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INFLUENCE OF LEFT VENTRICULAR DIMENSIONS ON β–ADRENERGIC–INDUCED CARDIAC REMODELING IN MALE SPONTANEOUSLY HYPERTENSIVE RATS

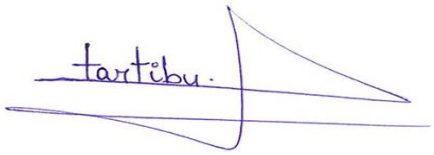
Linda Katanda Tartibu

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

Johannesburg, 2022

DECLARATION

I, Linda Tartibu Katanda, 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 dissertation, has not been submitted for any degree or examination in this university, or any other university.



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(Signature of candidate)

Signed on the 18th January 2022

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 2018/09/45B





……. …….

Prof Frederic Michel (supervisor 1) Dr Vernice Peterson (supervisor 2)

Date the 18th January 2022 Date the 18th January 2022

DEDICATION

I dedicate this dissertation to my husband, SAMUEL MATANDA, my sons NOAH MATANDA and YOHAN-LOUIS MATANDA, my parents JEAN-LOUIS TARTIBU NYEMBO and GEORGETTE NGALA TSHOBA, my brothers and sisters, OLIVIER BOMOLO, DEFI BARAKA, WILLY TARTIBU, LAGOUGE TARTIBU, LYSETTE TARTIBU, JAMIE DONATO, HERVE TARTIBU, SYLA KASH, ALICIA JOELLE, NEL TARTIBU, TONY TARTIBU, LAETITIA TARTIBU, CHARLES MILIKITO, NABI MBAYO and JOSEPH TARTIBU and to my nephews and nieces.

ABSTRACT

Heart failure progression in hypertension is characterised by compensated left ventricular hypertrophy (LVH) which may progress to cardiac dilation. Adrenergic activation is another process in the transition to cardiac dilation. Regression of LVH following antihypertensive therapy ameliorates the prognosis of heart failure. However, impact of LVH regression on cardiac remodelling following adrenergic activation is unknown. The aim of the study was to determine the effect of LVH regression on ß-adrenergic-induced cardiac remodelling in male spontaneously hypertensive rats (SHRs).

One-month-old male SHRs (21) and normotensive rats (8) were used in the study. During the first 6 months, SHRs were untreated, treated with: captopril (an angiotensin-converting enzyme inhibitor) or with hydralazine (a non-specific vasodilator). Normotensives were untreated. At 7 months, anti-hypertensive treatments were stopped and rats in the four groups received a daily isoproterenol (beta-adrenergic agonist) injection for 5 months. Blood pressure was measured every two weeks. Echocardiography was performed at 7 and 12 months of age.

At 7 months, systolic blood pressure (SBP) was significantly higher in the untreated hypertensive group compared to the other groups. Ventricular wall thickness in diastole (WTd), relative wall thickness (RWT) and calculated left ventricular mass (LVMc) were significantly greater in untreated hypertensive group compared to the other groups. Ventricular functions were similar between the four groups. At 12 months, SBP was significantly higher in the hypertensive groups compared to the normotensive group. At 12 months compared to 7 months, WTd and the ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/e' filling index) were significantly increased in the hypertensive group. At 12 months, WTd, RWT and LVMc were significantly greater in the hypertensive group compared to the normotensive group and the hypertensive group previously treated with captopril. Endocardial fractional shortening and E/e' filling index were significantly greater in the hypertensive group compared to the normotensive group. Left ventricular dimensions

and functions were similar between hypertensive rats untreated and hypertensive rats previously treated with hydralazine.

At 7 months, hypertensive rats developed concentric LVH without systolic or diastolic dysfunction, suggesting a compensation process. Both antihypertensive drugs reduced LVH to a similar extent as normotensive rats. At 12 months, hypertensive rats developed eccentric LVH with signs of diastolic dysfunction, suggesting a progression to cardiac dilation with ß-adrenergic-receptor activation. The regression of LVH with a non-specific vasodilator before ß-adrenergic-receptor activation did not hamper the development of eccentric LVH in hypertensive rats. However, the regression of LVH with an angiotensin-converting enzyme inhibitor before receptor activation prevented eccentric LVH in hypertensive rats to a similar extent than in normotensive rats. In conclusion, activation of the renin-angiotensin-aldosterone system independent of blood pressure may be detrimental in the development of ß-adrenergic-induced cardiac remodelling.

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Professor Frederic Michel and Dr. Vernice Peterson: for their supervision and all the valuable knowledge and skills they taught me. They have been good role models and inspired me a lot.

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LIST OF ABBREVIATIONS

2D	: two-dimensional
a’	: peak velocity during late (atrial) diastole
A wave	: trans-mitral blood flow velocity in the late (atrial-A) period of left Ventricular diastolic filling
ACE	: angiotensin-converting enzyme
AESC	: Animal Ethics Screening Committee
Ang II	: angiotensin II
AUC	: area under the curve
BP	: blood pressure
BW	: body weight
CAS	: central animal service
CHF	: chronic heart failure
cm	: centimeter
CTR	: cardiothoracic ratio
CVD	: cardiovascular diseases
DBP	: diastolic blood pressure
E’	: peak velocity during early diastole at the mitral annulus
E’/a’	: ratio of early-to-late mitral annulus velocity
E/A	: ratio of early-to-late diastolic filling
E/E’	: index of left ventricular filling pressures
E wave	: trans-mitral blood flow velocity in the early period of left ventricular diastolic filling
ECG	: electrocardiography
EF	: ejection fraction
FSend	: endocardial fractional shortening
g	: gram
HF	: heart failure
mm Hg	: millimeters of mercury
HHD	: hypertensive heart disease
HR	: heart rate
HT	: hypertensive rat untreated during the first six months of the study

HT+C : hypertensive rat treated with captopril
HT+H : hypertensive rat treated with hydralazine
HTN : hypertension
HW : heart weight
HW-BW : heart weight adjusted to body weight
IGF-1 : insulin growth factor-1
ISO : isoproterenol
IVSd : intraventricular septum thickness at end-diastole
IVSs : intraventricular septum thickness at end-systole
JNC : Joint National Committee
L/min : litres per minute
LV : left ventricle
LVEDD : left ventricular end diastolic diameter
LVEDV : left ventricular end diastolic volume.
LVEF : left ventricular ejection fraction
LVESD : left ventricular end systolic diameter
LVESV : left ventricular end systolic volume.
LVH : left ventricular hypertrophy
LVM : left ventricular mass
LVMc : left ventricular mass calculated
LVPWd : left ventricular posterior wall thickness at end-diastole
LVPWs : left ventricular posterior wall thickness at end-systole
LVW : left ventricular weight
LVW-BW : left ventricular weight adjusted to body weight
mg/kg : milligram per kilogram
MHz : mega hertz
MI : myocardial infarction
ml : millilitre
mm : millimeter
M-mode : motion mode
MV E’ : peak velocity of early diastolic mitral annular motion
MV A’ : peak velocity of diastolic mitral annular motion
MV S’ : peak velocity of systolic mitral annular motion
n : number or size of samples

NCDs : non-communicable diseases
NIBP : non-invasive blood pressure
NT : normotensive rat
p : probability value
RAA : renin-angiotensin-aldosterone
RWT : relative wall thickness
SBP : systolic blood pressure
SD : standard deviation
SHR : spontaneously hypertensive rat
SHRs : spontaneously hypertensive rats
T : time
WKY : wistar–kyoto

Chapter 1. Introduction

1.1. Hypertensive heart disease

Worldwide, cardiovascular disease (CVD) is the leading cause of death, contributing to approximately 17.3 million deaths (Alberts et al 2005, Kearney et al 2005, Mathers et al 2006, World Heart Federation, 2012). Although CVD is a major burden globally, high mortality rates are prevalent in high-income countries (Lopez et al 2006, Mayosi et al 2009). Amongst the 10 leading causes of disease worldwide, hypertension is the most significant modifiable and independent risk factor in occurrence of cardiovascular diseases like cerebrovascular accident, atherosclerotic heart disease, kidney failure and heart failure (Gaziano et al 2017) and for mortality worldwide (Allen et al 2012, Bonifonte et al 2015; GBD 2015 Risk Factors Collaborators, 2016; Sasai et al 2011). Considered as a silent killer because of the lack of symptoms in hypertensive patients (American Heart Association, 2015), hypertension is a rising health problem worldwide, observed in nearly 1 billion people with approximately 9.4 million annual deaths worldwide (Lim et al 2012, Poulter et al 2015).

In South Africa, hypertension, strokes and ischaemic heart disease are some of the most common non-communicable diseases (NCDs) leading to many premature adult deaths (Bradshaw et al 2005). Indeed, as a result of this, in South Africa, prevalence of heart failure is increased (Bradshaw et al 2003, Steyn et al 2006, Rayner, 2010, Mozaffarian et al 2015). This is attributed to a rising rate of cardiovascular diseases (such as coronary heart disease or cardiomyopathy, high blood pressure, obesity and diabetes mellitus), a consequence of urbanisation, globalisation and marked changes in lifestyle (Steyn et al 2006, Rayner, 2010, Maredza et al 2011).

Over the years, more notions into risk factors for CVD including obesity, alcohol intake, smoking, hypertension and diabetes mellitus have provided epidemiological evidence (Lopez et al 2006, Steyn et al 2005, O'Donnell et al 2010). However, hypertension is still the most common life-threatening risk factor for CVD in developing countries (Connor et al 2005). Hypertensive heart disease (HHD) can be defined as cardiac reaction to stress and afterload on left ventricle secondary to a gradually augmentation

of the blood pressure in arteries (Frohlich et al 1992). In other words, HHD is a set of abnormalities consisting of left ventricular hypertrophy (LVH), systolic and diastolic dysfunction, and their clinical events including arrhythmias and symptomatic heart failure (Naoyuki Hasebe, 2011). Hypertensive heart disease and heart failure are the most frequent cardiovascular diseases of Africans (Frohlich et al 1992). Moreover, in Africa, hypertensive heart disease and dilated cardiomyopathy are leading causes of heart failure (Gianluigi and Lund al 2017).

1.2. Hypertension

1.2.1. Definition

Hypertension (HTN) is defined as a persistent elevation of blood pressure (BP) ≥ 140/90 mmHg in accordance with the Eighth Joint National Committee (JNC 8) criteria and is the product of cardiac output and total peripheral vascular resistance. The optimal BP is a value < 130/85 mmHg. HTN is stratified into three grades, prehypertension, stage 1 hypertension and stage 2 hypertension, depending on severity, which is useful in defining the approach to treatment. High-normal is BP levels from 130–139 mmHg systolic and 85–89 mmHg diastolic. The high-normal group are at higher risk of developing CVD and are also at risk of developing HTN that does not require drug treatment.

Defined and recorded as an average of systolic blood pressure over diastolic blood pressure, blood pressure varies throughout the day. Systolic pressure is the pressure in the arterial system caused by the left ventricle contracting and pushing blood into the circulation and diastolic pressure is the pressure in arteries when the heart is resting between contractions. Although peripheral vasoconstriction contributes, systolic hypertension is mainly caused by an augmentation of aortic and central arterial stiffness (London et al 2002). The prescription of a certain therapy against hypertension depends on organ damaged by hypertension, coexistence of cardiovascular risk factors, fragility of elderly and particular resistance of each patient. To normalise BP, systolic BP between 120 and 140 mmHg and a diastolic BP between 70 and 80 mmHg should be the aim (Christian and Schmieder , 2021). Lower targets

are no longer suggested. Moreover, the decrease of BP in the elderly should be reached gradually over one month (Seedat et al 2014).

1.2.2 Prevalence of hypertension

Defined in the past as pathology of rich countries, hypertension is now prevalent among poor countries (Van de Vijver et al 2013). Low- and middle-income countries suffer two-thirds of the global burden of cardiovascular diseases (CVD), coupled to an inappropriate management of hypertension (Perkovic et al 2007). Indeed, cases of hypertensives patients are greater in low- and middle-income countries than in high in-come countries (Joffres et al 2013) with an insufficient health system because of low income, poor infrastructure and insufficient equipment. In Africa, prevalence of hypertension has been reported in many studies (Akpan et al 2015, Díaz et al 2015, Guwatudde et al 2015, Pires et al 2013). In addition, prevalence is not the same; in urban zones, prevalence is more than in rural zones. As an example, a rate of 25% and 9.4% among urban and rural Zulus of South Africa, respectively (Seedat et al 1982). In Nigeria, rates of 9.8% and 14.6% were found in rural and urban zones (non-communicable diseases in Nigeria-Final report of National Survey, 1997). In Sub-Saharan Africa (SSA), hypertension has become a major health problem in the population (Addo et al 2007, Ibrahim et al 2012) with an increase prevalence rate (Mills et al 2016). CVDs are considered to be a major cause leading to premature death in this part of Africa. Although affecting older population, the prevalence is now becoming increased in younger population (Bradshaw et al 2007).

Despite its high prevalence, both awareness and control are low among hypertensive subjects, with figures ranging between 19.0–56.0% and 4.0–33.0%, respectively (Palafox et al 2016, Peltzer and Phaswana-Mafuya,2013, Ataklte et al 2015) leading South African guidelines to recommend a frequent assessment of blood pressure every 3 months at most (Seedat et al 2011). Moreover, management of hypertension decreased the occurrence of complications, such as cerebrovascular accident, atherosclerotic heart disease and kidney failure (Beckett et al 2008, Ninomiya et al 2013, Cushman et al 2010). Without successful management of hypertension,

projections suggest a continuous rise in hypertension-associated CVD with an augmentation of mortality and disability (Mendis, 2003, Juma et al 2017).

1.2.3. Pathogenesis of hypertension

Development of essential high blood pressure is complex and depends on many factors such as genetics, obesity, an increased dietary salt intake and activation of neurohormonal systems such as sympathetic nervous system and renin-angiotensin-aldosterone system (Hall et al 2012). Blood pressure is the result of cardiac output multiplied by total peripheral vascular resistance. Many factors are implicated in short-term and long-term control of blood pressure for sufficient tissue perfusion:

Cardiac output and circulatory blood volume

Cardiac output is the product of stroke volume and heart rate. Many factors such as sodium intake, renal function and mineralocorticoids affect cardiac output (Hamrahian, 2017). The inotropic effects increase stroke volume, rate and contractility of the heart. However, circulating blood volume is controlled by both renal salt and water handling, an important fact in occurrence of salt-sensitive hypertension and into context of chronic kidney disease (Guyton et al 1972).

Vascular caliber, vascular elasticity, and reactivity

Elasticity of vessel walls refers to the ability to take back its normal shape after being tensile and compressed. Peripheral vascular resistance refers to compliance. Decreased arterial compliance increases the incident pressure wave and has an effect on reflected pressure waves leading to an increase in systolic pressure and ventricular afterload and then to left ventricular hypertrophy.

Humoral mediators effects on blood pressure

The humoral actions on peripheral vascular resistance are caused by mediators, such as vasoconstrictors (eg, endothelin [ET], angiotensin II [Ang II], catecholamine) or vasodilators (eg, nitric oxide [NO], prostaglandins, kinins) (Hamrahian, 2017).

Neural stimulation effects on blood pressure

Peripheral vascular resistance depends on sympathetic nervous system (SNS), humoral factors and local autoregulation. Indeed, vasculature is highly innervated by sympathetic fibers and SNS induces its effects via the vasoconstrictor alpha effect or the vasodilator beta effect (DiBona, 2013). Moreover, viscosity of blood, vascular wall stress and blood flow velocity have important effects with regard to control of blood pressure in humans by vascular and endothelial function (Hamrahian, 2017).

1.3 Left ventricular hypertrophy

High blood pressure is one of the most important risk factor of left ventricular hypertrophy. In particular, systolic hypertension is the primary cause of left ventricular hypertrophy (Richard et al 2011). During hypertension crisis, the left ventricle is not able to compensate for an acute elevation of systemic vascular resistance.

Age and a wide pulse pressure are risks factors of coronary heart disease. Indeed, in elderly, pulse pressure is augmented by elasticity of arteries and a stiffness of aorta. Waves reflect from early diastole to late systole (Foex and Sear, 2004). Following those factors, coronary perfusion pressure decreases while the consumption of oxygen in the myocardium increases leading to development of hypertrophy of left ventricle (Heilpern, 2008). However, in a young subject, left ventricle produces a low pulse pressure and reflected waves occur after the end of systole leading to an increase pressure during the early part of diastole and then an increase coronary perfusion (Foex and Sear, 2004).

Moreover, in left ventricular hypertrophy, increased afterload leads to some structural changes observed in the myocardium. Hypertrophy of myocytes following this increase

of pressure allows the heart to pump blood more strongly. Although, the contraction of left ventricle remains normal until later stages, the chamber lumen decrease limiting diastolic filling and stroke volume. Thus, diastolic function of left ventricle is deteriorated in long-standing elevation of blood pressure (Seyed, 2017). Exact factors of left ventricular diastolic dysfunction have not yet been demonstrated. However, possible mechanisms include an aberration, during diastole, in the passive relaxation of the left ventricle. In the Framingham heart study, isolated systolic hypertension is associated with an increase left ventricular wall thickness and impaired diastolic filling in the elderly (Sagie et al 1993). Thus, left ventricular hypertrophy may result in systolic and diastolic dysfunction, both leading to the development of heart failure (HF) (Haider et al 2003).

1.3.1 Definition of left ventricular hypertrophy

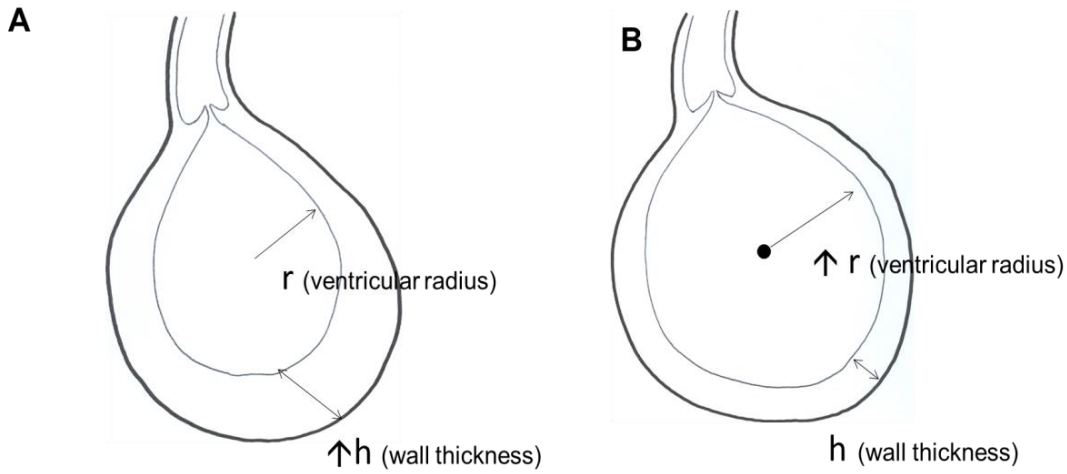
Known as a complication of systemic hypertension, hypertrophy of the left ventricle (LVH) is the most effective marker to study heart diseases that occur because of hypertension (Frohlich, 1987). For many years, LVH was a beneficial compensatory mechanism for sustaining normal wall stress of left ventricle (LV) under pressure or volume overload (Liebson et al 2001). Left ventricular hypertrophy is now recognized as both an important maladaptive response to chronic pressure overload and a major risk factor in hypertensive subjects. Its pathogenesis is highly correlated with systolic hypertension. Epidemiological studies using electrocardiography (ECG) and echocardiography have shown that LVH is a non-dependent and potent risk factor for congestive heart failure, coronary events, life threatening dysrhythmias and cardiac mortality (Levy et al 1989, Messerli et al 1984).

1.3.2. Classification of left ventricular hypertrophy

1.3.2.1. Cardiac hypertrophy and remodeling process

Cardiac hypertrophy was in early stages defined as an augmentation in cardiac mass associated to an increase in cardiomyocytes mass. Remodeling process under a

physiological or pathological stress involves, firstly, cardiomyocytes growth and on the other hand changes in other cell types such as changes in intracellular and extracellular structure, protein expression, signaling pathways, energy metabolism, vascularization and so forth. Thus, cardiac hypertrophy includes, by oversimplification, remodeling of myocardium and all others aspects of cardiac response to stress. Moreover, left ventricular hypertrophy (LVH) which is associated to heart failure, is a form of cardiac remodeling that can be classified as physiological or pathological remodeling (Richard et al 2011, Ankur et al 2012). Physiological hypertrophy is an increase in muscle mass and pumping capacity of myocardium after training (Mone et al 1996), exercise, pregnancy (Hill and Olson 2008; Shimizu and Minamino 2016; Weeks and McMullen 2011) in normal healthy individuals or in anaemia and thyrotoxicosis-induced cardiac hypertrophy (De Boer et al. 2003). Pathological hypertrophy is an increase in muscle mass and collagen accumulation in myocardium during hypertension, cardiovascular disease and genetic mutations (Lemitsu et al 2001, Sagara et al 2012). Left ventricular remodelling is not only an increase in the overall size of the heart, but also an alteration of the heart structure and shape. Changes in shape and size of the chamber are also accompanied by a further decrease in the overall pumping capacity of the heart (Kurrelmeyer et al 1998). The nature of the ventricular wall stress lead, both physiological and pathological hypertrophy to progress to concentric or eccentric hypertrophy. On the other side, fibrosis and the lack of inflammatory response make difference between physiological and pathological hypertrophy (Muller and Dhalla, 2013). In pathological hypertrophy, neuroendocrine factors are released to stimulate LVH that may progress to maladaptive forms such as concentric or eccentric hypertrophy (Jop et al 2013). Concentric LVH is due to pressure overload caused by hypertension or other diseases such as aortic stenosis. In this case, the size of the left ventricular cavity is normal, wall thickness, left ventricle mass and relative wall thickness are increased (Figure 1.1, diagram A). On the other hand, eccentric hypertrophy deals with volume overload due to a significant valvular regurgitation or high cardiac index. In this case, left ventricle wall thickness is normal, left ventricle cavity size and left ventricular mass are increased and relative wall thickness is decreased (figure 1.1, diagram B) (Judith et al 2018).



(School of Physiology, PHSL3006 Cardiovascular notes)

Figure 2.1: Diagram A illustrating the changes in ventricular radius (r) and wall thickness (h) as a result of concentric left ventricular hypertrophy and diagram B illustrating a normal left ventricle wall thickness (h) and an increase of left ventricle cavity size and left ventricular mass (r) as a result of eccentric left ventricular hypertrophy.

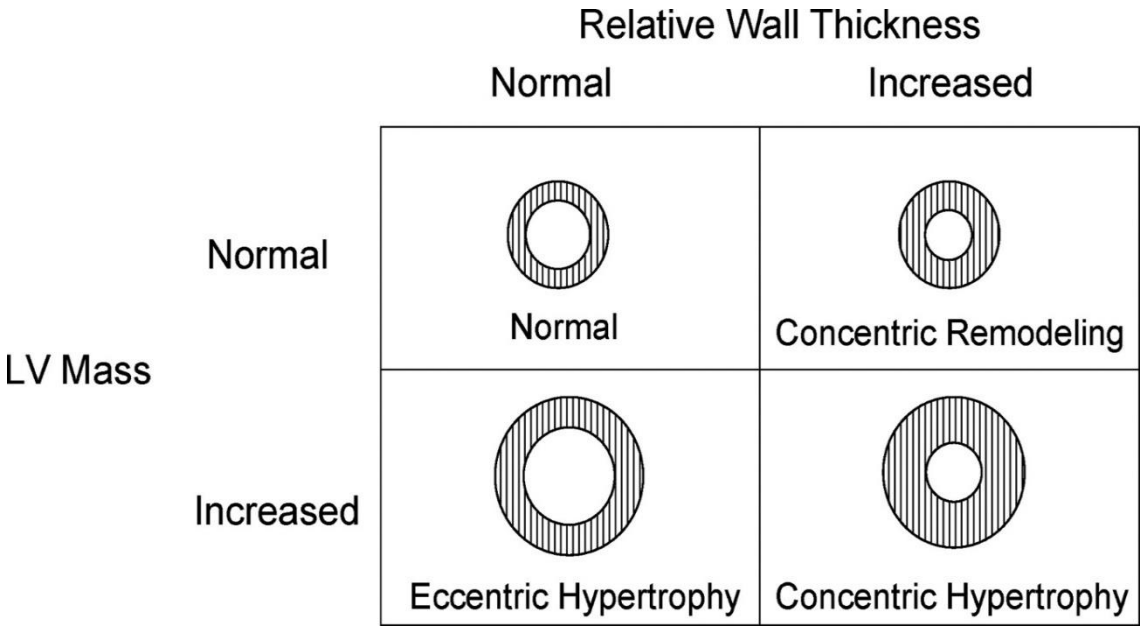
1.3.2.2 Understanding the process from concentric remodelling to systolic or diastolic dysfunction in hypertensive patients

The essential procedures involved in cardiac remodelling remain unclear. In some pathological conditions, the development of moderate concentric hypertrophy might be good if it could be improved (Lorell and Carabello, 2000).

Moreover, the absence of an augmentation of wall thickness to balance the augmentation of chamber radius leads progressively to an increase in diastolic stress that generates the remodelling process followed by left ventricular systolic dysfunction and increased morbidity and mortality (Lorell and Carabello, 2000). If one could outline the augmentation of wall thickness and restrict cardiac dilatation after infarction, experimental evidence proposes that remodelling would be restrained and beneficial (Litwin et al 1991). In addition, idea that a success adaptation of the heart to severe pressure overload by concentric remodelling and improved contractile function of myocardium without an important increase in left ventricular mass has been proved after a new trial models severe hypertension caused by the inhibition of nitric oxide synthesis (Bartunek et al 2000).

1.3.2.3 Different patterns of left ventricular hypertrophy as obtained by echocardiography

Some geometries types of left ventricular hypertrophy have been described in subjects with hypertension using echocardiographic procedure (Ganau et al 1992, Mayet et al 1997, Koren et al 1991). Left ventricular relative wall thickness (RWT) and left ventricular mass are used to define four geometric types: normal geometry, concentric remodeling, eccentric hypertrophy and concentric hypertrophy. Relative wall thickness (RWT) is defined as left ventricular posterior wall diastolic thickness × 2/ left ventricular end-diameter. Thus, we have, a normal geometry defined by RWT ≤0.42 and a normal LVM; a concentric remodelling defined by RWT >0.42 and a normal LVM; a concentric hypertrophy defined by RWT >0.42 and an increased LVM; an eccentric hypertrophy defined by RWT ≤0.42 and an increased LVM (Figure 1.2) (Lang et al 2015).



(Drazner MH. 2011)

Figure 1.2: Left ventricular geometry patterns based on left ventricular mass and relative wall thickness. Relative wall thickness is represented by the striped surface and the inner part of the circle represents left ventricular volume. The figure is adapted from Sehgal and Drazner (copyright © 2007, Elsevier) and Khouri et al (copyright © 2010, the American Heart Association).

These different types of left ventricular geometry have an effect on prognosis (Koren et al 1991). Although all different patterns of left ventricular geometry are associated with an increase cardiovascular disease risk, concentric hypertrophy has been shown as the structural parameter of the heart most strongly associated with incidence of cardiovascular disease risk (Muiesan et al 2004, Mancia et al 2007). Indeed, Koren et al found, in human study, that subjects with concentric hypertrophy had the highest risk of mortality and cardiovascular morbid events compared to subjects with normal geometry; and subjects with eccentric hypertrophy and concentric remodeling had a higher cardiovascular morbid event compared to subjects with normal left ventricle geometry; however, the risk to develop eccentric hypertrophy was lower than in subjects with concentric hypertrophy (Koren et al 1991).

In the United Kingdom, Mayet et al (Mayet et al 1997) found among hypertensive subjects, 40% of concentric hypertrophy, 32% of concentric remodelling, 6% of eccentric hypertrophy and 16% of normal geometry. In Africa, Aje et al (Aje et al 2006) found in a study of 100 newly diagnosed hypertensive patients that 72% of the subjects had abnormal left ventricular geometry (concentric hypertrophy-28%, concentric remodelling-26%, eccentric hypertrophy-18%).

1.3.3 Mechanical factors involved in the development of left ventricular hypertrophy

Elevated blood pressure and left ventricular wall stress are main mechanical factors involved:

1.3.3.1 Elevated blood pressure

Increase left ventricular mass precedes increase in blood pressure (Shimbo et al 2011).

Elevated high blood pressure generates pressure overload and if untreated, it increases the left ventricle walls stress that may affect cardiac output (Barry and Townsend 2010).

According to Laplace's law:

Wall tension = Pressure × Radius/2 × Wall thickness

Myocyte hypertrophy and increased wall thickness compensate an increase pressure overload to equalize the left ventricle wall stress (Grossman et al., 1975).

Hence, among factors involve in the development and occurrence of left ventricular hypertrophy, blood pressure is the most important haemodynamic factor and both haemodynamic and non-haemodynamic factors determine left ventricular mass (Levy et al 1988). In addition, cardiac pressure or volume overload are the main cause of an increase left ventricular mass. Pressure overload leads, within hours, to an augmentation of myosin heavy chain synthesis by 35%. In the early stages, this increase is predicted by an augmentation of translational efficiency (Imamura et al 1994). A common cause of pressure overload is pulmonary hypertension. In addition, pulmonary artery stenosis, pulmonic atresia, aortic stenosis, aortic coarctation are other causes leading to pressure overload (Abhinav and Gerald, 2007). On the other hand, volume overload increases LVM by reducing the myosin heavy chain degradation rate (Matsuo et al 1998). Common causes of volume overload are ventricular septal defects, mitral and aortic regurgitation and chronic anaemia. Other causes of volume overload include cirrhosis, kidney failure, nephrotic syndrome, premenstrual oedema, and pregnancy (Lewis 2020).

In Africa, many studies have found that poor hypertensive management leads to myocardial damage resulting in myocardial hypertrophy and ultimately a weakened myocardium (Lawal et al 1988, Attah et al 1977). These studies demonstrated that after the early thickening of myocardium, damage starts and continues up until total deterioration of cardiac muscle. Finally, myocardium is so deteriorated that it is no longer able to sustain a high blood pressure leading to a normal or subnormal blood pressure grade. At this stage the patient presents a myocardial disease of unknown origin. However, some patients present a blood pressure not fully stabilized and thought to be caused by heart failure instead of reactive hypertension. Further studies (Gradman et al 2006) of impact of hypertension on the heart have highlighted following events:

First of all, compared to a normal ventricle, a thickened left ventricle requires much blood supplied by the heart itself. This insures sufficient perfusion and performance of myocardium. If the thickening is mild and/or coronary vessels are healthy enough to

insure sufficient blood to the thickened myocardial wall, this process does not have any problem. However, if the thickening continues, either because of a poor or a lack of antihypertensive therapy, and if coronary vessels are diseased and not able to insure sufficient perfusion of the muscle wall, ischaemic of the entire mass wall will occur. The result is a scar tissue developed called reactive fibrosis (Gradman et al 2006, Diez et al 2001). Some ventricular muscle fibers may be deteriorated or fully destroyed in the process not only by hypertension but also by an inflammation of myocardium or immoderate consumption of alcohol and are in the same way substituted by scar tissue called reparative fibrosis.

The outcome of this reactive and reparative tissue is the same and is not conceived to contract or press blood as the original myocardium fibers. Therefore, this non-contractile scar tissue causes the initial stiffness of the cardiac muscle (diastolic dysfunction) and in Africa; it is assumed that the process weakened ability of the heart to contract (systolic dysfunction) and as a result, it leads to a cardiac dilatation and heart failure.

1.3.3.2. Left ventricular wall stress

The increase of left ventricular wall thickness may be influenced by some factors such as:

Familial and genetic predisposition

Several studies have demonstrated that genetics have some effects on left ventricular mass, without influence of other factors (Post et al 1997). Findings that augmentation of left ventricular mass may precede high blood pressure and that subjects with the same grade of high blood pressure may have significant differences in left ventricular mass suggest that genetic markers can stimulate and slow down occurrence of LVH. This is sustained by the augmented risk for LVH seen in middle-aged men with the DD genotype of the ACE gene (Schunkert et al 1994). The augmented risk correlated with

the DD genotype has been extended to subjects who went recently through transplantation of kidneys (Hernandez et al 1997).

Race and sex

Important racial dissimilarities exist with regard to left ventricular mass. Young blacks in good health have higher left ventricular wall thickness compared to whites (Hinderliter et al 1992). The incidence of left ventricular hypertrophy detected in black patients with mild hypertension is twice that founds in white patients (Hammond et al 1986). Gardin et al (Gardin et al 1995) also showed that left ventricular mass was greater in blacks. With regards to sex, males appear to have a 15-20% higher left ventricular mass index than women (Devereux et al 1984) because of the 15% lower lean body mass and maximal oxygen consumption observed in women compared with men (Goble et al 1992).

Salt intake

Salt intake is an important risk factor for high blood pressure and a potent determinant of left ventricular wall thickness and left ventricular mass. Studies (Elliott et al 1996, Staesen, 1991, Ogunlesi et al 1991, Olubodun et al 1997), sustaining the role of salt in the pathogenesis of elevated blood pressure, have found that there is a positive link between the excretion of salt in urine and the pressure of blood in arteries.

Insulin and Insulin Growth Factor-1 (IGF-1)

Insulin and glucose metabolism may influence left ventricular geometry (Celentano et al 1995). Insulin and IGF-1 are non-dependent factors of left ventricular mass and left ventricular geometry (Verdecchia et al 1999). Subjects with essential high blood pressure presented an IGF-1 augmented and associated to left ventricular mass. A

negative combination of insulin sensitivity and left ventricular wall thickness has been reported in these subjects (Hill, 1985). Moreover, insulin has a trophic effect on cardiac muscles cells. Thus, hypertrophy of the heart may be triggered by IGF-1 and insulin can induce muscle growth by binding to the IGF-1 receptors due to the structural similarity between the two molecules (Diez et al 1995).

Others factors including obesity (Lauer et al 1991), high alcohol consumption (Manolio et al 1991), plasma viscosity (Devereux et al 1984), physical activity (Savage et al 1990) and increase age (Dannenberg et al 1989) have all been shown to influence left ventricular mass (Post et al 1997).

1.3.4 Correlation between blood pressure and left ventricular mass in the development of left ventricular hypertrophy

There is a proof that elevated left ventricular mass may precede the development of manifest hypertension (Mahoney et al 1988, De Simone et al 1991, Iso et al 1994, Post et al 1994). Left ventricular mass is more closely correlated to 24-hour blood pressure than occasional blood pressure (Devereux et al 1983).
Increased left ventricular mass has been suggested to compensate an increase blood pressure (Schmieder et al 2000). Several factors may play a role in the occurrence of left ventricular mass in the context of hypertension: genetic factors (Arnett et al 2009; Mayosi et al 2008), excessive sympathetic nervous system activation and dysregulation of RAA system (Olsen et al 2002) and arterial stiffness. Possible mechanisms are: an increased left ventricular mass may aggravate itself mechanisms responsible for an increase blood pressure, an increase left ventricular mass goes together with an increase of stroke volume, cardiac output, and central blood volume; then these parameters decrease up to a normal level with an increase systemic vascular resistance (Schmieder et al 1995; Lutas et al 1985; Lund-Johansen, 1986). Finally, prehypertension may explain an increased left ventricular mass at baseline (Vasan et al 2001).

Diastolic blood pressure is related to left ventricular wall thickness, reflecting a pressure load while systolic blood pressure is related to left ventricular mass, suggesting an impact of both pressure and volume load. These two haemodynamic

factors play significant roles in the development and maintenance of hypertrophy of left ventricle (Kahan, 1998). Palmieri et al showed that patients with inappropriate increase left ventricular mass had higher ambulatory blood pressures, left ventricular relative wall thickness, and lower left ventricular systolic performance than those with normal left ventricular mass (Palmieri et al 1999).

1.3.5 Neurohormonal systems involved in the pathogenesis of LVH

Although directly related to systolic blood pressure, the pathogenesis of LVH suggests addition of important non-haemodynamic factors (Thomas et al 2005) such as stimulation of the renin angiotensin-aldosterone and sympathetic nervous systems. Indeed, it is now recognised that hypertrophy of the left ventricle is triggered not only by the mechanical stress of pressure overload, but also by several neurohormonal substances exerting independently trophic effects on cardiac muscle cells and no myocytes in the heart (Post et al 1994). The severity of left ventricular dysfunction has been shown to be dependent of neurohormonal activation in patients with heart failure (Benedict et al 1994).

1.3.5.1 Sympathetic nervous system activation

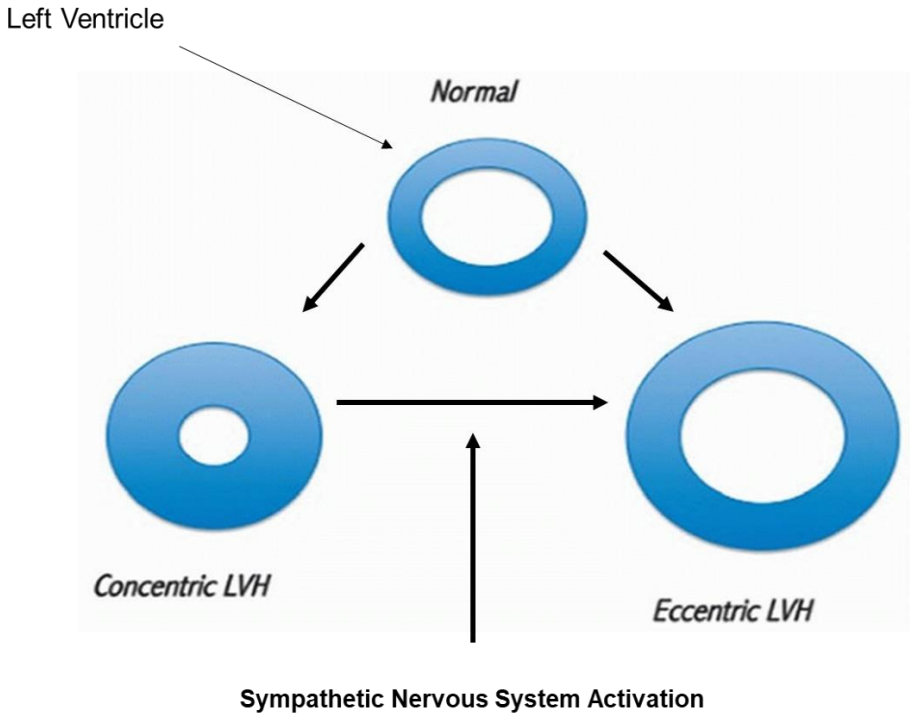
LVH plays a main role in the transition to clinical HF, resulting from increased diastolic wall stress, neurohormonal activation, cardiomyocyte injury and tissue fibrosis. The process leading to LVH remains no elucidated but hemodynamic and evidence of humoral factors stimuli are included (Patel et al 1991, Kelm, 1996). Sympathetic nervous system may play an important role especially via release circulating catecholamine's that have demonstrated to have trophic action (John et al 2001). A direct haemodynamic effect from sympathetic augmentation in hypertension contributes to the development of left ventricular hypertrophy (Klingbeil et al 2003). Thus, locally, an increased cardiac sympathetic neurotransmission plays a part in the increase in left ventricular mass in hypertension (Schlaich et al 2003). Indeed, it has been showed that in patients with hypertension, the presence of LVH is related to increase sympathetic nerve activity in muscles (John et al 2001). In pathological

hypertrophy, neuroendocrine factors are released to stimulate LVH that may progress to maladaptive forms such as concentric or eccentric hypertrophy (Jop et al 2013). There is evidence showing that adrenergic receptors play an important role in the activation of many intracellular pathways leading to nuclear responses of myocardiocyte in cardiac hypertrophy and vascular remodelling in the context of hypertension.

Moreover, excessive adrenergic activation is another important process involved in the transition to heart failure in the context of pressure overload, such as hypertension (Magubane et al 2017). Indeed, independently of blood pressure, increased susceptibility to β-adrenergic-induced cardiac dilation is developed in hypertensive subjects (Figure1.3) (Magubane et al 2017). It has been proven that male SHRs are more susceptible to adverse cardiac remodelling induced by beta-adrenergic stimulation in the context of hypertension.

1.3.5.2 The Renin–Angiotensin–Aldosterone (RAA) system activation

There is now increasing evidence that local cardiac (Raman et al 1995, Dostal et al 1999) and renal (Schunkert et al 1992) activation of renin and angiotensin is involved in the neurohormonal adaptation. Neurohormonal factors, particularly the renin-angiotensin system, plays a main role in occurrence of LVH in the context of hypertension (Alan et al 2006, Dzau, 1993). Indeed, It has been suggested in an experimental animal studies that in response to hypertension; endothelin plays a role in hypertrophy of the myocardium (Masaki et al 1991). Therefore, RAA system plays a prominent part in accelerating hypertensive organ damages (Sun et al 1993, Ronald, 2012). As it has been shown, independently of blood pressure variation or cardiac volume preloads, aldosterone receptor blockade may prevent the capacity of b-adrenergic receptor activation to promote transition from LVH to cardiac dysfunction in elevated blood pressure (Veliotes et al 2005), an independent change in the effects of blood pressure (Chan et al 2011).



(Burke and Maleszewski, 2016)

Figure 1.3: Mechanism of sympathetic nervous system activation leading to a transition from concentric LVH to cardiac dilation

1. Angiotensin II

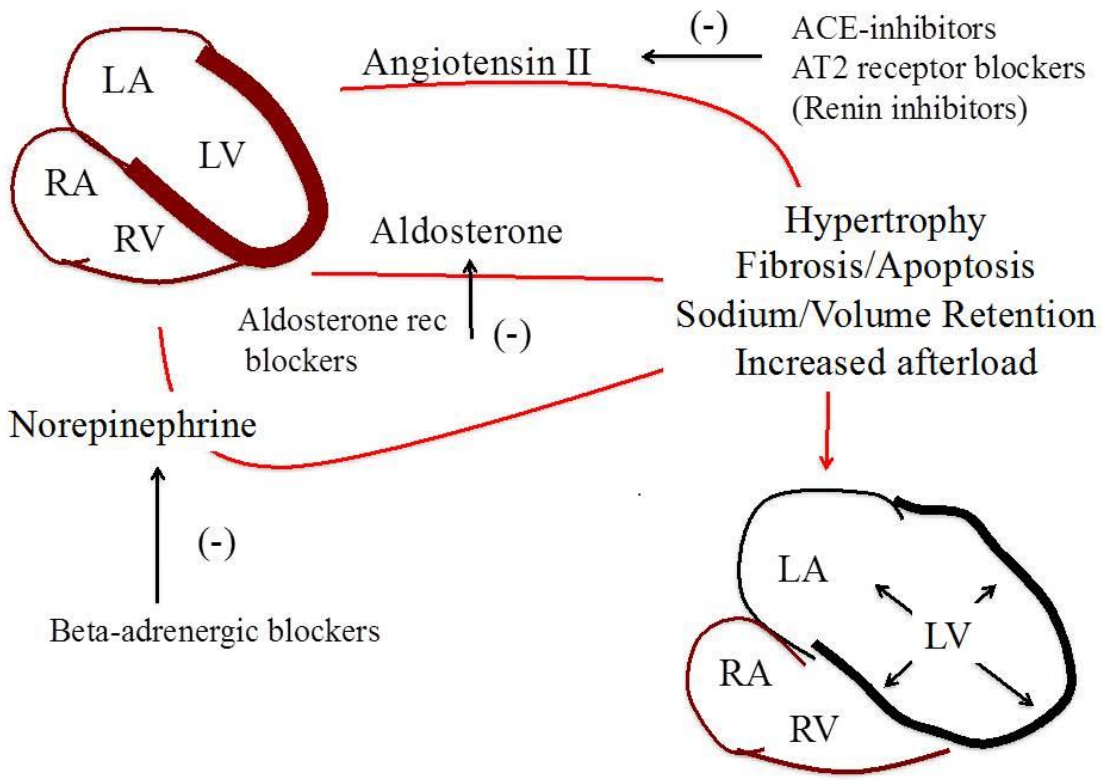
It has been suggested that induction of cardiac hypertrophy is mainly triggered by angiotensin II, via the ATI receptor because it can directly produce the molecular events of early cardiac growth (Figure1.4) (Sadoshima et al 1993). Harrap et al (Harrap et al 1996) showed a direct role of angiotensin II correlated to LVM in 84 young healthy subjects aged 16 to 24. Regression analysis also demonstrated that angiotensin II, renin and angiotensin converting enzyme (ACE) levels in the plasma were significantly correlated to LVM. In addition, the role of angiotensin in the pathogenesis of LVH in subjects with hypertension is also proposed indirectly following findings that an ACE inhibitor regresses LVH more than other antihypertensive medications (Schmieder et al 1996).

2. Endothelin II

It has been suggested in an experimental animal studies that in response to hypertension; endothelin plays a role in hypertrophy of the myocardium (Masaki et al 1991). There are three subtypes of endothelin: ET-1, ET-2, and ET-3. ET-1, produced in myocytes and endothelium, is a potent vasoconstrictor acting in the heart function as inotrope, chronotrope, and stimulator of the renin-angiotensin-aldosterone system (Kockskamper et al. 2008). Thus, as mentioned above, RAA system plays a main role in occurrence of LVH in the context of hypertension (Alan et al 2006, Dzau, 1993) and in response to hypertension; endothelin plays a role in hypertrophy of the myocardium (Masaki et al 1991).

3. Norepinephrine

Norepinephrine, as a myocardial hypertrophic neurohormone, has been suggested in some experimental and animal studies to play an essential role in the development of LVH (Figure1.4). Such evidence has not yet been conclusively validated in humans (Patel et al 2010, Simpson, 1983).



(Nayef Abouzaki and Antonio Abbate. 2016)

Figure 1.4: Neurohormonal Activation and Ventricular Remodeling. Mechanism of activation of renin-angiotensin-aldosterone (RAA) system leading to the development of hypertrophy, fibrosis/apoptosis, sodium/volume retention and increases afterload, and action of drugs such as ACE-Inhibitors, AT2 receptor blockers, Aldosterone receptor blockers and Beta-adrenergic blockers to prevent those events

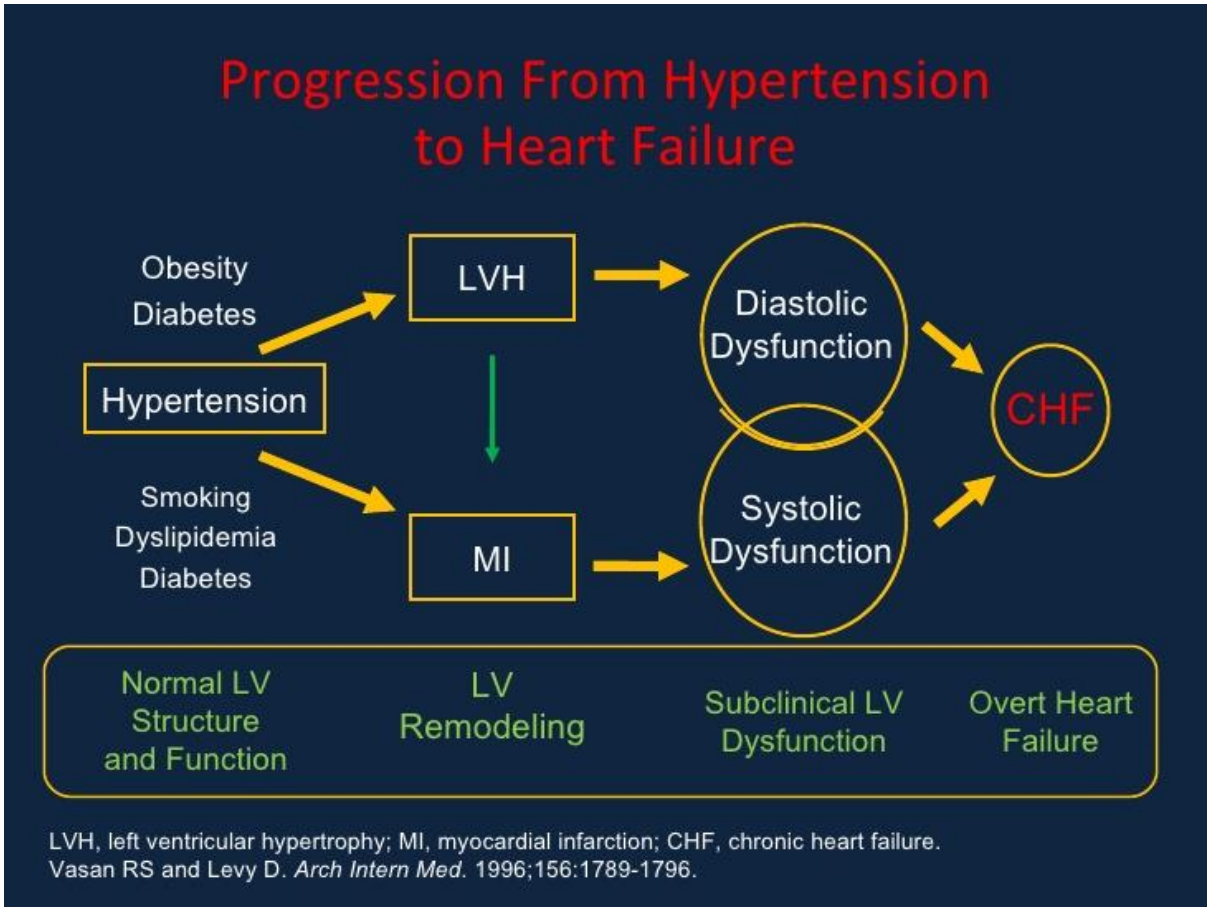
1.3.6 Progression from hypertension to heart failure

Left ventricular hypertrophy (LVH) and heart failure are important causes of high rate of morbidity and mortality (Chen et al. 2011). Classically, LVH secondary to high blood pressure was thought to be a compensatory response aimed to counterbalance systolic wall stress and prevent occurrence of heart failure (Levy et al 1990). However, the actual accepted notion is that pathological hypertrophy associated to ventricular dysfunction leads to heart failure (Crozatier et al 2015). Indeed, subjects with hypertension associated to hypertrophy of left ventricle have an increased risk of progressing to left ventricular systolic dysfunction and diastolic dysfunction, both leading to the development of heart failure (Levy et al 1996). Moreover, left ventricular diastolic dysfunction is the initial perceptible manifestation of heart disease (Messerli F.H. 1982).

In the context of hypertension, a sustained pressure overload progresses to decompensated concentric hypertrophy of the left ventricle, characterized by a left ventricular wall thickened with a reduced chamber size, and then progresses to hypertensive HF with preserved ejection fraction. On the other side, in sustained volume overload, left ventricular dilation progresses to a decompensate eccentric left ventricular hypertrophy and then to HF with reduced ejection fraction (HFrEF) (Messerli et al 2017). The presence of LVH is therefore a potent and non-dependent risk factor for poor outcome in heart disease, associated with an augmentation of cardiovascular morbidity and mortality (Verdecchia et al 2001, Lonn et al 2003).

It is assumed that patients with HHD and constant pressure overload often demonstrate a transition from a stage of compensated hypertrophy to heart failure (HF). Indeed, Veliotes et al showed, in an animal study using SHR, that the transition from left ventricular hypertrophy to cardiac dilation and pump dysfunction in the context of hypertension may be suppressed by aldosterone receptor blockade (Veliotes et al 2010). Moreover, Booysen et al showed that a discontinuation of chronic administration of β-adrenergic receptor activation may reverse effects of adrenergic-induced pump dysfunction, dilation of the heart and apoptosis of cardiomyocyte (Booysen et al 2011). However, the mechanism of this transition is still unclear.

In hypertension, β-adrenoreceptor activation promoting transition from cardiac hypertrophy to pump dysfunction and sympathetic over activation in left ventricular hypertrophy (Agabiti-Rosei et al 1987, Schlaich et al 2003) may be an important factor in the progression to heart failure. Indeed, without affecting loading terms, genetic alterations that decrease sympathetic activation attenuate (Esposito et al 2002) and chronic b-adrenoreceptor stimulation promotes (Badenhorst et al 2003) transition from LVH to heart dilatation and pump dysfunction in the context of hypertension.



(Vasan et al 1996)

Figure 1.5: Progression from hypertension to heart failure. LV: left ventricular, LVH: left ventricular hypertrophy, MI: myocardial infarction, CHF: chronic heart failure

1.3.7 Clinical presentation of left ventricular hypertrophy

Risks of cardiovascular morbidity and mortality are increased two-to-four-fold in patients with left ventricular hypertrophy compared to patients with normal left ventricular mass. Hence the detection and the measure of the extent of LVH is important (Kannel, 1983, Casale et al 1986). The proportion of hypertensive patients who developed HHD and heart failure is high in Africa (Falase et al 1983). The larger part of hypertensive patients seen in clinic for the first time are asymptomatic. Physical examination is the first step to diagnose left ventricular hypertrophy. By palpating the apex beat, indication of the presence of abnormal enlargement of the heart or left ventricular hypertrophy with or without distension, is given.

Imaging methods displaying non-invasively measurements of left ventricle are naturally the recommended methods for LVM assessment and LVH diagnosis (Ljuba Bacharova, 2014). A chest x-ray is still helpful in Africa as it allows a detection of cardiac and left ventricular enlargement at reduced cost. A cardiothoracic ratio (CTR) bigger than 50% is accepted as an index of cardiac enlargement while a displaced apex of the heart to the left and downwards sometimes below the diagram is an indication of left ventricular enlargement. Magnetic resonance imaging, thallium imaging and ultrafast CT scanner are other advanced methods of investigations very expensive and not widely available in Africa.

LVH detection by ECG remains a potent predictor of detrimental outcomes in epidemiological studies despite the fact that assessment of LVM using this method is less accurate (Verdecchia et al 1998, Levy et al 1994). On the other hand, assessment of LVM by echocardiographic method is also a potent indicator of a cardiovascular event and most commonly used to assess patient with hypertension in clinic because of its lower cost. Moreover, in the past years, quantitative echocardiography has been established as a strong and accurate method to assess left ventricular mass, left ventricular wall thickness and left ventricular architecture.

Furthermore, the advantage of this procedure is that it enables serial non-invasive measurements to be performed on live and sedated animals to assess cardiac structure and function, before and after therapy.

1.3.8 Management of left ventricular hypertrophy

It is clinically important to recognize that LVH is a modifiable risk factor and that management is more complex than just blood pressure control.

1.3.8.1 Regression of left ventricular hypertrophy and blood pressure

The context of pathological hypertrophy has led to the hypothesis that prevention of the development of hypertrophy may be associated with preservation of ventricular function. Studies underline the beneficial effect of this prevention on ventricular function (Braz, 1999). In the context of high blood pressure, it has long been proved that reduction of blood pressure is effective in the regression of left ventricular hypertrophy both in humans with essential high blood pressure (Ciulla et al 2009) and in different animal models of arterial high blood pressure, such as SHRs (Vapaatalo et al 2000, Vrankova et al 2009, Songcang Chen et al 1998). High blood pressure in this animal model has been shown to be similar to human hypertension and one of the similarities is the development of a stable left ventricular hypertrophy accompanied by a progression to heart failure (Boluyt et al 1995).

Experimental studies have showed that hypertrophy of myocardium is not necessary to conserve a normal function of the heart in the context of hypertensive stress (Schiattarella et al 2015). As a result, suppression of this hypertrophy has appeared as a possible approach to decrease pressure overload-induced organs deterioration (Wu et al 2015). The regression of left ventricular hypertrophy requires an efficient decrease of arterial blood pressure in 24 hours a day. Thus, antihypertensive drugs regressing left ventricular wall thickness and mass should be on the first line of hypertensive heart disease management in Africa (Schmieder et al 1996, Dahlof et al 1992). It is important to well choose an antihypertensive drug when it comes to treat a subject with hypertension associated to hypertrophy of the left ventricle. Among

antihypertensive drugs, the most effectives when it comes to regress the hypertrophy of the left ventricle are angiotensin-converting-enzyme inhibitors and the angiotensin II receptor blockers followed by calcium channel antagonists (Schmieder et al 1996, Liebson et al 1995, Gottdiener et al 2014, Tapp et al 2010).

In animal models of high blood pressure, the process employed to induce hypertension and methods used to decrease elevated BP are important determinants when it comes to regress LVH (Pfeffer, 1994). Despite equal effectiveness to reduce blood pressure, in genetic model hypertensive, LVH may either decrease (methyldopa) (Sen et al 1974) guanethidine, and angiotensin-converting-enzyme [ACE] inhibitors); increase (minoxidil) (Fenje et al 1985); or remain unaffected (hydralazine) (Pfeffer, 1994).

On the other hand, in hypertensive patient, non-pharmacological means have been demonstrated to regress or reduce blood pressure with an effective decrease of echocardiographic LVH e.g. weight reduction and reduction in salt intake (Hammond et al 1986). Regarding reduction of left ventricular mass, it can be obtained in hypertensive patients with LVH without decreasing left ventricular ejection fraction or cardiac output (Balogun et al 1991). It has been showed in many meta-analyses (Schmieder et al 1996, Dahlof et al 1992) that common classes of antihypertensive drugs reduce left ventricular mass except for direct acting vasodilators (hydralazine) and some beta blockers with intrinsic sympathomimetic action (Liebson et al 1987). Angiotensin-converting-enzyme inhibitors have been showed in a meta-analysis of 109 treatment studies including 2,357 hypertensive patients to be the most efficient antihypertensive medications to decrease left ventricular mass (Araoye, 1996).

This analysis demonstrated that angiotensin-converting enzyme inhibitors, beta-blockers, and calcium channel blockers decrease left ventricular mass by decreasing wall thickness; diuretics decrease left ventricular mass by decreasing left ventricular volume (Araoye, 1996); and, alpha-adrenergic blockers (Mesa et al 1999) and direct-acting vasodilators (Colan, 1997) do not decrease left ventricular mass.

1.3.8.2 Anti-Hypertensive drugs and their effects on left ventricular remodelling in hypertensive patient

Important target under antihypertensive therapy is to regress hypertrophy of the left

ventricle and some drugs as the angiotensin converting enzyme inhibitors or angiotensin receptor blockers (ACEI/ARBs) are now accessible (Dahlof et al 1992). Indeed, reduction of left ventricular mass has some beneficial results such as improvement of filling of left ventricle, augmentation of coronary reserve, reduction of cardiovascular mortality and morbidity, improvement of mid-wall fractional shortening and an augmentation of electrophysiological stability. Some authors suggested that regression of LVH should be obtained under aggressive control of blood pressure (Ogah et al 2006).

Processing of high blood pressure greatly attenuates the development of LVH and considerably reduces incidence of heart failure. Current management of hypertensive heart disease include antihypertensive drugs that attenuate or reverse the remodelling process. Therefore, the choice of antihypertensive drugs is very important targeting regression or attenuation of LVH (Emdin et al 2015). The most commonly prescribed are the angiotensin-converting enzyme inhibitors and the angiotensin II receptor blockers followed by calcium channel antagonists (Schmieder et al 1996, Liebson et al 1995, Gottdiener et al 2007, Tapp et al 2010). Angiotensin-converting-enzyme inhibitors and angiotensin II receptor blockers have significant beneficial effects in left ventricular remodelling and regression of LVH (James et al 2014) because direct trophic effect of angiotensin II has been proved in the development of left ventricular hypertrophy (Wachtell et al 2007, Iriarte et al 1995, Mathew et al 2001).

Another class of antihypertensive drugs, direct vasodilators, such as minoxidil and hydralazine, despite the fact that they are stimulating sympathetic nervous system activity and renin-angiotensin-aldosterone system and controlling blood pressure, do not have effect on the regression of left ventricular hypertrophy (Katholi, 2000). However, it has been found that the vasodilator hydralazine was able to reduce the increase of left ventricular distension, chamber eccentricity, myocardial necrosis, deleterious interstitial remodelling and signs of cardiac decompensation (Tsotetsi et al 2001). Tsotetsi et al in experimental animal study supported the notion that an antihypertensive drug that decrease blood pressure is more relevant than the one that especially has a capacity to regress the development of LVH but attempt to prevent occurrence of heart failure associated with a dilation of left ventricle, an eccentric left ventricle, an advanced deterioration of myocardium and a deleterious interstitial remodelling in the context of high blood pressure (Tsotetsi et al 2001).

In addition, various human studies have demonstrated that efficient and aggressive control of blood pressure in patients with high blood pressure may prevent the occurrence of LVH or regress it when present. In experimental control conducted in subjects with LVH, cardiac mass and blood pressure were reduced by weight loss, a dietary sodium reduction and antihypertensive drugs (Frohlich et al 1992, MacMahon et al 1986, Jula et al 1994, Schmieder et al 1996, Hinderliter et al 2002). Common anti-hypertensive drugs used like diuretics, calcium channel blockers; beta blockers and Angiotensin-converting-enzyme (ACE) inhibitors promote regression in LVH. In comparison, despite efficient effects on blood pressure, regression of left ventricular hypertrophy is largely not present with direct vasodilators (eg, hydralazine or minoxidil) and some calcium channel blockers (Schmieder et al 1996, Julien et al 1990; Leenen et al 1992). Absence of effects of these drugs can be explained by a stimulation reflex of hormones promoting the development of LVH such as norepinephrine and angiotensin II (Leenen et al 1992). Hence choice of treatment may be extremely crucial when it comes to treat patient with hypertension and LVH (Emdin et al 2015).

A meta-analysis was conducted on data from double-blind, randomized, experimental controlled performed up until September 2002 that assessed, using echocardiography, effects of diuretics, beta-blockers, calcium channel blockers, angiotensin-converting-enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) on LVM in patients with hypertension (Klingbeil et al 2003). Results demonstrated that treatment targeting RAA system leads the most to an important decrease of LVM. Indeed, increase levels of circulating angiotensin II and aldosterone have been showed to be positively associated with an augmentation of LVM and decrease function of the left ventricle (Schlaich et al 1998, Schlaich et al 2000, Klingbeil et al 2003). Especially, there is a proof that angiotensin II has a profibrotic action on the myocardium in patients with hypertension giving explanation on the choice of angiotensin-converting-enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) as the most effective drug to treat LVH. In addition, clinical studies using ACE inhibitors (Agabiti-Rosei et al 1995, Garavaglia et al 1988) or ARBs (Kahan, 1998, Wachtell et al 2002) to treat mild hypertension in patients have proved that regression of LVH in this case is independent of the ability of these drugs to decrease blood pressure. Thus, an interruption of RAA system by a pharmacological therapy is suggested as an efficient approach to prevent hypertension and regress LVH,

protecting other target organs from angiotensin-II-induced damage in patients with hypertension and LVH.

1.3.8.2.1 Angiotensin-Converting-Enzyme (ACE) Inhibitors

Angiotensin-converting-enzyme (ACE) inhibitors have long been proved to be efficient to reduce blood pressure and to regress LVH both in humans with essential elevation of blood pressure (Ciulla et al 2009) and in various animal models of arterial hypertension, such as SHRs (Vapaatalo et al 2000). Indeed, ACE inhibitors have major beneficial effects in left ventricular remodelling and regression of LVH (James et al 2014) as demonstrated by Prasanna et al in a pilot study on LVH that echocardiography is a technique of choice to detect LVH and ACE inhibitors is the most efficient drug to improve LVH (Prasanna et al 2016). Angiotensin-converting-enzyme inhibitors and angiotensin II receptor blockers are especially efficient because angiotensin II has been demonstrated to have a direct trophic effect in the mechanism leading to development of left ventricular hypertrophy (figure1.4) (Wachtell et al 2007, Iriarte et al 1995, Mathew et al 2001). Moreover, angiotensin-converting-enzyme (ACE) is responsible for the production of angiotensin II (Ang. II), which correlates to left ventricular hypertrophy (Klara et al 2015).

Kokubo and colleagues showed that cardiac hypertrophy in hypertensive rats began to develop at the age of 4 weeks and systolic and diastolic dysfunction were important at 2 to 3 months of age (Kokubo et al 2005). Prevention of the development of cardiac and vascular remodelling during this phase has been proven by transient treatment with captopril (ACE inhibitor) (Julia et al 2015). Treatment with captopril has been showed to prevent an augmentation of blood pressure and heart rate observed in SHRs and to reverse entirely the increase of left ventricular weight index. These findings confirmed anterior findings obtained in studies using SHRs as animal model (Vapaatalo et al 2000, Vrankova et al 2009). Blockade of RAA system (Figure1.4) has been shown to be beneficial in patients with hypertension, acute myocardial infarction, chronic systolic heart failure, stroke and diabetic renal disease (Terry et al 2010). And many studies have demonstrated that angiotensin-converting-enzyme inhibitors delay the development of congestive heart failure (Mathew et al 2001).

1.3.8.2.2 Direct acting vasodilators, hydralazine

Direct vasodilators, such as minoxidil and hydralazine, although efficient with regard of blood pressure reduction, are inefficient in regressing LVH despite their action in the stimulation of renin-angiotensin-aldosterone system and sympathetic nervous system (Katholi, 2000). In SHRs, it has been demonstrated that hydralazine decreased systolic blood pressure at the level of WKY control values during the entire period of the study (Tsotetsi et al 2001). Furthermore, hydralazine, a direct acting vasodilator, has been demonstrated to not have any effect on LVH despite its antihypertensive actions (Norton et al 1997). Indeed, it has been found in animal studies using SHRs, that at an antihypertensive dose, hydralazine does not prevent or attenuate LVH (Pegram et al 1982, Jespersen et al 1985). Similar findings were demonstrated in transgenic (m REN 2) 27 rats with overexpression of the mouse renin 2d gene. These rats have severe elevation of blood pressure and a hypertrophy of the heart (Zolk et al1998).
Conversely, in many experimental studies it was found that hydralazine exacerbates LVH, as demonstrated by an augmentation of cardiac weight and media thickness (Tsoporis et al 1988). The ineffectiveness of this drug on LVH or the possibility that LVH becomes worse despite the antihypertensive action may be justified by the stimulation of renin-angiotensin-aldosterone system and sympathetic nervous system induced by this drug (Van Zwieten, 2000).

On the other hand, in the context of hypertension, left ventricular dilatation and an eccentric geometry reflects inappropriate remodelling of the left ventricle, in which despite heart growth, wall thickness–to–internal radius ratios are reduced and the goal of hypertrophy is attenuated (Tsotetsi et al 2001). Indeed, it has been shown that hydralazine given to SHRs although unable to influence the development of LVH, attenuate the development of left ventricular dilatation, chamber eccentricity, myocardial necrosis, deleterious interstitial remodelling, and signs of decompensation of the heart (Tsotetsi et al 2001). Mechanisms explaining absence of hydralazine action on the weight of the heart in the context of high blood pressure have not yet been demonstrated. Possible mechanisms include conversion of pressure to volume-overload in hypertrophy, reflex sympathetic over-activity following excessive

vasodilatation, and stimulation of the renin-angiotensin-aldosteron system (Tsotetsi et al 2001).

1.4 Summary problem statements

Hypertensive heart disease and dilated cardiomyopathy are the leading causes of heart failure in Africa. Hypertension is the most important risk factor of heart failure. The progression to heart failure in systemic hypertension is characterised by the initial development of compensated left ventricular hypertrophy (LVH). Compensated LVH refers to an adaptive response to an increase in ventricular stress on the walls of the heart and characterised by cardiomyocyte growth secondary to haemodynamic stress and or injury of the myocardium (Abhinav and Gerald, 2007).

Independently of blood pressure, excessive adrenergic activation is involved in the transition from LVH to heart failure in hypertension (Schlaich et al 2003). Indeed, β-adrenergic receptor blockade prevents the transition to cardiac dilation (Chan et al 2011). Moreover numerous studies from our group have attempted to identify the pathophysiology associated with β-adrenergic receptor activation in hypertensive and normotensive rats (Woodiwiss et al 2001; Badenhorst et al 2003; Veliotes et al 2005; Osadchi et al 2007a; Osadchi et al 2007b; Osadchi et al 2007c; Veliotes et al 2010; Booysen et al 2012; Michel et al 2017).

A decrease in blood pressure reduces LVH and has appeared as a possible plan to decrease pressure overload-induced end organ deterioration (Ciulla et al 2009; Wu et al 2015). Antihypertensive therapy has been showed to regress left ventricular wall thickness and mass in hypertensive heart disease in Africa (Schmieder et al 1996, Dahlof et al 1992). Some studies showed that regression of LVH following antihypertensive therapy is associated with improved left ventricular systolic performance, diastolic filling and reduces cardiovascular morbidity and mortality and thus improves the prognosis of heart failure (Achtell et al 2002; Verdecchia et al 2003). LVH is then considered as a significant and independent risk factor for the development of heart failure. It is important to keep in mind that LVH is a modifiable risk factor. Although directly related to systolic hypertension, other factors such as stimulation of the renin angiotensin- aldosterone and sympathetic nervous systems

play an important role in the pathogenesis. Therefore, the treatment is more complex than just blood pressure control and furthermore, the regression of left ventricle to a normal level does not mean that the structure and composition of the ventricle is normal (Tarazi et al 1987).

To the best of our knowledge, no study has investigated the effect of antihypertensive drugs, an angiotensin-converting-enzyme inhibitor and a non-specific vasodilator, on beta-adrenergic induced cardiac dilation. Therefore, the present study is designed to better understand the cardiac remodelling process and the transition from LVH to heart failure in the context of hypertension under beta adrenergic activation.

1.4.1 Aim of the study

To determine whether chronic antihypertensive treatments, an angiotensin-converting-enzyme inhibitor (ACE inhibitor) and a non-specific vasodilator, impact the development of β-adrenergic-induced cardiac remodelling in male spontaneously hypertensive rats (SHRs).

1.4.2 Objectives of the study

To determine:

1. Whether 6-month treatment with captopril, ACE inhibitor, reduces systolic blood pressure and prevents or regresses LVH in SHRs.
2. Whether 6-month treatment with hydralazine, a non-specific vasodilator, reduces systolic blood pressure and prevents or regresses LVH in SHRs.
3. Whether the prevention or regression of LVH, using captopril or hydralazine for 6 months, impacts cardiac dilation induced by chronic beta-adrenergic activation using isoproterenol.

Chapter 2: Materials and Methods

2.1. Ethics

The Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand approved the experimental protocol of this study (AESC approval number 2018/09/45/B) (See Appendix).

2.2. Study design

Thirty-one (31) one (1)-month-old male spontaneously hypertensive rats (SHRs) and nine (9) one (1)-month-old normotensive counterpart rats were used in the study. Rats were provided by the Central Animal Service (CAS) of the University of Witwatersrand. Animals were housed individually in standard rat cages and kept in the Central Animal Service, in a temperature-controlled room (25ºC) with a 12-hour light-dark cycle for the duration of the study. Rats were fed with standard food and water *ad libitum*.

2.2.1. Part I

After 2 weeks of habituation, SHRs were divided into three groups (each SHR group have similar body mass). Antihypertensive drugs were administered daily until 7-month-old to 2 of the groups according to Figure 2.1 below. One group (n=10) was treated with captopril, an angiotensin-converting-enzyme inhibitor, at a dose of 40 mg/kg/day (Paul et al 2007). Another group (n=9) was treated with hydralazine, a non-specific vasodilator, at a dose of 20 mg/kg/day (Spijkers et al 2011). Anti-hypertensive drugs were dissolved in a gelatine cube. The last group of SHRs (n=11) and the normotensive counterpart (n=9) were given a plain gelatine cube daily.

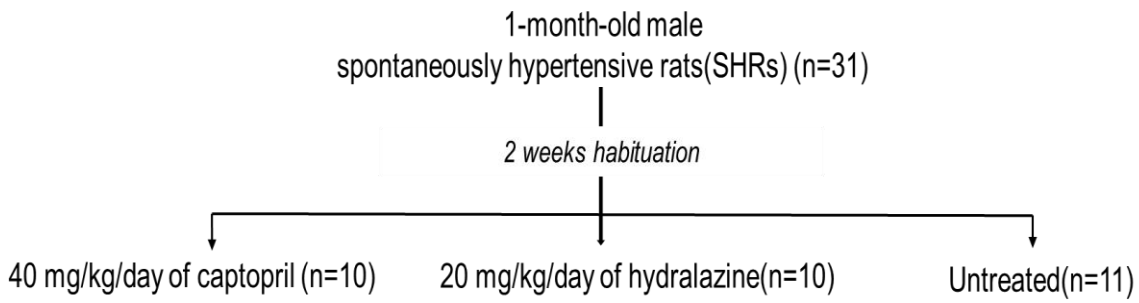


Figure 2.1: Study design: division of the 1-month-old and 2 weeks Spontaneously Hypertensive Rats into control group and antihypertensive treatment groups

The gelatine solution was prepared by adding 8 g of gelatine powder, 8 g of sugar (to help firm up the gels) and 10 ml of Bovril (to offer flavour to the gels) to 100 ml of boiled distilled water. The mixture was stirred to ensure that content was completely dissolved. Thereafter, concentration of each antihypertensive drug was added separately to quantities of the mixture and adjusted to body weight in order to ensure correct dosage (See Appendix B and C). The mixture was then left to cool and solidify at 4ºC, in an ice cube tray, before being administered to respective groups.

During this phase of the study, BP was measured in each rat once every 2 weeks using a tail-cuff system as indicated by Figure 2.2. At the end of the 6-month period, echocardiography was performed under anaesthesia.

2.2.2. Part II

At 7 months of age, treatment was stopped. Rats in each group received isoproterenol intraperitoneally (i.p.) for 5 months at a dose of 0.03 mg/kg/day to achieve chronic adrenergic activation. Due to the sensitivity to adrenergic stimulation (and to avoid deaths), rats were habituated to lower doses of isoproterenol. For the first week, rats received isoproterenol at a dose of 0.005 mg/kg/day, the second week 0.01 mg/kg/day, the third week 0.02 mg/kg/day; thereafter, a dose of 0.03 mg/kg/day was administered for a further 5 months. During this period, BP was measured in each rat once every 2 weeks using a tail-cuff system as indicated by Figure 2.2. At 12 months of age, echocardiography was performed under anaesthesia. Following 5 months of isoproterenol administration, rats were terminated at 12 months of age.

Part II:

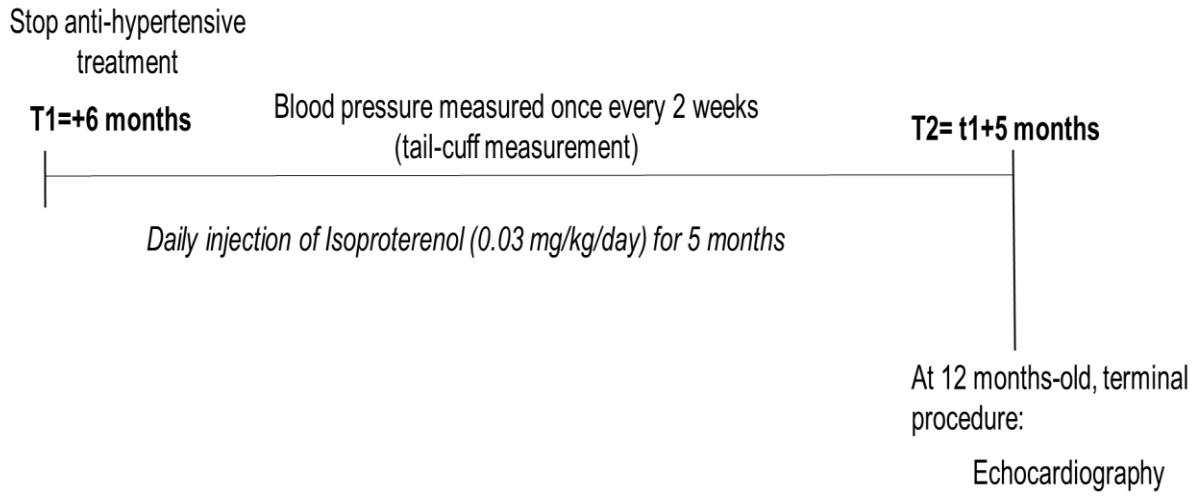


Figure 2.2: Timeline of daily injection of isoproterenol after stopping antihypertensive treatment and measurements performed in part II of the study (blood pressure measured once every 2 weeks and echocardiography after 5 months of daily injection of isoproterenol, at 12 month-old)

2.3. Mortality report

Thirty (30) 1-month-old male spontaneously hypertensive rats (SHRs) and nine (9) three-month-old normotensive rats (Wistar) were used in our study. Among rats that did not survive the entire period of our study and were not included in the sample numbers given in our results, five (5) HT died at between 33 to 45 weeks of age, three (3) HT+C died at between 39 weeks to 45 weeks of age, one (1) HT+H died at the age of 42 weeks of age and one (1) normotensive rat died at 35 weeks of age. Recorded rat deaths occurred during the second part of our study, and causes of death were related to isoproterenol administration (see Table 2.1). Therefore, these rats were excluded from the study.

Table 2.1 Mortality report indicating strain, age and cause of death in animals during the study

STRAIN	AGE	CAUSES OF DEATH
HT	33 weeks	Severe pulmonary oedema and cardiac compromise
HT	39 weeks	Haemorrhagic enteritis, cardiac hypertrophy
HT	40 weeks	Respiratory failure
HT	45 weeks	Pancreatitis and cardiopulmonary compromise
HT	40 weeks	Severe concentric hypertrophy of left ventricle with congestion of pulmonary tissue and diffuse centrilobular hepatosis
HT+C	41 weeks	Lung compromise and pancreatitis
HT+C	45 weeks	Pulmonary congestion and hemothorax
HT+C	39 weeks	Pulmonary petechiation and congestion, pancreatitis, corneal ulcer
HT+H	42 weeks	Dilated cardiomyopathy with pulmonary congestion and exudative pleuritic
NT	35 weeks	Cardiopulmonary compromise and pancreatitis

NT: Normotensive rat. HT: hypertensive rat untreated during the first 6 months of the study. HT+H: hypertensive rat previously treated with hydralazine. HT+C: hypertensive rat previously treated with captopril.

2.4 Measurements

2.4.1 Body, Heart and Left Ventricular weight

Weekly body weight was taken using a portable waterproof scale (Clover Scales PTY LTD, SA).

At termination, heart and left ventricular weights were obtained using a scale. Firstly, atrial and ventricles were emptied of post-mortem clots. Thereafter, aorta and pulmonary artery were dissected above their origin without including pericardium, mediastinal fat or lung. Measurement of left ventricle chamber size has been obtained after cross section at the midventricular level without including the septum Intraventricular. Heart weight and left ventricular weight adjusted to the body weight (HW/BW and LVW/BW) that mean the number of grams of heart tissue or left ventricular tissue respectively for every kilogram of body weight of the rat.

2.4.2 Blood pressure measurement

Blood pressures (BP) were assessed on conscious rats using a non-invasive blood pressure (NIBP) measurement tail-cuff system (NIBP250 BIOPAC Systems, CA, Inc.). BP was measured at midday (to avoid diurnal variation) once every two weeks from 8 weeks of age until 52 weeks of age (termination). Before the first measurement, rats were habituated to the procedure for 20 minutes per day 3 times a week in a Perspex restrainer. Thereafter, blood pressure was recorded while rats were restrained, using a tail-cuff sensor placed on the tail. Tail was kept warm (34 ºC) using a heating pad. NIBP tail-cuff system has a built-in pump that automatically inflates the tail-cuff to occlude the tail vessel. Once the inflation point is reached, the pump slowly deflates the cuff, resulting in a linear fall in pressure. Blood pressure and heart rate were recorded from at least two consecutive readings obtained for each rat.

2.4.3 Echocardiography

Echocardiography was at 7 months of age, after 6 months of antihypertensive treatment and at 12 months of age, after 5 months of isoproterenol injection. Prior to non-invasive echocardiographic measurement, animals were anaesthetized using isoflurane (2-5% oxygen (O_2)). Animals were kept unconscious under isoflurane at a constant concentration of 1%-2.5% for 20 to 30 minutes. For the procedure, rats were placed in the left lateral decubitus position on a heating pad to maintain a constant body temperature at 37 ºC, with the anterior chest hair shaved.

Echocardiographic measurements were acquired using the Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.) coupled with a high-resolution 10 MHz paediatric linear array transducer and ECG. Left ventricular dimensions were measured through two-dimensional (2D) guided M-mode echocardiography in the parasternal long-axis view, M-Mode images were obtained in the short axis of the heart as close to the mitral valve leaflet tips as possible in all rats. Left ventricular internal dimensions (leading edge method) and posterior wall thickness at the end of systole and diastole were analysed according to the American Society of Echocardiography convention (Sahn et al. 1981) (Figure 2.3). Left ventricular systolic chamber function was evaluated using endocardial fractional shortening (FSend), as per the equation below:

$$FSend = \frac{(LVEDD-LVESD)}{LVEDD} \times 100$$

Where

LVEDD= Left Ventricular End-Diastolic Diameter

LVESD= Left Ventricular End-Systolic Diameter

The Teichholz method was used to calculate left ventricular ejection fraction (EF) using end-systolic and end-diastolic volumes:

$$\mathrm{LVESV} = \frac{7\ \mathrm{LVESD}^3}{[2.4 + \mathrm{LVESD}]}, \quad \mathrm{LVEDV} = \frac{7\ \mathrm{LVEDD}^3}{[2.4 + \mathrm{LVEDD}]},$$

$$\mathrm{LV} - \mathrm{EF}_{\mathrm{Teich}}(\%) = \left[\left(\frac{\mathrm{LVEDV} - \mathrm{LVESV}}{\mathrm{LVEDV}}\right)\right] \times 100$$

Where LVEDV= Left ventricular end diastolic/systolic volume and LVESV = Left ventricular end diastolic/systolic volume (Jörg Stypmann et al 2009).

Left ventricular mass (LVM) was calculated from M-mode measurements using the Devereux formula (Devereux et al 1986): (LVM = 0.8 x [1.04 (Left ventricular end diastolic diameter + Left ventricular end diastolic septal wall thickness + Left ventricular end diastolic posterior wall thickness)^3 – (Left ventricular end diastolic diameter)^3] + 0.6).

Wall thickness (WT) was calculated as the sum of Intraventricular Septum Thickness (IVS) and Left Ventricular Posterior Wall Thickness (LVPW), in diastole or systole divided by 2. LV relative wall thickness (RWT) was calculated as the sum of IVS and LVPW in diastole divided by left ventricular end-diastolic diameter.

Moreover, LV diastolic function was assessed using a pulsed wave Doppler exam to determine mitral valve flow velocity at rest and tissue Doppler indexes (TDI). Pulse wave Doppler imaging was used to obtain the trans-mitral velocity measures during early (E) and late (atrial contraction) (A) period of LV diastolic inflow. Mitral inflow velocity as E/A was used as an index of myocardial relaxation (Figure 2.4). Left ventricular posterior wall Doppler early (E') and atrial (A') velocity of myocardial lengthening at the level of the mitral valve were determined. Left myocardial relaxation was indexed by E', left ventricular filling pressure was indexed by E/E' and left ventricular myocardial stiffness was indexed by E'/A' (Figure 2.5). E/A and E/E' represent two echocardiographic parameters to assess diastolic function. E' is more sensitive to relaxation than on preload and E/E' eliminates influence of relaxation on E wave explaining the sensitivity of E' to preload (Popovic et al 2018). Abnormal cut-offs values to identify a diastolic dysfunction are E': septal E' < 7 cm/sec, lateral E' < 10 cm/sec, average E/e' ratio > 14 (Nagueh et al 2016).

2.5 Data analysis

Data were expressed as means ± SD. Data analysis was performed with SAS software version 9.4 (SAS Institute, Cary, North Carolina). Body weight, heart weight, left ventricular weight, left ventricular weight adjusted for body weight and heart weight adjusted for body weight were analysed using one way ANOVA. The mixed model was used to analyse parameters measured between groups at various time points and interactive effect. Differences were considered statistically significant at $p \leq 0.05$.

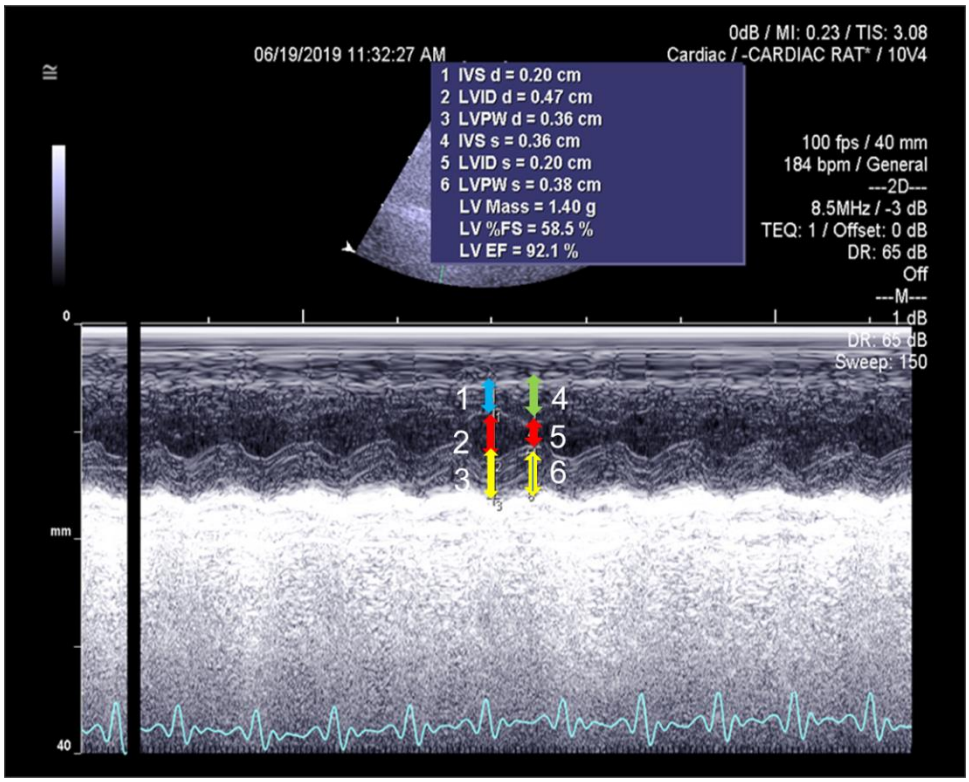


Figure 2.3: Two-dimensional M-mode guided echocardiographic view illustrating left ventricular dimensions and left ventricular systolic function. 1= intraventricular septum thickness at end-diastole (IVSd), 2= left ventricular internal dimension at end diastole (LVIDd), 3= left ventricular posterior wall thickness in diastole (LVPWd), 4= intraventricular septum thickness at end-diastole (IVSs), 5= left ventricular internal dimension at end systole (LVIDs), 6= LV Mass: left ventricular mass. LV%FS: endocardial fractional shortening. LVEF: left ventricular ejection fraction

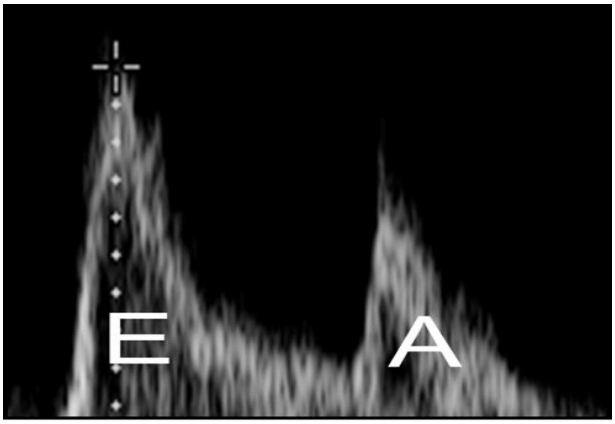


Figure 2.4: Example of an echocardiographic image of pulsed Doppler of the transmitral velocity showing the early (E) and late (atrial contraction) (A) period of LV diastolic inflow.

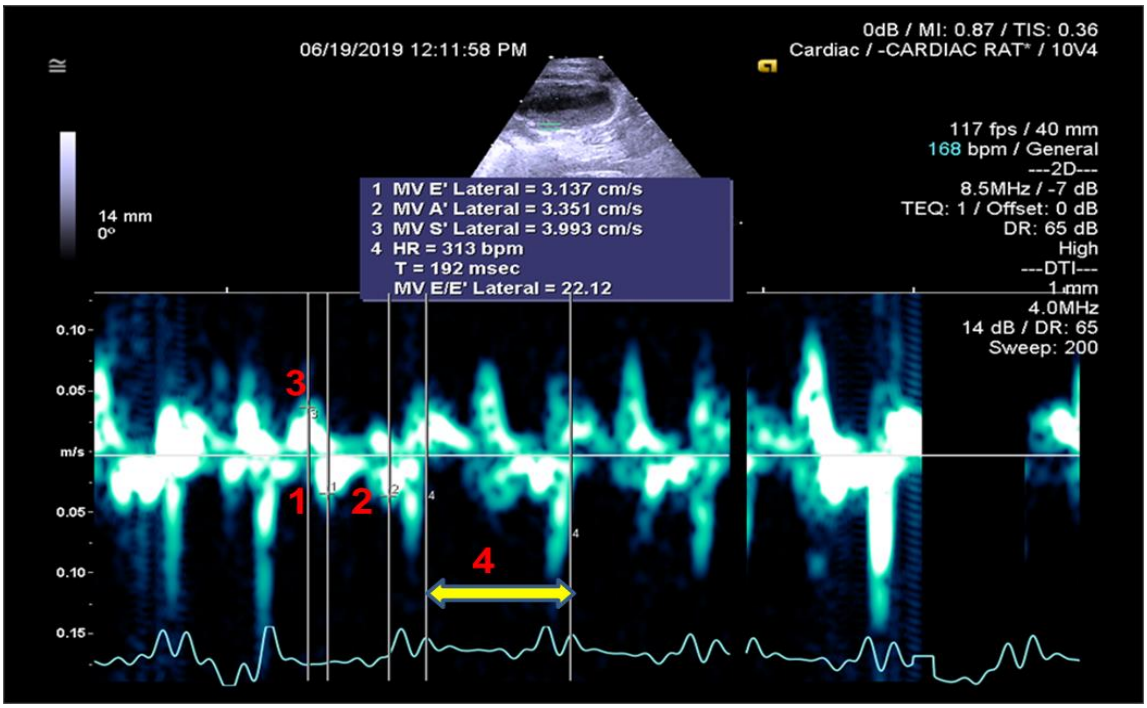


Figure 2.5: Septal and lateral mitral annular velocity measurements using tissue Doppler imaging (TDI) showing peak velocities during early (E') and late (atrial contraction) (A') diastole. HR: heart rate (beat per minute). T: time in mini second. MV E/E': MV E velocity divided by mitral annular E′ velocity. MV E': peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler**.** MV A': peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler**.** MV S': peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler.

Chapter 3: Results

3.1 Systolic or diastolic blood pressures in 7-month-old and 12-month-old normotensive and hypertensive rats

Table 3.1, 7-month-old systolic and diastolic blood pressures were significantly higher in hypertensive rats than normotensive rats ($p<0.001$). Area under the curve of the continuous measurement of systolic and diastolic blood pressures was significantly greater in hypertensive rats compared to normotensive rats ($p<0.001$). Systolic and diastolic blood pressures were significantly higher in hypertensive rats untreated compared to hypertensive rats treated with hydralazine or captopril ($p<0.001$). Systolic and diastolic blood pressures were similar in hypertensive rats treated with hydralazine or captopril compared to normotensive rats ($p<0.001$). Area under the curve of the continuous measurement of systolic and diastolic blood pressures were significantly greater in hypertensive rats compared to hypertensive rats treated with hydralazine or captopril ($p<0.001$).

The 12-month-old systolic and diastolic blood pressures were significantly higher in hypertensive rats compared to normotensive rats ($p<0.001$) (Table 3.1). Area under the curve of the continuous measurement of systolic and diastolic blood pressures were significantly greater in hypertensive rats compared to normotensive rats ($p<0.001$). Systolic and diastolic blood pressures were similar in hypertensive rats previously treated with hydralazine or captopril compared to hypertensive rats untreated during the first six months of the study. However, systolic and diastolic blood pressures were significantly higher in hypertensive rats previously treated with hydralazine or captopril compared to normotensive rats ($p<0.001$). Area under the curve of the continuous measurement of systolic and diastolic blood pressures were significantly greater in hypertensive rats compared to hypertensive rats previously treated with hydralazine or captopril ($p<0.001$).

At 7-months versus 12-months, in Table 3.1, area under the curve of the continuous measurement of systolic and diastolic blood pressures were significantly greater in the 4 experimental groups at 12 months compared to 7 months ($p<0.001$).

Table 3.1 Effects of six months antihypertensive treatment and 5 months of chronic administration of isoproterenol on blood pressure in 7-month-old and 12-month-old normotensive and hypertensive rats

	NT	**HT**	**HT+H**	**HT+C**
7-month-old	n=8	n=7	n=8	n=7
SBP (mmHg)	124 ± 8	185 ± 8[a]	122 ± 9	127 ± 6
DBP (mmHg)	76 ± 11	101 ± 10[a]	73 ± 109	77 ± 8
SBP (AUC)	2466 ± 59	3569 ± 108[a]	2421 ± 147	2507 ± 97
DBP (AUC)	1504 ± 95	2186 ± 114[a]	1535 ± 62	1565 ± 132
12-month-old	n=8	n=5	n=8	n=6
SBP (mmHg)	121 ± 5[a]	186 ± 10	186 ± 11[c]	182 ± 15[c]
DBP (mmHg)	72 ± 5[a]	112 ± 10	109 ± 8[c]	112 ± 11[c]
SBP (AUC)	5156 ± 124[c]	7576 ± 215[a, c]	6238 ± 180[b, c]	6182 ± 153[b, c]
DBP (AUC)	3090 ± 86[c]	4655 ± 124[a, c]	3824 ± 68[b, c]	3819 ± 180[b, c]

Data are expressed as mean ± SD. NT. Normotensive rat; HT, hypertensive rat untreated during the first six months of the study; HT+H, hypertensive rat treated with hydralazine; HT+C, hypertensive rat treated with captopril; SBP, systolic blood pressure; DBP, diastolic blood pressure; AUC, area under the curve. Mm Hg: millimeter of mercury. [a] $p<0.001$ vs other groups (mixed model). [b] $p<0.001$ vs HT (mixed model). [c] $p<0.001$ vs 7-month-old (mixed model).

3.2 Effects of chronic (5 months) isoproterenol administration on body weight, heart weight, left ventricular weight, left ventricular weight/body weight ratio and heart weight/body weight ratio in normotensive and hypertensive rats

In Table 3.2, 12-month-old normotensive rats were significantly heavier than hypertensive rats ($p<0.0001$). Heart weight and left ventricular weight in normotensive rats were lower compared to hypertensive rats ($p<0.01$ both). Heart and left ventricle weights were significantly greater in hypertensive rats previously untreated compared to normotensive or hypertensive rats previously treated with hydralazine or captopril ($p<0.01$ and $p<0.05$, respectively). Left ventricular weight adjusted to body weight (LVW-BW) and heart weight adjusted to body weight (HW-BW) were significantly greater in hypertensive rats compared to normotensive rats ($p<0.01$ both). Left ventricular weight adjusted to body weight (LVW-BW) and heart weight adjusted to body weight (HW-BW) were significantly lower in hypertensive rats treated with captopril compared to hypertensive rats ($p<0.05$).

Table 3.2 Effects of chronic (5 months) isoproterenol (ISO) administration on body weight, heart weight, left ventricular weight, left ventricular weight/body weight ratio and heart weight/body weight ratio in 12-month-old normotensive rats and hypertensive rats

	NT	**HT**	**HT+H**	**HT+C**
12-month-old	n=8	n=5	n=8	n=6
BW (g)	428±26[a]	370±30	363±28	368±34
HW (g)	1.21±0.06[a]	1.78±0.23	1.60±0.15[b]	1.51±0.11[b]
LVW (g)	0.56±0.02[a]	0.86±0.09	0.76±0.09[b]	0.69±0.04[b]
HW-BW	0.28±0.03[a]	0.48±0.06	0.44±0.05	0.41±0.02[b]
LVW-BW	0.13±0.01[a]	0.23±0.02	0.21±0.03	0.19±0.02[b]

Data are expressed as mean ± SD. NT: Normotensive rat. HT: hypertensive rat untreated during the first six months of the study. HT+H: hypertensive rat treated with hydralazine. HT+C: hypertensive rat treated with captopril. BW: body weight. HW: heart weight. LVW: left ventricular weight. LVW-BW: left ventricular weight adjusted to body weight. HW-BW: heart weight adjusted to body weight. [a] ,$p<0.01$ vs other groups (1-way ANOVA). [b] ,$p<0.05$ vs HT (1-way ANOVA).

3.3 Left ventricular chamber dimension in 7 and 12-month-old normotensive and hypertensive rats.

In Table 3.3, left ventricular mass calculated (LVMc) was significantly greater in hypertensive rats untreated during the first six months of the study compared to the three other groups ($p<0.0001$). Left ventricular end diastolic and systolic diameters, as well as wall thickness in systole were similar between the 4 experimental groups. Furthermore, wall thickness in diastole was significantly greater in hypertensive rats untreated during the first six months of the study compared to the three other groups ($p<0.0001$), as shown in Figure 3.1 panel A. Relative wall thickness was similar between the 4 experimental groups, as shown in Figure 3.2 panel A.

In Table 3.3, at 12 months of age, LVMc was significantly greater in hypertensive rats untreated during the first six months of the study compared to the three other groups ($p<0.0001$). Furthermore, LVMc was significantly greater in hypertensive rats previously treated with hydralazine compared to normotensive rats and hypertensive rats previously treated with captopril ($p=0.0038$ and $p=0.0131$, respectively). Left ventricular end-diastolic diameter was similar between the 4 experimental groups. However, left ventricular end-systolic diameter was significantly greater in hypertensive rats previously treated with captopril compared to hypertensive rats untreated during the first six months of the study ($p=0.05$), and in normotensive rats compared to hypertensive rats untreated during the first six months of the study and hypertensive rats previously treated with hydralazine ($p=0.0015$ and 0.0017, respectively). In systole, wall thickness was significantly greater in hypertensive rats untreated during the first six months of the study and hypertensive rats previously treated with hydralazine compared to normotensive rats and hypertensive rats previously treated with captopril. Wall thickness in diastole and relative wall thickness, as shown in panel B of Figure 3.2 and Figure 3.3, respectively, were significantly greater in hypertensive rats untreated during the first six months of the study and hypertensive rats previously treated with hydralazine compared to normotensive rats ($p<0.0001$ and $p<0.0001$; $p=0.0008$ and $p=0017$, respectively).

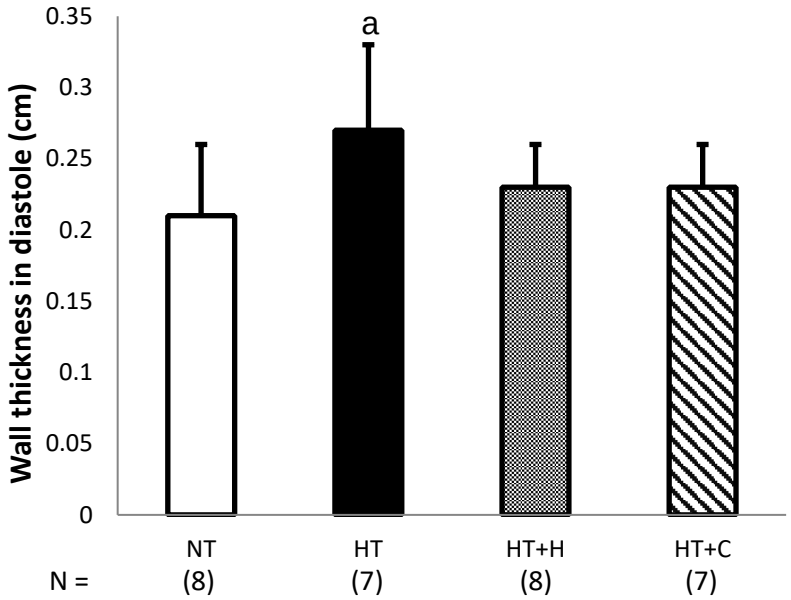
At 7-months versus 12-months, Table 3.3, LVMc was significantly increased in hypertensive rats untreated during the first six months of the study and hypertensive rats previously treated with hydralazine ($p<0.0001$ and $p<0.0001$, respectively). Left ventricular mass calculated was significantly lower in hypertensive rats previously treated with captopril ($p=0.05$). Moreover, wall thickness in systole was significantly increased at 12 months in hypertensive rats untreated during the first six months of the study and hypertensive rats previously treated with hydralazine ($p=0.038$ and $p<0.0001$, respectively). Left ventricular end-systolic diameter was significantly increased at 12 months compared to 7 months in normotensive rats and hypertensive rats previously treated with captopril ($p<0.0001$ and $p<0.0001$, respectively). Furthermore, left ventricular end-diastolic diameter was significantly increased in the 4 groups ($p<0.0001$). In Figure 3.1, wall thickness in diastole was significantly increased in the hypertensive rats untreated during the first six months of the study and hypertensive rats previously treated with hydralazine ($p=0.0109$ and $p=0.0003$, respectively).

Table 3.3 Effect of 6 months antihypertensive treatment followed by 5 months of chronic administration of isoproterenol on left ventricular dimensions assessed in vivo in 7 month-old and 12 month-old normotensive and hypertensive rats

	NT	HT	HT+H	HT+C
7-month-old	n=8	n=7	n=8	n=7
LVEDD (cm)	0.65±0.08	0.63±0.06	0.60±0.04	0.57±0.05
LVESD (cm)	0.35±0.05	0.29±0.05	0.34±0.05	0.28±0.05
WTs (cm)	0.32±0.06	0.37±0.06	0.32±0.06	0.34±0.05
LVMc (g)	0.74±0.03	0.91±0.08[a]	0.74±0.05	0.78±0.04
12-month-old	n=8	n=5	n=8	n=6
LVEDD (cm)	0.77±0.05[c]	0.69±0.09[c]	0.67±0.10[c]	0.67±0.08[c]
LVESD (cm)	0.52±0.05[b, c, d]	0.37±0.05	0.39±0.06	0.48±0.08[b, c]
WTs (cm)	0.29±0.05[b, d]	0.40±0.05[c]	0.39±0.06[c]	0.31±0.05[b, d]
LVMc (g)	0.69±0.06[d]	1.01±0.1[a, c]	0.89±0.04[c]	0.70±0.06[c, d]

Data are expressed as mean ± SD. NT: Normotensive rats. HT: hypertensive rat untreated during the first six months of the study. HT+H: hypertensive rat treated with hydralazine. HT+C: hypertensive rat treated with captopril. LVEDD: Left Ventricular End Diastolic Diameter. WTs: Wall Thickness in systole. LVMc: Left Ventricular Mass Calculated. LVESD: Left Ventricular End Systolic Diameter. [a] ,$p<0.05$ vs other groups (mixed model). [b] $p<0.05$ vs HT (mixed model). [c] $p<0.05$ vs 7-month-old (mixed model). [d] $p<0.05$ HT+H (mixed model).

A



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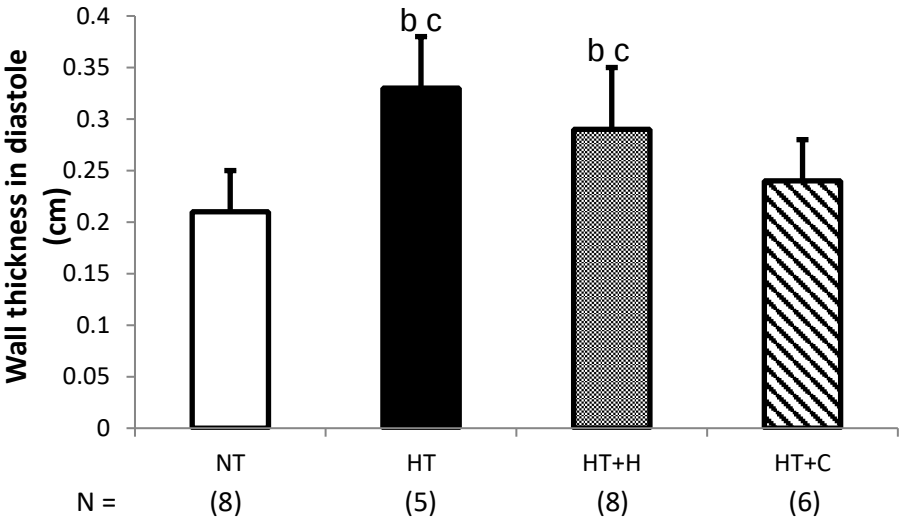
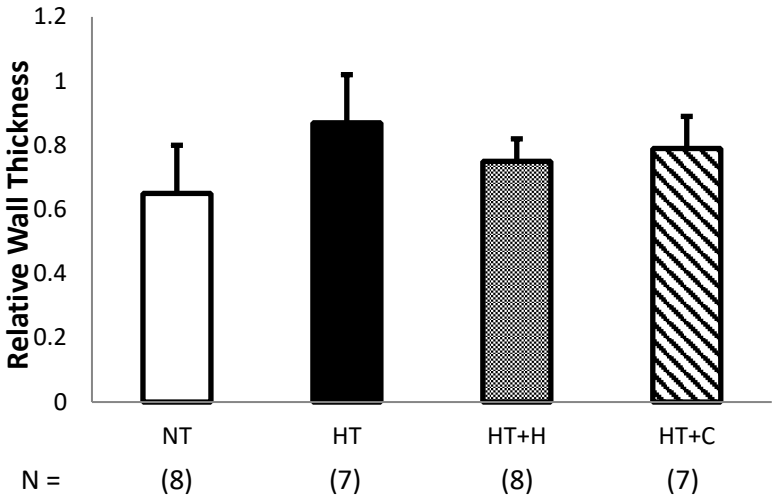


Figure 3.1: Effects of 6 months antihypertensive treatment and 5 months of chronic administration of isoproterenol on wall thickness in diastole. Data are expressed as means ± SD. Panel A represents wall thickness in diastole assessed in vivo in 7-month-old normotensive and hypertensive rats after 6 months of antihypertensive treatment. Panel B represents wall thickness in diastole assessed in vivo in 12-month-old normotensive and hypertensive rats after 5 months of chronic administration of isoproterenol. NT: Normotensive rat. HT: hypertensive rat untreated during the first six

months of the study. HT+H: hypertensive rat treated with hydralazine. HT+C: hypertensive rat treated with captopril. [a] $p<0.05$ vs other groups (mixed model). [b] $p<0.05$ vs NT (mixed model). [c] $p<0.05$ vs 7-month-old (mixed model).

A



B

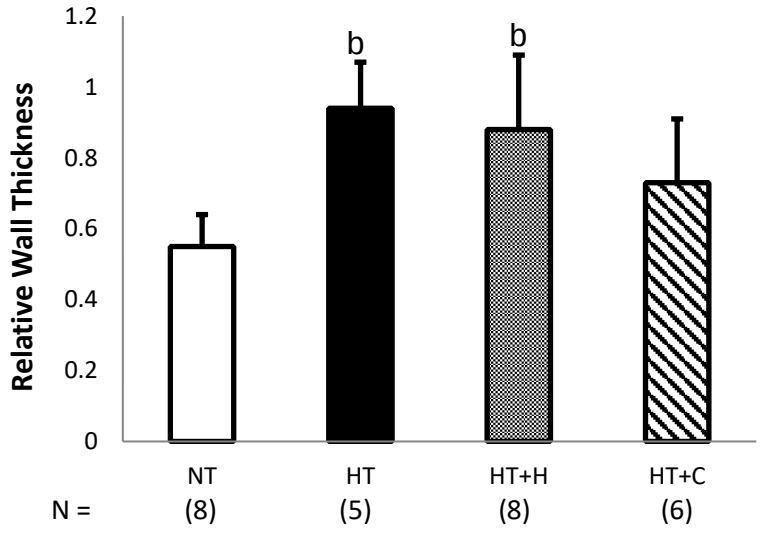


Figure 3.2: Effects of 6 months antihypertensive treatment and 5 months of chronic administration of isoproterenol on relative wall thickness in diastole. Data are expressed as means ± SD. Panel A represents relative wall thickness in diastole assessed in vivo in 7-month-old normotensive and hypertensive rats after 6 months of antihypertensive treatment. Panel B represents relative wall thickness in diastole assessed in 12-month-old normotensive and hypertensive rats after 5 months of chronic administration of Isoproterenol. NT: Normotensive rat. HT: hypertensive rat

untreated during the first six months of the study. HT+H: hypertensive rat treated with hydralazine. HT+C: hypertensive rat treated with captopril. [b] $p<0.05$ vs NT (mixed model).

3.4 Left ventricular systolic function in 7 and 12-month-old normotensive and hypertensive rats.

At 7 months of age, ejection fraction and endocardial fractional shortening were similar in all 4 groups following antihypertensive treatment (Figure 3.3 and Table 3.4). Interestingly, following isoproterenol administration at 12 months of age, the ejection fraction was significantly lower in hypertensive rats previously treated with captopril and hypertensive rats previously treated with hydralazine compared to hypertensive rats untreated during the first six months of the study ($p=0.0138$ and $p=0.0013$, respectively).Furthermore, ejection fraction was significantly lower in normotensive rats compared to hypertensive rats previously treated with hydralazine ($p=0.044$) (Table 3.4). Figure 3.3, panel B demonstrates that at 12 months of age, following isoproterenol administration, endocardial fractional shortening was significantly lower in hypertensive rats previously treated with captopril compared to hypertensive rats untreated during the first six months of the study and hypertensive rats previously treated with hydralazine ($p=0.0034$ and $p=0.0214$, respectively). In addition, endocardial fractional shortening was significantly lower in normotensive rats compared to hypertensive rats untreated during the first six months of the study and hypertensive rats previously treated with hydralazine ($p=0.0141$ and $p=0.0975$, respectively).

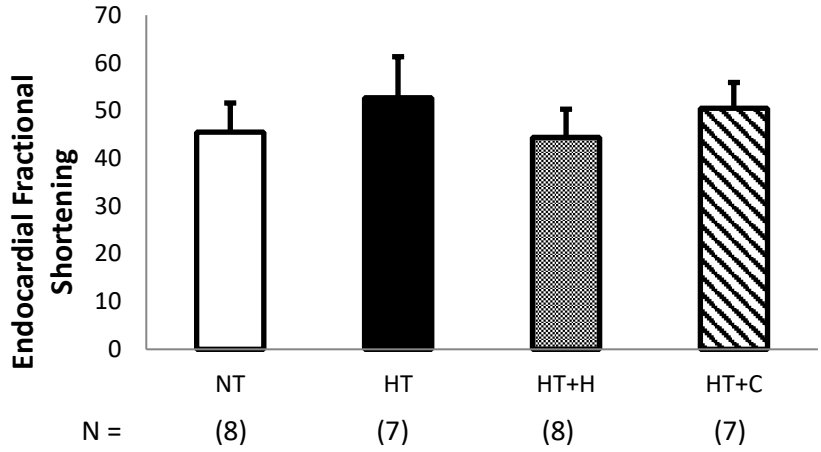
When comparing 7 versus 12 months of age, ejection fraction was significantly lower in the 4 experimental groups at 12 months compared to 7 months ($p<0.0001$) (Table 3.4) and endocardial fractional shortening was significantly lower in normotensive and hypertensive rats previously treated with captopril ($p<0.0001$ and $p<0.0001$, respectively) (Figure 3.3).

Table 3.4 Effects of 6 months antihypertensive treatment followed by chronic (5 months) isoproterenol (ISO) administration on ejection fraction in 7 and 12-month-old normotensive and hypertensive rats.

	NT	HT	HT+H	HT+C
7-month-old	n=8	n=7	n=8	n=7
EF (%)	82.2±4.8	84.8±6.6	81.9±4.1	83.4±7.6
12-month-old	n=8	n=5	n=8	n=6
EF (%)	31.9±6.2[b, c]	39.7±1.5[c]	41.4±5.2[c]	26.5±6.2[a, b, c]

Data are expressed as mean ± SD. NT: Normotensive rats. HT: hypertensive rat untreated during the first six months of the study. HT+H: hypertensive rat treated with hydralazine, HT+C: hypertensive rat treated with captopril, EF: Ejection Fraction. [a] $p<0.05$ vs HT (mixed model). [b] $p<0.05$ vs HT + H (mixed model), [c] $p<0.05$ vs 7-month-old (mixed model).

A



B

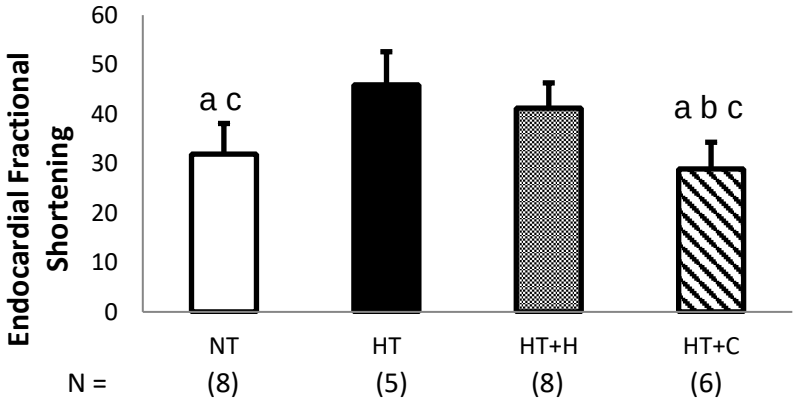


Figure 3.3: Effect of 6 months antihypertensive treatment and 5 months of chronic administration of isoproterenol on Endocardial Fractional Shortening. Data are expressed as means ± SD. Panel A represents Endocardial Fractional Shortening assessed in vivo in 7-month-old normotensive and hypertensive rats after 6 months of antihypertensive. Panel B represents Endocardial Fractional Shortening assessed in 12-month-old normotensive and hypertensive rats after 5 months of chronic administration of isoproterenol. NT: Normotensive rats. HT: hypertensive rats untreated during the first six months of the study. HT+H: hypertensive rat treated with hydralazine. HT+C: hypertensive rat treated with captopril. Fsend: Endocardial Fractional Shortening. %: percentage. [a] $p<0.05$ vs HT (mixed model). [b] $p<0.05$ vs HT + H (mixed model). [c] $p<0.05$ vs 7-month-old (mixed model).

3.5 Left Ventricular diastolic function in 7 and 12-month-old normotensive and hypertensive rats

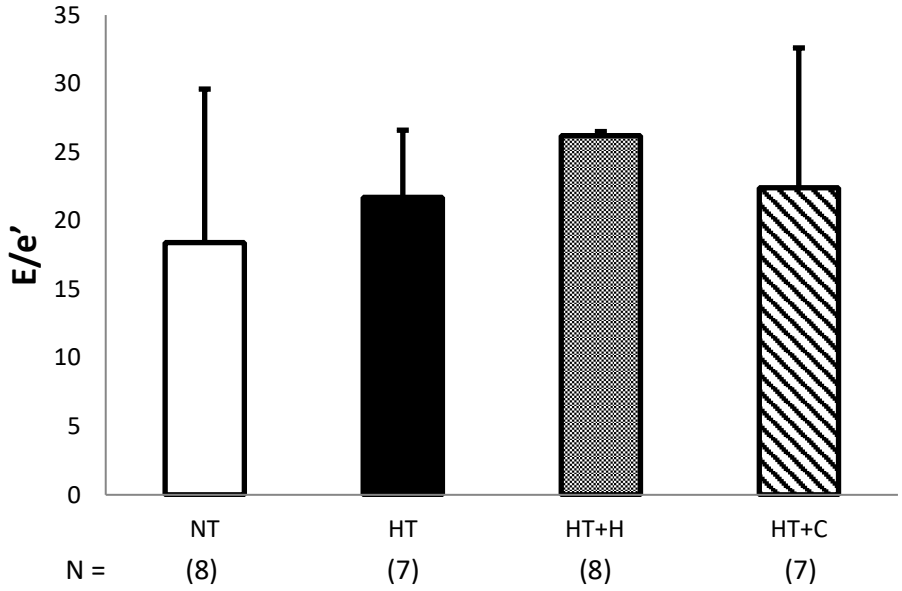
At 7 months of age, following 6 months antihypertensive treatment, the ratio of early (E) to late (atrial contraction) (A) mitral inflow (E/A) (index of myocardial relaxation), the peak velocity during early (e') and late (atrial contraction) (a') diastole and index of left ventricular filling pressures (E/e') were similar between the 4 experimental groups (Table 3.5 and Figure 3.4 panel A). At 12 months of age, following chronic isoproterenol administration, index of left ventricular filling pressure (E/e') was significantly greater in hypertensive rats untreated during the first six months of the study compared to normotensive rats ($p<0.05$) as shown in Figure 3.4 panel B. When comparing 7 versus 12 months of age, index of left ventricular filling pressure was significantly increased in hypertensive rats untreated during the first six months of the study at 12 months versus 7 months of age ($p=0.02$) as shown in Figure 3.4.

Table 3.5 Effects of 6 months antihypertensive treatment followed by chronic (5 months) isoproterenol (Iso) administration on E/A and e'/a' in 7 and 12-month-old normotensive and hypertensive rats.

	NT	**HT**	**HT+H**	**HT+C**
7-month-old	n=8	n=7	n=8	n=7
E/A	2.99±0.87	2.95±0.55	2.59±0.53	2.69±0.78
e'/a'	1.04±0.95	1.17±0.59	1.12±0.30	1.07±0.63
12-month-old	n=8	n=5	n=8	n=6
E/A	3.42±0.57	2.69±0.79	3.72±1.14	2.63±0.27
e'/a'	1.72±1.06	0.58±0.08	1.07±0.81	1.32±0.46

Data are expressed as mean ± SD. NT: Normotensive rat. HT: hypertensive rat untreated during the first six months of the study. HT+H: hypertensive rat treated with hydralazine. HT+C: hypertensive rat treated with captopril. E/A: ratio of early-to-late diastolic filling. e'/a': ratio of early-to-late mitral annulus velocity.

A



B

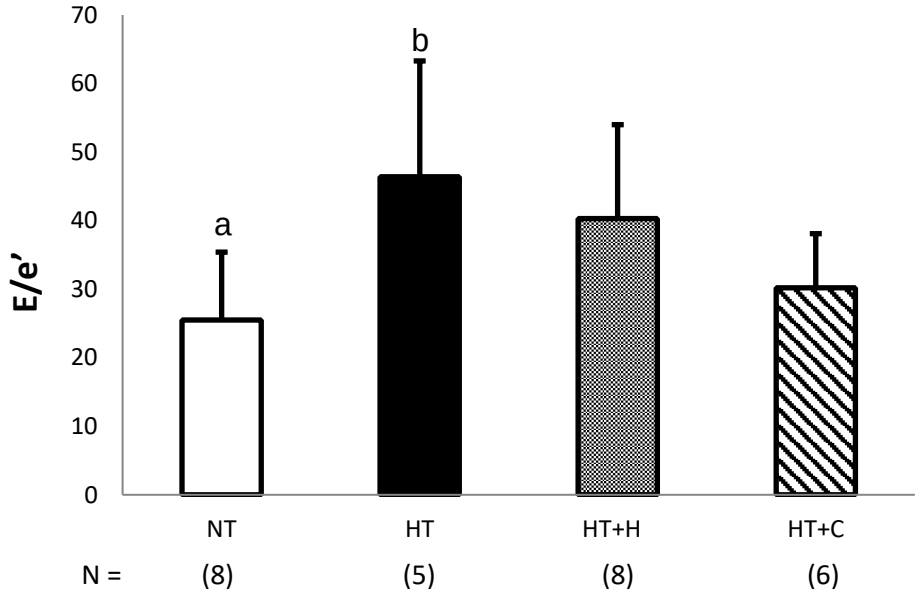


Figure 3.4: Effect of 6 months antihypertensive treatment and 5 months of chronic administration of isoproterenol on index of left ventricular filling pressures (E/e'). Data are expressed as means ± SD. Panel A represents E/e' assessed in vivo in 7-month-old normotensive and hypertensive rats after 6 months of antihypertensive treatment. Panel B represents E/e' assessed in 12-month-old normotensive and hypertensive rats

after 5 months of chronic administration of isoproterenol. NT: Normotensive rats. HT: hypertensive rat untreated during the first six months of the study. HT+H: hypertensive rat treated with hydralazine. HT+C: hypertensive rat treated with captopril. E/e': index of left ventricular diastolic filling pressure. [a] p=0.05 vs HT (mixed model). [b] p=0.02 vs 7-month-old (mixed model).

Chapter 4: Discussion

Our study was the first to evaluate effects of antihypertensive medication on ß-adrenergic-induced LVH and the progression to cardiac dilation. The main findings of the current study show that 7-month-old hypertensive rats developed LVH without systolic or diastolic dysfunction, suggesting a compensation process. Both antihypertensive drugs reduced blood pressures and maintained left ventricle to a similar extent than normotensive rats, confirming that blood pressure is the main factor involved in developing compensated LVH. At 12-months of age, hypertensive rats continued to develop LVH but with signs of diastolic dysfunction, suggesting a progression to cardiac dilation with ß-adrenergic-receptor activation. Regression of LVH with a non-specific vasodilator before ß-adrenergic-receptor activation did not hamper the development of LVH in hypertensive rats. However, regression of LVH with an angiotensin converting enzyme inhibitor before Beta-adrenergic receptor activation prevented the development of LVH in hypertensive rats to a similar extent as in normotensive rats. These results together suggest that activation of RAA system independent of blood pressure may be detrimental in the development of ß-adrenergic-induced cardiac remodeling.

Defined as an adaptive response of the cardiac muscle to increase the workload of the heart, the development of LVH stabilizes wall tension with a normal systolic and diastolic function of the ventricle (Rowlands, 1981, Devereux et al 1983, White et al 1989, Verdecchia et al 1990, Mancia et al 1997). Human studies have shown that hypertrophy of the LV is causally related to high blood pressure (Satish et al 2004). In 5 or 6-week-old SHRs, blood pressure increases to reach systolic blood pressures of 180–200 mm Hg. In untreated SHRs, LVH is found in all studies, and many rats progress to heart failure between 18 and 24 months old (Yigal al 1998). In agreement with the concept of compensated hypertrophy, the present study demonstrated the development of LVH with normal systolic and diastolic functions in hypertensive rats at 7 months. The development of systolic and diastolic dysfunction, however, is more controversial. In human studies, Pavlopoulos et al showed that diastolic dysfunction was present early before the occurrence of hypertensive disease and before any

remodelling of the left ventricle or important modification in left ventricular mass (Pavlopoulos et al 2008). Aeschbacher et al were in agreement with this previous study showing that diastolic dysfunction may be present before any LVH in patients in the context of systemic hypertension (Aeschbacher et al 2001; Wachtell et al 2000). Systolic dysfunction is not frequent with a worse prognosis (Anguita Sanchez 1999).

The regression of LVM with an antihypertensive drug depends on the type of therapy used. In several human studies, adequate and aggressive BP control in hypertensive patients may prevent the development of LVH and even reverse it when developed (Satish et al 2004). In the present study, administration of an ACE inhibitor decreased blood pressure and the development of LVH. Activation of RAA system has been shown, in many studies, to participate in high blood pressure and the development of LVH (Klara et al 2015, Alan et al 2006, Dzau, 1993). Consequently, ACE inhibitors have been clinically used as one of the main anti-hypertensive treatments (Ciulla et al 2009). Indeed, in addition to drop blood pressure, ACE inhibitors induce regression of LVH) independently of the ability of these drugs to decrease blood pressure (Agabiti-Rosei et al 1995, Garavaglia et al 1988. Similar results have been found in SHR (Vapaatalo et al 2000).

Vasodilators such as hydralazine are efficient blood pressure and peripheral vascular resistance reducing agents due to their direct action via arterial dilation on smooth muscles of arterioles probably by preventing oxidation of nitric oxide (Li Y et al 2010; Mary Anna Labato, 2015). However, they are ineffective in regression of LVH (Katholi, 2000). Indeed, human studies demonstrate that regression of LVH is largely absent with hydralazine, despite adequate BP control (Schmieder et al 1996, Julien et al 1990, Leenen et al 1992). The ineffectiveness of hydralazine in these studies is likely due to reflex stimulation of norepinephrine and angiotensin II, which may directly induce LVH (Dzau. 1993, Leenen et al 1992). In a SHR study, it has been demonstrated that hydralazine decreased systolic blood pressure at the level of WKY control values during the entire period of the study (Tsotetsi et al 2001). Numerous rodent studies have shown that an antihypertensive dose of hydralazine does not prevent or attenuate LVH (Pegram et al 1982, Jespersen et al 1985, Norton et al 1997). In contrast, numerous experimental studies have found that hydralazine exacerbates LVH, as demonstrated by an augmentation of cardiac weight and media thickness (Tsoporis et al 1988). The ineffectiveness of this drug on LVH or the possibility that LVH becomes

worse despite the antihypertensive action may be justified by stimulation of the RAA system and SNS (Van Zwieten, 2000). In our study, hydralazine given to hypertensive rats at an antihypertensive dose decreased blood pressure and LVH regressed in hypertensive rats at the same level than normotensive rats. Indeed, wall thickness and left ventricular mass were not different than those of normotensive rats before administration of isoproterenol. Interaction of an antihypertensive drug with RAA system, SNS, and decreased blood pressure impacts the regression of LVH. In addition, LVH regression or prevention are determined not only by the initial blood pressure values but also by the duration and intensity of therapy as well as the degree of LVH at the beginning of therapy (Van Zwieten, 2000). In our study, LVH regression obtained with hydralazine may be explained by the level of blood pressure at the beginning of our treatment and by the duration and intensity of our treatment. The latter has not been assessed in our study and should be investigated for better understanding.

In pressure overload, it has been shown that mechanisms involved in the development of cardiac dilation follow compensated hypertrophy. Progression of cardiac hypertrophy to cardiac dilation involves beta-adrenergic receptor activation independent of BP (Badenhorst et al 2003). Isoproterenol, a ß-adrenergic receptor agonist, is well known to produce cardiac hypertrophy (Tipnis et al 1989; Teerlink et al 1994). In normotensive rats, a single injection of a necrotic dose (that do not induce a myocardial necrosis) of isoproterenol progressively induces an enlargement of the left ventricle out of the normal proportion mass (Teerlink et al 1994). In addition, a chronic non-necrotic dose of isoproterenol induces left ventricular dilatation in normotensive rats (Woodiwiss et al 2001; Boyssen et al 2011). Indeed, Michel and colleagues showed that chronic administration of isoproterenol (0.04 mg/kg/day for 6 months) induced in male SHRs cardiac hypertrophy and dilation of the heart (Michel et al 2017). In normotensive rats, a single injection of isoproterenol progressively induces an enlargement of the left ventricle out of the normal proportion mass (Teerlink et al 1994). In addition, a chronic non-necrotic dose of isoproterenol induces left ventricular dilatation in normotensive rats (Woodiwiss et al 2001; Boyssen et al 2011). Moreover, Osadchii and colleagues demonstrated for the first time that chronic ß-adrenergic-receptor activation in a normal heart produces cardiac dysfunction due to left ventricular dilatation primarily (Osadchii et al 2007). Left ventricular dilatation is a

precursor of cardiac dysfunction induced by ß-adrenergic-receptor activation in normal heart. Chronic ß-adrenergic-receptor activation induced a cardiac dysfunction independently of intrinsic myocardial contractility changes and necrosis but through a mechanism associated with interstitial and chamber remodelling which is left ventricular dilatation (Osadchii et al 2007). Hence, our data are in agreement with previous animal studies. Indeed, in the present study, normotensive rats developed left ventricular dilatation following chronic ß-adrenergic-receptor activation that can explain a cardiac dysfunction noted after 5 months of isoproterenol injection. This effect is independent of blood pressure effect in normotensive rats.

In the present study, chronic ß-adrenergic-receptor activation, using isoproterenol, worsened LVH already developed during the first 6 months of the study in hypertensive rats untreated during the first 6 months of the study. In addition, chronic ß-adrenergic-receptor activation induces eccentric remodelling as noted by increased LVEDD in hypertensive rats. These results are in agreement with the concept stipulating that β-adrenoreceptor activation promotes the transition from cardiac hypertrophy to cardiac dysfunction in hypertension (Badenhorst et al 2003). Sympathetic nervous system may play an important role especially via release circulating catecholamine that has demonstrated to have trophic action (John et al 2001). Locally, an increased cardiac sympathetic neurotransmission plays a part in increase left ventricular mass in hypertension (Schlaich et al 2003). Moreover, it has been showed that in patients with hypertension, the presence of LVH is related to increase sympathetic nervous activity in muscles (John et al 2001). In pathological hypertrophy, neuroendocrine factors released stimulate LVH that may progress to maladaptive forms such as concentric or eccentric hypertrophy (Jop et al 2013). Thus, adrenergic receptors may activate many intracellular pathways leading to nuclear responses of myocardiocyte in cardiac hypertrophy and vascular remodelling in the context of hypertension.

However, the same dose of isoproterenol, that produced dilation of the left ventricle in normotensive, only worsen hypertrophy in hypertensive rats suggesting that SHRs are less sensitive than normotensive rats to ß-adrenergic-receptor activation. One possible explanation is that left ventricular hypertrophy may protect against the development of dilation induced by isoproterenol. Chronic ß-adrenergic-receptor activation also induced systolic dysfunction as noted by the decreased EF. However, ventricular contraction indexed by FSend was still normal. Despite the fact that it is difficult to

dissociate effects of chronic ß-adrenergic-receptor activation from those of an increased afterload induced by hypertension, chronic ß-adrenergic-receptor activation may be responsible for the decrease systolic cardiac function as shown in normotensive. Badenhorst and colleagues have suggested that cardiac dysfunction is caused primarily by chronic β adrenergic activation on chamber remodelling in hypertensive LVH (Badenhorst et al 2003). Their results sustained the first concept proposed by Cohn (Cohn, 1995) and thereafter outcomes of human and animal studies stipulating that dilatation of the heart is the first step in the occurrence of left ventricular dysfunction (Vasan et al 1997, Norton et al 2002).

In the present study, discontinuation of treatment led blood pressure to increase at the same level than what observed in hypertensive rats with both anti-hypertensive drugs. This observation is in agreement with data on SHR showing an increase in blood pressure after discontinuation of hydralazine treatment (Smeda et al 1988). Studies in human and rodent have shown a prolonged decrease of blood pressure and a persistence suppression of left ventricular hypertrophy after discontinuation of captopril treatment (Songcang et al 1998, Harrap et al 1990, Wu et al 1993). The persistent low blood pressure may be related to a reduction in peripheral vascular resistance without any changes in the level of circulating renin and angiotensin (Harrap et al 1990). Accumulation of antihypertensive factors during the early period of treatment may also explain the persistent low blood pressure. Indeed, accumulation of bradykinin after using ACE inhibitor may explain a prolonged decrease in blood pressure and an improvement of cardiac, vascular and structural changes observed in SHR (O'Sullivan et al 1995). In our study, discontinuation of captopril followed by a chronic ß-adrenergic-receptor activation, using isoproterenol, led to an increase blood pressure. Indeed, isoproterenol, via a beta-adrenergic receptor stimulation, activates renin-angiotensin-aldosteron system, one of the factor involved in the pathogenesis of hypertension, (Himori et al 1980), leading to an increase peripheral resistance and then hypertension which may explain the increase of blood pressure at a hypertensive level after discontinuation of captopril and under isoproterenol injection.

Following the regression of LVH obtained with hydralazine in the present study, chronic ß-adrenergic-receptor activation induced eccentric left ventricular hypertrophy with systolic dysfunctions in a similar way than what observed in hypertensive rats untreated during the first 6 months of the study. It suggests that regression of LVH

obtained with hydralazine does not prevent the development of eccentric LVH and cardiac dilation in hypertensive rats previously treated with hydralazine.

LVH has been defined as a risk factor for the development of cardiac dilation; and reduction of LVH may improve prognosis of patients (Achtell et al 2002; Verdecchia et al 2003). However, the present study suggests that reduction of LVH via reduction of BP, using a non-specific vasodilator, does not ameliorate prognosis in SHR. On the other hand, under an antihypertensive therapy using an angiotensin-converting-enzyme inhibitor that reduced blood pressure and concentric LVH to a normotensive level, hypertensive rats developed a similar phenotype under isoproterenol injection than what observed in normotensive rats. The development of cardiac dilation following ß-adrenergic-receptor activation in hypertensive rats previously treated with captopril is evident despite an increase in blood pressure after stopping treatment suggesting that isoproterenol effect is blood pressure independent as previously stated (Himori et al 1980).

Overall, these results also suggest that reducing LVH with an ACE inhibitor, as opposed to hydralazine, protects not only from a blood pressure effect but also from a blood pressure-independent effect. Although the reduction of LVH indexed by echocardiography measurements was similar in SHRs after treatment with captopril; captopril may have protected the heart from neurohormonal damage associated with the development of hypertrophy in the context of hypertension. The protection associated with an ACE inhibitor may have continued during chronic ß-adrenergic-receptor activation which may explain the similar phenotype than normotensive despite increase of blood pressure after stopping captopril. Then, renin-angiotensin-aldosterone system may be involved in the development to ß-adrenergic-induced cardiac remodelling in hypertensive rats as previously shown (Veliotes et al 2010). In addition, the present study demonstrated that the effect of activation of the renin-angiotensin-aldosterone system may be in part independent of blood pressure.

There are several possible limitations in the current study. First, the study was designed to investigate cardiac effects of 5 months of administration of isoproterenol after 6 months of antihypertensive therapy. However, a large number of rats, mainly in untreated hypertensive group, died. Causes of death were related to heart disease and possible heart attack. As a result, echocardiographic results for untreated hypertensive

group may have been biased as the sample size was reduced. Secondly, the study requires further investigation to elaborate on RAA system, systemic and cardiac, mechanisms as pathways involved may attest if RAA system is further inhibited even after discontinuation of treatment in various groups. Therefore, further investigation is needed to support our findings.

In conclusion, our study showed that the regression of LVH obtained with hydralazine does not prevent the development of eccentric LVH following chronic ß-adrenergic-receptor activation in hypertensive rats. The prevention of LVH by angiotensin-converting-enzyme inhibitor in hypertensive rats does not protect against the development of cardiac dilation induced by ß-adrenergic-receptor activation. Lastly, the current results suggest that activation of RAA system independent of blood pressure may be detrimental to the development of cardiac remodelling associated with ß-adrenergic-receptor activation in hypertensive rats. Further studies are needed to explain the involvement of LVH in the development of cardiac dilation.

APPENDICES

Appendix A: Ethical clearance certificates

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2018/09/45/B

APPLICANT: Ms LK Tartibu

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: Influence of left ventricular dimensions on B-adrenergic-induced cardiac remodeling in male spontaneously hypertensive rats

Number and Species

36X1 month old male SHR rats

Approval was given for the use of animals for the project described above at an AESC meeting held on 2018/09/25. This approval remains valid until 2020/10/03.
Unreported changes to the application may invalidate the clearance given by the AESC
An annual progress report must be provided

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 is subject to any additional conditions listed below:

Signed: _____ (Chairperson, AESC) Date: 9th OCTOBER 2018

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: _____ (Registered Veterinarian) Date: 05 october 2018

cc: Supervisor: Dr F Michel
Director: CAS

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Appendix B: Concentration of captopril in the mixture of gelatine adjusted to body weight

The concentration of captopril [C] adjusted to body weight [BWa] was dissolved in 2 ml of the mixture of gelatine and given per day according to the required concentration of 40 mg of captopril [C1] to rat of 1kg or 1000g [BWr] as explained in the formula below:

$$[C] = \frac{[C1] \times [BWa]}{[BWr]}$$

Example:

C1	BWr	Bwa	C
40mg	1000g	100g	5mg
40mg	1000g	125g	6mg
40mg	1000g	150g	7mg
40mg	1000g	175g	8mg
40mg	1000g	200g	9mg
40mg	1000g	225g	10mg
40mg	1000g	250g	11mg
40mg	1000g	275g	12mg
40mg	1000g	300g	13mg
40mg	1000g	325g	14mg
40mg	1000g	350g	15mg
40mg	1000g	375g	16mg
40mg	1000g	400g	17mg
40mg	1000g	425g	18mg

C: concentration of captopril adjusted to body weight dissolved in 2 ml of the mixture of gelatine. C1: concentration of 40 mg of captopril. BWa: body weight of rat adjusted. BWr: body weight of rat taken as reference.

Appendix C: Concentration of hydralazine in the mixture of gelatine adjusted to body weight

The concentration of hydralazine [C] adjusted to body weight [BWa] was dissolved in 2 ml of the mixture of gelatine and given per day according to the required concentration of 20 mg of hydralazine [C1] to rat of 1kg or 1000g [BWr] as explained in the formula below:

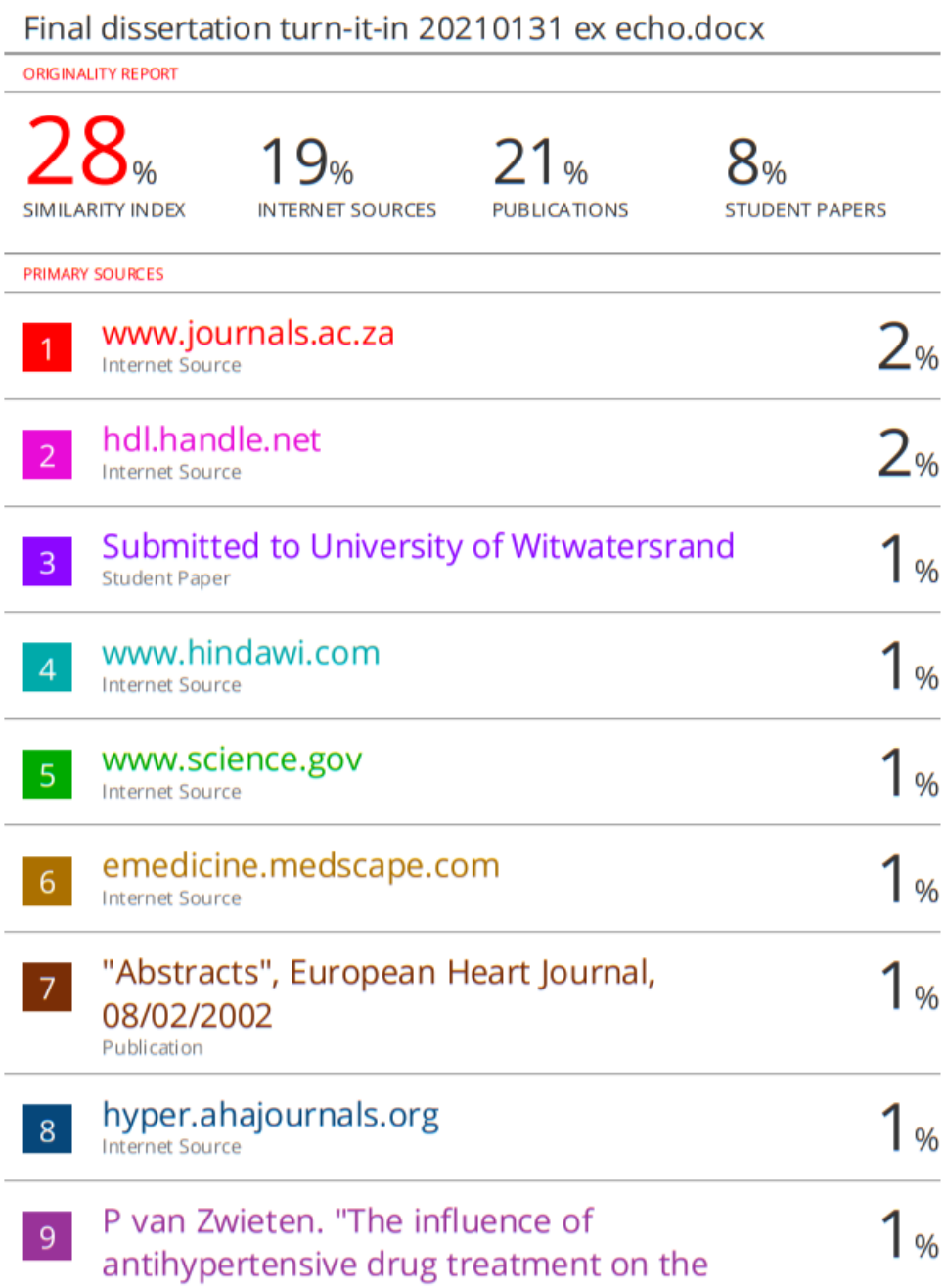
$$[C] = \frac{[C1] \times [BWa]}{[BWr]}$$

Example:

C1	BWr	Bwa	C
20mg	1000g	100g	2mg
20mg	1000g	125g	2.5mg
20mg	1000g	150g	3mg
20mg	1000g	175g	3.5mg
20mg	1000g	200g	4mg
20mg	1000g	225g	4.5mg
20mg	1000g	250g	5mg
20mg	1000g	275g	5.5mg
20mg	1000g	300g	6mg
20mg	1000g	325g	6.5mg
20mg	1000g	350g	7mg
20mg	1000g	375g	7.5mg
20mg	1000g	400g	8mg
20mg	1000g	425g	8.5mg

C: concentration of hydralazine adjusted to body weight dissolved in 2 ml of the mixture of gelatine. C1: concentration of 20 mg of hydralazine. BWa: body weight of rat adjusted. BWr: body weight of rat taken as reference.

Appendix D: “Turn-it-in” Plagiarism report



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