al. 1991) and patients exposed to chronic pathological loading (Topol et al. 1985, Carrol et al. 1992, Villari et al. 1992, Aurigemma et al. 1994, Douglas et al. 1995, & Villari et al. 1995). In this regard, Devereux et al. (1998) reported that both normotensive and hypertensive women have an enhanced chamber function compared to men. In addition, evidence from elderly patients under pressure overload induced with aortic stenosis, also demonstrated that the systolic function is more often depressed in men than in women (Carroll et al. 1992, & Aurigemma et al. 1994). Moreover, young women with aortic stenosis demonstrated ventricular concentric hypertrophy with a normal to supernormal systolic function; whereas young men had a reduced systolic function (Villari et al. 1995).

The gender-related differences in systolic function found in my study are also consistent with animal studies, which have also reported that female rats have an enhanced

systolic function compared to male rats when exposed to either pressure (Pfeffer et al. 1982, Weinberg et al. 1999, & Chan et al. 2011) or volume (Gardner et al. 2002, Brower et al 2003, & Dent et al. 2010) overload conditions. Moreover, in normal physiological conditions both male and female rats have the same systolic LV function, however under pressure overload this parameter is reduced in male rats but not in female rats (Monasky et al. 2008). In addition, studies of ageing SHRs have reported that male rats had systolic dysfunction that coincided with the structural progression to eccentric hypertrophy (Tamura et al. 1999, & Chan et al. 2011). Furthermore, *ex vivo* cardiac function assessments in Wistar rats have shown that the female rat hearts have a normal systolic function that was markedly reduced in the hearts of the male rats after pressure overload (Weinberg et al. 1999). Importantly, it is suggested that the enhanced myocardial contractility in female rats is attributed to a greater shortening of the papillary muscle than in the male rats (Capasso et al. 1983). This notion was also supported by Gao et al. (2003) in that female mice have a greater myocardial contractility in the absence of β-adrenergic activation, whereas male mice subjected to β-adrenergic activation progress to systolic dysfunction. Therefore these studies suggest that a gender difference in myocardial contractility is largely attributed to the different activities in the mediators of myocardial contractility, including calcium and actinomyosin ATPase (Malhotra et al. 1981). In the male rats, delay in the rate of calcium release and reuptake result in decreased calcium activated actomyosin and myosin ATPase activity, and hence a depressed myocardial contractility (Capasso et al. 1983).

In the current study the female SHRs had greater cardiac function and smaller end-diastolic and systolic volumes, despite similar systolic and diastolic blood pressure values. It is unknown whether these differences result from intrinsic differences in the cardiomyocyte molecular adaptation to pressure overload between male and female rats or are related to

other factors extrinsic to the myocardium. Therefore, gender effects on cardiomyocyte death were assessed as a possible mechanism that may explain gender-related differences.

4.3.3 Gender differences on cardiomyocyte apoptosis and necrosis

It has been shown that β-adrenergic receptor stimulation induces cardiomyocyte apoptosis (Saito et al. 2000, Kim et al. 2006, & Osadchii et al. 2007). The current study failed to show gender differences in cardiomyocyte apoptosis after five days of ISO administration. However, it should be noted that there was a trend ($p=0.08$) for an ISO effect in both the male and female SHRs. In conjunction with the current study, previous work from our laboratory has shown that five days of β-adrenergic activation induces cardiomyocyte apoptosis in both the male and the female Wistar-Kyoto (WKY) rats (C.Mielke, MSc dissertation). Importantly, as the current study showed no gender differences in cardiomyocyte apoptosis, this suggests that cardiomyocyte apoptosis is not responsible for the differences in LV dimensions and systolic pump dysfunction between the males that received ISO and the other groups.

The current study also failed to show gender differences in pathological scores ($p=0.08$), which are an index of necrosis. Hence, necrosis is also not responsible for the differences in LV dimension and systolic pump dysfunction between male and female rats receiving ISO. It is important to note, that the current study is the first study to our knowledge to evaluate the impact of gender on β-adrenergic receptor induced necrosis in SHRs.

If neither apoptosis nor necrosis are responsible for the cardiac remodelling and dysfunction in male rats, what other factors could play a role. Several studies have reported that structural factors such as the collagen network of the ventricular wall are important

determinants of left ventricular systolic function in patients subjected to pressure overload (Doering et al. 1988, Krayenbuehl et al. 1989, Brilla et al. 1991, & Villari et al. 1993). Although not demonstrated in this study it is acknowledged that the collagen network plays a key role in the regulation and adaptation of left ventricular function under pressure overload conditions (Villari et al. 1993, & Woodiwiss et al. 2001). ISO-induced cardiac remodelling and dysfunction are associated with alterations in the characteristics of myocardial collagen (Woodiwiss et al. 2001, & Badenhorst et al. 2003). Moreover, Villari et al. (1995) demonstrated that in 27 patients with aortic stenosis, 93% of all males and in 38% of all females had collagen disarrangement and myocardial dysfunction as determined *in vivo*. Therefore, it can be suggested that the collagen network arrangement may account for the gender-related differences in the left ventricular remodelling and function subjected to pressure overload.

4.4 The potential clinical importance of the findings of the present study.

The present study provides substantial evidence to indicate that in the settings of hypertension, males are more susceptible to the adverse effects of chronic adrenergic stimulation on LV cavity dimensions and systolic chamber function compared to females. Moreover, these data highlight the clinical significance of gender differences and the mechanistic cause for developing HF in hypertensive patients. However, there is still a need for a better understanding for the role of sex steroids in the development of heart failure through β-adrenergic stimulation and the presence of hypertension. At present, it can be suggested that different therapeutic strategies may need to be employed regarding HF in different hypertensive genders. A subsequent study should be conducted to assess the role of sex steroids in the development of heart failure in the presence of hypertension. Furthermore, other suggested mechanisms for the progressive HF should be employed in assessing the

gender differences in adverse cardiac remodelling mediated by β-adrenergic stimulation in the presence hypertension.

4.5 Limitations

The possible limitations to this study are as follows; firstly, there was a low sample size in each group for the short-term β-AR stimulation study. Although, there was a noticeable trend for an ISO effect compared to saline vehicle groups in both the male and the female SHRs, the low sample sizes failed to produce statistical power for an ISO effect in each gender. It may be easily assumed that with an increase in the sample size from each group a statistical significance for an ISO effect would have been attained. Nevertheless, the data clearly show no differences between male and female rats and hence rule out cardiomyocyte apoptosis as a mechanism for ISO-induced dilatation and pump dysfunction in the male rats. Secondly, the dose of ISO administered to these rats may have had less effect in females compared to males. There is debate in the literature regarding gender differences in cardiac β-AR responsiveness. Studies in rat atrial and ventricular myocytes have shown that males show an enhanced β-adrenergic contractile response compared to females (Schwertz et al. 1999, Vizgirda et al 2002, Curl et al. 2003, & McIntosh et al 2011). In contrast, other studies in isolated rat trabeculas (Monasky et al. 2008) and rat papillary muscle (Chu et al. 2005) reported no gender differences in β-adrenergic contractile responses. It is suggested that these difference may be due to the doses of ISO used. Moreover, McIntosh et al (2011) indicated that gender differences exist in the β-adrenergic contractile response at low doses of ISO. Therefore, the dose response study in isolated perfused male and female hearts of the SHR model can be performed to assess an appropriate dose for each gender. Nevertheless, if high dose of ISO is required in female rats compared to male rats, this would support the gender differences shown in the current study.

Conclusions

In conclusion, as compared to female SHR, male SHR are more susceptible to the adverse effects of chronic β-AR activation on LV cavity dimensions and systolic chamber function. These results suggest that the higher prevalence of LV dilatation and systolic chamber dysfunction in males than in females with LVH may be attributed to an increased susceptibility to the adverse effects of β-adrenergic stimulation in males. The latter is important as it sheds light for the impact of gender on β-adrenergic-induced cardiac dilatation and systolic dysfunction in SHRs. Therefore, subsequent studies should be conducted to assess the impacts of castration and ovariectomy on β-adrenergic-induced cardiac dilatation and systolic dysfunction in SHRs.

Future Studies

As LVH is associated with diastolic dysfunction, I would suggest that future studies assess the impact of gender. Moreover, as there was no evidence to indicate that cardiomyocyte death was a mechanistic cause of cardiac dilatation in my studies, future studies should assess changes in fibrosis and collagen cross linkage as a possible cause of cardiac dilatation in this model.

Chapter Five - References

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2010/21/04

APPLICANT: DR F Michel

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: **Gender comparison of the progression of compensated hypertrophy to heart failure in isoproterenol-treated spontaneously hypertensive rats**

Number and Species

30 F spontaneously hypertensive rats (SHR), 30 M SHR, 30 F SHR

Approval was given for the use of animals for the project described above at an AESC meeting held on 30.03.2010. This approval remains valid until 30.03.2012.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

Echocardiography to be performed at the end of the study only.
Animals to receive saline injections initially for approximately 2 weeks before starting the incremental doses of Isoproterenol. This will be required to habituate rats to the procedures

Signed: _____ Date: 16/04/2010
(Chairperson, AESC)

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

Signed: _____ Date: 16/04/2010
(Registered Veterinarian)

cc: Supervisor:
Director: CAS

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