Left Ventricular Diastolic Dysfunction in Hypertension.

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, for the degree of Doctor of Philosophy

Johannesburg, South Africa

2019

Abstract

Heart failure with a preserved ejection fraction (HFpEF) accounts for half of all admissions for heart failure. However, there are no therapeutic approaches with proven benefits. Hypertension is a major risk factor for HFpEF, but the development of HFpEF may often occur in hypertensives irrespective of the degree of blood pressure (BP) control. Better insights into the identification of those at risk for and appropriate approaches to managing hypertensives at risk of HFpEF are therefore required. In the present thesis I assessed several aspects of the functional changes in the left ventricle (LV) (diastolic dysfunction [DD]), thought to antedate the development of hypertensive HFpEF.

Patients with hypertensive heart disease and associated underlying coronary artery or other diseases, often require β-adrenergic receptor (AR) blocker therapy. However, whether sympathetic-induced β-AR stimulation (which often accompanies heart failure) has beneficial effects on LV diastolic function in those with hypertensive LV DD, is unknown. I therefore assessed the impact of acute administration of the β-AR stimulant, isoproterenol (ISO) on LV diastolic function in rat models of hypertension. As compared to normotensive rats, or Dahl salt-sensitive (DSS) rats not receiving NaCl in the drinking water, Spontaneously Hypertensive (SHR) and DSS rats receiving NaCl in the drinking water had a reduced myocardial relaxation as indexed by lateral wall e' (early diastolic tissue velocity at the level of the mitral annulus) and an increased LV filling pressure as indexed by E/e'. However, LV ejection fraction and deformation and motion were preserved in both SHR and DSS rats. The administration of ISO resulted in a marked increase in ejection fraction and decrease in LV filling volumes in all groups; and an increase in e' in SHR, but not DSS rats. However, after ISO administration, although E/e' decreased in DSS rats in association with a reduced filling volume, E/e' in SHR remained unchanged. These data suggest that the hypertensive heart is indeed characterised by reductions in myocardial relaxation and increases in filling pressures, but β-AR activation fails to improve myocardial relaxation and when this occurs, does not reduce LV filling pressures.

Although the development of LV dysfunction in hypertension has traditionally been viewed as a transition process from a phase of structural LV remodelling to dysfunction, the extent to which LV mass (LVM) and remodelling account for BP-associated alterations in LV diastolic function is uncertain. **:** In 709 randomly selected participants from a community sample with a high prevalence of hypertension (49.6%), in product of coefficient mediation analysis, I therefore determined the extent to which LVM index (LVMI) or relative wall thickness (RWT) account for relations between BP and LV diastolic function. Although with adjustments for confounders, LVMI and RWT were independently associated with E/A, e'/a', e' and E/e', in product of coefficient mediation analysis, LVM and RWT failed to account for most BP-associated changes in diastolic function. Thus, most BP-associated decreases in LV diastolic function are likely to be a transition process independent of LV hypertrophy or concentric remodelling.

Although obesity-associated metabolic abnormalities (insulin resistance-IR) may not play as marked a role in determining LV DD as hypertension, the impact of combinations of these risk factors on DD is unknown. In 704 randomly selected participants from a community sample with a high prevalence of hypertension (50.6%) and obesity (46.5%), I determined the impact of adiposity indices or the homeostasis model (HOMA-IR) on LV diastolic function in hypertension. HOMA-IR was independently associated with lateral wall e' and E/e' as well as a diagnosis of DD. Importantly however, an enhanced relationship between HOMA-IR and E/e' in hypertensives (n=356) as compared to normotensives (n=348) was noted. Consequently, as compared to normotensives, with adjustments for confounders, hypertension was independently associated with DD only in those with the highest tertile of HOMA-IR, whilst in those with the lowest tertile of HOMA-IR, hypertension failed to show a higher prevalence of DD. Thus, IR enhances the impact of hypertension on LV DD.

In conclusion, in the present thesis I provide evidence to suggest that β-adrenergic receptor stimulation is not beneficial to hypertensive LV DD; that structural remodeling does not account for BP-associated alterations in LV DD; and that insulin resistance enhances the impact of hypertension on LV DD. Thus, the present thesis provides further insights into the

identification of those at risk and appropriate management approaches that may be employed in hypertensives at risk of HFpEF.

Declaration

I declare that this is my own unaided work; it is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences, University of the Witwatersrand Johannesburg. The work contained in this thesis has not been submitted for any degree or examination in this University, or any other University.

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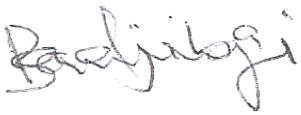
Adamu Jibril Bamaiyi ……31st…… day of …July…., 2019

I certify that all human and animal studies contained in this thesis have the approval of the Committee for Research on Human Studies and the Animal Ethics Screening Committee of the University of the Witwatersrand respectively. The clearance numbers are M17-02-71, M17-04-01, M12-04-108, M07-04-69, M02-04-72, 2016/03/08/A, 2016/06/28/B.



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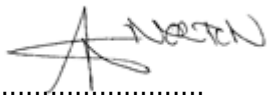
..... 

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Angela J Woodiwiss (supervisor) Gavin R Norton (supervisor)

Date.....31/07/2019..... Date.....31/07/2019.....

Dedication:

To all of the participants who contributed to the present study and to everyone who has had to surmount all odds to acquire quality education, wherever they may be, all over the world.

Publications

In support of the present thesis, the work included has resulted in the following publications in international journals.

1. Bamaiyi AJ, Norton GR, Peterson V, Norman G, Mojiminiyi FB, Woodiwiss AJ. Limited impact of β-adrenergic receptor activation on left ventricular diastolic function in rat models of hypertensive heart disease. *J Cardiovasc Pharmacol* 2018;72(5):242-251
2. Bamaiyi AJ, Norton GR, Peterson V, Libhaber CD, Sareli P, Woodiwiss AJ. Limited contribution of left ventricular mass and remodelling to the impact of blood pressure on diastolic function in a community sample. *J Hypertens* 2019;37(6):1191-1199
3. Bamaiyi AJ, Woodiwiss AJ, Peterson V, Gomes M, Libhaber CD, Sareli P, Norton GR. Insulin resistance influences the impact of hypertension on left ventricular diastolic dysfunction in a community sample. *Clin Cardiol* 2019; 42(2): 305-311

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Acknowledgements

I give glory to Allah, who makes everything possible. To my supervisors, Professor Gavin Robert Norton and Professor Angela Jill Woodiwiss (Mrs Norton), I most sincerely appreciate the tutoring, mentoring and provision of all of the support required to enable me to become a cardiovascular physiologist. To the members of the Cardiovascular Pathophysiology Research Unit (CPRU) to whom I am greatly thankful for their assistance throughout the course of my studies. To my wife, Zainab Muhammad, and to my children, Gbente, Asma'u, Sultan and Mahmoed for their love and understanding through the course of this difficult time. I really appreciate the unflinching support and love of my parents, Alhaji Adamu Balasi and Amina Idris and my brothers and sisters. I pray almighty Allah to bless their lives. I am most appreciative of the concern, encouragement, and advice from mentors and friends in Nigeria, South Africa and elsewhere across the globe during the course of my higher degree. To them I say, your efforts have been worthwhile! I am very grateful to the Usmanu Danfodiyo University, Sokoto and through its Needs Assessment Scheme. Without your support this undertaking would not have been possible.

List of Abbreviations

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
Adj	adjustment
AJW	angela jill woodiwiss
Akt	protein kinase B
ALT-711	advanced glycosylation end-product
ANOVA	analysis of variance
AR	adrenergic receptor
ARBs	angiotensin receptor blockers
ASCOT	anglo-scandinavian cardiac outcome trial
ASE	american society of echocardiography
ATP	adenosine triphosphate
B	beta
β-AR	beta adrenergic receptor
BMI	body mass index
B-mode	bright mode
BP	blood pressure
BSA	body surface area
CDL	carlos david libhaber
CI	confidence interval
Clin Cardiol	clinical cardiology
Cm	centimeters
cm/s	centimeter per second
DBP	diastolic blood pressure
DD	diastolic dysfunction

DM	diabetes mellitus
DSS	dahl salt-sensitive rat
e’	peak velocity during early diastole at the mitral annulus
E/A	ratio of E wave to A wave velocity
E/e’	index of LV filling pressures
EAE	european association of echocardiography
ECG	electrocardiogram
EDV	end diastolic volume
ED	end diastole
EDP	end diastolic pressure
EF	ejection fraction
E wave	trans-mitral blood flow velocity in the early period of left ventricular diastolic filling
FSend	endocardial fractional shortening
FSmid	midwall fractional shortening
g	gram
g/m	gram per meter
HbA1c	percentage glycated haemoglobin
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HOMA-IR	homeostatic model assessment of insulin resistance
HT	hypertension
iso	isoproterenol
J Cardiovasc Pharmacol	journal of cardiovascular pharmacology
J Hypertens	journal of hypertension
kg	kilogram
kg/m^2	kg per $meter^2$
LA	left atrial
LAV	left atrial volume

LV	left ventricular
LV DD	left ventricular diastolic dysfunction
LVEDD	left ventricular end diastolic diameter
LVESD	left ventricular end systolic diameter
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass indexed for height$^{2.7}$
LVMI-ht	left ventricular mass indexed to height
LVMI-BSA	left ventricular mass indexed to body mass area
MAP	mean arterial pressure
Mm	millimeters
mmHg	millimeter of mercury
ml/m^2	millimeter per meter square
µU/ml	micro unit per milliliters
mmol/L	micromole per liter
MRC	medical research council
n	number
NaCl	sodium chloride
NT	normotensive
OR	odd ratio
PE	phenylephrine
PCr/ ATP	phosphocreatine to adenosine triphosphate ratio
PI3K	phosphatidylinositol 3-kinase
p	p-value or statistical significance
PWED	left ventricular posterior wall thickness at end diastole
PWES	left ventricular posterior wall thickness at end systole
RV	right ventricle
r^2	goodness of fit
r	correlation coefficient

RWT	relative wall thickness
SAS	statistical analysis system
SEM	standard error of mean
SD	standard deviation
sec^{-1}	per second
SHR	spontaneously hypertensive rat
TDI	tissue doppler imaging
TOPCAT	treatment of preserved cardiac function heart failure with an aldosterone antagonist
USA	United States of America
VVI	velocity vector imaging
vs	versus
WC	waist circumference
WKY	wistar kyoto

List of Symbols

>	greater than
<	less than
≥	greater than or equal to
%	percentage

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Preface

Whilst striking progress has been made in the prevention and management of cardiovascular events in general, heart failure remains a major burden in most countries and in particular on the African continent. Importantly, heart failure is a leading cause of cardiovascular disease in Africa. An important reason for the burden of heart failure anywhere in the world is an increasing recognition of heart failure associated with a preserved ejection fraction (HFpEF), a form of heart failure where there are no therapeutic approaches with proven benefit and which may account for half of all admissions for heart failure. Although it is well recognized that hypertension is the most important risk factor for HFpEF, the ability to identify those hypertensives at risk of HFpEF and the most appropriate management approaches is uncertain. Consequently, I conducted the studies included in the present thesis to further our understanding of management approaches and risk identification of HFpEF. In this regard, I employed both human studies and where necessary (for ethical reasons) animal models of hypertensive heart disease to assess various aspects of the factors associated with preclinical cardiac diastolic dysfunction, the pathophysiological change thought to be primarily responsible for HFpEF.

The present thesis consists of a series of semi-independent chapters, each with its own introduction, methods, results and discussion section. The thesis begins with a chapter where I critically review the available evidence in the field and lead the reader through a series of arguments in support of conducting the studies described in the present thesis. Furthermore, the present thesis concludes with a summary chapter which consolidates the findings of each chapter and underscores the novelty of the findings by placing the studies in the context of our present understanding of the field. In support of the present thesis, the data presented in chapters 2 to 4 have either been published or are in-press in the well-respected international journals, the *Journal of Cardiovascular Pharmacology* (Bamaiyi et al 2018), the *Journal of Hypertension* (Bamaiyi et al 2019, in-press), and the journal *Clinical Cardiology* (Bamaiyi et al 2019).

CHAPTER 1

INTRODUCTION

Pathophysiology of Cardiac Diastolic Dysfunction with a Focus on Hypertension

1.1 **Introduction**

Whilst significant progress has been made in the prevention and management of cardiovascular events in general (Buonacera et al 2018; Borlaug & Redfield 2011) heart failure remains a major burden to society in most countries and in particular on the African continent. The prevalence of heart failure is still on the rise (Ebong et al 2014; Bloomfield et al 2013) and is a leading cause of cardiovascular disease in Africa (Ogah et al 2015; Owolabi et al 2014; Appiah et al 2017). Whilst the prevalence of heart failure is estimated to be 2-3% of cardiovascular events in industrialized countries, in sub-Saharan Africa the prevalence is reported to be as high as between 25.6%-88.3% of admissions for cardiovascular disease (Ebong et al 2014; Appiah et al 2017). Much of the reason for the burden of heart failure in all countries is because heart failure with a preserved ejection fraction (HFpEF), a form of heart failure that accounts for approximately 50 % of heart failure cases, presents with distinct phenotypes which still elude treatment (Lewis et al 2017; Borlaug & Paulus 2011; Shah et al 2016). Advancement in the diagnosis of HFpEF has led to an increasing rate of detection of HFpEF (Borlaug & Redfield 2011; Shah et al 2016). Coupled with ageing of the African demographic structure, the concomitant burden of hypertension has contributed to an increasing prevalence of heart failure in Africa (Akinyemi et al 2018; Borlaug & Redfield 2011). Indeed, HFpEF has at its cornerstone, uncontrolled hypertension, an entity explained in Africa to be associated with urbanization and changes in lifestyle (Twinamasiko et al 2018; Bintabara & Mpondo 2018). Hypertensive heart disease is the commonest cause of HFpEF in sub-Saharan Africa (Agbor et al 2018; Tadic. et al 2018; Ojji et al 2013) and heart failure is estimated to be as high as 15% of hypertensives in sub-Saharan Africa (Ogah et al 2015). In this regard, in sub-Saharan Africa hypertension control is reported to be abysmally low among patients taking antihypertensive medications and in some countries in sub-Saharan Africa over 70% of the hypertensive population are unaware that they have the disorder (Beaney et al 2018; Ogah et al 2015; Twinamasiko et al 2018).

As will be underscored in the present chapter, although much is understood of the cellular mechanisms that explain the functional disturbances of the heart in HFpEF, little of this

information has translated into the clinical arena, largely because it is only in the more recent past that HFpEF has been acknowledged as a clinical entity. In this regard, there are several outstanding questions which remain from a clinical perspective, such as whether the compensatory neurohumoral and other changes in this form of heart failure, as with heart failure with a reduced ejection fraction (HFrEF), have deleterious effects. Moreover, what the primary changes responsible for myocardial functional disturbances are in response to risk factors in HFpEF is uncertain. In addition, the exact contribution of some of the risk factors that herald the transition to the myocardial functional changes responsible for HFpEF is unknown. The importance of these questions prompted me to address several of these issues in the present thesis. Therefore, in the present chapter I will review the topic of HFpEF and the structural and functional changes (diastolic dysfunction) thought to produce this form of heart failure, highlighting the missing evidence that prompted me to perform the studies described in the present thesis.

1.2 Heart failure with a preserved versus reduced ejection fraction

Clinically, heart failure is described as a disorder identified from a group of symptoms and signs that are a consequence of raised pressures in the heart during filling or a reduced ability of the heart to generate a normal blood flow (Borlaug & Redfield 2011; Yancy et al 2013). The types of heart failure can be classified in different ways. The less commonly used classification of heart failure, namely the ACC/AHA heart failure classification. According to this classification, heart failure is described as having four stages which are: A, patients identified as being at risk due to the presence of diabetes mellitus or coronary artery disease; B, patients with structural heart disease (ie. LV hypertrophy or reduced LV ejection fraction or chamber enlargement) who have not yet developed symptoms of heart failure; C, patients who have developed clinical heart failure; D, patients with refractory heart failure which requires intervention such as LV assist device, pacemakers or transplant (Yancy et al 2013). A more clinically relevant classification for patients requiring heart failure therapy, which is based on a measure of cardiac systolic chamber function, that is left ventricular (LV) ejection fraction (EF)

(Hundely et al 2001; Lee et al 2009). In this classification, two major forms of heart failure are described, namely HFrEF in which heart failure is associated with a decreased ejection fraction (EF<40%), and HFpEF in which ejection fraction is normal (≥50%). These two forms of heart failure fit into the stage C category according to the ACC/AHA heart failure classification. More recently, an intermediate phenotype of heart failure has been proposed in which the ejection fraction lies between 40 and 50% (Nadar & Tariq 2018; Andronic et al 2016; Sato et al 2017), but this is at present a poorly understood entity. Heart failure with a reduced ejection fraction, is also termed systolic heart failure as it is a consequence of systolic dysfunction of the LV. Heart failure with a reduced ejection fraction is associated with cardiac dilatation (marked eccentric LV remodeling when the radius of the chamber increases more than the wall thickens). Systolic heart failure is commonly seen in men who are elderly (Federmann & Hess 1994; Masoudi et al 2003; Bhatia et al 2006; Borlaug & Redfield 2011); is a frequent occurrence following myocardial infarction, and also commonly occurs with no apparent cause (idiopathic dilated cardiomyopathy) (Francone 2014; Slavich et al 2011). It may occur as a consequence of hypertension or any other cardiac pathology that has progressed to an advanced stage including disorders which start off with marked concentric LV remodeling such as hypertrophic obstructive cardiomyopathy (Slavich et al 2011; McNally & Mestroni 2017). Alternatively, HFpEF, is also termed diastolic heart failure as it is characterised by LV diastolic dysfunction. Heart failure with a preserved ejection fraction is more commonly associated with hypertension, obesity and diabetes mellitus, and is more frequently found in women who are elderly (Borlaug & Redfield 2011). Indeed, in large community-based studies, patients with a diagnosis of HFpEF are more commonly elderly women; infrequently have coronary artery disease, although coronary artery disease may be a cause, and more often present with hypertension and obesity (Masoudi et al 2003; Redfield et al 2003; Bhatia et al 2006; Bursi et al 2006; Owan et al 2006; Lee et al 2009). In comparison to HFrEF where LV dilatation is common, HFpEF is often associated with concentric LV hypertrophy or remodeling (Borlaug & Redfield 2011), where the wall thickens more than the chamber enlarges and wall thickness to radius ratio is increased.

The proportion of patients with HFpEF as opposed to HFrEF is rising progressively, with an annual increase of about 1% in the prevalence of HFpEF as compared to HFrEF

(Federmann & Hess 1994; Owan et al 2006; Fonarow et al 2007; Borlaug & Redfield 2011; Borlaug & Paulus 2011; Scantlebury & Borlaug 2011). Most newly diagnosed cases of heart failure are likely to be HFpEF (Borlaug & Redfield 2011; Bhatia et al 2006) and HFrEF and HFpEF may exist in a 1:1 ratio (Borlaug & Paulus 2011; Adeniran 2015). Thus, whilst the disease burden of HFrEF is decreasing, the prevalence of HFpEF is on the rise (Shah et al 2016). The increased prevalence of HFpEF is probably a consequence of longevity and escalating prevalence of hypertension, diabetes and obesity and/or an increased identification of HFpEF by clinicians (Owan et al 2006). As the world's population becomes increasingly industrialized and westernized with a related increase in the prevalence of obesity, diabetes mellitus and hypertension (Twinamasiko et al 2018; Bloomfield et al 2013), and through reductions in infectious diseases and malnutrition, live to an older age, the prevalence of HFpEF has grown beyond the rate at HFrEF (Akinyemi et al 2018; Bloomfield et al 2013). Moreover, the increasing prevalence of coronary artery disease in black populations may also predispose to HFpEF (Shah et al 2016; Mohammed et al 2012; Wan et al 2014).

1.2.1 Cardiac dysfunction in HFpEF versus HFrEF

The functional changes in the heart of patients with HFrEF are fairly well known and this knowledge is the foundation of current approaches to the therapeutic management of heart failure in general (Harjola et al 2017). In essence the myocardium develops a reduced ability to generate an adequate force of contraction in systole, hence HFrEF is often called systolic heart failure. The decreased force of contraction in HFrEF is attributed to several changes. First myocardial damage or dysfunction may occur through cardiomyocyte apoptosis, necrosis or decreased function of the ability of the myocyte to cycle calcium or a reduced ability of myofilaments to effectively shorten (Pitt et al 2014; Sadej et al 2014). The LV initially maintains stroke volume by increasing filling volumes, which promote an enhanced Frank-Starling effect, but at the same time increase filling pressures. The increased filling pressures transmit backward to the left atrium and hence pulmonary capillaries causing increases in pulmonary capillary hydrostatic pressures and pulmonary congestion. The increased left atrial pressures

also increase pulmonary artery pressures and hence enhance the afterload to the right ventricle causing right heart failure. The LV is thought with time to maintain normal filling pressures at higher filling volumes by dilating, a process that involves cardiomyocyte slippage (Kapelko et al 1991). The cardiac dilatation, although maintaining normal filling pressures initially, will with time increase wall tension through LaPLace's Law (T=Pr/2h, where T is tension, P is pressure, r is radius and h is wall thickness). The high wall tension reduces systolic function even further and the heart develops terminal decreases in stroke volume and increases in filling pressures. Although cardiac dilatation is thought to be a consequence of cell damage and contractile disturbances there is significant evidence that cardiac dilatation without contractile disturbances are sufficient to reduce ejection fraction (Norton et al 2002; Norton et al 2008). Importantly, cardiac dilatation may occur through chronic neurohumoral activation (Norton et al 2002; Norton et al 2008). Consequently, the principle change associated with an improved ejection fraction with heart failure therapy is reverse remodeling of a dilated LV (Booysen et al 2012). Current approaches that reverse remodel the LV incorporate the administration of agents that block the renin-angiotensin-aldosterone system (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and aldosterone receptor blockers) or the sympathetic nervous system (β-adrenergic receptor blockers), which target systems that are activated in chronic heart failure and which are involved in dilating the LV. Importantly, it has been shown repeatedly in large multicenter clinical trials that the risk of death or hospitalization for heart failure in patients with HFrEF can be diminished by the use of these current therapeutic practices (Borlaug & Redfield 2011). Consequently, in patients with HFrEF, survival has increased steadily (Roger et al 2004).

In comparison to HFrEF, the functional changes in the heart of patients with HFpEF is less well understood, and therefore therapeutic practices largely rely on an understanding of the pathophysiology of HFrEF. In this regard however, these therapeutic options have been unsuccessful in increasing survival in patients with HFpEF (Borlaug & Redfield 2011; Owan et al 2006). Although various functional changes such as decreases in contractile reserve, chronotropic reserve, vasodilatory reserve and endothelial function (Borlaug et al 2006; Brubaker et al 2006; Borlaug[a] et al 2010; Borlaug[b] et al 2010) are likely to contribute to the transition to HFpEF, the essential abnormality of cardiac function in HFpEF is an abnormality of

the diastolic period of the cardiac cycle. Hence, HFpEF is often called diastolic heart failure. In other words, the characteristic changes in function of the LV in HFpEF are those of LV diastolic dysfunction (LV DD) (Burke et al 2014; Lee & Cooper 2009; Russo et al 2011), In this regard, decreases in the active relaxation and an enhanced passive stiffness of the LV are fundamental features of LV DD (Phan et al 2009; Selby et al 2011; Westermann et al 2008; Zile et al 2004). Indeed, increases in ventricular stiffness (a left shift in the ventricular pressure-volume relationship at end diastole) and decreases in myocardial relaxation occur in patients with HFpEF (Burke et al 2014; Mohammed et al 2012; Shah et al 2014; Westerman et al 2008; Zile et al 2004). The effect of these changes is enhanced filling pressures in the LV for a given filling volume, the consequences of which are several fold. First, increased filling pressures cause increased left atrial pressures and hence enhanced pulmonary capillary hydrostatic pressures, the result being pulmonary congestion. Second, the high left atrial pressures increase pulmonary artery pressures, afterload to the right ventricle and hence produce right heart failure. In addition, the ability to fill the LV during exercise is reduced and hence increases in stroke volume during exercise are limited (do Prado & Rocco 2017; Rommel et al 2018) an effect that may contribute to the exercise intolerance in HFpEF. Therefore, HFpEF may be characterized by several functional changes that may be determined using non-invasive assessments. What are the commonly noted alterations in LV function in HFpEF?

First, an increased LV filling pressure (raised end diastolic pressure [EDP]) may be indexed by increases in the ratio (E/e') of early diastolic transmitral blood flow velocity (E)/velocity of myocardial tissue lengthening during early diastole (e'). In this regard, whilst E reflects the degree of myocardial relaxation, it also increases when filling pressures increase. In contrast, e' is a preload independent index of myocardial relaxation. Thus the ratio of E/e' is an index of filling pressures and this increases in HFpEF. In addition, in HFpEF reductions in indices of myocardial relaxation including e' also occur. As filling pressures in the LV increase, these pressures are transmitted back to the left atrium, which dilates and hence increased left atrial volumes (LAV) similarly occur. As the afterload to the right ventricle (RV) increases, the RV fails and hence changes in indices of right sided pressures such as increases in tricuspid regurgitant velocities also occur in HFpEF (Bar et al 2018; Mascherbauer et al 2017).

Although HFpEF is characterized by alterations in the diastolic period of the cardiac cycle, LV systolic dysfunction without decreases in EF may occur together with diastolic dysfunction in HFpEF (Wan et al 2014; Borlaug & Paulus 2011). In this regard decreases in systolic myocardial function including LV segmental strain, strain rate, displacement or velocity may frequently occur (Adeniran et al 2015). In the presence of a normal EF however, exactly how these changes in systolic function contribute to heart failure in patients with HFpEF is uncertain.

1.2.2 Current therapeutic approaches to treating HFpEF

Beneficial therapeutic approaches to treating HFrEF all rely heavily on blockade of neurohumoral activation and increased loading conditions (Abraham et al 2015; Zhang & Anderson 2014) and thus largely target the deleterious effects described in aforementioned section. However, neurohumoral activation may also promote myocardial fibrosis and overload the heart irrespective of the cause of heart failure. Thus, as indicated in the aforementioned sections, clinically, the treatment approaches to patients with HFpEF are primarily founded upon knowledge of the pathophysiology of HFrEF (Federmann & Hess 1994; Yusuf et al 2003; Cleland et al 2006; Massie et al 2008; Hernandez et al 2009; Borlaug & Redfield 2011). Nevertheless, data from large multicenter clinical trials that have employed agents that target activation of the renin-angiotensin-aldosterone system have revealed that the rate of death or hospitalization for heart failure in patients with HFpEF is not reduced by any of the current therapeutic approaches (Owan et al 2006; Paulus & van Ballegoij 2010; Borlaug & Paulus 2011; Borlaug & Redfield 2011; Li et al 2013; Pitt et al 2014). Moreover, to-date the clinical outcomes of more novel agents designed to treat the pathophysiological changes in the myocardium thought to be responsible for HFpEF have also been unsatisfactory. Importantly, agents designed to reduce myocardial fibrosis, the purported primary pathophysiological mechanism underpinning HFrEF (see discussion below), have proved to be ineffective (Nanayakkara & Kaye 2015). Indeed, although, the aldosterone receptor antagonist, spironolactone has been reported to improve diastolic function (Edelmann et al 2013), a large outcomes-based study

(TOPCAT) failed to show improved survival in HFpEF as a consequence of spironolactone treatment (Pitt et al 2014). Moreover, the more novel aldosterone receptor antagonist, eplerenone, had no effect on conventional measures of diastolic function and only modest reductions in LV filling pressures (Deswal et al 2011) and other approaches to reduce fibrosis (such as the use of sildenafil, a phosphodiesterase 5 inhibitor) have failed to show improvements in clinical measurements or measurements of cardiac structure (Redfield et al 2013). Moreover, the advanced glycosylation end-product breaker (ALT-711) has failed to improve measurements of diastolic function (Little et al 2005). Although several alternative approaches are being explored to treat HFpEF, none have provided early data to suggest possible benefits.

As current therapeutic options for the management of patients with HFpEF are ineffective (Borlaug & Redfield 2011), and the burden of disease of HFpEF is high (Bhatia et al 2006; Yancy et al 2006; Fonarow et al 2007; Lam et al 2007; Borlaug & Redfield 2011; Chirinos et al 2012; Nichols et al 2015), further approaches that will successfully decrease mortality and morbidity due to HFpEF are required. Improving our knowledge of the pathophysiology of LV diastolic dysfunction (DD), which is thought to be the predecessor of HFpEF may assist in the prevention of the development of HFpEF and may provide insights into the pathophysiological process responsible for heart failure in HFpEF. Consequently, in the present thesis I focused on the determinants of preclinical LV DD and aspects of the functional changes in the LV in preclinical LV DD. In the subsequent section I will therefore discuss aspects of the determinants of and pathophysiological changes in LV DD and several notions of LV DD that are poorly understood that prompted me to perform the studies in my thesis.

1.2.3 Cellular mechanisms responsible for DD in HFpEF

There are several cellular mechanisms responsible LV diastolic dysfunction in HFpEF. The increased resting tension in cardiomyocytes in HFpEF may be due to incomplete relaxation as a consequence of reduced calcium reuptake by the sarcoplasmic reticulum (van Heerebeek et al 2006); or when the stiffer isoform of the macromolecule titin is noted to be increased (van

Heerebeek et al 2006). Alternatively, interstitial changes may produce a stiffer myocardium (changes in passive properties). With respect to alterations in relaxation processes, ion channel remodelling and a lower resting energy reserve, changes which may reduce the ability to sequester calcium, may reduce active myocardial relaxation and hence contribute to diastolic dysfunction (Adeniran et al 2015; Lamb et al 1999; Phan et al 2009; Selby et al 2011; Shah et al 2014). Importantly, pharmacological agents that influence cardiomyocyte calcium handling including ryanodine receptor stabilisers (Sacherer et al 2012), inhibitors of the sodium-calcium exchanger (Kamimura et al 2012), and strategies that improve calcium uptake by the sarcoplasmic reticulum (Sedej et al 2014), can improve diastolic function. Furthermore, interventions that shift energy substrate utilization important for promoting calcium sequestration, improve cardiac PCr/ATP ratio and diastolic function (Abozguia et al 2010; Beadle et al 2015; Fragasso et al 2006). However, in these studies improved cardiac diastolic properties were noted in forms of cardiac disease that are caused primarily by abnormalities of systolic rather than diastolic function (Abozguia et al 2010; Beadle et al 2015; Fragasso et al 2006; Sedej et al 2014). Consequently, whether an improvement in active processes involved in myocardial relaxation reduce filling pressures in cardiac pathology causing primarily decreases in diastolic function, remains uncertain.

With respect to changes in passive properties, the proliferation of myocardial collagen (fibrosis) related to enhanced activation of the renin-angiotensin-aldosterone system (thought to mainly be mediated by aldosterone effects) and/or enhanced collagen cross-linking (commonly related to the increase in advanced glycation end-products in diabetes mellitus or to an enhanced oxidative stress) result in an increased myocardial stiffness (Borlaug & Paulus 2011; Norton et al 1996; Kasner et al 2011; Lopez et al 2012; van Heerebeek et al 2006). Even in the absence of diabetes mellitus, the expression of the protein lysyl oxidase (which mediates the cross-linking of collagen) is increased in patients with HFpEF in association with an increase in myocardial fibrosis (Kasner et al 2011; Lopez et al 2012). The pathophysiological changes described above have been the foundation upon which pharmacological agents have been developed. Although pharmacological agents that enhance myocardial sarcoplasmic reticulum calcium re-uptake; reduce collagen deposition or decrease collagen cross-linking have been

developed, only a few of these agents have advanced from pre-clinical studies to clinical trials and none have translated into evidence for improved clinical outcomes in HFpEF.

More recently, a complex microvascular endothelial change, which through inflammatory substances causes cellular changes responsible for abnormalities of diastolic function has been suggested as a cause of HFpEF. This hypothesis posits that risk factors cause chronic low-grade inflammation and through the inflammatory substances tumour necrosis factor-α and interleukin-6 consequently produce rarefaction of the coronary microvasculature (Paulus & Tschope 2013; Franssen et al 2016; Mohammed et al 2015). Inflammatory-induced microvascular changes in turn through the liberation of various chemical substances produce oxidative stress and hence influence all of the cellular changes responsible for DD (Franssen et al 2016; Paulus & Tschope 2013; Matyas et al 2017; van Heerebeek et al 2012). This hypothesis provides an additional potential target for HFpEF. That is, it is possible that approaches that reduce the inflammatory changes responsible for the cellular alterations causing DD may prove to be beneficial. However, as yet this has not translated into clinical trials and it is possible that extensive work is still required at a preclinical level before taking this information to a clinical level.

1.2.4 Neurohumoral activation and the implications thereof in HFpEF versus HFrEF

Heart failure is widely reported to be associated with increased sympathetic activation (Zhang & Anderson 2014; Abraham et al 2015; Ramchandra et al 2014), a state that may have some compensatory effects (Leite-Moreira et al 2001). In this regard, adrenergic activation will promote both inotropy and lusitropy, thus simultaneously improving cardiac output and promoting increases in filling volumes at lower filling pressures. However, as a failing heart with a reduced EF decompensates, β_1-adrenergic receptor-induced signaling downregulates (Talan et al 2011; Klabunde 2012) and the heart then depends more on β_2 adrenergic receptor signaling, a far less effective pathway for promoting inotropy and lusitropy. Ultimately, when EF decreases below 40% even β_2 adrenergic receptor signaling is impaired (Lahaye Sle et al 2010). Whilst sympathetic activation in HFrEF may initially produce some compensatory

inotropic and lusitropic effects, the long term effects are markedly deleterious. Indeed, through activation of apoptotic and necrotic pathways; through several interstitial changes (activation of matrix metalloproteinases and reductions in collagen cross-linking); and through cardiomyocyte lengethening, chronic β_1-adrenergic receptor activation produces further decreases in cardiac function and at the same time induces cardiac dilatation (adverse remodelling), thus contributing to reductions in EF. Thus, the early beneficial effects of sympathetic activation in HFrEF are offset by the chronic adverse effects. Consequently, based on several large clinical trials, it is now well-recognized that β-adrenergic receptor blocking agents are some of the most effective agents at preventing death and hospitalisation in chronic heart failure. Nevertheless, this has only been demonstrated for HFrEF (Abraham et al 2015; Zhang & Anderson 2014; Parker et al 1995). What of the possible impact of sympathetic activation on HFpEF?

Hypertension and coronary artery disease are common causes of HFpEF (Mohammed et al 2015; Desai et al 2013; Kamimura et al 2012), and these disorders often require β-adrenergic receptor blocking agents for their management (Zhang & Anderson 2014). Furthermore, HFpEF, particularly that caused by hypertension, may be associated with atrial fibrillation, a condition that also benefits from rate control produced by the use of β-adrenergic receptor blocking agents (Hernandez et al 2009; Reiken et al 2003). It is therefore important to understand whether sympathetic activation 1) occurs in HFpEF; 2) produces acute benefits to diastolic function in those with LV DD and 3) produces adverse effects on LV DD when activated chronically. In this regard, if neurohumoral activation occurs in HFpEF and is beneficial for DD, then β-adrenergic receptor blocking agents may promote the development and extent of HFpEF.

Importantly, sympathetic activation is a characteristic finding in both HFpEF and HFrEF (Chang et al 2013; Grimm & Brown 2010; Woo & Xiao 2012; Akhter et al 1997). In this regard, β-adrenergic receptor activation in cardiomyocytes is thought to improve both inotropy as well as lusitropy (Chang et al 2013; Pepe et al 2004; Leite-Moreira et al 2001). Both β_1 and β_2 adrenergic receptors predominate in mammalian (including human) cardiomyocytes (Zheng et al 2005; Shizukuda & Buttrick 2002) and in HFpEF, β_2-adrenergic receptors may be over-expressed leading to phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt)-induced

anti-apoptotic cardio-protection (Zheng et al 2004; Shizukuda & Buttrick 2002). Thus, sympathetic activation in HFpEF may not only promote lusitropic effects, but via β_2-adrenoreceptors and PI3K and Akt activation may improve LV DD (Devic et al 2001; Florea & Blatter 2012; Leone et al 2002; Leite-Moreira et al 2001), thus preventing the progression from LVDD to HFpEF. On the other hand, however, chronic β-adrenergic stimulation could stimulate β_1-adrenergic receptors and through calcium-calmodulin dependent protein kinase II signaling, result in an enhanced mitochondrial pore permeability and unfavorable cardiac remodeling, including apoptosis and hypertrophy (Chang et al 2013; Grimm & Brown 2010). Importantly, β_2-adrenergic receptor-mediated cardio-protection (Florea & Blatter 2012; Chang et al 2013; Devic et al 2001) may dominate in HFpEF (Woo & Xiao 2012; Zheng et al 2004). Indeed, both animal (Leite-Moreira et al 2001; Talan et al 2011) and clinical studies (Jensen et al 2014) suggest improvement in both systolic and diastolic function of the LV in LV DD and HFpEF following selective β-adrenoreceptor stimulation. Thus, selective β_2-adrenergic receptor activation may provide a novel modality for the treatment of HFpEF (Jensen et al 2014; Grimm & Brown 2010; Brodde et al 2006). In summary, as with HFrEF, neurohumoral activation is indeed associated with HFpEF. As β-adrenergic receptor blocking agents have no proven benefit in HFpEF (Williams et al 2017; Leite-Moreira et al 2001; Lahye et al 2010), and indeed selective β-adrenergic receptor stimulation may protect the heart in LV DD, it is unlikely that chronic sympathetic activation in HFpEF promotes adverse effects on the heart. Thus, the question that remains is whether sympathetic activation in DD and HFpEF improves LV diastolic function? Importantly, β_1 adrenergic receptor-induced effects on chronotropic and inotropic properties of the LV are preserved in HFpEF (Klabunde, 2012, Lahaye Sle et al., 2010). However, whether at rest or during physical activity, β-AR-induced lusitropic effects have benefits to the heart with DD (thus accommodating increased filling volumes at normal filling pressures) and β-adrenoreceptor blockers may thus attenuate these beneficial effects, is unknown. Furthermore, whether sympathetic activation in DD, through β-AR-induced lusitropic effects and increases in filling volumes at normal filling pressures limits the transition to clinical HFpEF, is unknown. Importantly, in hearts with DD, an attenuated sympathetic-induced LV suction (an effect mediated by relaxation of the LV in early diastole) occurs (Ohara et al 2012). If a decreased

sympathetic-induced LV suction (Ohara et al 2012) translates into a limited impact of adrenergic activation on LV filling pressures in DD, under these circumstances the use of β-adrenoreceptor blocking agents may have little deleterious effect on DD either at rest or during physical activity and sympathetic activation cannot be viewed as a beneficial effect in the transition to clinical HFpEF. In the present thesis I therefore assessed the extent to which β-adrenoreceptor stimulation improves LV relaxation abnormalities and hence decreases Doppler indexes of LV filling pressure in Spontaneously Hypertensive (SHR) and Dahl salt-sensitive (DSS) hypertensive rat models of LV DD. I selected to study animal models of DD as the impact of maximal adrenergic stimulation cannot be ethically assessed in humans and in humans with DD data will be confounded by the presence of agents employed to manage risk factors. I assessed diastolic function using contemporary non-invasive echocardiographic approaches consistent with the clinical scenario rather than invasive approaches, but selected animal models (SHR) to study in which our group have previously demonstrated diastolic dysfunction using gold-standard end diastolic pressure-dimensions and stress-strain relations (Norton et al 1997). The data for this study have been published in *J Cardiovasc Pharmacol* (Bamaiyi et al, 2018) and are described and discussed in chapter 2 of the present thesis.

1.2.5 Clinical presentation of HFpEF versus HFrEF

Both HFpEF and HFrEF may present with common clinical features including breathlessness, exercise intolerance and signs of right heart failure (Phan et al 2009; Bhatia et al., 2006, Borlaug & Paulus, 2011). However, the underlying mechanisms are different (Borlaug & Paulus, 2011; Bhatia et al 2006; Phan et al 2009). In HFrEF exercise intolerance is due the decrease in the LV's ability to eject sufficient blood to meet the requirements for physical exertion (Abraham et al., 2015, Rengo et al 2014) whilst breathlessness is due to an increased LV end diastolic volume and hence pressure which will lead to pulmonary congestion (Bhatia et al 2006; Borlaug & Paulus 2011). In HFpEF, a reduced LV relaxation and increased passive stiffness increases LV filling pressure (Adeniran et al., 2015, Zile et al., 2004, Leite-Moreira et al., 2001), consequently leading to pulmonary congestion, breathlessness and exercise

intolerance (Phan et al 2009; Leite-Moreira et al 2001). In this regard, increased filling pressures in DD have indeed been shown to contribute to exercise intolerance (Paulus 2010). In this regard, exercise-induced pulmonary hypertension and an elevation of pulmonary capillary wedge pressures occurs in patients with exertional dyspnea and DD (Borlaug et al 2010). Importantly exercise activates the sympathetic nervous system and in a normal LV enhances myocardial relaxation, thus reducing filling pressures and accommodating greater filling volumes (thus allowing for a greater Frank-Starling effect). Sympathetic activation in HFpEF may similarly limit the symptoms of heart failure by promoting reduced filling pressures. Attenuation of sympathetic-induced increases in myocardial relaxation with the use of β-adrenoreceptor blocking agents in those with LV DD or HFpEF could therefore result in an LV that does not accommodate a higher filling volume at rest or during exercise, thus reducing the Frank-Starling mechanism and contributing to exercise intolerance or increasing filling pressures at rest. However, there are presently no studies that have evaluated whether sympathetic activation in LV DD produces benefits to LV diastolic function. Thus, as indicated in the aforementioned section in the present thesis I therefore assessed the extent to which β-adrenoreceptor stimulation improves LV relaxation abnormalities and hence decreases Doppler indexes of LV filling pressure in Spontaneously Hypertensive (SHR) and Dahl salt-sensitive (DSS) hypertensive rat models of LV DD. As also indicated these data have been published in *J Cardiovasc Pharmacol* (Bamaiyi et al, 2018) and are described and discussed in chapter 2 of the present thesis.

1.3 Preclinical cardiac diastolic dysfunction

Several large community-based studies have demonstrated a high prevalence of LV DD prior to the development of HFpEF and these functional changes in the LV are strongly related to several acknowledged risk factors for HFpEF, including age, hypertension, and obesity (Burke et al 2014; Libhaber et al 2014; Mohammed et al 2012; Russo et al 2011; Owan et al 2006; Bursi et al 2006; Zile et al 2004; Redfield et al 2003). These findings therefore provide an ideal setting to better study the impact of risk factors on LV diastolic function. Importantly, there

is an association between LV DD and HFpEF (Shah et al 2016; Paulus & Tschope 2013; Hamdani et al 2013; Lamb et al 1999). Moreover, LV DD or indexes of an abnormal LV relaxation or increased filling pressures precede or predict the development of heart failure or HFpEF (Bella et al 2002; Wan et al 2014; Wang et al 2003; Zile et al 2002; Westermann et al 2008; Aurigemma et al 2001; Kane et al 2011; Lam et al 2011; Redfield et al 2003; Schillaci et al 2002). Moreover, a decreased LV end-diastolic volume, which often accompanies DD, predicts the progression to HFpEF (Burke et al 2014).

1.3.1 Hypertension as a risk factor for preclinical LV DD and HFpEF

There is considerable evidence to substantiate a role for hypertension as an important cause of heart failure. Approximately 48% of patients diagnosed with heart failure in primary care facilities have hypertension (Cleland et al 2002). Hypertension is one of the most frequent comorbidities identified in patients with heart failure (Levy et al 1996), and in addition, hypertension is one of the four most frequently cited comorbidities in clinical trials of heart failure (Krum & Gilbert 2003). The importance of blood pressure in the development of heart failure is underscored by the substantial evidence showing the prevention of heart failure development with the use of antihypertensive therapy (Dahlof et al 1991; Kostis et al 1997; MRC Working Party Medical Research Council 1992). Although hypertension may play a role in either HFrEF or HFpEF, hypertension may be a more important risk factor for HFpEF than HFrEF. What is the evidence to support this notion?

There is substantial evidence from preclinical animal models of hypertension that hypertension results in decreases in diastolic function of the LV (either increases in stiffness or decreases in relaxation) well before systolic abnormalities are observed and that these are prevented with adequate blood pressure (BP) control (Norton et al 1997). Moreover, many studies have shown associations between systolic BP (Abhayaratna et al 2008; Chung et al 2010; Hsu et al 2010; Masugata et al 2005; Pavlopoulos et al 2008) or diastolic BP (AlJaroudi et al 2012; Hwang et al 2012; Tsioufis et al 2008; Libhaber et al 2014) and LV E/A, a preload-dependent index of LV relaxation. Importantly, the BP that most strongly relates to E/A or LV

DD in some large studies is diastolic rather than systolic BP (AlJaroudi et al 2012; Hwang et al 2012; Tsioufis et al 2008; Libhaber et al 2009) and more recently diastolic BP has been shown to be correlated with E/A independent of systolic BP whereas systolic BP is not correlated with E/A independent of diastolic BP (Libhaber et al 2014). In comparison to relationships between BP and E/A, E/e' (an index of LV filling pressures) or the preload-independent index of myocardial relaxation (e') are more frequently associated with systolic BP, but not diastolic BP (Abhayaratna et al 2008; Chung et al 2010; Hsu et al 2010; Libhaber et al 2014; Mottram et al 2005; Pavlopoulos et al 2008; Russo et al 2010). However, one study showed that both systolic and diastolic BP were independently associated with E/e' (Hwang et al 2012). Importantly, regardless of which brachial BP (systolic or diastolic) is better related to measures of LV diastolic function in cross-sectional studies, in several clinical studies including major clinical trials such as the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) (Tapp et al 2010) antihypertensive therapy has been demonstrated to produce beneficial effects on tissue Doppler measures of DD (E/e' and e') and systolic BP is the BP that is best related to these improvements in diastolic function (Almuntaser et al 2009). Also noteworthy is the finding in cross-sectional analysis of a community-based study with a high prevalence of obesity, that BP contributed substantially more to LV DD than did any other risk factor except for age (Millen et al 2014).

With respect to evidence that hypertension is an important risk factor for HFpEF, as previously highlighted, HFpEF is frequently related to hypertension (Masoudi et al 2003; Redfield et al 2003; Owan et al 2006; Desai et al 2009; Kane et al 2011). In this regard, hypertension is an independent determinant of the progression to HFpEF (Mohammed et al 2012; Schillaci et al 2002) and a more rapid progression to LV DD occurs in hypertensives than normotensives (Bella et al 2002). Heart failure with a preserved ejection fraction has also been reported to be the dominant phenotype of heart failure in hypertensive heart disease (Tadic et al 2018).

1.3.2 Role of cardiac hypertrophy and remodeling in hypertensive LV DD and heart failure.

Traditionally, the development of LV dysfunction and consequently heart failure in hypertension has been viewed as the evolution from a phase of structural LV remodeling (concentric remodeling and hypertrophy) to LV decompensation. Indeed, well-recognised determinants of a reduced LV diastolic function are LV hypertrophy and concentric LV remodelling (as indexed by relative wall thickness) (Chahal et al 2010). Consequently, the presence of LV hypertrophy is thought to be an essential prelude to LV dysfunction, including LV DD. Indeed, LV DD may be seen in up to 84% of hypertensive individuals with LV hypertrophy (Wachtell et al 2000). Conversely, it has been estimated that only 11% to 20% of hypertensive patients have LV DD without exhibiting LV hypertrophy (Dini et al 2013; Phillips et al 1989). However, these estimates were obtained at a time when more contemporary non-invasive approaches to determining LV diastolic function were not available. Nevertheless, DD may be worse in patients with LV hypertrophy as compared to those without LV hypertrophy (Kattel et al 2016). Moreover, LV hypertrophy is well-recognised as progressing to both DD and heart failure and concentric LV hypertrophy is thought to progress to HFpEF (Drazner 2011).

Although there is substantial evidence to support the fact that LV hypertrophy and concentric LV remodelling mediate LV DD, there is also considerable evidence to oppose this view. Indeed, animal models of DD without LVH are well described (Norton et al 1996). Furthermore, even in animal models of hypertensive LVH, antihypertensive agents that reduce pressures without modifying LV mass are equally as effective at preventing LV DD as agents that regress LV hypertrophy (Norton et al 1997). It is also now well recognised that many patients with HFpEF do not have LV hypertrophy (Lam et al 2007) despite the fact that hypertension is the dominant risk factor for this form of heart failure. Moreover, it is recognised that LV DD without LV hypertrophy is an early manifestation of hypertensive heart disease (Messerli et al 2017). Furthermore, a dissociation has been noted between ethnicity and LV mass versus DD in a recent large echocardiographic study (Shantsila et al 2018), and the limited contribution of concentric LV hypertrophy to DD in hypertensives recently described (Nazário Leão et al 2018). In addition, it is mainly those with LV hypertrophy who, in addition to a structural change in the LV, have biomarker evidence of increased loading conditions or myocardial damage produced presumably by increased loads, that progress to heart failure

(Seliger et al 2015). Further, assigning those with LV hypertrophy to concentric versus eccentric subtypes only moderately differentiates participants at increased risk of heart failure with a preserved versus reduced ejection fraction (Ho et al 2013). Also in the ASCOT, the marked differences in treatment groups in improvements in LV diastolic function with antihypertensive therapy were unaffected by adjustments for LV mass index (Tapp et al 2010). Importantly, as indicated in preceding sections, it has been well-recognised for several decades (González et al 2018) with even more novel mechanisms continuing to emerge (González et al 2018; Paulus & Tschope 2013) of a role for several cellular changes induced by hypertension that are unrelated to the hypertrophic process and which determine LV diastolic function. Consequently, the value of measures of LV hypertrophy or the remodelling process as effective indices that herald the presence of LV DD is unclear. As part of the present thesis I therefore, in a large community-based study with a high prevalence of untreated hypertension, using product of coefficient mediation analysis, also aimed to determine the extent to which LV mass or relative wall thickness accounts for the impact of BP on indices of LV diastolic function. These data have been accepted for publication in *J Hypertens* (Bamaiyi et al, 2019, in-press) and are described and discussed in chapter 3 of the present thesis. The answer to this question is essential to obtaining a better understanding of those factors that may better identify hypertensives at risk of DD and hence of HFpEF,

1.4 Obesity as a risk factor for heart failure.

Frequently observed comorbid conditions are hypertension and obesity. In this regard, obesity is a well-recognized risk factor for hypertension. A steady rise in the global prevalence of obesity has been observed (Ebong et al 2014; Jonk et al 2007) with one-third of adults in the United States, (Buonacera et al 2018) and a similar proportion of adult women in developing countries such as South Africa (Beaney et al 2018) being obese. Although hypertension has over several decades been documented as a cause of heart failure (Mickerson 1959; Mancia et al 1993), it is only in the more recent past that obesity has been observed to be an independent risk factor for heart failure beyond all conventional risk factors including hypertension, diabetes

mellitus, dyslipidaemia and prior myocardial infarction. Indeed, a nested case-control study and a number of prospective studies have shown an independent association between the extent of adiposity and the development of heart failure (Chen et al 1999; He et al 2001; Johansson et al 2001; Wilhelmsen et al 2001; Kenchaiah et al 2002; 2009; Ingelsson et al 2005a & 2005b; Nicklas et al 2006; Bahrami et al 2008; Spies et al 2009). This relationship has been shown in the general population (He et al 2001; Kenchaiah et al 2002; Ingelsson et al 2005b; Bahrami et al 2008), in the middle-aged (Ingelsson et al 2005a), in the elderly (Chen et al 1999; Nicklas et al 2006), in women (He et al 2001), in men (Wilhelmsen et al 2001; Ingelsson 2005b), in general practice (Johansson et al 2001), in physicians (Kenchaiah et al 2009) and in persons with established coronary artery disease (Spies et al 2009).

Independent of conventional cardiovascular risk factors, a body mass index (BMI) of between 25 and 30 kg/m^2 (overweight) may increase the risk of heart failure by 39-49% compared to a BMI between 20 and 25 kg/m^2 (normal) (Bahrami et al 2008; Kenchaiah et al 2009). Similarly, a BMI greater than or equal to 30 kg/m^2 (obese) independently increases the risk of heart failure by 83-180% (Johansson et al 2001; Kenchaiah et al 2002; Bahrami et al 2008; Kenchaiah et al 2009). Importantly, the independent risk for heart failure attributed to overweight or obese is quantitatively similar to the effects of the presence of hypertension or diabetes mellitus noted in the same study.

Although the strength of the ability of indices of an excess adiposity to predict the risk of heart failure is influenced by statistical adjustments for traditional cardiovascular risk factors, as well as by adjustments for coronary events, the effect of these adjustments is surprisingly minimal. Indeed, in overweight and obese persons, the risk for heart failure is decreased by only 1-13% with these adjustments (He et al 2001; Ingelsson et al 2005a; 2005b; Nicklas et al 2006; Spies et al 2009) and by only 55% in obese individuals despite obesity increasing the risk for heart failure by 180% (Kenchaiah et al 2009). Hence, although there is no doubt that targeting modifiable cardiovascular risk factors with lifestyle interventions and medication is a crucial approach to prevent overweight/obesity-induced heart failure, the large remaining risk for heart failure after adjustments for conventional cardiovascular risk factors indicates that this is probably not the most effective approach. Importantly, while obesity is responsible for 11-14%

of heart failure in the United State of America, (Ebong et al 2014) it is reported to be responsible for 13.7-19.4% of heart failure in sub-Saharan Africa (Adeboye et al 2012). Thus, targeting obesity may be a key element to reducing the high prevalence of heart failure in Africa.

1.4.1 Relationship between obesity or associated insulin resistance and preclinical LV DD

Although hypertension has been well-recognised for several decades as a cause of LV DD (Shkhvatsabaia et al 1979; Dreslinski et al 1981) as with heart failure, it is also only more recently that the comorbidity, obesity has been recognized in large cross-sectional studies as being independently associated with LV DD (Tsioufis et al 2008; Libhaber et al 2009; Russo et al 2011; AlJaroudi et al 2012; Cil et al 2012). Moreover, in a meta-analysis and systematic review of 23 studies evaluating the impact of bariatric surgery on cardiac function and structure in obese patients, enhancements in LV diastolic function as well as decreases in relative wall thickness and LV mass were noted (Cuspidi et al 2014). Importantly however, more recent evidence from our group suggests that the impact of BP on LV diastolic function as assessed from contemporary measures of function at a community level is more crucial than the impact of obesity effects on LV diastolic function (Millen et al 2014). Nevertheless, assuming that obesity does play an important role in mediating DD, what are the potential mechanisms of this effect?

In obesity, insulin signaling may be defective with associated insulin resistance occurring. However, in the early stages of insulin-resistance, euglycaemia is maintained through compensatory hyperinsulinaemia. Prior to the development of diabetes mellitus and poor blood glucose control, insulin resistance may induce adverse cardiac changes through a number of mechanisms. Importantly, insulin resistance is not restricted to skeletal muscle, but also affects myocardial muscle tissue (Nikolaidis et al 2004, Ouwens et al 2005, Coort et al 2007). In myocardial muscle, insulin resistance downregulates glucose uptake and thereby prevents the energetic advantage provided by glucose compared to free fatty acid oxidation (Nikolaidis et al 2004, Ouwens et al 2005). In addition, insulin resistance is associated with intracellular accumulation of triacylglycerol resulting in activation of apoptotic pathways (lipotoxicity) (Coort

et al 2007). Hence, by reducing the efficiency of energy utilization or by promoting cell death (lipotoxicity), obesity could decrease myocardial relaxation thereby producing LV DD. Evidence for a role for insulin resistance in promoting obesity-associated LV DD has accumulated over the years from many small studies. However, the evidence from studies in unselected populations with a large sample size is less convincing. In this regard, in the Jackson Heart Study (2399 participants), the homeostasis model of insulin resistance (HOMA-IR) was assessed in women with a normal fasting blood glucose, and no relations with LV diastolic function determined using trans-mitral velocity and tissue Doppler assessments were reported on (Fox et al 2011). We therefore assume that no independent relations were noted in that study (Fox et al 2011). In participants being evaluated for health checks (n=1599), although decreases in E/A and increases in E/e' were observed with increasing quartiles of HOMA-IR, whether adjustments for indices of excess adiposity had any impact on these relationships was not reported on (Hwang et al 2012). In contrast to the lack of impact of adiposity indices on DD as demonstrated by our group (Millen et al 2014), in a more recent study conducted by our group, in 430 randomly recruited participants from a community sample, a more concentrically remodeled LV determined whether insulin resistance as determined from HOMA-IR, was associated with LV diastolic function (Peterson et al 2016). Importantly, in that study (Peterson et al 2016), relations between HOMA-IR and more contemporary measures of LV diastolic function (tissue Doppler indices) were modest at best and robust relations were only noted in those with a more concentrically remodeled LV. As hypertension is a strong determinant of concentric LV remodeling, the possibility exists that even if obesity or associated insulin resistance considered alone have only a modest impact on LV diastolic function, that obesity or its metabolic consequences may determine the extent to which DD occurs in hypertension. However, the extent to which adiposity indices or the metabolic abnormalities associated with obesity enhance the impact of hypertension on LV diastolic function has not been assessed. Consequently, as part of the present thesis I aimed to determine whether adiposity indices or insulin resistance influence the extent to which DD occurs in hypertension in a community sample with a high prevalence of hypertension and obesity. These data have been accepted for publication in *Clin Cardiol* (Bamaiyi et al, 2019) and are described and discussed in chapter 4 of

the present thesis The answer to this question is likely to better identify those hypertensives at risk of DD and hence HFpEF and perhaps offer an additional therapeutic target to prevent the transition from hypertension to HFpEF.

1.5 Problem statement

The prevalence of HFpEF is becoming increasingly more prevalent in all countries and is a major cause of hypertensive heart failure in Africa. As there are no therapeutic approaches with proven treatment benefits for HFpEF, a better understanding of the factors that influence its development is required. As HFpEF is caused by DD, a thorough understanding of the determinants of DD is essential. Central to DD is hypertension, but there are several outstanding questions regarding hypertensive DD that remain unanswered. In this regard, β-adrenergic receptor blockers are often essential for the management of hypertension, particularly in those patients with underlying coronary artery disease or atrial fibrillation. Although neurohumoral (including sympathetic) activation occurs in HFpEF, there is little understanding as to whether through well-recognised lusitropic properties this is beneficial to DD and hence whether β-adrenergic receptor blocker therapy will worsen DD. Furthermore, although classical principles promote the notion that the transition to LV dysfunction, including DD, occurs through concentric LV hypertrophy, several lines of evidence suggest that this concept is outdated. If so, specifically targeting LV hypertrophy would no longer be an acceptable approach to preventing or reversing DD in hypertension. In addition, not all hypertensives develop LV DD, and hence a better understanding of those at risk is essential. In this regard, obesity or its metabolic consequences, which are common co-morbid conditions in hypertension, although contributing comparatively less to DD than hypertension may enhance the impact of hypertension on DD.

1.6 Aims

Therefore, in the present thesis I aimed to determine;

1. Whether in hypertensive rat models (SHR and Dahl Salt-sensitive rats) associated with impaired LV diastolic but normal systolic function, β-adrenergic receptor stimulation improves LV relaxation and consequently decreases LV filling pressure. These data have been published in *J Cardiovasc Pharmacol* (Bamaiyi et al 2018) and are described and discussed in chapter 2.
2. Using product of coefficient mediation analysis, the extent to which LV mass and concentric LV remodelling account for the impact of blood pressure on LV diastolic function and LV DD in a community sample with a high prevalence of uncontrolled hypertension. These data have been accepted for publication in *J Hypertens* (Bamaiyi et al, in-press) and are described and discussed in chapter 3.
3. The extent to which adiposity indices or the homeostasis model of insulin resistance add to the impact of hypertension on LV diastolic function and DD in a community sample with a high prevalence of obesity and its metabolic consequences. These data have been published in *Clin Cardiol* (Bamaiyi et al 2019) and are described and discussed in chapter 4.

CHAPTER 2

Limited Impact of β-Adrenergic Receptor Activation on Left Ventricular Diastolic Function in Rat Models of Hypertensive Heart Disease.

The data in this chapter have been published in the *Journal of Cardiovascular Pharmacology*

Adamu J Bamaiyi, Gavin R Norton, Vernice Peterson, Glenda Norman, Frank B Mojiminiyi, Angela J Woodiwiss. Limited Impact of β-Adrenergic Receptor Activation on Left Ventricular Diastolic Function in Rat Models of Hypertensive Heart Disease,

J Cardiovasc Pharmacol. 2018;72(5):242-251

2.0 Abstract

Background: Hypertension is a major cause of left ventricular (LV) diastolic dysfunction (DD). Although β-adrenergic receptor (β-AR) blockers are often employed to manage hypertension, the impact of β-AR activation on LV lusitropic effects and hence filling pressures in the hypertensive heart with LV DD, is uncertain.

Methods: Using tissue Doppler imaging and Speckle tracking software we assessed LV function in isoflurane anesthetised Spontaneously Hypertensive (SHR) and Dahl salt sensitive (DSS) rats before and after β-AR activation (isoproterenol [ISO] administration).

Results: As compared to normotensive Wistar Kyoto control (WKY) rats, or DSS rats not receiving NaCl in the drinking water, SHR and DSS rats receiving NaCl in the drinking water had a reduced myocardial relaxation as indexed by lateral wall e' (early diastolic tissue velocity at the level of the mitral annulus) and an increased LV filling pressure as indexed by E/e'. However, LV ejection fraction and deformation and motion were preserved in both SHR and DSS rats. The administration of ISO resulted in a marked increase in ejection fraction and decrease in LV filling volumes in all groups; and an increase in e' in SHR, but not DSS rats. However, after ISO administration, although E/e' decreased in DSS rats in association with a reduced filling volume, E/e' in SHR remained unchanged and SHR retained greater values than WKY.

Conclusions: The hypertensive heart is characterised by reductions in myocardial relaxation and increases in filling pressures, but β-AR activation may fail to improve myocardial relaxation and when this occurs, does not reduce LV filling pressures.

Key words: hypertensive heart disease, left ventricular diastolic function, myocardial relaxation, adrenergic activation.

2.1 Introduction

Hypertension is a well-recognised cause of left ventricular (LV) diastolic dysfunction (DD) and consequently heart failure with a preserved ejection fraction (EF) (Borlaug & Paulus 2011; Borlaug & Redfield 2011; Lee et al 2009). Heart failure with a preserved EF contributes to close to half of all admissions for heart failure, and the outcomes may be equally as poor as heart failure with a reduced EF (Bhatia et al 2006; Owan et al 2006; Pitt et al 2014). Included in those agents that may be employed to manage hypertension are β-adrenergic receptor (β-AR) blockers. However, sympathetic activation, such as may occur with physical activity, has well-recognised physiological benefits to LV diastolic function, with the ability to enhance lusitropy through β-AR stimulation. Thus, the use of β-AR blockers to treat hypertension may worsen DD during periods of physical activity. However, the impact of β-AR stimulation on LV diastolic function in hypertensives with LV DD, is unclear.

Decreases in myocardial active properties (which influence relaxation) contribute to LV DD (Zile et al 2004; Lewis et al 2017; Shah et al 2016; Tschöpe et al 2017; Shah et al 2014) and are thought to play a major role in determining increases in filling pressures in hypertensive DD (Shah et al 2014).Consequently, during physical activity, β-AR-induced lusitropic effects may have benefits to the heart with DD (thus accommodating increased filling volumes at normal filling pressures) and β-AR blockers may attenuate these beneficial effects. However, in hearts with DD, an attenuated sympathetic-induced LV suction (an effect mediated by relaxation of the LV in early diastole) occurs (Ohara et al 2012). If a decreased sympathetic-induced LV suction (Ohara et al 2012) translates into a limited impact of adrenergic activation on LV filling pressures in DD, under these circumstances the use of β-AR blocking agents may have little deleterious effect on DD during physical activity. Nevertheless, the impact of β-AR stimulation on LV filling pressures in DD, is unknown. In the present study we therefore assessed the extent to which β-AR stimulation improves LV relaxation abnormalities and hence decreases Doppler indexes of LV filling pressure in Spontaneously Hypertensive (SHR) and Dahl salt-sensitive (DSS) hypertensive rat models of LV DD.

2.2 Methods

The Animal Ethics Screening Committee of the University of the Witwatersrand approved the protocol (approval numbers 2016/03/08/A and 2016/06/28/B). Forty one rats were evaluated in the present study. Seven month old male SHR (n=14) and age-matched Wistar Kyoto control (WKY) (n=11) rats, and 5 month old male Dahl salt-sensitive rats (DSS) (originally sourced from Charles River Co, Washington DC, USA) (n=16) were evaluated. Of the DSS rats, 7 received 0.6% NaCl in the drinking water for 2 months and 9 received normal tap water. All DSS rats were assessed at 7 months of age. Prior to echocardiographic and invasive hemodynamic assessments, awake restrained rats had tail cuff blood pressures (BP) determined using standard techniques (BIOPAC systems, Inc. NIBP250 blood pressure amplifier). Rats were habituated to these measurements by placing them in restrainers for 10 minutes at a time on 3 separate occasions and allowing them to undergo the BP measurement procedure before BP values were recorded. Fourteen SHR, 5 WKY rats and all DSS rats had hemodynamic assessments performed at baseline and then following the administration of a β-adrenoreceptor agonist (isoproterenol). Six WKY rats had hemodynamic assessments performed at baseline and then after increases in BP produced by administration of an α-adrenoreceptor agonist, phenylephrine. This approach was adopted to ensure that differences in function between hypertensives and normotensives were not attributed to the effects of BP afterload on the LV. At the end of the study post-mortem examinations were performed to identify the presence of clinical features of heart failure (Tsotetsi et al 2001).

2.2.1 Hemodynamics. Anesthesia was induced with isoflurane (5%) in oxygen and maintained with 2% isoflurane. Rats were placed on a heating pad for the duration of the study. To assess the impact of isoproterenol on hemodynamic values in anesthetised rats, baseline echocardiography was performed and then again after 4 doses of 0.1 mg/kg per rat isoproterenol at which point heart rate no longer increased (a total of 0.4 mg/kg per rat). To assess hemodynamic values in WKY at higher afterloads, echocardiography was performed at baseline and following 7 doses of phenylephrine (PE) (0.5 mg/kg intraperitoneal) at which point BP no longer increased (total of 3.5 mg/kg per rat). Data were obtained over a maximum of 30

minutes from the time of anesthetic induction in rats receiving isoproterenol and the average time (mean±SD) taken to collect all data was similar between groups of rats receiving isoproterenol (WKY=19 minutes and 20 seconds±4 minute and 52 seconds; SHR=23 minutes and 36 seconds±4 minutes and 18 seconds; DSS-no additional NaCl=21 minutes and 22 seconds±5 minutes and 38 seconds; DSS receiving additional NaCl=22 minutes and 35 seconds±5 minutes and 22 seconds). As a catheter was inserted into WKY rats receiving PE, the time taken from anesthetic induction to final data collection was 40 minutes and 42 seconds±8 minutes and 41 seconds.The average time taken between baseline measurements and measurements obtained in the presence of isoproterenol stimulation was also similar between groups of rats (WKY=14 minutes±1 minute and 32 seconds; SHR=17 minutes and 25 seconds±3 minutes and 36 seconds; DSS-no additional NaCl=18 minutes and 17 seconds±3 minutes and 30 seconds; DSS receiving additional NaCl=17 minutes and 25 seconds±4 minutes and 50 seconds). In a pilot study performed with no interventions in normotensive rats (n=4), no significant changes in hemodynamic parameters occurred from baseline over a 30 minute assessment period.

To determine pressure changes in rats receiving PE, carotid pressures (assumed to be only 1 mm Hg higher than aortic pressure and hence employed as a surrogate thereof) were recorded using a PP-50 heparinised saline-filled polyethylene catheter, coupled to a pressure transducer. The catheter was inserted into the left common carotid artery, advanced 1.5 to 2.0 cm until the tip of the catheter was close to the origin of the carotid artery from the aorta, and subsequently the catheter was tied in place. The amplitude-frequency response of the catheter-transducer dome combination employed was uniform to 10 Hz. Pressure recordings were obtained on a Powerlab recording system (4/30 model ML866, Adinstruments system, Australia).

2.2.2 Echocardiography. Echocardiographic measurements were performedby an experienced observer (AJW) with the rats (anterior chest hair shaved) in the left lateral decubitus position using an echocardiogram (Acuson SC2000 Diagnostic ultrasound system, Siemens Medical Solutions, USA, Inc.) equipped with a 10 MHz linear array transducer and ECG. A frame rate of >110 frames per second was employed for all B-mode and M-mode

images. Left ventricular dimensions were determined using two-dimensional directed (parasternal long axis view) M-mode echocardiography obtained in the short axis of the heart as close to the tip of the mitral valves as possible in all rats. The LV dimensions were measured only when appropriate visualization of both the right and the left septal surfaces occurred and where the endocardial surfaces of both the septal and posterior wall were clearly visible. LV end systolic (LVESD) and end diastolic (LVEDD) internal diameters (leading edge method) and wall thickness values were obtained from M-Mode images and these recordings were analyzed according to the American Society of Echocardiography convention (Sahn et al 1979). Left ventricular systolic chamber function was determined from LV endocardial fractional shortening (FSend) calculated from LV (EDD-ESD)/EDD expressed as a percentage and LV ejection fraction (EF) calculated using the Teichholz method.

To further evaluate changes in LV systolic function, LV strain, strain rate, displacement and velocity measurements were obtained from digital dynamic cine-loops of long axis (longitudinal) and short axis (circumferential or rotational) B-mode images using vendor's software (velocity vector imaging, VVI). Images selected for analysis had well-defined endocardial and epicardial borders and no substantial image artefacts. Image analysis was performed according to manufacturer's instructions. At least five consecutive cardiac cycles were selected for analysis. The endocardium and epicardium were traced semi-automatically using vendor's software (VVI) beginning at the mitral valve annulus (free wall edge), extending to the apex, and returning to the mitral valve annulus (septal edge) for the long-axis images, and beginning at the anteroseptum and returning to the original location in a clockwise direction for the short axis images. The traces were manually adjusted to ensure adequate tracking of the endocardial and epicardial borders throughout the cardiac cycle. Velocity, displacement, strain, and strain rate were calculated in the longitudinal and circumferential planes.

Left ventricular diastolic function was assessed from a pulsed wave Doppler examination of the mitral inflow at rest and tissue Doppler indexes (TDI). Pulse wave Doppler recordings of trans-mitral velocity were obtained with the sample volume at the tip of the mitral valve in the apical four-chamber view. Trans-mitral velocity measurements were obtained during the early (E) and late (atrial contraction) (A) period of LV diastolic inflow and care was taken to avoid the

overshoot in the spectral image often noted in rat imaging when velocity assessments are determined. In this regard, the leading (outer) edge or the most dense, or brightest, portion of the spectral image was identified as the actual velocity waveform. To perform TDI, the velocity of myocardial tissue lengthening at the level of the mitral annulus was recorded in the apical four-chamber view. The sample volume was positioned at the lateral corner of the mitral annulus. To determine diastolic function using TDI, peak velocities during early (e') and late (atrial contraction)(a') diastole were measured. Data were expressed as the E/e' ratio (an index of LV filling pressures) or as e' alone, e'/a' and E/A (indexes of myocardial relaxation). Because mitral annular velocity (e') remains constant and trans-mitral flow velocity (E) increases with an increased filling pressure, E/e' ratio correlates well with left ventricular filling pressures in rat hearts (Leite et al 2015).

2.2.3 Data analysis. For comparisons of resting values, an unpaired Student's t-test was performed. For comparisons of values before and after interventions, a paired Student's t-test (effect of phenylephrine in WKY rats) or repeated measures two-way ANOVA with a Tukey *post hoc* test (effect of isoproterenol on hypertensives and controls) was performed where appropriate.

2.3 Results

*2.3.1 Blood pressures, LV remodelling and evidence of heart failure***.** SHR and DSS rats receiving NaCl in the drinking water had increased tail cuff (awake, restrained) BP values as compared to controls (Table 2.1). Although SHR had similar absolute heart and LV weights as WKY, heart weight or LV weight/body weight ratios were markedly increased in SHR (Table 2.1). DSS rats receiving NaCl in the drinking water however, did not have increased heart and LV weights as compared to DSS rats receiving no additional NaCl (Table 2.1). As compared to WKY rats, SHR had concentric LV remodelling as indexed by increases in relative wall thickness (septal+posterior wall thickness at end diastole/EDD) and decreases in internal diameters (Table 2.1). In contrast, DSS rats receiving NaCl in the drinking water had no obvious

Table 2.1. Blood pressures, cardiac weight and geometry and left ventricular (LV) function in hypertensive rat models.

	WKY (n=11)	SHR (n=14)	DSS (n=9)	DSS-NaCl (n=7)
Tail cuff blood pressure				
Systolic blood pressure (mm Hg)	147±10	214±4**	163±9	233±5**
Cardiac weight and geometry				
Body weight (BW) (g)	467±8	359±10**	432±7	402±8*
Total heart weight (g)	1.33±0.03	1.34±0.03	1.65±0.05	1.65±0.02
Heart weight/BW x 10^3	2.85 ±0.06	3.73±0.10**	3.82±0.12	4.1.2±0.11
Left ventricular (LV) weight (g)	1.00±0.04	1.01±0.01	1.31±0.03	1.33±0.04
LV/BW x 10^3	2.13±0.20	2.80±0.10*	3.04±0.09	3.32±0.13
LV end diastolic (ED) diameter (mm)	0.70±0.03	0.60±0.02*	0.77±0.02	0.77±0.04
LVED posterior wall thickness (mm)	0.19±0.01	0.20±0.01	0.18±0.02	0.14±0.02
LVED relative wall thickness	0.52±0.03	0.67±0.04*	0.49±0.03	0.40±0.06
LV function				
Endocardial fractional shortening (%)	35.4±3.6	31.6±1.8	33.4±3.2	35.7±3.9
Midwall fractional shortening (%)	20.0±0.02	19.0±0.01	18.9±1.9	21.8±3.0
Longitudinal strain rate (sec^{-1})	-3.38±0.70	-3.85±0.48	-5.72±1.44	-8.25±1.60
Longitudinal displacement (cm)	0.27±0.07	0.24±0.06	0.35±0.07	0.33±0.15
Longitudinal velocity (cm/sec)	0.79±0.11	0.83±0.16	0.91±0.13	0.63±0.36
Transmitral E(cm/s)	88±8	120±4**	78±12	111±25
e'/a'	1.15±0.16	1.26±0.08	1.25±0.21	0.80±0.18
E/A	2.29±0.18	2.88±0.18*	2.47±0.32	2.76±0.63

SHR, Spontaneously Hypertensive Rats; WKY, Wistar Kyoto control rats; DSS, Dahl salt-sensitive rats; E,transmitral blood flow velocity in the early phase of diastole; e', velocity of myocardial tissue lengthening in the early phase of diastole at the level of the mitral annulus; a', velocity of myocardial tissue lengthening in the atrial contraction phase of diastole at the level of the mitral annulus; A, transmitral blood flow velocity in the atrial contraction phase of diastole. *p<0.01, **p<0.005 versus control group.

concentric LV remodelling as compared to controls (Table 2.1). On post mortem examination, none of the rats had evidence of left atrial thrombi, or pleuro-pericardial effusions and none of the SHR or NaCl-loaded Dahl SS rats had lung weights that were two standard deviations above the mean value of WKY or non-NaCl-loaded Dahl SS rats.

2.3.2 Left ventricular systolic function at baseline. No differences were noted in indexes of systolic chamber function (FSend and EF) at baseline between SHR or DSS rats receiving NaCl in the drinking water and their respective control groups (Table 2.1, Figure 2.1). Furthermore, neither indexes of LV myocardial deformation (longitudinal or circumferential strain or strain rate) nor motion (longitudinal or circumferential/rotational displacement or velocity) were reduced in SHR or DSS rats receiving NaCl in the drinking water as compared to their respective control groups at baseline (Tables 2.1 and 2.2, Figure 2.1). Indeed, DSS rats receiving additional NaCl had a marked increase in longitudinal strain as compared to controls (Figure 2.1).

2.3.3 Left ventricular diastolic function at baseline. As compared to controls, at baseline, SHR and DSS rats receiving NaCl in the drinking water had a diminished LV relaxation as indexed by decreases in lateral wall e' (Figure 2.1). In addition, as compared to controls, at baseline, SHR and DSS rats receiving NaCl in the drinking water had increases in the Doppler index of LV filling pressures, E/e' (Figure 2.1). Consistent with a restrictive filling pattern in a concentrically remodelled LV, E and E/A values were increased in SHR as compared to WKY (Table 2.1). However, in keeping with the lack of concentric LV remodeling in DSS rats receiving NaCl in the drinking water, no significant differences in E or E/A were observed as compared to controls (Table 2.1).

2.3.4 Load-induced effects on left ventricular function in WKY. The administration of phenylephrine produced marked increases in BP in WKY rats with no significant change in indexes of systolic LV chamber function, LV myocardial deformation or motion (Tables 2.3 and 2.4). Phenylephprine administration did however produce a trend for improvements in LV relaxation (e') and decreases in LV filling pressure (E/e') (Table 2.3).

2.3.5 Impact of β-adrenergic receptor stimulation on LV systolic function. Isoproterenol administration produced marked and consistent increases in FSend, FSmid, and EF as well as

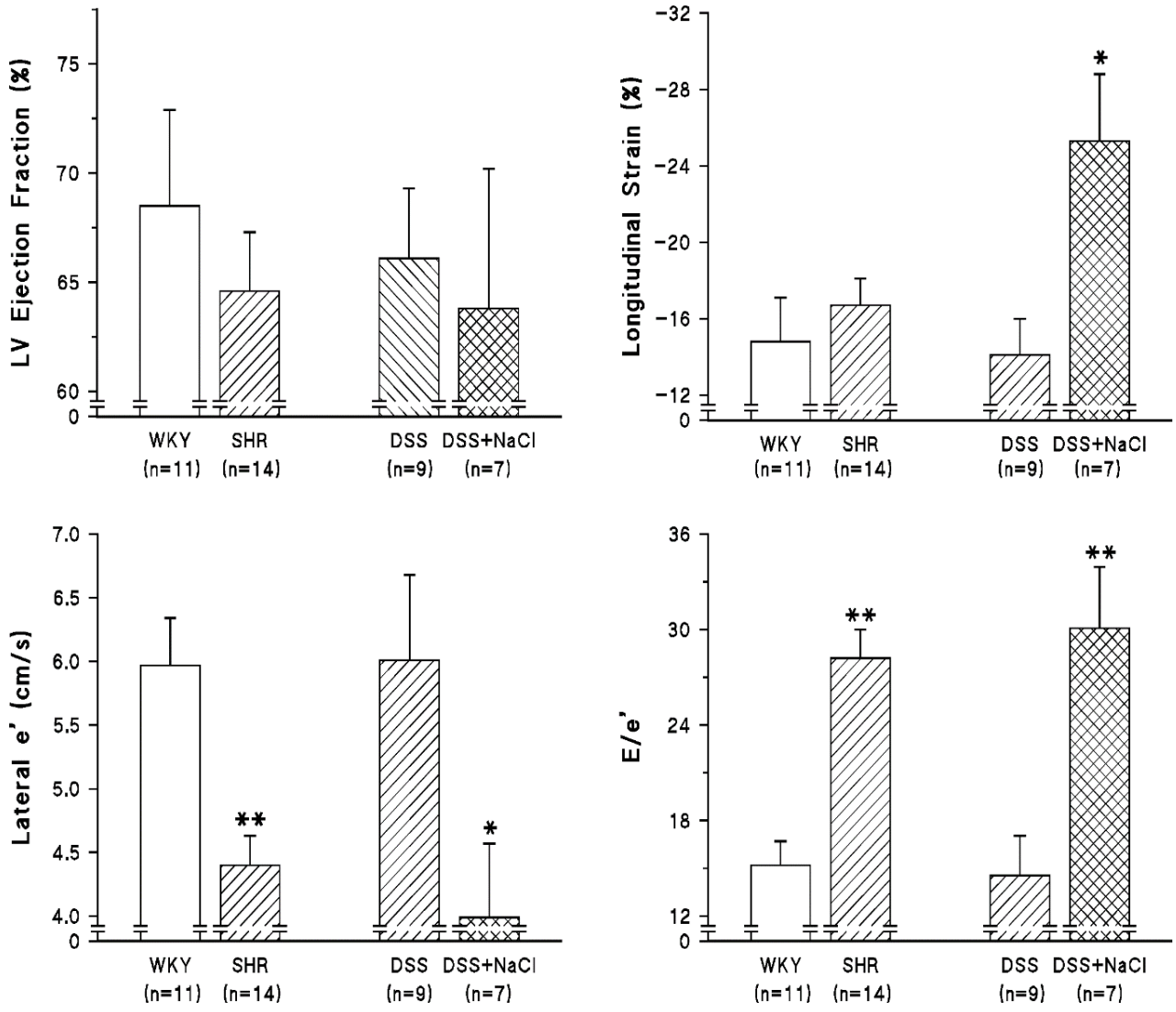

Figure 2.1. Left ventricular (LV) systolic and diastolic function in hypertensive rat models. See table 3 for other LV parameters. *p<0.05,**p<0.005 vs WKY or DSS. SHR, Spontaneously Hypertensive Rat; WKY, Wistar Kyoto control rats; DSS, Dahl salt sensitive rats; lateral e', velocity of myocardial tissue lengthening in the early phase of diastole in the lateral wall of the LV at the level of the mitral annulus; E, transmitral blood flow velocity in the early phase of diastole; E/e', index of filling pressure.

Table 2.2. Left ventricular (LV) systolic function in the circumferential plane in hypertensive rat models.

	WKY (n=11)	SHR (n=14)	DSS (n=9)	DSS-NaCl (n=7)
Circumferential strain (%)	-23.3±3.8	-21.1±2.9	-27.2±7.0	-12.4±10.0
Circumferential strain rate (sec^{-1})	-5.92±1.68	-3.77±0.59	-7.90±2.00	-6.07±1.15
Rotational displacement (degree)	3.60±0.69	3.51±0.66	3.75±1.39	1.80±0.84
Rotational velocity (degree/sec)	99±17	102±12	126±39	83±15

SHR, Spontaneously Hypertensive Rats; WKY, Wistar Kyoto control rats; DSS, Dahl salt-sensitive rats.

Table 2.3. Impact of the administration of phenylephrine (PE) on blood pressures and left ventricular function in Wistar Kyoto control rats (WKY) (n=6).

	Before PE	After PE
Carotid systolic blood pressure (mm Hg)	120±3	188±6**
Carotid diastolic blood pressure (mm Hg)	94±1	136±5**
Left ventricular ejection fraction (%)	72.0±7.8	69.6±6.5
Endocardial fractional shortening (%)	38.7±6.3	36.0±4.4
Midwall fractional shortening (%)	19.8±4.5	21.0±3.1
Longitudinal strain (%)	-14.6±4.3	-12.6±3.4
Longitudinal strain rate (sec^{-1})	-3.38±0.70	-2.69±0.69
Longitudinal displacement (cm)	0.25±0.21	0.22±0.06
Longitudinal velocity (cm/sec)	0.86±0.19	0.70±0.16
Lateral e' (cm/s)	5.66±0.66	8.92±1.72*
Transmitral E (cm/s)	77.0±7.7	79.1±6.8
e'/a'	0.96±0.16	1.59±0.46
E/A	2.44±0.31	2.20±0.32
E/e'	14.1±1.7	8.8±1.5*

See table 2.1 for abbreviations. *p=0.05, ** p<0.0001 versus before PE.

Table 2.4. Impact of the administration of phenylephrine (PE) on left ventricular systolic function in the circumferential plane in Wistar Kyoto control rats (WKY) (n=6).

	Before PE	After PE
Circumferential strain (%)	-26.3±7.1	-18.6±5.7
Circumferential strain rate (sec^{-1})	-5.92±1.68	-4.57±1.01
Rotational displacement (degree)	4.55±1.29	3.59±1.03
Rotational velocity (degree/sec)	123±34	98±35

See table 2.1 for abbreviations.

rotational velocity in both SHR and DSS rats and their respective control groups (Tables 2.5 and 2.6). Isoproterenol administration also increased rotational displacement in DSS rats, but not in WKY or SHR (Tables 2.7 and 2.8). However, isoproterenol administration decreased circumferential strain in WKY, SHR and control DSS rats (Tables 2.5 and 2.6), but increased circumferential strain and circumferential strain rate in DSS rats receiving NaCl in the drinking water (Tables 2.6 and 2.8). Therefore, whilst SHR and WKY rats had similar systolic function after isoproterenol administration, DSS rats receiving NaCl in the drinking water had a greater longitudinal and circumferential strain and strain rate after isoproterenol administration than DSS rats receiving no additional NaCl in the drinking water (Tables 2.5, 2.6, 2.7 and 2.8).

2.3.6 Impact of β-adrenergic receptor stimulation on LV diastolic function. Isoproterenol administration produced a decrease in end diastolic volume (EDV), but no change in E, e', e'/a' or E/A in WKY rats (Figure 2.2, Table 2.5). However, in SHR, isoproterenol administration resulted in a decreased EDV and an increased LV relaxation as indexed by increases in E and e', but not e'/a' or E/A (Figure 2.2, Table 2.5). As isoproterenol improved myocardial relaxation, as indexed by the load-independent measure, e', in SHR, but not WKY, relaxation properties (e') were similar between SHR and WKY after isoproterenol administration (Figure 2.2). A correlation between isoproterenol-induced increases in e' and decreases in filling pressures (E/e') was noted in WKY and SHR (r^2=0.48, p<0.001). Despite this correlation and the finding that isoproterenol administration resulted in reductions in EDV in both SHR and WKY rats, in neither WKY, nor SHR did isoproterenol administration alter filling pressures (E/e') (Figure 2.2). Hence filling pressures in SHR remained elevated as compared to WKY rats (Figure 2.2).

Isoproterenol administration produced a decrease in end diastolic volume (EDV), but failed to improve myocardial relaxation as indexed by E, e', e'/a' or E/A in either DSS rats receiving NaCl in the drinking water or controls (Figure 2.3, Table 2.6). However, in DSS rats receiving NaCl in the drinking water isoproterenol administration reduced filling pressure (E/e') (Figure 2.3). The isoproterenol-induced decrease in filling pressures in DSS rats receiving NaCl in the drinking water was attributed to reductions in EDV. Indeed, isoproterenol-induced decreases in E/e' correlated with reductions in EDV (r^2=0.44, p<0.001).

Table 2.5. Left ventricular function in Spontaneously Hypertensive Rats (SHR) versus Wistar Kyoto control rats (WKY) before and after β-adrenergic receptor stimulation with isoproterenol (ISO).

	WKY (n=5)		SHR (n=14)	
	Before ISO	After ISO	Before ISO	After ISO
Heart rate (min^{-1})	345±10	442±12**	314±10†	442±9**
Ejection fraction (%)	50.2±2.4	85.4±4.9**	64.6±2.7	89.2±1.6**
Endocardial fractional shortening (%)	26.9±3.0	44.5±11.3**	31.6±1.8	59.5±2.6**
Midwall fractional shortening (%)	15.6±2.1	24.4±4.5**	18.6±1.3	32.5±1.5**
Longitudinal strain (%)	-14.9±4.4	-8.9±4.5	-16.7±1.4	-10.7±2.4
Circumferential strain (%)	-20.4±2.5	-9.4±4.9*	-21.1±2.9	-8.0±1.3**
Rotational velocity (degree/sec)	70±6	199±58**	102±12	191±39**
Transmitral E (cm/s)	101±13	127±19	120±4	163±9**
e'/a'	1.38±0.30	1.40±0.16	1.26±0.08	1.37±0.12
E/A	2.11±0.21	2.52±0.13	2.88±0.17†	1.79±0.09**‡

See table 2.1 for abbreviations. *p<0.05 **p<0.005 versus before ISO; †p<0.05 versus WKY before ISO; ‡p<0.05 versus WKY after ISO.

Table 2.6. Left ventricular function in Dahl salt-sensitive rats (DSS) before and after β-adrenergic receptor stimulation with isoproterenol (ISO).

	DSS (n=9)		DSS-NaCl (n=7)	
	Before ISO	After ISO	Before ISO	After ISO
Heart rate (min^{-1})	355±13	418±9**	352±11	382±14**
Ejection fraction (%)	66.1±3.2	91.3±1.9**	63.8±6.4	92.0±2.0**
Endocardial fractional shortening (%)	33.4±3.2	55.3±5.7**	35.7±3.9	53.3±4.0**
Midwall fractional shortening (%)	18.9±1.9	26.2±3.9**	21.8±3.0	29.7±3.1**
Longitudinal strain (%)	-14.1±1.9	-9.9±2.2	-25.3±3.5†	-24.2±5.0‡
Circumferential strain (%)	-27.2±7.0	-11.0±4.5*	-12.4±10.0	-45.7±11.7*‡
Rotational velocity (degree/sec)	126±39	345±93**	83±15	348±60**
Transmitral E (cm/s)	78±11	64±17	111±25	99±14
e'/a'	1.25±0.21	0.91±0.14	0.80±0.18	0.89±0.13
E/A	2.47±0.32	1.46±0.30*	2.76±0.63	1.51±0.43*

See table 2.1 for abbreviations. *p<0.05, **p<0.005 versus before ISO, †p<.05 versus DSS or DSS-NaCl before ISO, ‡p<0.05 versus DSS after ISO.

Table 2.7. Additional left ventricular systolic functional parameters in Spontaneously Hypertensive Rats (SHR) versus Wistar Kyoto control rats (WKY) before and after β-adrenergic receptor stimulation with isoproterenol (ISO).

	WKY (n=5)		SHR (n=14)	
	Before ISO	After ISO	Before ISO	After ISO
Longitudinal strain Rate(sec^{-1})	-3.16±1.00	-4.41±1.52	-3.85±0.48	-3.97±0.73
Circumferential Strain rate (sec^{-1})	-3.84±0.70	-4.06±1.89	-3.77±0.59	-2.74±0.74
Longitudinal displacement (cm)	0.32±0.07	0.32±0.14	0.24±0.06	0.13±0.03*
Rotational displacement (degree)	3.45±1.14	4.20±2.59	3.51±0.66	2.38±0.68
Longitudinal velocity (cm/sec)	0.70±0.14	1.09±0.31	0.83±0.16	0.88±0.13

See table 2.1 for abbreviations. *p<0.05 versus before ISO.

Table 2.8. Additional left ventricular systolic functional parameters in Dahl salt-sensitive rats (DSS) before and after β-adrenergic receptor stimulation with isoproterenol (ISO).

	DSS (n=9)		DSS-NaCl (n=7)	
	Before ISO	After ISO	Before ISO	After ISO
Longitudinal strain rate (sec^{-1})	-5.72±1.44	-3.71±1.10	-8.25±1.60	-9.94±2.06‡
Circumferential strain rate (sec^{-1})	-7.90±2.01	-7.39±1.81	-6.1±1.15	-13.0±1.9*‡
Longitudinal displacement (cm)	0.35±0.07	0.23±0.06	0.33±0.15	0.37±0.14
Rotational displacement (degree)	3.75±1.39	10.96±3.42*	1.80±0.84	7.34±2.44*
Longitudinal velocity (cm/sec)	0.91±0.13	0.89±0.29	0.63±0.36	1.50±0.33

See table 2.1 for abbreviations. *$p<0.05$ versus before ISO, ‡$p<0.05$ versus DSS after ISO.

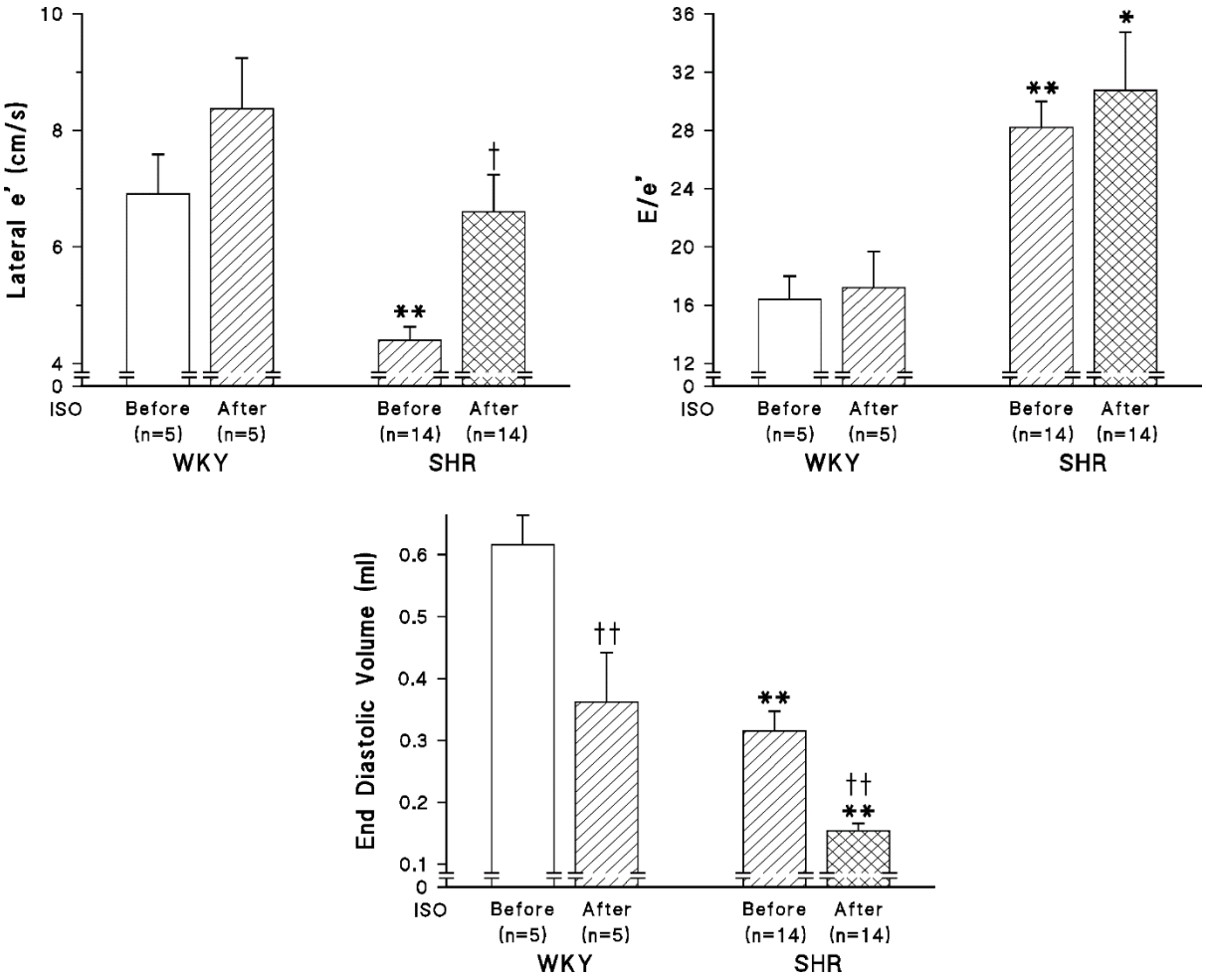

Figure 2.2. Impact of β-adrenergic receptor stimulation (isoproterenol administration [ISO]) on left ventricular diastolic function in Spontaneously Hypertensive Rats (SHR) versus Wistar Kyoto control rats (WKY). *p<0.05, **p<0.005 vs WKY; †p<0.05, ††p<0.005 vs before ISO. See Figure 2.1 for abbreviations.

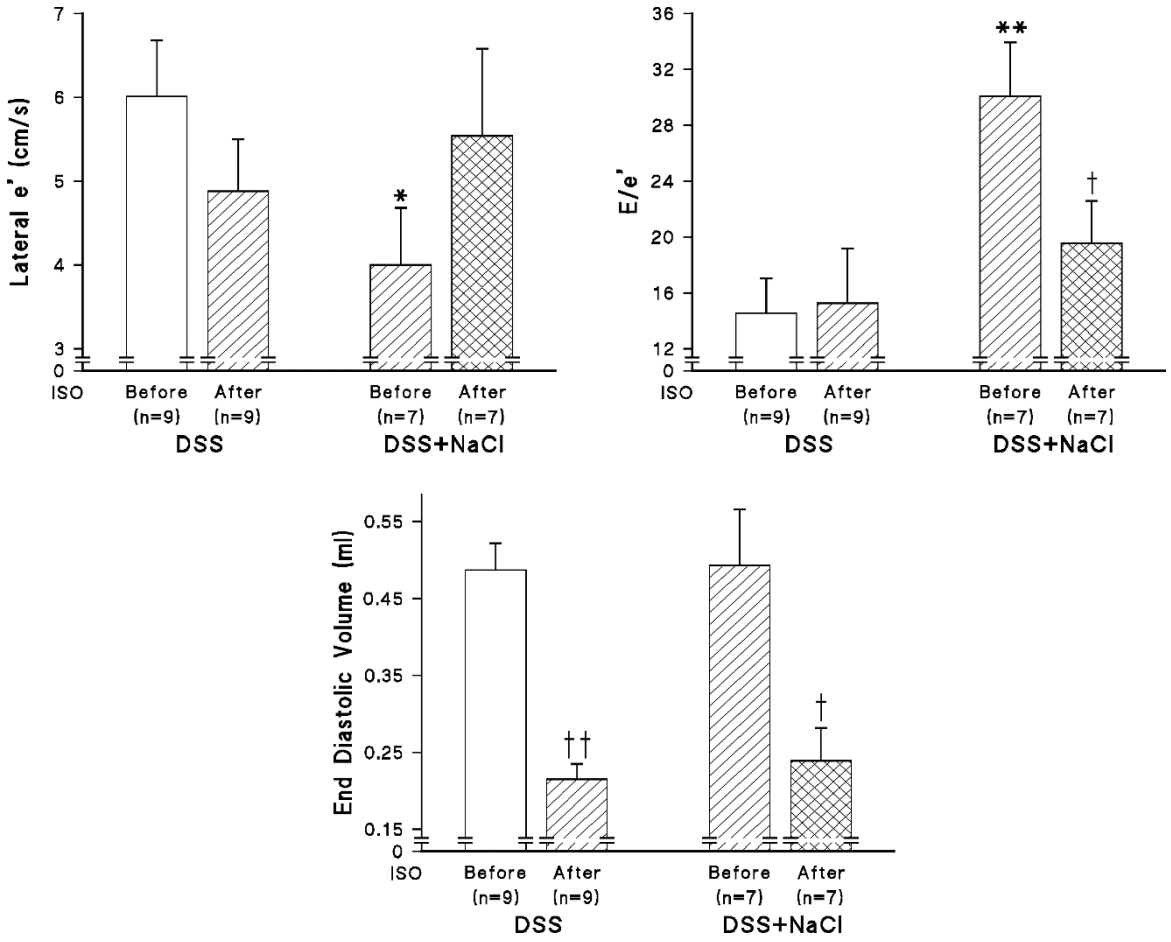

Figure 2.3. Impact of β-adrenergic receptor stimulation (isoproterenol administration [ISO]) on left ventricular diastolic function in Dahl salt-sensitive (DSS) rats. *p<0.05, **p<0.005 vs DSS; †p<0.05, ††p<0.005 vs before ISO. See Figure 2.1 for abbreviations.

2.4 Discussion

The main findings of the present study are as follows: As compared to control rats, as determined under anesthetic conditions the LV of SHR and DSS rats receiving additional NaCl, is characterized by a reduced LV relaxation (decreased e'), and increased filling pressures (increased E/e'). These diastolic abnormalities were noted despite preserved indexes of LV chamber and myocardial (deformation or motion) systolic function. Importantly, through increases in ventricular ejection with consequent reductions in filling volumes, but not through lusitropic effects (e'), β-adrenergic receptor stimulation decreased filling pressures (E/e') in DSS rats. In contrast however, although adrenergic stimulation in SHR induced lusitropic effects (e'), and through increases in rotational velocity increased EF and reduced filling volumes, these changes failed to translate into decreases in filling pressures (E/e').

2.4.1 β-AR-induced changes in LV diastolic function in hypertensive hearts. Abnormalities in LV diastolic function in hypertensive rats (decreases in myocardial relaxation as indexed by e' and increases in filling pressures as indexed by E/e') in the present study are consistent with those previously reported in SHR at a similar age (Shah et al 2014). Importantly, the impact of hypertension in SHR at a similar age on LV diastolic function (as determined from diastolic pressure-dimension relations) and on myocardial collagen changes has been well described by our group on several previous occasions (Tsotetsi et al 2001; Norton et al 1997). In this regard, SHR have marked changes in the interstitium which may in-part explain reductions in diastolic function. However, a number of cellular changes (Lewis et al 2017; Shah et al 2016; Tschöpe et al 2017) may contribute to diastolic dysfunction, alterations which influence either active or passive processes. Ion channel remodeling and a lower resting energy reserve, changes which may decrease the ability to sequester calcium, reduce active myocardial relaxation and hence contribute to DD (Adeniran et al 2015; Lamb et al 1999; Phan et al 2009; Selby et al 2011). Importantly, alterations in cardiomyocyte calcium release and sequestration are well-described in the heart of SHR (Shah et al 2014). Consequently, sympathetic activation during physical activity, could improve myocardial relaxation and thus in

hypertensive heart disease, maintain normal LV filling pressures whilst filling volumes increase. However, in the present study we show that despite abnormalities in myocardial relaxation in hypertensive rat models, β-AR activation of an LV with DD does not improve myocardial relaxation in some forms of DD (DSS rats), and despite promoting lusitropic effects in SHR, fails to reduce LV filling pressures. Thus, in the management of hypertensives with LV DD, the use of agents that block β-ARs are unlikely to oppose any beneficial actions of sympathetic-induced lusitropic effects that may occur during physical activity, on LV filling pressures.

When SHR or Dahl rats progress to heart failure, an important mechanism responsible for this change is early LV dilatation and systolic chamber dysfunction, rather than heart failure with solely a preserved ejection fraction (Tsotetsi et al 2001; Norton et al 1997). However, under these circumstances LV diastolic abnormalities always occur secondary to systolic functional abnormalities rather than primarily as a consequence of diastolic abnormalities. To avoid the confounding effects of systolic functional abnormalities on diastolic function, in the present study we therefore focused on rats with preclinical diastolic dysfunction and a preserved systolic function, rather than overt heart failure. Indeed, in the present study neither systolic chamber function, nor more sensitive indexes of myocardial systolic function (deformation or motion) were modified in either SHR or Dahl rats. Importantly, preclinical diastolic dysfunction is now well recognized as progressing to overt heart failure. However, further studies are required to evaluate the impact of adrenergic stimulation on LV diastolic function in rat models of heart failure with a normal systolic function.

The inability of β-AR activation to reduce LV early diastolic filling pressures in SHR in the present study, despite promoting lusitropic effects (increased e’), could be explained by a reduced adrenergic-induced increase in LV suction forces, previously noted to occur in patients with DD (Ohara et al 2012). In this regard, decreases in β-AR-induced early diastolic suction in patients with DD are attributed to reduced inertial acceleration between the mid-LV and LV apex (Ohara et al 2012). These suction forces are therefore likely to generate enhanced filling because of reductions in LV early diastolic pressures. The consequence of these reduced

suction forces is therefore likely to be an inability of β-AR activation to reduce filling pressures in the early phase of LV filling (as indexed by E/e') as demonstrated in SHR.

In the present study, although β-AR activation failed to improve lusitropic effects (lateral wall e' remained unchanged) in DSS rats receiving NaCl in the drinking water, it reduced filling pressures. This may be attributed to increases in ejection and consequent decreases in filling volumes (EDV). These findings highlight the marked adverse effects of adrenergic stimulation in SHR on diastolic properties, where even though lusitropy (e') improved and EF increased markedly with consequent decreases in filling volumes, filling pressures retained values as high as before adrenergic stimulation. Importantly, an explanation for the differential impact of β-AR stimulation on lusitropic properties in SHR and DSS rats is not apparent. An increased heart rate contributes to the normal response to β-AR stimulation in-part through the relaxation-frequency relation (Izawa et al 1997) and this may be retained in those with DD (Wachter et al 2009). As heart rate increased less in DSS rats after β-AR stimulation (despite the same increases in EF) as compared to SHR, it is therefore possible that lusitropic effects occur in the LV with DD only in the presence of more marked β-AR-induced chronotropic effects.

2.4.2 Potential impact of limited β-AR-induced changes in LV diastolic function in hypertensive hearts. Increased filling pressures in DD contribute to exercise intolerance (Paulus 2010). Indeed, exercise-induced pulmonary hypertension and an elevation of pulmonary capillary wedge pressures occurs in patients with exertional dyspnea and DD (Borlaug et al 2010). Importantly exercise activates the sympathetic nervous system and in a normal LV enhances myocardial relaxation, thus reducing filling pressures and accommodating greater filling volumes (thus allowing for a greater Frank-Starling effect). Although we did not assess the impact of adrenergic stimulation after enhancing filling volumes, such as occurs with exercise-induced increases in preload, the present study suggests that in hypertensive heart disease, adrenergic activation either fails to improve myocardial relaxation (DSS rats), or if it increases myocardial relaxation, this fails to translate into reduced filling pressures, despite reductions in filling volumes (SHR). The consequence could be that the LV does not accommodate a higher filling volume during exercise, thus reducing the Frank-Starling mechanism and contributing to

exercise intolerance. The limited beneficial effect of β-AR activation on DD in hypertensive hearts suggests that the use of β-AR blocking agents in hypertension will have no deleterious actions on DD during physical activity.

2.4.3 LV systolic function before and after β-AR stimulation in hypertensive hearts. A reduced LV systolic deformation has been well described in hypertensive heart disease (Tadic et al 2018) including in SHR (Shah et al 2014). These changes have been suggested to occur in combination with diastolic LV abnormalities and to be a consequence of concentric remodeling (Tadic et al 2018). Importantly, SHR in the present study had marked concentric LV remodeling, but failed to show reductions in LV deformation or motion. These data suggest that DD precedes systolic dysfunction and a lack of causal relationship between concentric LV remodeling and decreases in LV systolic deformation. Hence, reductions in longitudinal LV systolic deformation in hypertensive heart disease (Shah et al 2014; Tadic et al 2018) occur after diastolic dysfunction occurs and are more likely to reflect long-term damage to the myocardium produced by BP effects rather than an adverse effect of LV remodeling *per se*.

In the present study whilst β-AR stimulation produced marked increases in LV systolic chamber function (EF and FSend), neither longitudinal, nor circumferential strain, or longitudinal displacement were increased by β-AR stimulation. Moreover, in keeping with reports on adrenergic-induced decreases in LV strain at higher heart rates (Weidemann et al 2002), in the present study rats showed a decline in circumferential strain with β-AR stimulation in most groups. However, rotational velocity was increased by β-AR stimulation to an equivalent extent in all groups. It is therefore possible that β-AR stimulation enhances the speed of LV rotation and that this is sufficient to promote an increased EF.

2.4.4 Animal models of preclinical hypertensive LV dysfunction. An important question that arises is whether the current animal models studied appropriately reflect the human condition. In this regard it is now well recognized that hypertensive heart disease is often associated with LV diastolic dysfunction, including decreases in e' and increases in E/e', with little change in systolic function (Tadic et al 2018). Moreover, these abnormalities may be all that is noted in the LV of patients with overt heart failure (Lee et al 2009; Zile et al 2004; Lewis

et al 2017) and are included in guidelines to assist in identifying patients with heart failure with a preserved ejection fraction (Nagueh et al 2016). The ability of the hypertensive heart to maintain systolic function is explained by an increase in LV relative wall thickness (wall thickness-to-radius ratio [h/r])(concentric LV remodeling)(Tsotetsi et al 2001; Norton et al 1997), which according to LaPlace's Law of the heart, adequately maintains a normal wall stress despite high pressure loads. Although overt heart failure may occur in hypertensive humans in the absence of systolic chamber dysfunction, it is only when LV dilatation begins to occur and h/r decreases, that SHR decompensate. In this regard, we have previously described the decrease in h/r in the transition to heart failure in the SHR (Tsotetsi et al 2001). At the point where heart failure occurs, despite markedly greater increases in LV weight in older SHR, h/r decreases relative to values in younger SHR without heart failure, but who have only modest increases in LV weight (Tsotetsi et al 2001). In other words, as with preclinical human diastolic LV dysfunction and in heart failure with a preserved ejection fraction, before heart failure occurs in SHR, despite markedly high pressure loads, systolic function is preserved, an effect attributed to concentric LV remodeling (Tsotetsi et al 2001; Norton et al 1997). The higher E/A in SHR confirms the impact of the marked concentric LV remodeling, where this change is considered to represent a restrictive filling pattern. With respect to the DSS rats we have noted that after a further month (3 months in total) of salt in the drinking water, that DSS rats quickly develop evidence of heart failure. This is likely to occur because DSS rats do not adequately remodel the LV at very high pressures (h/r was unchanged), a finding which may relate to the fact that they do not have markedly high BP values from a young age (as with SHR). Hence, in the present study we only maintained the salt load for 2 months before final data collection occurred.

2.4.5 Study limitations. Although in the present study we explored the impact ofβ-AR activation on LV diastolic function in the hypertensive heart, we are unable to assess function of the LV in SHR and DSS during physical activity. Consequently, whether β-AR blockade modifies LV diastolic function during physical activity in hypertensives requires further study. Second, the use of anesthesia is likely to have influenced LV function. However, we employed maximal effective doses of isoproterenol titrated against heart rate and EF and report on an

inability rather than reduction in ability of β-AR stimulation to improve lusitropy in DSS rats, and a lack of effect rather than reduction in effect of β-AR stimulation to reduce filling pressures in SHR despite achieving lusitropic effects. Importantly, the inability of β-AR stimulation to produce lusitropic effects in DSS rats and decreases in filling pressures in SHR despite lusitropic effects, were noted despite marked increases in systolic function (EF) with β-AR stimulation. Third, we employed non-invasive approaches rather than catheter-based systems to assessing DD, as in rats we have noted decreases in e' (relaxation) following the insertion of fluid-filled catheters through the aortic valve. However, tissue Doppler indices of filling pressures have previously been well correlated with filling pressures in rats (Sahn et al 1979). Fourth, although SHR demonstrated marked increases in LV relative wall thickness and an increased LV-to-body weight ratio, absolute LV weights were similar to normotensive controls. Moreover, salt-loaded Dahl SS rats did not show LV remodelling. As LV remodelling is thought to contribute to LV diastolic dysfunction, it may be argued that we could have underestimated the impact of hypertension on LV diastolic function. However, we have previously demonstrated a limited contribution of LV hypertrophy to diastolic function in SHR (Tsotetsi et al 2001; Norton et al 1997), and in the present study we demonstrated similar changes in LV diastolic function in both SHR and Dahl SS rats as those previously described in SHR (Shah et al 2014). Moreover, the diastolic functional abnormalities described in both SHR and salt-loaded DSS rats are typical of changes in diastolic function noted in humans with heart failure with a preserved ejection fraction (Nagueh et al 2016). Thus, it is likely that factors other than LV hypertrophy play an essential role in mediating LV diastolic dysfunction in hypertension and that the LV diastolic functional changes noted in the present study reflect typical changes noted in human hypertension. Fifth, we employed standard rat diets to assess the impact of hypertension in Dahl SS rats on LV function. In this regard, this rat strain is sensitive to several dietary components which were not controlled for and which may have contributed to the development of hypertension and the cardiorenal syndrome in these rats. Nevertheless, we assessed two rat strains of hypertension, one of which was the SHR, a rat strain that is not sensitive to dietary components, and yet similar data were obtained using either strain.

2.5 Conclusions

In conclusion, in the present study we show that under isoflurane anesthesia, the LV of rat models with hypertension (SHR and DSS rats) and a preserved systolic function (in both the longitudinal and circumferential planes), have a reduced myocardial relaxation and increased filling pressures. Although β-AR stimulation markedly enhances EF and hence reduces filling volumes and pressures in anesthetised DSS rats, decreases in filling pressures are not attributed to lusitropic effects. Although β-AR stimulation improves lusitropy in anesthetised SHR, these changes do not reduce filling pressures. These findings indicate that acute β-AR-mediated lusitropic effects may have little physiological benefit to an LV with DD and may even enhance filling pressures for a given filling volume. These findings caste light on the potential impact of β-AR blocking effects on sympathetic-induced changes in LV diastolic function during physical activity in hypertensives with LV DD.

CHAPTER 3

Limited Contribution of Left Ventricular Mass and Remodelling to the Impact of Blood Pressure on Diastolic Function in a Community Sample.

The data in this chapter have been accepted for publication in the Journal of Hypertension

Adamu J Bamaiyi, Gavin R Norton, Vernice Peterson, Carlos D Libhaber, Pinhas Sareli, Angela J Woodiwiss. Limited Contribution of Left Ventricular Mass and Remodelling to the Impact of Blood Pressure on Diastolic Function in a Community Sample.

J Hypertens. 2019;37(6):1191-1199

3.0 Abstract

Background: Although the development of left ventricular (LV) dysfunction in hypertension has traditionally been viewed as a transition process from a phase of structural LV remodelling to dysfunction, the extent to which LV mass (LVM) and remodelling account for blood pressure (BP)-associated alterations in LV diastolic function is uncertain. In product of coefficient mediation analysis, we aimed to determine the extent to which LVM index (LVMI) or relative wall thickness (RWT) account for relations between BP and LV diastolic function,

Methods: In 709 randomly selected participants from a community sample with a high prevalence of hypertension (49.6%), we determined BP and LVMI, RWT and several indices of diastolic function from transmitral blood flow and myocardial tissue Doppler (E/A, e'/a', e' and E/e') and left atrial volume using standard echocardiographic techniques.

Results: With adjustments for confounders, LVMI ($p<0.001$-0.0001) and RWT ($p<0.05$-0.001) were independently associated with E/A, e'/a', e' and E/e'. However, in product of coefficient mediation analysis, LVM and RWT failed to account for most BP-associated changes in diastolic function. Indeed, whilst a one SD increase in diastolic or systolic BP (13 and 22 mmHg respectively) translated into a 0.07, 0.13 and 0.53 decrease in E/A, e'/a', e' and a 0.73 increase in E/e' respectively, in mediation analysis LVMI accounted for only 0.0005, 0.0017, 0.05 and 0.08 of the impact of a one SD effect of LVMI on E/A, e'/a', e' and E/e' respectively. Similar contributions of RWT as for LVMI to BP-associated LV diastolic functional changes were noted and the contribution of LVMI or RWT to BP-related alterations in diastolic function was similar in those participants not receiving antihypertensive therapy.

Conclusions: Although structural LV remodelling is independently associated with changes in LV diastolic function, LVMI and RWT account for only a minor proportion of the impact of BP on diastolic function. Thus, most BP-associated decreases in LV diastolic function are likely to be a transition process independent of LV hypertrophy or concentric remodelling.

Key words: left ventricular hypertrophy, left ventricular remodelling, left ventricular diastolic function, hypertension.

3.1 Introduction

Heart failure with a preserved ejection fraction may contribute to close to half of all admissions for heart failure, and the outcomes may be equally as poor as heart failure with a reduced ejection fraction (Borlaug & Redfield 2011; Lee et al 2009; Owan et al 2006; Bhatia et al 2006). There is presently little evidence for proven treatment benefits for heart failure with a preserved ejection fraction (Borlaug & Redfield 2011; Borlaug & Paulus 2011; Pitt et al 2014). Hence, a better understanding of the pathophysiological mechanisms involved, is essential. Decreases in left ventricular (LV) diastolic function are central to the pathophysiology and outcomes of heart failure with preserved ejection fraction (Zile et al 2004; Westermann et al 2008; Burke et al 2014; Mohammed et al 2012; Shah et al 2014) and pre-clinical LV diastolic dysfunction predicts the progression to heart failure with a preserved ejection fraction (Wan et al 2014). Although it is now well-recognised that sustained hypertension is a major determinant of LV diastolic dysfunction (DD) and the development of heart failure with a preserved ejection fraction, the transition process from a compensated LV to LV DD in hypertension is unclear.

Traditionally, the development of LV dysfunction and consequently heart failure in hypertension has been viewed as the evolution from a phase of structural LV remodeling (concentric remodeling and hypertrophy) to LV decompensation. Consequently, the presence of LV hypertrophy (LVH) is thought to be an essential prelude to LV dysfunction, including LV DD. Indeed, LV DD may be seen in up to 84% of hypertensive individuals with LVH (Wachtell et al 2000). Conversely, it has been estimated that only 11% to 20% of hypertensive patients have LV DD without exhibiting LVH (Dini et al 2013; Phillips et al 1989). However, these estimates were obtained at a time when more contemporary non-invasive approaches to determining LV diastolic function were not available. Importantly, it has been well-recognised for several decades (González et al 2018) with even more novel mechanisms continuing to emerge (González et al 2018; Paulus & Tschope 2013) of a role for several cellular changes induced by hypertension that are unrelated to the hypertrophic process and which determine LV diastolic function. In this regard, many patients with heart failure with a preserved ejection fraction do not

have LVH (Lam et al 2007) despite the fact that hypertension is the dominant risk factor for this form of heart failure. Moreover, LV DD without LVH is an early manifestation of hypertensive heart disease (Messerli et al 2017). Consequently, the value of measures of LVH or the remodeling process as effective indices that herald the presence of LV diastolic dysfunction is unclear. In the present study we therefore evaluated in a reasonably large community-based study with a high prevalence of untreated hypertension, using product of coefficient mediation analysis, the extent to which LV mass (LVM) or relative wall thickness (RWT) account for the impact of BP on indices of LV diastolic function.

3.2 Methods

3.2.1 Study sample. The present study was approved by the University of the Witwatersrand Committee for Research in Human Subjects (approval number M02-04-72 renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. The study design has previously been described (Woodiwiss et al 2008; Libhaber et al 2014; Woodiwiss et al 2009; Peterson et al 2016; Redelinghuys et al 2010). 1044 Participants of nuclear families of black African descent with siblings older than 16 years were randomly recruited from the South West Township of Johannesburg, South Africa for echocardiographic studies. Random recruitment of families living in formal dwellings (but not institutions) was performed based on the national census figures of 2001 (Department of Home Affairs) and a participation rate of 72% was obtained (Woodiwiss et al 2008; Libhaber et al 2014; Woodiwiss et al 2009; Peterson et al 2016; Redelinghuys et al 2010). No subjects of mixed, Asian, or European ancestry and no Khoi-San subjects were recruited. Tissue Doppler measures of myocardial function were obtained in a sub-study conducted in 709 participants from the time that these measures became routinely available.

3.2.2 Demographic and clinical information. A standardized questionnaire was administered to obtain demographic and clinical data (Woodiwiss et al 2008; Libhaber et al 2014; Woodiwiss et al 2009; Peterson et al 2016; Redelinghuys et al 2010). Height, weight, and

waist circumference (WC) were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2, and obese if their BMI was ≥30 kg/m^2. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men). Laboratory blood tests including percentage glycated haemoglobin (HbA_{1c}) were performed. Diabetes mellitus was defined as the use of insulin or oral hypoglycaemic agents or a glycated haemoglobin (Roche Diagnostics, Mannheim, Germany) value greater than 6.5%.

Nurse-derived conventional BP was measured according to guidelines using a mercury sphygmomanometer after five minutes of rest in the seated position as previously described (Woodiwiss et al 2009). Five consecutive BP readings were obtained using an appropriately sized cuff, 30 to 60 seconds apart. The average of the five readings was taken as the BP. None of the visits had fewer than the planned BP recordings. Hypertension was defined as the use of antihypertensive medication or if the mean of the five conventional BP measurements was >140 (systolic BP) or 90 (diastolic BP) mm Hg in those not receiving medication.

3.2.3 Echocardiography. Echocardiographic measurements were performed as previously described (Woodiwiss et al 2008; Libhaber et al 2014; Peterson et al 2016) by two experienced observers (AJW and CDL) with the participants in the partial left decubitus position. All potential participants were assessed for mitral valve abnormalities as determined using 2-dimensional and colour Doppler imaging and excluded if significant valve abnormalities were present. The LV dimensions were measured on M-Mode images only when appropriate visualization of both the right and the left septal surfaces occurred and where the endocardial surfaces of both the septal and posterior wall were clearly visible. Pulse wave Doppler recordings of trans-mitral velocity were obtained with the sample volume at the tip of the mitral valve in the apical four-chamber view. To perform TDI, the velocity of myocardial tissue lengthening at the level of the mitral annulus was recorded in the apical four-chamber view. The sample volume was positioned at the septal and lateral corners of the mitral annulus. Left atrial volume was calculated using the area-length method, where length was defined as the shortest of the two long axes measured in the apical four-chamber and two-chamber views. Left atrial

area was measured by planimetry in the apical four-chamber and two-chamber views at left ventricular end systole (maximum left atrial dimensions).

Left ventricular dimensions were determined using two-dimensional directed M-mode echocardiography in the short axis view and these recordings were analyzed according to the American Society of Echocardiography convention (Sahn et al 1978). Left ventricular mass was determined using a standard formula (Devereux et al 1986) and due to the high prevalence of obesity and hypertension in the community studied, indexed (LVMI) to both height$^{1.7}$ as well as body surface area (BSA). Left ventricular RWT was assessed using standard M-mode approaches. Left ventricular ejection fraction was calculated using the biplane Simpson method. Left ventricular diastolic function was determined from a pulsed wave Doppler examination of the mitral inflow at rest (early [E] and late [atrial contraction-A] velocity) and using tissue Doppler indices (TDI)(early [e'] and late [atrial contraction a'] velocity) as well as left atrial volumes (LAV) (Peterson et al 2016). Data were expressed as E/A, e'/a' and the E/e' ratio (an index of LV filling pressures). Left atrial volume was indexed to body surface area.

The correlation coefficients, variances and differences in echocardiographic parameters determined for the calculation of LVMI within and between observers, and for E, A and e' within observers has previously been reported on (Maseko et al 2013; Millen et al 2014). In this regard, all variables showed little difference or variation between (inter-observer variability) or within (intra-observer variability) observers (Maseko et al 2013; Millen et al 2014). Intra-observer variability studies conducted on 26 subjects on whom repeat echocardiographic measurements were performed within a two week period of the initial measurements showed Pearson's correlation coefficients for LAV and a' of 0.78 and 0.97, variances (mean % difference ± SD) of 0.72±8.55% and 0.12±5.86%, and no significant differences between repeat measurements (paired t-test)(p=0.60 and p=0.96). Inter-observer variability studies conducted on 29 participants showed Pearson's correlation coefficients for LAV, E, A, e' and a' of 0.77, 0.96, 0.95, 0.98 and 0.95, variances (mean % difference ± SD) of 0.07±8.47%, 0.74±6.81%, -1.61±8.99%, 0.05±5.68% and -0.06±7.89%, and no significant differences between measurements (unpaired t-test) (p=0.73, p=0.94, p=0.80, p=0.86 and p=0.99 respectively).

As several approaches to identifying DD have been advocated and not all of those individuals identified as having DD according to one method have DD using an alternative method, we employed two approaches to diagnosing DD. In this regard, at the time of initiating our study, pulmonary artery pressures were not advocated, and we were unable to obtain consistent E and A data during the Valsalva manoeuvre. Hence, we employed modified approaches to that advocated by guidelines (Nagueh et al 2016) and to that originally suggested (Redfield et al 2003). Based on current guidelines, in those participants with an EF>50%, DD was therefore identified by the presence of at least two of the following: a lateral e'<10 cm/s or a septal e'<8 cm/s, E/e'>14, or LAV ≥34ml/m^2 (Nagueh et al 2016). For those participants with an EF <50%, we identified DD if participants had an E/A>2.0 (restrictive filling pattern) or an E/A 0.8-2.0 with both E/e'>14 and LAV ≥34ml/m^2 (Nagueh et al 2016). We further identified the presence of either mild, moderate or severe DD using previous criteria if E/A was <0.75 (mild DD), E/A was between 0.75 and 1.5 and E/e' was >10 (moderate DD), or if E/A was >1.5 and E/e' was >10 (severe restrictive filling pattern)(Redfield et al 2003). Although age-specific criteria for LV diastolic dysfunction have recently been described (Nayor et al 2018), these criteria were not used in the current study for the following reasons: they have not yet been included in guidelines and recommendations; they have been defined in largely Caucasian populations and hence may not be applicable as the participants studied in my thesis were all of African ancestry; and criteria for LAV and septal e' have not been described.

3.2.4 Data analysis. Database management and statistical analyses were performed with SAS software, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Data from individuals were averaged and expressed as mean±SD or SEM. To improve on the distribution of data lateral e', septal e', E/e', E/A, e'/a' and left atrial volume were logarithmically transformed. To determine independent relations, multivariate adjusted linear (continuous data) or logistic (discrete data) regression analysis was performed. Adjustments were performed for all factors correlated with BP, indices of LV remodeling, or indices of LV diastolic function. As we have previously demonstrated differential relations between SBP and DBP and various indices of LV diastolic function (Libhaber et al 2014), in relations with e' or E/e', SBP was

employed and in relations with E/A and e'/a', DBP was employed as the BP index. To determine the relative contribution of factors toward LV diastolic function, multivariate stepwise regression analysis was performed and factors not independently associated with LV diastolic function were forced into the model. To determine the contribution of LVMI or RWT to the impact of BP on LV diastolic function, multivariate adjusted product of coefficient mediation analysis, which accounts for hierarchical causal structures, was performed. Multivariate adjusted product of coefficient mediation analysis was conducted as for the following example where the contribution of LVMI to SBP-related increases in E/e' was assessed. The regression coefficient (slope) for the multivariate adjusted regression relationship between SBP and LVMI represents the change in LVMI per mm Hg. This regression coefficient has a value which we designate *x*. The regression coefficient (slope) for the multivariate adjusted regression relationship between LVMI and E/e' represents the change in LVMI per unit change in E/e'. This regression coefficient has a value which we designate *y*. From these two regression coefficients, we know that LVMI increases per mm Hg by *x* units and that for every unit increase in LVMI, E/e' increases by *y* units. Thus, per mm Hg, LVMI must cause E/e' to increase by the product of *x* and *y* or *xy* units. This value (*xy*) therefore identifies the mm Hg contribution of LVMI to E/e' elevation with SBP. The second regression (ie LVMI versus E/e'), with slope *y* must be adjusted for SBP, such that the potential influence of SBP (the key independent variable) is not accounted for twice as an independent variable in the multiplicative series. This analysis was performed to determine the contribution of either LVMI or RWT to relationships between either SBP or DBP and all indices of LV diastolic function. For the derivation of probability values, further adjustments were made for non-independence of family members using the mixed procedure as outlined in the SAS package. Sensitivity analysis was conducted in those not receiving antihypertensive therapy and in sex-specific and obese and non-obese subgroups.

3.3 Results

3.3.1 Characteristics of study sample. Table 3.1 gives the demographic and clinical characteristics of the participants. More women than men participated in the study and a high

Table 3.1. Characteristics of the study sample.

Sample number (% female)	709 (67.6)
Age (years)	47.2±18.1
Body mass index (kg/m^2)	30.0±7.9
% Overweight/obese	22.9/46.5
Waist circumference (WC) (cm)	94.4±18.0
% Abnormal waist circumference	53.7
Regular tobacco (% subjects)	16.2
Regular alcohol (% subjects)	19.5
% Diabetes mellitus or an HbA_{1c}>6.5%	13.1
% Hypertensive	49.6
% Treated for hypertension	29.3
% Uncontrolled blood pressure	35.0
Brachial SBP/DBP (mm Hg)	128±22/83±13
Lateral e' (cm/s)	11.3±4.1
E/A	1.26±0.51
e'/a'	1.40±0.70
E/e'	7.5±4.2
Left atrial (LA) volume index (ml/m^2)	19.7±7.5
LVM indexed to height (LVMI-ht$^{1.7}$)(g/m$^{1.7}$)	62.7±23.1
LVM indexed to body surface area (LVMI-BSA) (g/m^2)	68.9±27.6
LV relative wall thickness (RWT)	0.36±0.08

HbA_{1c}, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic BP; E/A, transmitral early/atrial blood flow velocity; e', myocardial tissue lengthening in early diastole at the mitral annulus; a', myocardial tissue lengthening in late diastole at the mitral annulus; E/e', transmitral early blood flow velocity/velocity of the mean value of lateral and septal wall myocardial tissue lengthening in early diastole at the mitral annulus; LVM, left ventricular mass.

proportion of participants were overweight, or obese and had central obesity. A high proportion of participants were hypertensive and were not receiving antihypertensive therapy and hence the proportion of participants with uncontrolled hypertension was high. As compared to participants recruited prior to TDI becoming available, participants in whom echocardiography was performed once routine TDI became available, were modestly older with more abdominal obesity (Table 3.2). Based on current guidelines, 15.4% of the participants had DD and this was largely determined by a combination of either reductions in lateral or septal e' and increases in E/e' (11.6%). Based on previous criteria, 28.1% of the participants had DD and this was largely determined by mild (12.8%) or moderate (11.3%) DD. No participants had an ejection fraction<40% and 4.1% had an ejection fraction<50%. Of the sample 39.6% had LVH (LVMI>80 $g/m^{1.7}$ for men and >60 $g/m^{1.7}$ for women) and 18.1% had concentric LV remodeling (relative wall thickness>0.42). A greater proportion of hypertensives than normotensives had LVH and concentric LV remodeling.

3.3.2 Factors related to LVMI or RWT. Independent of age, sex, regular smoking or alcohol intake, or the presence of diabetes mellitus, systolic BP and indices of adiposity (WC or BMI) were associated with LVMI ($p<0.0001$). In addition, independent of confounders systolic BP ($p=0.05$) and BMI ($p<0.01$) were associated with RWT.

3.3.3 Independent relations with LV diastolic function. With adjustments for confounders, systolic BP (Tables 3.3 and 3.4), and either WC (Tables 3.3 and 3.4) or BMI (data not shown) were independently associated with lateral wall e' and E/e'. Moreover, with adjustments for confounders, diastolic BP (Tables 3.4 and 3.5), and either WC (Tables 3.3 and 3.6) or BMI (data not shown) were independently associated with E/A and lateral wall e'/a'. With the inclusion of LVMI-$ht^{1.7}$ or RWT in the regression models, although both LVMI or RWT were independently associated with lateral wall e' or E/e' (Tables 3.3 and 3.4), or E/A or e'/a' (Tables 3.5 and 3.6), the impact of alternative risk factors on these indices of diastolic function was hardly modified. The use of LVMI-BSA rather than LVMI-$ht^{1.7}$ produced essentially the same results (Table 3.7). The lack of impact of adjustments for LVMI or RWT on relations between BP and indices of LV diastolic function was reproduced in men and women (see Table 3.8 for LVMI

Table 3.2. Characteristics of community sample without tissue Doppler imaging (TDI).

Sample number (% female)	335 (65.3)
Age (years)	43.5±18.1**
Body mass index (kg/m^2)	29.1±7.4
% Overweight/obese	24.5/42.5
Waist circumference (WC) (cm)	89.9±15.5**
% Abnormal waist circumference	43.3**
Regular tobacco (% subjects)	12.6
Regular alcohol (% subjects)	21.3
% Diabetes mellitus or an HbA_{1c}>6.5%	13.8
% Hypertensive	45.2
% Treated for hypertension	23.1*
% Uncontrolled blood pressure	36.4
Brachial SBP/DBP (mm Hg)	130±22/84±12

HbA_{1c}, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic BP. *p<0.05, **p<0.005 vs with TDI.

Table 3.3. Relative contribution (standardized β-coefficient) of risk factors toward indices of left ventricular diastolic function in a community sample (n=709).

	Log lateral wall e'					**Log E/e'**				
	β-coef±SEM (p-value)		β-coef±SEM (p-value)		β-coef±SEM (p-value)	β-coef±SEM (p value)		β-coef±SEM (p-value)		β-coef±SEM (p-value)
LVMI-ht$^{1.7}$	-	-	-0.100±0.032 (<0.005)	-	-	-	-	0.112±0.039 (<0.005)	-	-
RWT	-	-	-	-	-0.067±0.030 (<0.05)	-	-	-	-	0.099±0.036 (<0.01)
SBP	-0.147±0.032 (<0.0001)		-0.131±0.032 (<0.0001)		-0.142±0.032 (<0.0001)	0.189±0.039 (<0.0001)		0.171±0.040 (<0.0001)		0.181±0.039 (<0.0001)
WC	-0.159±0.034 (<0.0001)		-0.127±0.036 (<0.0005)		-0.156±0.034 (<0.0001)	0.130±0.042 (<0.005)		0.095±0.044 (<0.05)		0.126±0.042 (<0.005)
Age	-0.401±0.039 (<0.0001)		-0.387±0.039 (<0.0001)		-0.386±0.039 (<0.0001)	0.169±0.047 (<0.0005)		0.153±0.047 (<0.002)		0.147±0.048 (<0.005)

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are sex, regular tobacco use, and regular alcohol consumption, treatment for hypertension, diabetes mellitus and pulse rate. For models with LVMI-BSA see table 3.7.

Table 3.4. Relative contribution (standardized β-coefficient) of risk factors toward indices of left ventricular diastolic function in those having never received antihypertensive therapy from a community sample (n=501).

	Log lateral wall e'			**Log E/e'**		
	β-coef±SEM (p-value)	β-coef±SEM (p-value)	β-coef±SEM (p-value)	β-coef±SEM (p value)	β-coef±SEM (p-value)	β-coef±SEM (p-value)
LVMI-ht$^{1.7}$	- -	-0.081±0.040 (<0.05)	- -	- -	0.079±0.047 (=0.09)	- -
RWT	- -	- -	-0.076±0.037 (<0.05)	- -	- -	0.066±0.044 (=0.14)
SBP	-0.168±0.040 (<0.0001)	-0.151±0.041 (<0.0005)	-0.159±0.040 (<0.0001)	0.172±0.047 (<0.0005)	0.156±0.048 (<0.002)	0.165±0.048 (<0.001)
WC	-0.124±0.042 (<0.005)	-0.095±0.044 (<0.05)	-0.114±0.042 (<0.01)	0.161±0.049 (<0.002)	0.133±0.052 (<0.02)	0.153±0.050 (<0.005)
Age	-0.427±0.045 (<0.0001)	-0.421±0.045 (<0.0001)	-0.415±0.045 (<0.0001)	0.142±0.053 (<0.01)	0.136±0.053 (<0.02)	0.132±0.054 (<0.02)

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are sex, regular tobacco use, and regular alcohol consumption, diabetes mellitus and pulse rate.

Table 3.5. Relative contribution (standardized β-coefficient) of risk factors toward indices of left ventricular diastolic function in a community sample (n=709).

	Log lateral wall e'/a'						**Log E/A**					
	β-coef±SEM (p-value)			β-coef±SEM (p-value)		β-coef±SEM (p-value)	β-coef±SEM (p value)			β-coef±SEM (p-value)		β-coef±SEM (p-value)
LVMI-ht$^{1.7}$	-	-		-0.070±0.027 (<0.01)	-	-	-	-		-0.024±0.030 (=0.44)	-	-
RWT	-	-		-	-	-0.042±0.025 (=0.10)	-	-		-	-	-0.035±0.029 (=0.22)
DBP	-0.137±0.025(<0.0001)			-0.134±0.025(<0.0001)		-0.135±0.025 (<0.0001)	-0.124±0.029 (<0.0001)			-0.123±0.029 (<0.0001)		-0.122±0.029 (<0.0001)
WC	-0.185±0.029 (<0.0001)			-0.162±0.031 (<0.0005)		-0.183±0.029 (<0.0001)	-0.099±0.033 (<0.005)			-0.091±0.035 (<0.01)		-0.097±0.033 (<0.005)
Age	-0.551±0.031(<0.0001)			-0.537±0.031(<0.0001)		-0.541±0.032 (<0.0001)	-0.563±0.035 (<0.0001)			-0.558±0.036 (<0.0001)		-0.554±0.036 (<0.0001)

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are sex, regular tobacco use, and regular alcohol consumption, treatment for hypertension, diabetes mellitus and pulse rate. For models with LVMI-BSA see table 3.7.

Table 3.6. Relative contribution (standardized β-coefficient) of risk factors toward indices of left ventricular diastolic function in those having never received antihypertensive therapy from a community sample (n=501).

	Log lateral wall e'/a'						**Log E/A**					
	β-coef±SEM (p-value)		β-coef±SEM (p-value)		β-coef±SEM (p-value)		β-coef±SEM (p value)		β-coef±SEM (p-value)		β-coef±SEM (p-value)	
LVMI-ht$^{1.7}$	-	-	-0.055±0.032 (=0.09)		-	-	-	-	-0.020±0.036 (=0.57)		-	-
RWT	-	-	-	-	-0.059±0.030 (<0.05)		-	-	-	-	-0.035±0.034 (=0.31)	
DBP	-0.154±0.031	(<0.0001)	-0.150±0.031	(<0.0001)	-0.152±0.031	(<0.0001)	-0.131±0.035	(<0.0005)	-0.130±0.035	(<0.0005)	-0.130±0.035	(<0.0005)
WC	-0.172±0.035	(<0.0001)	-0.152±0.037	(<0.0001)	-0.164±0.035	(<0.0001)	-0.078±0.038	(<0.05)	-0.071±0.041	(=0.09)	-0.073±0.039	(=0.06)
Age	-0.591±0.035	(<0.0001)	-0.584±0.035	(<0.0001)	-0.580±0.035	(<0.0001)	-0.611±0.039	(<0.0001)	-0.608±0.039	(<0.0001)	-0.604±0.039	(<0.0001)

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are sex, regular tobacco use, and regular alcohol consumption, treatment for hypertension, diabetes mellitus and pulse rate.

Table 3.7. Relative contribution (standardized β-coefficient) of left ventricular mass indexed to body surface area (LVMI-BSA) and blood pressure to indices of left ventricular diastolic function.

	β-coef±SEM (p-value)	β-coef±SEM (p-value)	β-coef±SEM (p value)	β-coef±SEM (p-value)
	Log lateral wall e'		**Log E/e'**	
LVMI-BSA	-	-0.111±0.031 (<0.0005)	-	0.123±0.038 (<0.002)
SBP	-0.147±0.032 (<0.0001)	-0.131±0.032 (<0.0001)	0.189±0.039 (<0.0001)	0.170±0.039 (<0.0001)
	Log lateral wall e'/a'		**Log E/A**	
LVMI-BSA	-	-0.086±0.026 (<0.002)	-	-0.069±0.029 (<0.02)
DBP	-0.137±0.025 (<0.0001)	-0.136±0.025 (<0.0001)	-0.124±0.029 (<0.0001)	-0.123±0.028 (<0.0001)

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are age, sex, waist circumference, regular tobacco use, and regular alcohol consumption, treatment for hypertension, diabetes mellitus and pulse rate.

Table 3.8. Relative contribution (standardized β-coefficient) of left ventricular mass index (LVMI) and blood pressure to indices of left ventricular diastolic function in men and women.

	Men (n=230)		Women (n=479)	
	β-coef±SEM (p-value)	β-coef±SEM (p-value)	β-coef±SEM (p value)	β-coef±SEM (p-value)
Log lateral wall e'				
LVMI-ht$^{1.7}$	-	-0.099±0.052 (=0.06)	-	-0.109±0.041 (<0.01)
SBP	-0.144±0.051 (<0.01)	-0.124±0.052 (<0.02)	-0.144±0.042 (<0.0001)	-0.129±0.042 (<0.005)
Log E/e'				
LVMI-ht$^{1.7}$	-	0.131±0.065 (<0.05)	-	0.102±0.050 (<0.05)
SBP	0.209±0.063 (<0.005)	0.183±0.064 (<0.01)	0.178±0.051 (<0.001)	0.164±0.052 (<0.002)
Log lateral wall e'/a'				
LVMI-ht$^{1.7}$	-	-0.104±0.042 (<0.02)	-	-0.059±0.035 (=0.09)
DBP	-0.212±0.042 (<0.0001)	-0.215±0.042 (<0.0001)	-0.099±0.032 (<0.005)	-0.094±0.032 (<0.005)
Log E/A				
LVMI-ht$^{1.7}$	-	-0.076±0.045 (=0.09)	-	0.005±0.041 (=0.90)
DBP	-0.149±0.045 (<0.002)	-0.152±0.045 (<0.002)	-0.123±0.038 (<0.005)	-0.123±0.038 (<0.005)

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are age, waist circumference, regular tobacco use, and regular alcohol consumption, treatment for hypertension, diabetes mellitus and pulse rate.

in the model with RWT showing similar data) and in obese versus non-obese (see Table 3.9 for LVMI in the model with RWT showing similar data) participants. Systolic BP was modestly and independently associated with LAV index in the whole group (Table 3.10), but not in the group having never received antihypertensive therapy (Table 3.11). Neither WC (Tables 3.10 and 3.11) nor BMI (data not shown) were independently associated with LAV index. Left ventricular mass index, but not RWT was independently associated with LAV index. With the inclusion of LVMI, but not RWT in the regression models, systolic BP was no longer independently associated with LAV index (Table 3.10).

3.3.4 Product of coefficient mediation analysis. Adjustments for LVMI-ht$^{1.7}$ or LVMI-BSA failed to modify the impact of a one standard deviation (SD) effect of BP on e', E/e', E/A or e'/a' (Figures 3.1 and 3.2) and in mediation analysis, LVMI-ht$^{1.7}$ or LVMI-BSA failed to account for a significant proportion of the impact of a one SD effect of BP on either e', E'/e, E/A or e'/a' (Figures 3.1 and 3.2). Similarly, adjustments for RWT failed to modify the impact of a one standard deviation (SD) effect of BP on e', E/e', E/A or e'/a' (Figures 3.1 and 3.2) and in mediation analysis, RWT failed to account for a significant proportion of the impact of a one SD effect of BP on either e', E'/e, E/A or e'/a' (Figures 3.1 and 3.2).

3.3.5 Relations with LV diastolic dysfunction. Irrespective of the criteria employed to diagnose DD, systolic BP ($p=0.005$), LVMI ($p<0.0005$) and RWT ($p<0.001$), were independently associated with LV DD. However, with adjustments for LVMI-ht$^{1.7}$, LVMI-BSA or RWT, the independent relations between systolic BP and DD were unchanged (Figures 3.3 and 3.4).

3.4 Discussion

The main findings of the present study are as follows: In a reasonably large community-based study with a high prevalence of untreated and uncontrolled hypertension we show that although both LVMI and RWT are independently associated with several characteristic changes in LV diastolic function, they account for little of the relationship between BP and LV diastolic function. Indeed, in product of coefficient mediation analysis, the contribution of either LVMI or

Table 3.9. Relative contribution (standardized β-coefficient) of left ventricular mass index (LVMI) and blood pressure to indices of left ventricular diastolic function obese and non-obese participants.

	Body mass index≥30 kg/m^2 (n=330)		Body mass index<30 kg/m^2 (n=379)	
	β-coef±SEM (p-value)	β-coef±SEM (p-value)	β-coef±SEM (p value)	β-coef±SEM (p-value)
		Log lateral wall e'		
LVMI-ht$^{1.7}$	-	-0.122±0.052 (<0.02)	-	-0.076±0.040 (=0.06)
SBP	-0.117±0.053 (<0.05)	-0.105±0.053 (<0.05)	-0.185±0.043 (<0.0001)	-0.171±0.043 (<0.0001)
		Log E/e'		
LVMI-ht$^{1.7}$	-	0.080±0.060 (=0.18)	-	0.117±0.051 (<0.05)
SBP	0.148±0.061 (<0.02)	0.140±0.061 (<0.05)	0.226±0.054 (<0.0001)	0.203±0.054 (<0.0005)
		Log lateral wall e'/a'		
LVMI-ht$^{1.7}$	-	-0.100±0.045 (<0.05)	-	-0.060±0.035 (=0.08)
DBP	-0.181±0.043 (<0.0001)	-0.177±0.042 (<0.0001)	-0.115±0.035 (<0.002)	-0.114±0.035 (<0.005)
		Log E/A		
LVMI-ht$^{1.7}$	-	-0.024±0.051 (=0.64)	-	-0.061±0.035 (=0.08)
DBP	-0.176±0.049 (<0.0005)	-0.175±0.049 (<0.0005)	-0.107±0.036 (<0.005)	-0.106±0.035 (<0.005)

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are age, sex, regular tobacco use, and regular alcohol consumption, treatment for hypertension, diabetes mellitus and pulse rate.

Table 3.10. Relative contribution (standardized β-coefficient) of risk factors toward left atrial volume index in a community sample (n=709).

	β-coef±SEM (p-value)	β-coef±SEM (p-value)	β-coef±SEM (p-value)
LVMI-ht$^{1.7}$	- -	0.265±0.041 (<0.0001)	- -
RWT	- -	- -	0.058±0.041 (=0.15)
SBP	0.089±0.043 (<0.05)	0.045±0.042 (=0.29)	0.085±0.043 (<0.05)
WC	0.053±0.046 (=0.25)	-0.026±0.047 (=0.57)	0.050±0.046 (=0.28)
Age	0.170±0.052 (<0.002)	0.129±0.051 (<0.02)	0.159±0.053 (<0.005)

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are sex, regular tobacco use, and regular alcohol consumption, treatment for hypertension, diabetes mellitus and pulse rate.

Table 3.11. Relative contribution (standardized β-coefficient) of risk factors toward left atrial volume index in those having never received antihypertensive therapy from a community sample (n=501).

	β-coef±SEM (p-value)		β-coef±SEM (p-value)		β-coef±SEM (p-value)	
LVMI-$ht^{1.7}$	-	-	0.232±0.050 (<0.0001)		-	-
RWT	-	-	-	-	0.079±0.049 (=0.11)	
SBP	0.062±0.052 (=0.23)		0.013±0.052 (=0.81)		0.055±0.052 (=0.29)	
WC	0.095±0.034 (=0.08)		0.017±0.056 (=0.76)		0.083±0.055 (=0.13)	
Age	0.106±0.058 (=0.07)		0.088±0.057 (=0.13)		0.098±0.058 (=0.09)	

β-coef, standardized β-coefficient (slope). See table 3.1 for other abbreviations. Also included in the models are sex, regular tobacco use, and regular alcohol consumption, diabetes mellitus and pulse rate.

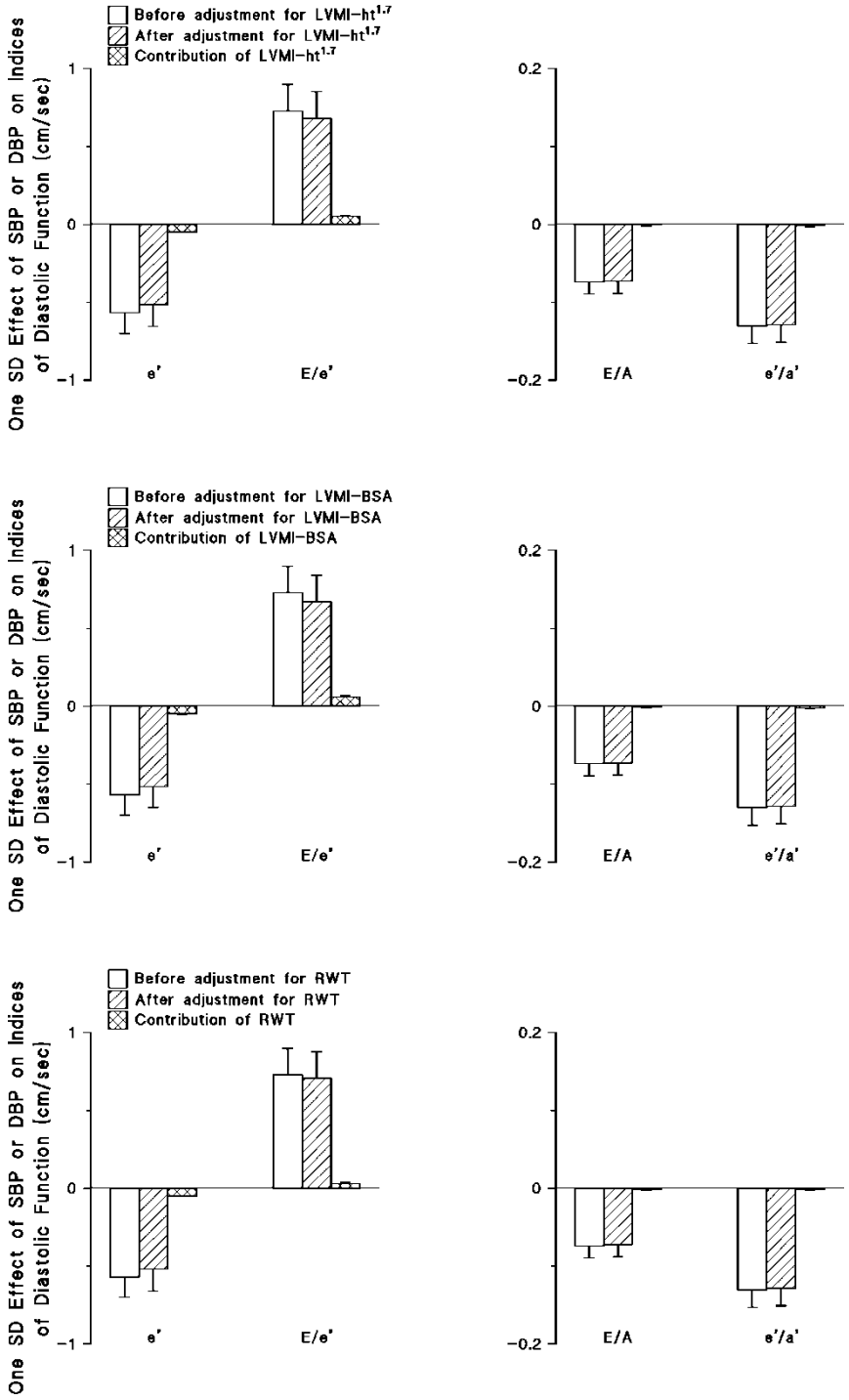

Figure 3.1. Contribution of left ventricular mass index (LVMI) or geometric remodelling (relative wall thickness [LVMI]) to the relationship between systolic (SBP) or diastolic (DBP) blood pressure and indices of left ventricular diastolic function in a community sample (n=709). Figures show one standard deviation (SD) effect of BP before and after adjustments (adj.) for LVMI or RWT on LV diastolic function and the contribution of LVMI or RWT to the one SD effect of BP on LV diastolic function (product of coefficient mediation analysis). See table 3.1 for abbreviations. Adjustments are for LVMI or RWT as indicated and age, sex, waist circumference, the presence of diabetes mellitus, treatment for hypertension, regular tobacco use, regular alcohol consumption and pulse rate.

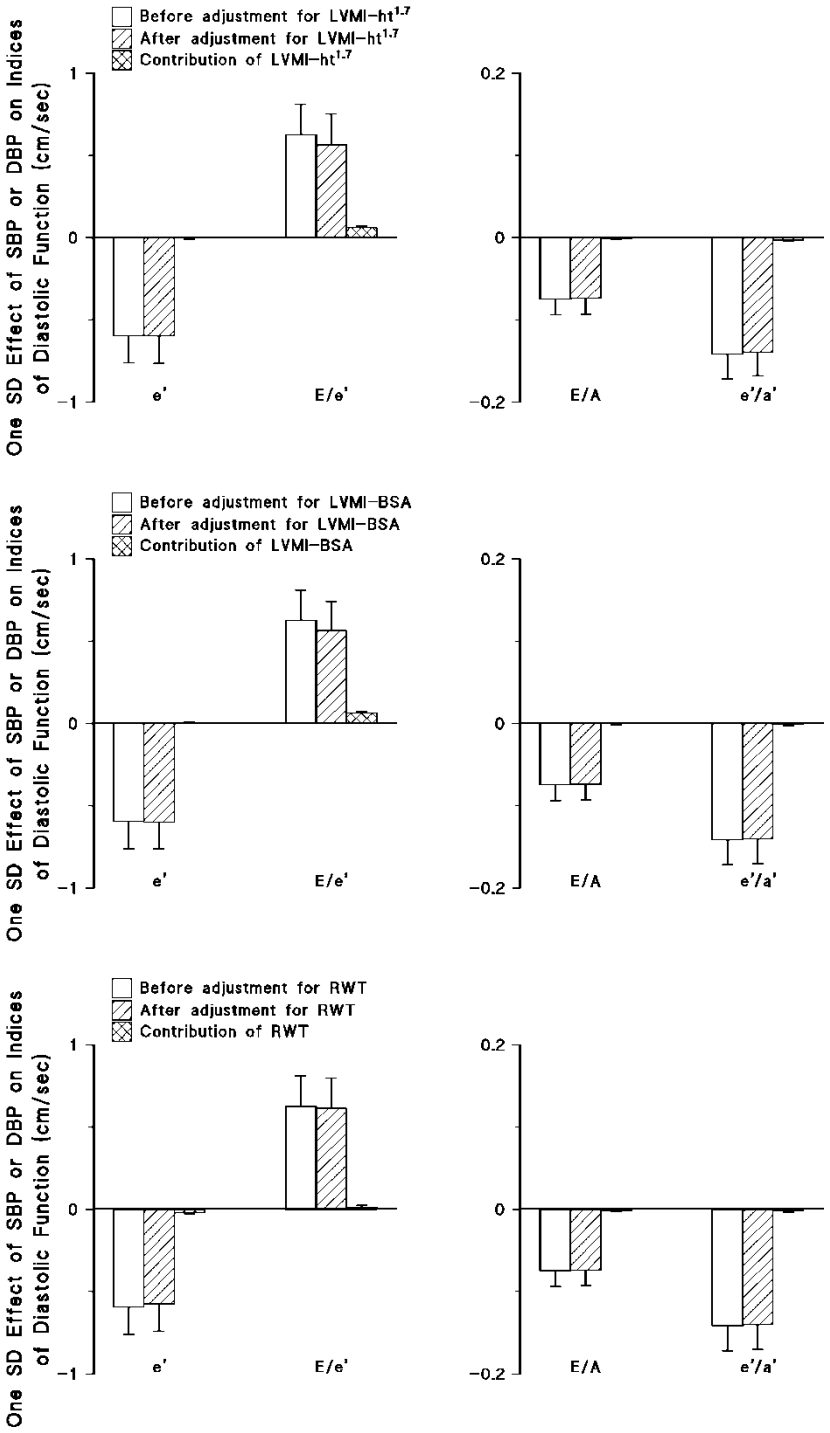

Figure 3.2. Contribution of left ventricular mass index (LVMI) or geometric remodeling (relative wall thickness [RWT]) to the relationship between systolic (SBP) or diastolic (DBP) blood pressure and indices of left ventricular diastolic function in those having never received antihypertensive treatment in a community sample (n=501). Figures show one standard deviation (SD) effect of BP before and after adjustments (adj.) for LVMI or RWT on LV diastolic function and the contribution of LVMI or RWT to the one SD effect of BP on LV diastolic function (product of coefficient mediation analysis). See table 3.1 for abbreviations. Adjustments are for LVMI or RWT as indicated and age, sex, waist circumference, the presence of diabetes mellitus, regular tobacco use, regular alcohol consumption and pulse rate.

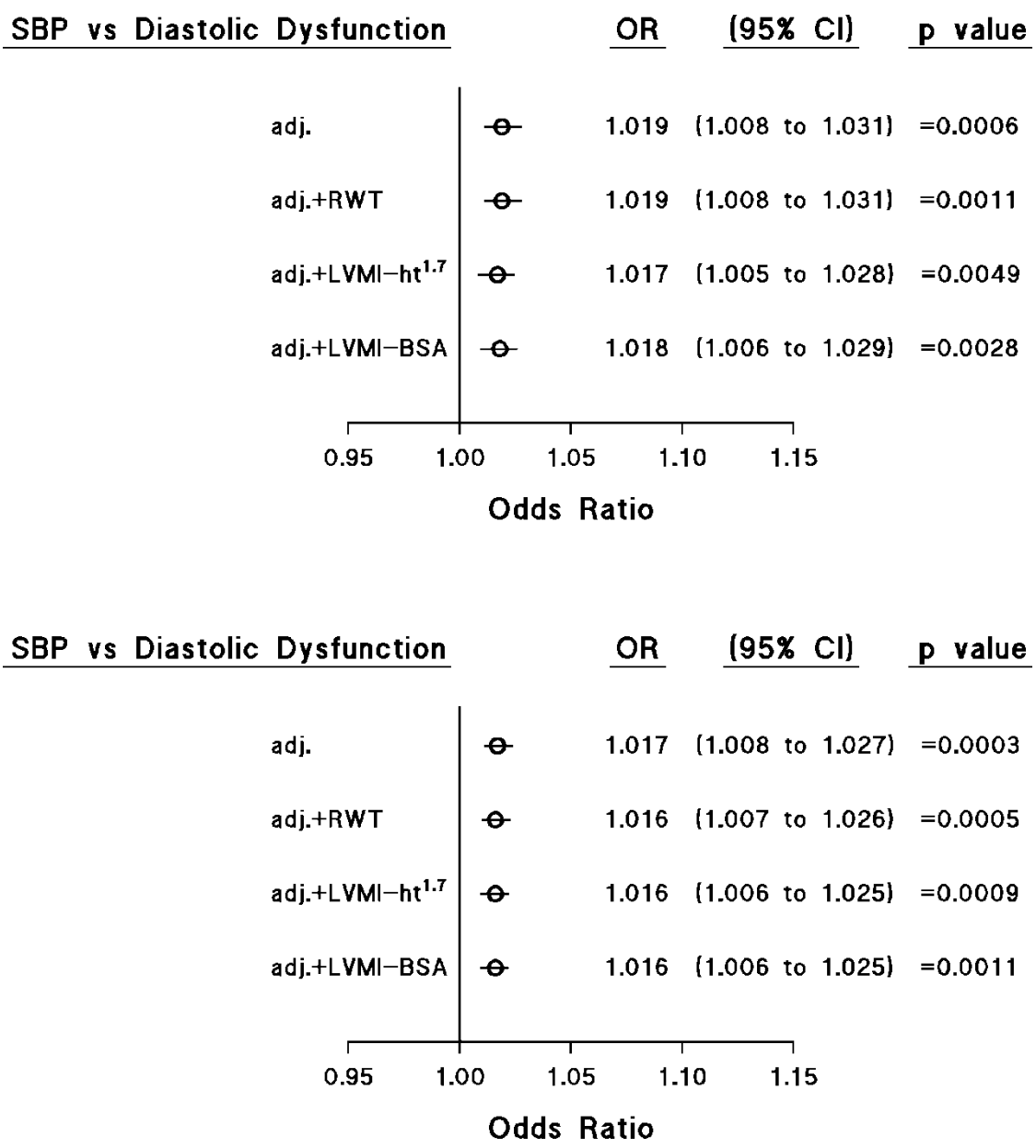

Figure 3.3. Impact of adjustments for left ventricular mass index (LVMI) or relative wall thickness on the independent associations between systolic blood pressure (SBP) and the presence of LV diastolic dysfunction (DD) in a community sample (n=709). Adjustments are for LVMI or RWT as indicated and age, sex, waist circumference, the presence of diabetes mellitus, treatment for hypertension, regular tobacco use, regular alcohol consumption and pulse rate. Upper panel shows relations with DD determined using criteria from current guidelines (Nagueh et al 2016) and the lower panel shows relations with DD determined using previously described criteria (Redfield et al 2003).

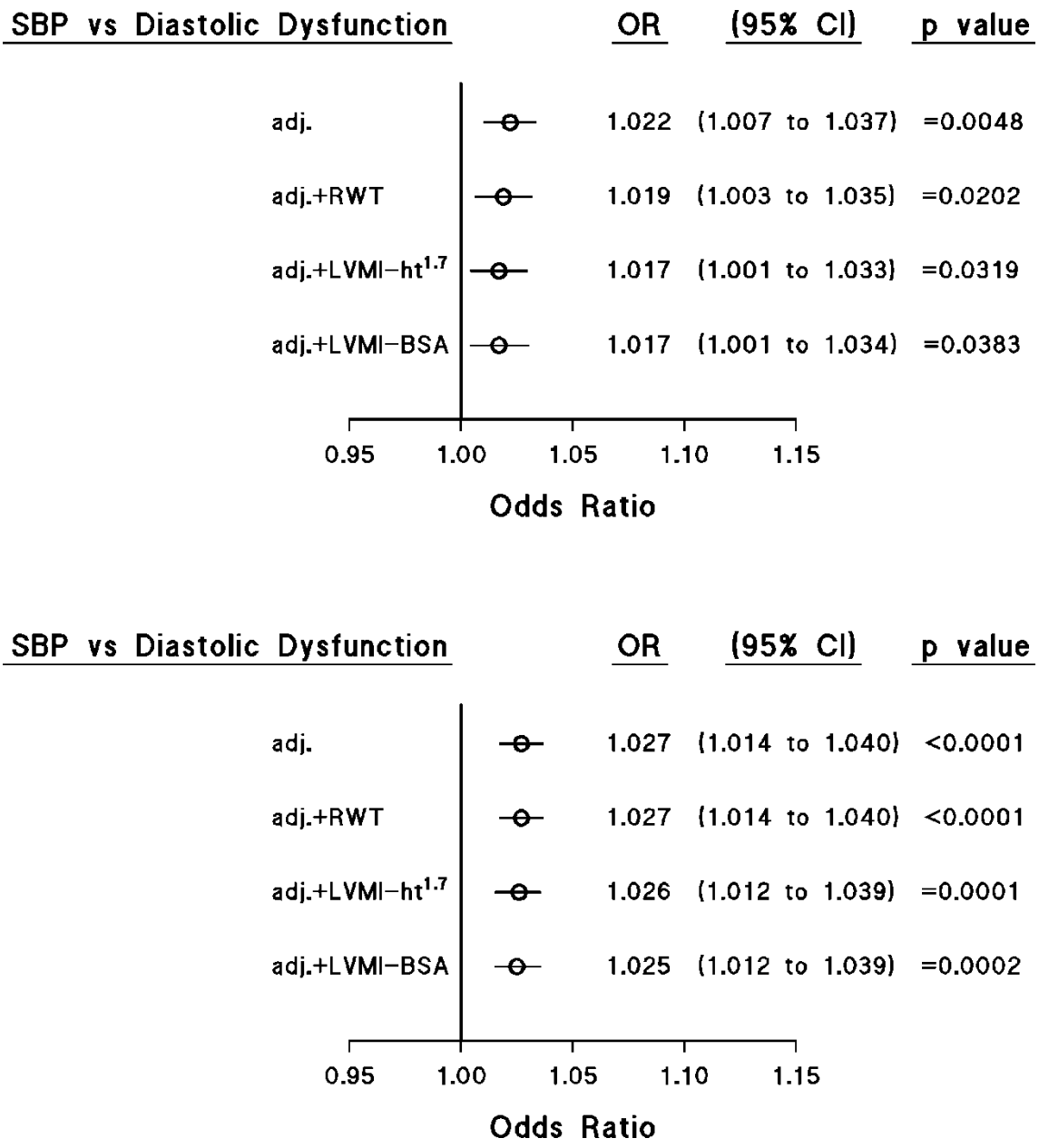

Figure 3.4. Impact of adjustments for left ventricular mass index (LVMI) or relative wall thickness (RWT) on the independent associations between systolic blood pressure (SBP) and the presence of LV diastolic dysfunction (DD) in those having never received antihypertensive treatment in a community sample (n=501). Adjustments are for LVMI or RWT as indicated and age, sex, waist circumference, the presence of diabetes mellitus, regular tobacco use, regular alcohol consumption and pulse rate. Upper panel shows relations with DD determined using criteria from current guidelines (Nagueh et al 2016) and the lower panel shows relations with DD determined using previously described criteria (Redfield et al 2003).

RWT to relationships between BP and lateral wall e', E/e', E/A and e'/a' was minor at best and adjustments for LVMI or RWT failed to significantly modify relationships between BP and either indices of LV diastolic function or the presence of DD. These effects were noted irrespective of whether all participants were considered or only those having never received antihypertensive agents.

Left ventricular hypertrophy and remodeling have traditionally been viewed as an almost necessary prelude to LV dysfunction and heart failure in hypertension. In this regard, concentric LV remodeling and hypertrophy are thought to progress to LV DD and eccentric hypertrophy to LV systolic dysfunction. Using less contemporary approaches to identifying LV DD, 84% of hypertensive patients with LVH have previously been noted to have LV DD (Wachtell et al 2000), and it is estimated that only 11% to 20% of hypertensive patients have LV diastolic dysfunction without exhibiting LVH (Dini et al 2013; Phillips et al 1989). However, these estimates were obtained at a time when more recent non-invasive approaches to determining LV diastolic function were not available. In contrast however, it is also well recognised that many patients with heart failure with a preserved ejection fraction do not have LVH (Lam et al 2007) despite the fact that hypertension is the dominant risk factor for this form of heart failure. Moreover, LV DD without LVH is an early manifestation of hypertensive heart disease (Messerli et al 2017). Indeed, the cellular changes responsible for LV DD in hypertension are often independent of the hypertrophic process (González et al 2018; Paulus & Tschope 2013). In this regard, myocardial fibrosis is correlated with diastolic dysfunction determined using tissue Doppler imaging in hypertensive patients irrespective of LVM, and myocardial fibrosis may precede LVH in the evolution of hypertensive heart disease (Muller-Brunotte et al 2007). In support of the notion that neither LVMI nor RWT explain BP-related decreases in LV diastolic function, little of the impact of BP on several aspects of LV diastolic function noted in the present study could be accounted for by LVMI or RWT. Importantly, these findings were noted when assessing relations between BP and diastolic function or dysfunction with as compared to without adjustments for LVMI or RWT and in product of coefficient mediation analysis. These data are in-part consistent with a dissociation noted between ethnicity and LVM versus DD in a

recent large echocardiographic study (Shantsila et al 2018), and the limited contribution of concentric LVH to DD in hypertensives recently described (Nazário Leão et al 2018). In this regard, the present study suggests that neither LVH nor the LV remodeling process contribute to any significant degree to BP-associated changes in LV diastolic function.

Although the results of the present study suggest that LVMI and RWT are not necessary preludes to LV DD in hypertension, they do not suggest that those with LVH or concentric LV remodeling are not at risk of LV DD or the development of heart failure with a reduced ejection fraction. Indeed, DD may be worse in patients with LVH as compared to those without LVH (Kattel et al 2016), and in the present study LVH and RWT were independently associated with DD. Moreover, LVH is well-recognised as progressing to both DD and heart failure and concentric LVH is thought to progress to heart failure with a preserved ejection fraction (Drazner 2011; Aurigemma et al 2001). The present study nevertheless raises the question of whether DD in LVH or the progression to DD in those with LVH is attributed to the impact of structural remodeling of the LV or rather separately to BP effects. Indeed, it is mainly those with LVH who, in addition to a structural change in the LV, have biomarker evidence of increased loading conditions or myocardial damage produced presumably by increased loads, that progress to heart failure (Seliger et al 2015). Moreover, assigning those with LVH to concentric versus eccentric subtypes only moderately differentiates participants at increased risk of heart failure with a preserved versus reduced ejection fraction (Ho et al 2013). Consequently, LVH and RWT alone may not be strong phenotypes for detecting the risk for heart failure or heart failure subtypes in hypertension. In this regard, the present study adds to this notion by suggesting the neither LVMI nor RWT are strong phenotypes for identifying the presence of BP-associated decreases in LV diastolic function. Nevertheless, whether as recently suggested, the combination of LVH and DD is a worse cardiac phenotype in hypertensive heart disease than either considered separately (Messerli et al 2017) requires further study.

A further question of importance that arises from the present study is whether in the treatment of hypertension, LV diastolic functional parameters improve in close association with on-treatment reductions in LVMI. Although several studies, including major clinical trials (Tapp

et al 2010) have demonstrated improvements in contemporary measures of LV diastolic function following antihypertensive therapy, to the best of our knowledge we can find no reported data to suggest the extent to which regression of LVH explains the benefits of antihypertensive therapy on LV diastolic function. However, in the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), the marked differences in treatment groups in improvements in LV diastolic function were unaffected by adjustments for LVMI (Tapp et al 2010). Hence, in-keeping with the present study, on-treatment reductions in LVMI in the ASCOT study (Tapp et al 2010) are unlikely to have contributed significantly to improvements in LV diastolic function. Although in the ASCOT study little difference in brachial BP was noted between treatment groups (Tapp et al 2010), marked differences in central aortic BP did occur between the treatment groups. Thus, on-treatment differences in the improvement of LV diastolic function in the ASCOT study (Tapp et al 2010) are likely to have been attributed to differences in the impact of treatment on pulsatile loads.

The criteria for the diagnosis of LV DD have been debated over several decades. As recently highlighted (Nagueh et al 2016), tissue Doppler indices of diastolic dysfunction (e' and E/e'), LA volume and pulmonary artery pressures, are recommended for the diagnosis of DD in the presence of a normal EF. Although we determined 3 of the 4 recommended measures of DD (lateral and septal wall e', E/e' and LA volumes), at the time of initiating the present study, we did not determine pulmonary artery pressures. As the diagnosis of DD in those with a normal EF requires more than 2 of the 4 criteria to be present (Nagueh et al 2016), we may have included indeterminate participants as having DD. We nevertheless also showed similar relations with DD when employing the original criteria proposed several years prior to the recent guidelines (Redfield et al 2003). However, using the previously described criteria for identifying DD (Redfield et al 2003), pseudo-normalisation of E/A may be determined from changes noted with the Valsalva manoeuvre, an approach which we were unable to reproducibly perform. Nevertheless, according to these original criteria (Redfield et al 2003) moderate DD may also be identified from E/e' >10 (Redfield et al 2003), an approach employed in the present study for the diagnosis of DD.

There are several additional limitations to the present study. This is a cross-sectional study and hence we cannot draw conclusions regarding causality. However, the causal relationship between BP and indices of LV diastolic function has been well described. In addition, concentric LV remodelling is more prevalent in groups of African descent than other origins, and female gender and obesity are well recognised risk factors for DD. In this regard, the present findings were noted in a community sample of black African ancestry with a high prevalence of obesity and where more women than men volunteered to participate. Hence, it is important to consider the possibility that the findings are specific to groups of African ancestry and to obese females. Nevertheless, although we were not statistically powered to perform analysis on relations with a diagnosis of DD in subgroups, the lack of contribution of LVMI or RWT to BP-associated changes in the individual criteria for DD were reproduced in men and women and in obese versus non-obese participants of the present study. Further, the lack of impact of adjustments for LVM on the ability of decreases in BP to modify tissue Doppler indices of diastolic function previously described (Tapp et al 2010) was observed in a clinical trial conducted largely in patients of European ancestry with an equivalent male as compared to female distribution and whom had a low prevalence of obesity. Moreover, relations between circulating concentrations of procollagen type I and indices of diastolic function beyond LVM (Norton et al 1997) were previously described in largely male Caucasians with a low prevalence of obesity. Thus, the ability of BP and BP-associated changes in myocardial properties to associate with diastolic function beyond LVM is likely to be consistent across ethnic groups, genders and levels of body size.

Although in the present study we show a limited contribution of LVM to BP effects on LV diastolic function, we failed to provide evidence for a mechanism that may explain these effects. In this regard, as indicated in the aforementioned paragraph, circulating concentrations of procollagen type I are correlated with tissue Doppler indices of diastolic function (Muller-Brunotte et al 2007). Importantly however, it is now well recognised that several mechanisms explain relationships between hypertension and diastolic dysfunction including alterations in myocardial calcium cycling, titin expression, collagen cross-linking (mediated by oxidative

stress) and coronary microvascular alterations (González et al 2018; Paulus & Tschope 2013; Norton et al 1997). Indeed, unequivocal preclinical evidence challenges the role of both myocardial fibrosis and LVH independently mediating DD in hypertension (Norton et al 1997). Hence, only biopsy studies will comprehensively identify the mechanisms of BP-mediated myocardial DD.

3.5 Conclusions

In a relatively large community-based sample with a high prevalence of untreated hypertension, we show in product of coefficient mediation analysis, that independent of confounders, LVM and the extent of concentric LV remodelling account for little of the adverse effects of BP on LV diastolic function. Thus, most BP-associated decreases in LV diastolic function should be viewed as a transition process to LV dysfunction independent of LVH or concentric remodelling and hence that measures of LVM or LV remodelling offer little insight into the adverse effects of BP on LV diastolic function.

CHAPTER 4

Insulin Resistance Influences the Impact of Hypertension on Left Ventricular Diastolic Dysfunction in a Community Sample.

The data in this chapter have been published in the journal *Clinical Cardiology*

Adamu J Bamaiyi, Angela J Woodiwiss, Vernice Peterson, Monica Gomes, Carlos D Libhaber, Pinhas Sareli, Gavin R Norton. Insulin Resistance Influences the Impact of Hypertension on Left Ventricular Diastolic Dysfunction in a Community Sample.

Clin Cardiol. 2019; 42(2):305-311.

4.0 Abstract

Background: Although obesity-associated metabolic abnormalities (insulin resistance-IR) may not play as marked a role in determining left ventricular (LV) diastolic dysfunction (DD) as hypertension, the impact of combinations of these risk factors on DD is unknown. We hypothesized that IR influences the impact of hypertension on DD.

Methods: In 704 randomly selected participants from a community sample with a high prevalence of hypertension (50.6%) and obesity (46.5%), we determined adiposity indices, IR from the homeostasis model (HOMA-IR) and LV diastolic function using standard echocardiographic techniques.

Results: HOMA-IR was independently associated with lateral wall e' and E/e' ($p<0.05$ to $p<0.005$) as well as a diagnosis of DD ($p<0.02$). Importantly however, an enhanced relationship between HOMA-IR and E/e' in hypertensives (n=356, partial r=0.15, $p<0.005$) as compared to normotensives (n=348, partial r=0.02 p=0.75) was noted. Consequently, as compared to normotensives, with adjustments for confounders, hypertension was independently associated with DD only in those with the highest tertile of HOMA-IR (Odds ratio=2.65, 95% confidence interval=1.29 to 5.42, $p<0.01$), whilst in those with the lowest tertile of HOMA-IR, hypertension failed to show a higher prevalence of DD (p=0.22).

Conclusions: Insulin resistance enhances the impact of hypertension on LV DD. Thus DD is more likely to occur with the combination of hypertension and IR.

Key words: obesity, insulin resistance, left ventricular diastolic function, hypertension.

4.1 Introduction

Heart failure with a preserved (normal) ejection fraction may contribute to close to half of all admissions for heart failure, and the outcomes may be equally as poor as heart failure with a reduced ejection fraction (Borlaug & Redfield 2011; Lee et al 2009; Owan et al 2006; Bhatia et al 2006). There is presently little evidence for proven treatment benefits for heart failure with a normal ejection fraction (Borlaug & Redfield 2011; Borlaug & Paulus 2011; Pitt et al 2014). Although diastolic dysfunction (DD) is central to the pathophysiology and outcomes of heart failure with a normal ejection fraction (Zile et al 2004; Westermann et al 2008; Burke et al 2014; Mohammed et al 2012; Shah et al 2014) and pre-clinical DD predicts the progression to heart failure with a normal ejection fraction (Wan et al 2014), it is possible that the pathophysiological mechanisms responsible for DD may differ depending on the risk factors involved. In order to better identify therapeutic targets for heart failure with a preserved ejection fraction, an improved understanding of the role of the risk factors for DD is required. In this regard, the impact on DD of the combination of the commonly occurring co-morbidities, hypertension and obesity or the associated metabolic disturbances, is uncertain.

Some prior studies conducted in elderly populations, in patients referred for echocardiography, or in samples with a high proportion of participants receiving therapy, report on an equal or greater impact of obesity as compared to hypertension on left ventricular (LV) diastolic function (Rusco et al 2011; Çil et al 2012; Aljaroudi et al 2012). However, in studies conducted across the full adult age range in a community sample with a high proportion of obesity and hypertension, but a low proportion of participants who were receiving antihypertensive therapy, blood pressure (BP) was noted to be the main determinant of DD (Millen et al 2014). Nevertheless, more recent evidence suggests that a more concentrically remodeled LV determines whether obesity-related insulin resistance (IR) is associated with LV diastolic function (Peterson et al 2016). As hypertension is a strong determinant of concentric LV remodeling, the possibility exists that even if obesity or associated IR have only a modest impact on LV diastolic function, that obesity or its metabolic consequences may determine the

extent to which DD occurs in hypertension. Consequently, in the present study we aimed to determine whether adiposity indices or IR influence the extent to which DD occurs in hypertension in a community sample with a high prevalence of hypertension and obesity.

4.2 Methods

4.2.1 Study sample. The present study was approved by the University of the Witwatersrand Committee for Research in Human Subjects (approval number M02-04-72 renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. The study design has previously been described (Woodiwiss et al 2008; Libhaber et al 2014; Woodiwiss et al 2009; Redelinghuys et al 2010). 1044 Participants of nuclear families of black African descent with siblings older than 16 years were randomly recruited from the South West Township of Johannesburg, South Africa for echocardiographic studies. Tissue Doppler measures of myocardial function were obtained in a sub-study conducted in 704 participants from the time that these measures became routinely available.

4.2.2 Demographic and clinical information. A standardized questionnaire was administered to obtain demographic and clinical data (Woodiwiss et al 2008; Libhaber et al 2014; Woodiwiss et al 2009; Redelinghuys et al 2010). Height, weight, and waist circumference (WC) were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2, obese if their BMI was ≥30 kg/m^2 and morbidly obese of their BMI was ≥35 kg/m^2. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men). Laboratory blood tests including percentage glycated hemoglobin (HbA_{1c}) were performed. Fasting plasma insulin concentrations were determined from an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA). Diabetes mellitus or an abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or a glycated hemoglobin (Roche Diagnostics, Mannheim, Germany) value greater than 6.5%.

Insulin resistance was estimated by the homeostasis model assessment of IR (HOMA -IR) using the formula (insulin [μU/ml] x glucose [mmol/l])/22.5.

Nurse-derived conventional BP was measured according to guidelines using a mercury sphygmomanometer after five minutes of rest in the seated position as previously described (Woodiwiss et al 2009). Five consecutive BP readings were obtained using an appropriately sized cuff, 30 to 60 seconds apart. The average of the five readings was taken as the BP. None of the visits had fewer than the planned BP recordings. Hypertension was defined as the use of antihypertensive medication or if the mean of the five conventional BP measurements was >140 (systolic BP) or 90 (diastolic BP) mm Hg in those not receiving medication.

4.2.3 Echocardiography. Echocardiographic measurements were performed as previously described (Millen et al 2014; Peterson et al 2016; Woodiwiss et al 2008; Libhaber et al 2014) by two experienced observers (AJW and CDL) with the participants in the partial left decubitus position. All potential participants were assessed for mitral valve abnormalities as determined using 2-dimensional and colour Doppler imaging and excluded if significant valve abnormalities were present. Left ventricular dimensions were determined using two-dimensional directed M-mode echocardiography in the short axis view and these recordings were analyzed according to the American Society of Echocardiography convention (Shah et al 1978). The LV dimensions were measured only when appropriate visualization of both the right and the left septal surfaces occurred and where the endocardial surfaces of both the septal and posterior wall were clearly visible. Left ventricular ejection fraction was calculated using the biplane Simpson method. Left ventricular mass (LVM) was determined using a standard formula (Devereux et al 1986) and indexed (LVMI) to height$^{1.7}$.

Left ventricular diastolic function was assessed from a pulsed wave Doppler examination of the mitral inflow at rest and using tissue Doppler indices (TDI) as well as left atrial volumes (LAV) (Nagueh et al 2016). Pulse wave Doppler recordings of trans-mitral velocity were obtained with the sample volume at the tip of the mitral valve in the apical 4-chamber view. Trans-mitral velocity measurements were obtained during the early (E) period of left ventricular diastolic inflow. To perform TDI, the velocity of myocardial tissue lengthening at the level of the

mitral annulus was recorded in the apical four-chamber view. The sample volume was positioned at the septal and lateral corners of the mitral annulus. To determine diastolic function using TDI, peak velocities during early (e') diastole were measured. Data were expressed as the E/e' ratio (an index of LV filling pressures). Because mitral annular velocity (e') remains constant and trans-mitral flow (E) increases with an increased filling pressure, E/e' ratio correlates well with left ventricular filling pressures. Left atrial volume indexed to body surface area, was calculated using the area-length method, where length was defined as the shortest of the two long axes measured in the apical four-chamber and two-chamber views. Left atrial area was measured by planimetry in the apical four-chamber and two-chamber views at left ventricular end systole (maximum left atrial dimensions). As no participants had a reduced EF, LV DD was identified by the presence of at least two of the following: a lateral e'<10 cm/s or a septal e'<8 cm/s, E/e'>14, or LAV index ≥34ml/m^2 (Nagueh et al 2016).

4.2.4 Data analysis. Database management and statistical analyses were performed with SAS software, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Data from individuals were averaged and expressed as mean±standard deviation (SD) or the standard error of the mean (SEM). To improve on the distribution of data, HOMA-IR, lateral e', septal e', E/e' and LAV index were logarithmically transformed. To determine independent relations, multivariate adjusted linear (continuous data) or logistic (discrete data) regression analysis was performed. Indexes of diastolic LV function were adjusted for several confounders associated with diastolic function noted in bivariate analysis. Relationships (partial r values) were compared with z-statistics.

4.3 Results

4.3.1 Characteristics of study sample. Table 4.1 gives the demographic and clinical characteristics of the normotensive and hypertensive participants. More women than men participated in the study and a high proportion of participants, particularly the hypertensives, were overweight, obese or morbidly obese and had central obesity. As compared to participants

Table 4.1. Characteristics of the study sample.

	All	Normotensives	Hypertensives
Sample number (% female)	704 (67.3%)	348 (65.8%)	356 (68.8%)
Age (years)	47.2±18.1	37.8±14.5	56.5±15.5**
Body mass index (BMI) (kg/m^2)	30.1±8.1	27.9±8.0	32.2±7.6**
% Overweight/obese/morbidly obese	23.0/20.2/26.3	27.0/16.1/16.1	19.1/24.2/36.2**
Waist circumference (WC) (cm)	93.9±18.2	87.6±17.2	100.0±17.0**
% Abnormal waist circumference	52.3	39.2	65.1**
Regular tobacco (% subjects)	16.5	18.1	14.9
Regular alcohol (% subjects)	19.5	21.8	17.1
% Diabetes mellitus or an HbA_{1c}>6.5%	14.4	5.7	22.7**
% Treated for hypertension	29.3	0	57.9**
HOMA-IR	2.51±3.92	2.13±3.74	2.88±4.07*
Brachial SBP/DBP (mm Hg)	128±21/83±13	114±11/76±8	141±22/89±13**
E/e’	7.5±4.2	7.0±3.3	9.2±4.3**
Lateral e’ (cm/s)	11.3±4.1	13.2±3.9	9.5±3.4**
Septal e’ (cm/s)	9.6±3.6	11.2±3.5	8.0±3.0**
Left atrial volume (LAV) index (ml/m^2)	19.7±3.6	18.6±7.0	20.9±7.8**
Left ventricular mass index (g/m$^{1.7}$)	62.7±23.1	55.9±20.3	69.2±23.8**
LV relative wall thickness	0.36±0.08	0.34±0.07	0.38±0.08**
% with diastolic dysfunction	15.3	5.5	25.0**

HbA_{1c}, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic BP; HOMA-IR, homeostasis model of insulin resistance; e’, myocardial tissue lengthening in early diastole at the mitral annulus; E/e’, transmitral early blood flow velocity/velocity of the mean value of lateral and septal wall myocardial tissue lengthening in early diastole at the mitral annulus; LV, left ventricle. *p<0.01, **p<0.0001 versus normotensives.

recruited prior to TDI becoming available, participants in whom echocardiography was performed once routine TDI became available, were modestly older with more abdominal obesity, but a lower HOMA-IR and LVMI and more were receiving treatment for hypertension (Table 4.2). 5.5% of the normotensives and 25.0% of the hypertensives had DD and this was largely determined by a combination of either reduction in lateral or septal e' and increases in E/e' (75%). No participants had an ejection fraction<40% and 4.4% had an ejection fraction<50%. Of the sample 39.3% had LV hypertrophy (LVH) (LVMI>80 $g/m^{1.7}$ for men and >60 $g/m^{1.7}$ for women) and 18.1% had concentric LV remodelling (relative wall thickness>0.42). A greater proportion of hypertensives than normotensives had LVH and concentric LV remodelling.

4.3.2 Factors associated with LV diastolic function. With adjustments for confounders, systolic BP, and either WC, HOMA-IR, or BMI were independently associated with lateral and septal wall e' and E/e' (Table 4.3). Whilst WC and systolic BP were independently associated with LAV index, HOMA-IR and BMI were not (Table 4.3). However, with adjustments for confounders systolic BP and HOMA-IR, but not WC or BMI were independently associated with the presence of LV DD (Table 4.4). Consistent with the effects of IR as opposed to hyperglycaemia, plasma insulin ($p<0.05$ to $p<0.01$) but not blood glucose ($p=0.08$ to $p=0.83$) was independently associated with LV diastolic function and the presence of LVDD.

4.3.3 Impact of IR on LV diastolic function in hypertensives and normotensives. In both normotensives and hypertensives, WC (and BMI) as well as HOMA-IR were independently associated with lateral wall e' (Table 4.5). However, in normotensives, but not in hypertensives, WC or BMI were independently associated with E/e', whilst in hypertensives, but not normotensives, HOMA-IR was independently associated with E/e' (Table 4.5). Importantly, as compared to normotensives, this translated into an independent effect of hypertension on E/e' (and lateral wall e') only in those hypertensives with a HOMA-IR in the upper two tertiles (Figure 4.1). Although hypertension was also only independently associated with an increased E/e' (and lateral wall e') in the upper two tertiles of BMI this effect failed to show a stepwise relationship and hypertension was also only independently associated with an increased E/e' (and lateral

Table 4.2. Characteristics of community sample without tissue Doppler imaging (TDI).

		p value versus with TDI
Sample number (% female)	340 (65.3)	(0.53)
Age (years)	43.5±18.1	0.002
Body mass index (kg/m^2)	29.1±7.4	0.06
% Overweight/obese/morbidly obese	24.6/20.1/22.5	0.19
Waist circumference (cm)	89.9±15.5	<0.001
% Abnormal waist circumference	42.4	0.007
Regular tobacco (% subjects)	12.6	0.10
Regular alcohol (% subjects)	21.3	0.51
% Diabetes mellitus or an HbA_{1c}>6.5%	13.8	0.85
% Treated for hypertension	23.0	0.03
HOMA-IR	3.23±3.28	0.002
Brachial SBP/DBP (mm Hg)	130±23/84±12	0.16/0.23
Left ventricular mass index (g/m$^{1.7}$)	73.0±21.6	<0.0001

HbA_{1c}, glycated hemoglobin; SBP, systolic blood pressure; DBP, diastolic BP; HOMA-IR, homeostasis model of insulin resistance; LV, left ventricle.

Table 4.3. Relative contribution (standardized β-coefficient) of the homeostasis model of insulin resistance (HOMA-IR), waist circumference (WC) or body mass index (BMI)versus alternative risk factors toward indices of left ventricular diastolic function in a community sample (n=704).

Models with→	HOMA-IR	WC	BMI	HOMA-IR	WC	BMI
	β-coef±SEM (p-value)	β-coef±SEM (p-value)	β-coef±SEM (p-value)	β-coef±SEM (p value)	β-coef±SEM (p value)	β-coef±SEM (p value)
		Log lateral wall e'		**Log E/e'**		
HOMA-IR	-0.103±0.031 (<0.001)	-	-	0.083±0.036 (0.020)	-	-
WC	-	-0.159±0.036 (<0.0001)	-	-	0.147±0.044 (<0.001)	-
BMI	-	-	-0.126±0.034 (<0.0005)	-	-	0.121±0.040 (0.003)
Age	-0.471±0.037 (<0.0001)	-0.421±0.041 (<0.0001)	-0.447±0.037 (<0.0001)	0.249±0.043 (<0.0001)	0.217±0.050 (<0.0001)	0.226±0.044 (<0.0001)
SBP	-0.168±0.033 (<0.0001)	-0.152±0.033 (<0.0001)	-0.153±0.033 (<0.0001)	0.208±0.038 (<0.0001)	0.188±0.041 (<0.0001)	0.195±0.038 (<0.0001)
Female	-0.002±0.033 (0.96)	0.040±0.035 (0.25)	0.049±0.035 (0.17)	0.088±0.039 (0.023)	0.063±0.043 (0.14)	0.043±0.041 (0.30)
		Log septal wall e'		**Log LA volume index**		
HOMA-IR	-0.069±0.029 (0.016)	-	-	0.040±0.039 (0.30)	-	-
WC	-	-0.157±0.038 (<0.0001)	-	-	0.067±0.047 (=0.16)	-
BMI	-	-	0.110±0.035 (0.002)	-	-	0.078±0.040 (0.07)
Age	-0.495±0.034 (<0.0001)	-0.451±0.044 (<0.0001)	-0.484±0.038 (<0.0001)	0.187±0.046 (<0.0001)	0.136±0.054 (0.01)	0.162±0.047 (<0.001)
SBP	-0.114±0.030 (<0.0005)	-0.087±0.035 (0.014)	-0.091±0.033 (0.007)	0.107±0.041 (0.010)	0.092±0.043 (0.03)	0.096±0.041 (0.02)
Female	-0.003±0.039 (0.91)	0.031±0.037 (0.40)	0.020±0.036 (0.57)	-0.083±0.042 (0.05)	-0.089±0.046 (0.06)	-0.116±0.045 (0.01)

β-coef, standardized β-coefficient (slope). See table 4.1 for other abbreviations. Also included in the models are regular tobacco use, and regular alcohol consumption, treatment for hypertension, diabetes mellitus and pulse rate.

Table 4.4. Multivariate adjusted associations between risk factors and left ventricular (LV) diastolic dysfunction (DD) in a community sample (n=704).

Models with→	HOMA-IR		Waist circumference (WC)		Body mass index (BMI)	
LV DD versus	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
HOMA-IR/WC/BMI	1.428 (1.078 to 1.890)	0.013	1.013 (0.995 to 1.031)	0.16	1.039 (0.999 to 1.080)	0.06
Age	1.038 (1.017 to 1.059)	<0.0005	1.035 (1.015 to 1.056)	<0.001	1.037 (1.017 to 1.058)	<0.0005
Systolic BP	1.021 (1.010 to 1.033)	<0.0005	1.020 (1.008 to 1.031)	<0.001	1.019 (1.008 to 1.031)	0.001
Female gender	1.663 (0.863 to 3.202)	0.13	1.319 (0.679 to 2.563)	0.41	1.193 (0.590 to 2.412)	0.62

See table 4.1 for abbreviations. Additional adjustors include regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus, and pulse rate.

Table 4.5. Impact of hypertension on multivariate adjusted relationships between the homeostasis model of insulin resistance (HOMA-IR), waist circumference or body mass index and indexes of left ventricular diastolic function in a community sample (n=704).

	Normotensives (n=348)			Hypertensives (n=356)		
	β-coef±SEM	Partial r (95% CI)	p value	β-coeff±SEM	Partial r (95% CI)	p value
HOMA-IR versus						
Log lateral wall e'	-0.018±0.008	-0.12 (-0.23 to -0.02)	0.024	-0.017±0.008	-0.11 (-0.21 to -0.002)	0.046
Log septal wall e'	-0.010±0.008	-0.07 (-0.17 to 0.03)	0.19	-0.013±0.008	-0.08 (-0.18 to 0.02)	0.13
Log E/e'	0.003±0.011	0.02 (-0.09 to 0.12)	0.75	0.031±0.010†	0.15 (0.05 to 0.25)	0.004
LA volume index	0.009±0.010	0.05 (-0.06 to 0.15)	0.39	0.006±0.010	0.03 (-0.07 to 0.14)	0.57
Waist circumference versus						
Log lateral wall e'	-0.0018±0.0004	-0.23 (-0.34 to -0.12)	<0.0001	-0.0011±0.0005	-0.13 (-0.24 to -0.02)	0.020
Log septal wall e'	-0.0021±0.0006	-0.22 (-0.32 to -0.10)	<0.0005	-0.0013±0.0005	-0.15 (-0.26 to -0.04)	0.008
Log E/e'	0.0023±0.0006	0.22 (0.11 to 0.32)	<0.0005	0.0009±0.0007	0.08 (-0.03 to 0.19)	0.16
LA volume index	0.0014±0.0006	0.14 (0.03 to 0.25)	0.015	0.0004±0.0006	0.004 (-0.11 to 0.12)	0.95
Body mass index versus						
Log lateral wall e'	-0.0032±0.0009	-0.19 (-0.29 to -0.09)	<0.0005	-0.0017±0.0011	-0.08 (-0.18 to 0.02)	0.13
Log septal wall e'	-0.0033±0.0010	-0.17 (-0.27 to -0.07)	0.001	-0.0017±0.0011	-0.08 (-0.18 to 0.02)	0.12
Log E/e'	0.0035±0.0012	0.16 (0.05 to 0.26)	0.004	0.0023±0.0014	0.09 (-0.02 to 0.19)	0.11
LA volume index	0.0010±0.0012	0.05 (-0.06 to 0.15)	0.39	0.0024±0.0013	0.10 (-0.01 to 0.21)	0.06

See table 4.1 for abbreviations. *Adjustments are for age, sex, systolic blood pressure, regular tobacco use, regular alcohol consumption, diabetes mellitus, and pulse rate. †$p<0.05$ versus β-coefficient in normotensives.

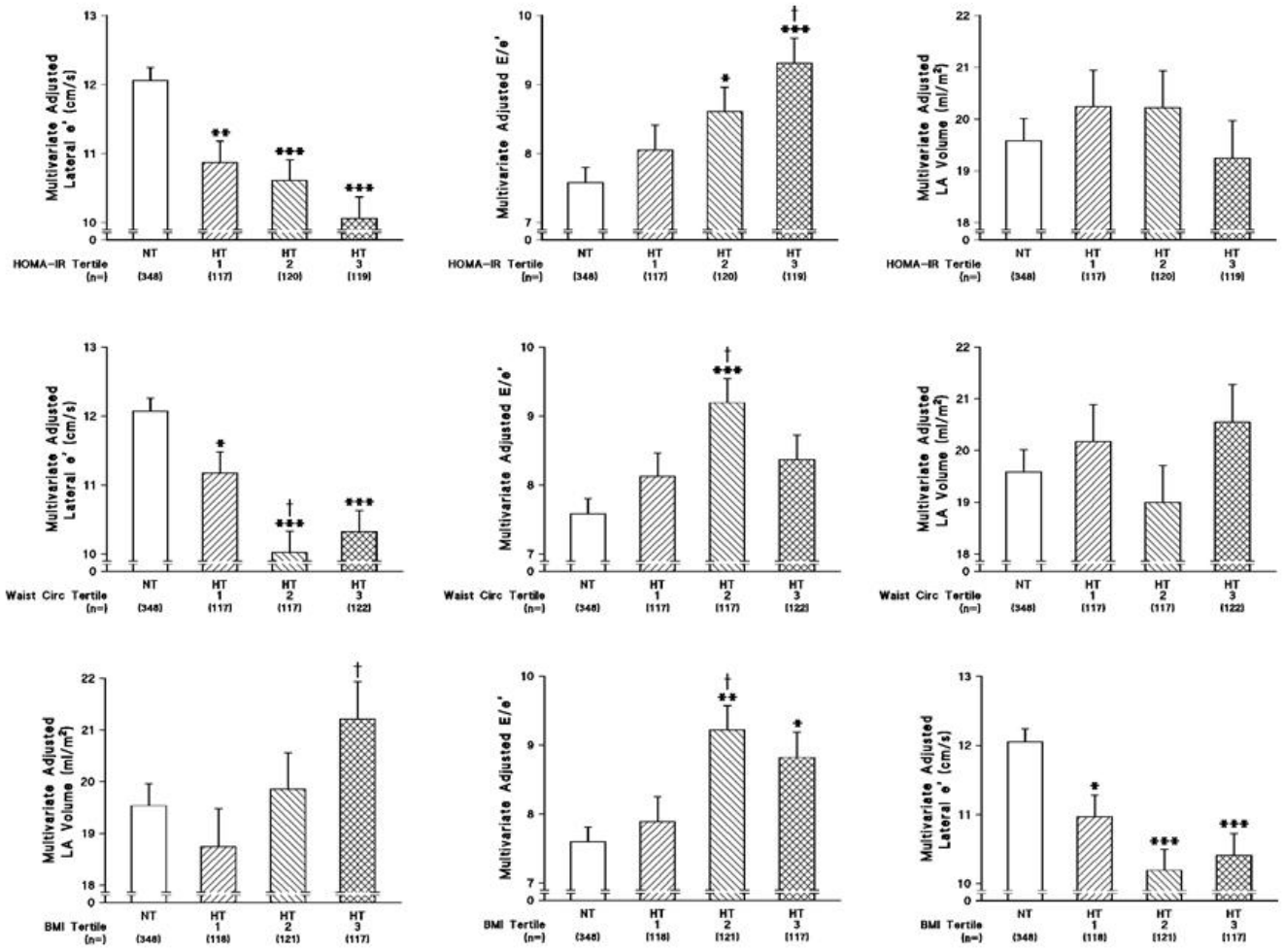

Figure 4.1. Multivariate adjusted indices of left ventricular diastolic function in normotensives and across tertiles of the homeostasis model of insulin resistance (HOMA-IR) (upper panel), waist circumference (WC) (middle panel) or body mass index (BMI) (lower panel) in hypertensives of a community sample. Adjustments are for age, sex, systolic blood pressure, pulse rate, regular smoking, regular alcohol consumption and diabetes mellitus. Abbreviations are given in Table 4.1. Tertiles of HOMA-IR, WC and BMI are defined in Table 4.6. *p<0.02, **p<0.001, ***p<0.0001 versus normotensives. †p<0.05 versus hypertensives HOMA-IR tertile 1 or versus hypertensives waist circumference tertile 1.

wall e') in the second tertile of WC (Figure 4.1). Although more hypertensives had DD across all tertiles of HOMA-IR, WC or BMI (Figure 4.2), these effects were largely attributed to age differences. Indeed, beyond age and other confounders hypertension was only independently associated with an increased odds of DD in those with the higher tertiles of HOMA-IR, WC or BMI (Figure 4.3). Importantly, a stepwise increase in the odds of DD occurred across tertiles of HOMA-IR, but inconsistent effects were noted for WC and BMI (Figure 4.3).

4.4 Discussion

The main findings of the present study are as follows: In a community sample with a high prevalence of obesity, indices of excess adiposity and IR were independently associated with indices of LV diastolic function (e' and E/e'). However, whilst HOMA-IR was independently associated with lateral wall e' in both hypertensives and normotensives, HOMA-IR was independently associated with E/e' in hypertensives, but not in normotensives. Consequently, HOMA-IR determined whether hypertensives developed LV DD as compared to normotensives. In contrast, adiposity indices were associated with indices of diastolic function less well in hypertensives as compared to normotensives and adiposity indices were consequently not independently associated with DD.

Although several large studies have demonstrated that indices of excess adiposity are strongly and independently associated with indices of LV DD (Rucso et al 2011; Çil et al 2012; Aljaroudi et al 2012), these studies have been confounded by the use of predominantly elderly populations (where age is the principle determinant of DD), in select patients referred for echocardiography, or in samples with a high proportion of participants receiving antihypertensive therapy (Rucso et al 2011; Çil et al 2012; Aljaroudi et al 2012). In contrast, in an alternative study conducted in a much smaller cohort of the present community, but across the adult age range and in a sample with a high prevalence of obesity and hypertension, where antihypertensive therapy was employed in only half the hypertensives (Millen et al 2014), BP was noted to contribute far more to DD than adiposity indices. In keeping with this prior study

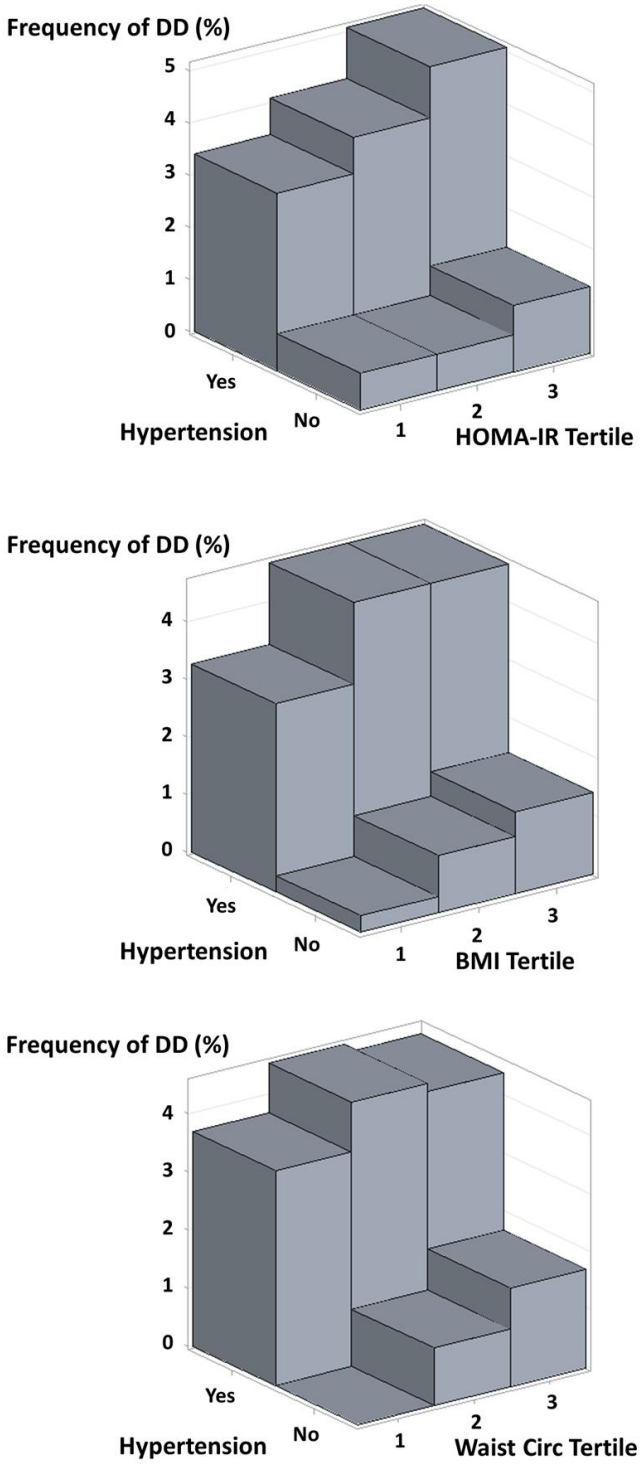

Figure 4.2. Prevalence of left ventricular diastolic dysfunction (DD) in hypertensives as compared to normotensives across tertiles of the homeostasis model of insulin resistance (HOMA-IR), WC or BMI in the whole group and across similar age ranges. Tertiles of HOMA-IR, WC and BMI are defined in Table 4.6.

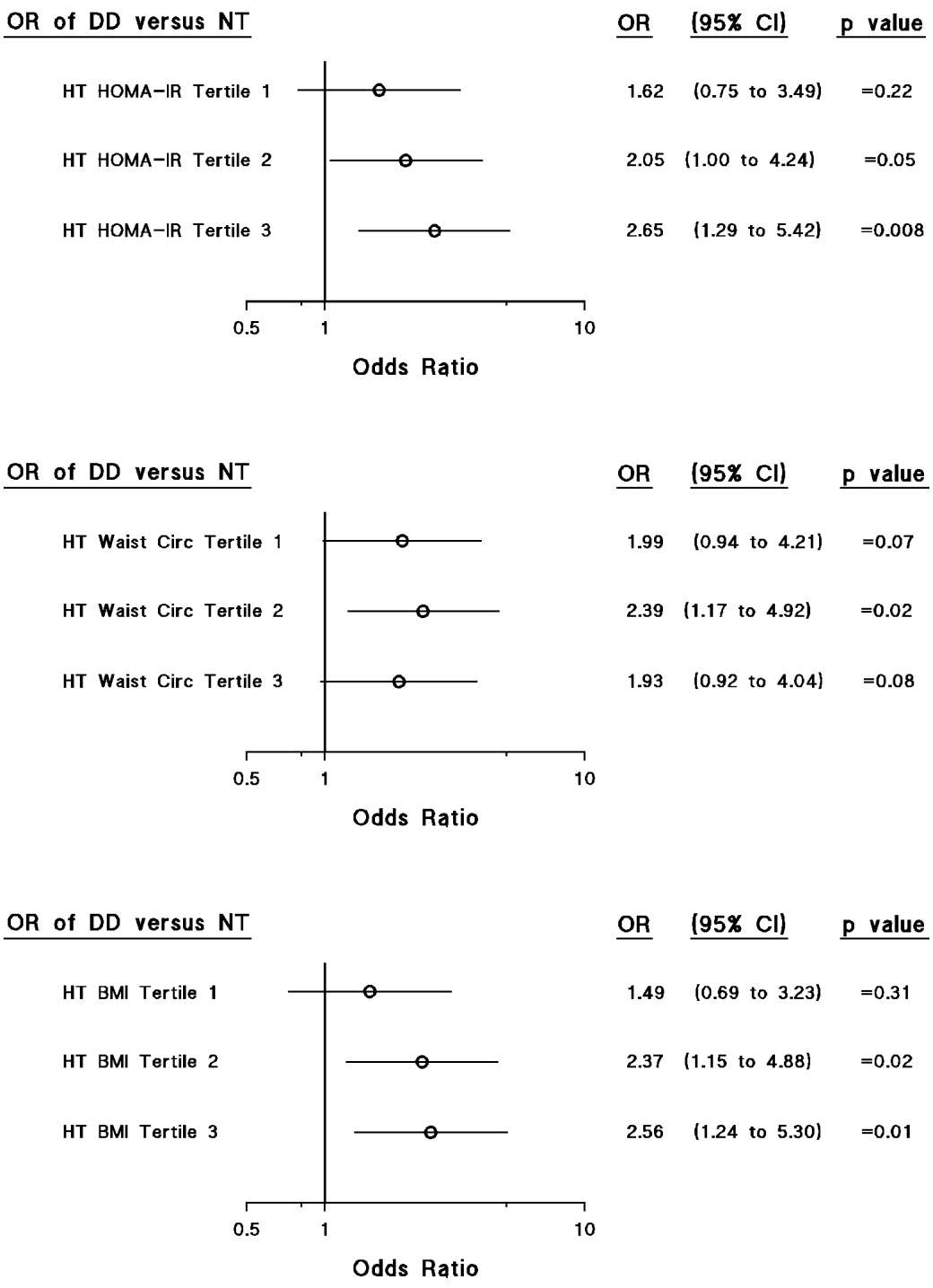

Figure 4.3. Impact of insulin resistance (homeostasis model-HOMA-IR), waist circumference (WC) or body mass index (BMI) on the odds of independent associations between hypertension and left ventricular diastolic dysfunction. Adjustments in the left panels are for age, sex, pulse rate, regular smoking, regular alcohol consumption and diabetes mellitus. Abbreviations are given in Table 4.1. Tertiles of HOMA-IR, WC and BMI are defined in Table 4.6.

Table 4.6. Ranges of tertiles of the homeostasis model of insulin resistance (HOMA-IR), waist circumference (WC) and body mass index (BMI) in normotensives and hypertensives.

	HOMA-IR	WC	BMI
Normotensives Tertile 1	<0.92	<79	<23.51
Normotensives Tertile 2	0.92 to 2.87	79 to 95	23.51 to 29.86
Normotensives Tertile 3	≥2.87	≥95	≥29.86
Hypertensives Tertile 1	<1.36	<94	<28.26
Hypertensives Tertile 2	1.36 to 6.18	94 to 108	28.26 to 35.55
Hypertensives Tertile 3	≥6.18	≥108	≥35.55

(Millen et al 2014) in the present study conducted in a much larger study sample of the same community we show that whilst BP and HOMA-IR translated into DD, adiposity indices failed to do so. The ability of HOMA-IR to associate with DD whilst adiposity indices did not, we attribute to an impact of HOMA-IR, but not adiposity indices on E/e' in the hypertensive, but not in the normotensive BP range. These data therefore suggest that IR is an important contributor to DD, but mainly in those with hypertension.

An important caveat of the present study is that the results do not suggest that IR mediates LV DD through hypertension. Indeed, relations between adiposity indices or HOMA-IR and LV diastolic functional parameters were independent of systolic BP. Rather, the present study suggests that the impact of hypertension on relations between HOMA-IR and DD should be viewed as an additive effect with systolic BP effects alone being more important, but with IR influencing whether hypertension translates into DD.

The criteria for the diagnosis of LV DD have been debated over several decades. As recently highlighted (Nagueh et al 2016), in those with a normal ejection fraction, tissue Doppler indices of DD (e' and E/e'), LAV index and the extent of tricuspid regurgitation, an index if pulmonary artery pressures, are recommended for the diagnosis of DD. Although we determined 3 of the 4 recommended measures of DD (lateral and septal wall e', E/e' and LAV index), at the time of initiating the present study, we did not determine the extent of tricuspid regurgitation. To diagnose DD we nevertheless employed 2 of 3 criteria, whilst current guidelines recommend 2 of 4 (50%) criteria. Although there are no large studies that have demonstrated a relationship between adiposity indices and the extent of tricuspid regurgitation, it is therefore possible that we underestimated the prevalence of obesity-associated DD in the present study. However, the present study is the first to assess relations between adiposity indices and DD determined according to contemporary guidelines (Nagueh et al 2016) which do not include E/A if ejection fraction is within a normal range. In this regard, because of pseudo-normalization of E/A, E/A is only recommended for use for the diagnosis of DD in those with a reduced ejection fraction. In this regard, the present and previous studies (Rucso et al 2011; Çil et al 2012; Aljaroudi et al 2012) were conducted in participants with a largely normal ejection

fraction. Hence, relations between HOMA-IR or indices of excess adiposity and DD, as described in the present study, are more likely to reflect relations between an excess adiposity and actual DD than those previously described (Rucso et al 2011; Çil et al 2012; Aljaroudi et al 2012).

An explanation for the impact of HOMA-IR on E/e' (an index of LV filling pressures) and hence DD in hypertensives, but not normotensives in the present study, is unclear. It is possible that because hypertension is associated with concentric LV remodeling, and as recently demonstrated, a more concentrically remodeled LV determines the impact of IR on E/e' (Peterson et al 2016), that IR only contributes to LV filling pressures when the LV is more concentrically remodeled. Importantly, however, the presence of hypertension in the present study did not influence the association between HOMA-IR and lateral wall e', an index of LV relaxation, whilst previous work does show an impact of relative wall thickness on relations between HOMA-IR and lateral wall e' (Peterson et al 2016). Hence, the impact of hypertension on relations between HOMA-IR and E/e' may not be accounted for just by an effect of the extent of concentric LV remodeling, but by an alternative as yet unidentified factor.

There are several potential implications of the findings of the present study. First, in contrast to adiposity indices, which had less of an ability to determine the impact of hypertension on DD, the assessment of IR may better characterize hypertensives at risk of developing DD and hence heart failure with a preserved ejection fraction. In this regard, longitudinal studies are required to assess this question. Indeed, a recent study reported that insulin resistance at baseline or during follow-up predicted worsening of E/e' after 4.7 years of follow-up (Cauwenberghs et al 2018). Second, targeting IR as opposed to an excess adiposity *per se* with behavioral modification in hypertensives may have marked benefits to preventing the development of DD and hence heart failure with a preserved ejection fraction. In this regard, intervention studies are required to assess these hypotheses.

The limitations of the present study are as follows: First, this is a cross-sectional study and hence we cannot draw conclusions regarding causality. Whether the development of IR influences that of LV DD in those with hypertension rather than normotension therefore requires

further study. Second, we are not statistically powered to perform sex-specific analysis and hence it is possible that as more women than men volunteered for the study, the results may relate mainly to women.

4.5. Conclusions

In a relatively large community-based sample with a high prevalence of obesity and hypertension, we show that independent of confounders, the extent of IR influences whether hypertension translates into diastolic dysfunction. These data suggest that from a clinical perspective, hypertensives with IR may be particularly prone to the development of LV DD and thus possibly the progression to heart failure with a preserved ejection fraction. Consequently, targeting IR in hypertension may have marked benefits for preventing the development of heart failure.

CHAPTER 5

CONCLUSIONS

5.1 Introduction

As highlighted in the introduction to the present thesis, the prevalence of HFpEF is increasing. Whilst morbidity and mortality for HFrEF is decreasing in many countries, because no therapeutic approaches have been demonstrated to improve outcomes in HFpEF, morbidty and mortality in this condition remains high (Borlaug & Paulus 2011; Tamaki et al 2013). Despite several mechanisms demonstrated to contribute to HFpEF, therapeutic agents designed to target these cellular changes have failed to show clinical benefits. It is therefore important to better understand the transition in patients with risk factors for HFpEF to heart failure itself. In this regard, dysfunction in the diastolic period of the cardiac cycle (diastolic dysfunction or DD) characterizes HFpEF and hence in the present thesis I evaluated several outstanding questions related to LV DD. The findings of these studies published in several international journals have been described and discussed in extensive detail in chapters 2 to 4. However, in the present chapter I will place these findings in the context of the wider implications. A perspective will be provided for the implications of my findings for the possible screening of patients at risk, and interventions that may be instituted to prevent the transition to HFpEF. In the present thesis I focused on hypertension and LV DD as hypertension is the principle risk factor associated with LV DD and HFpEF.

5.2 Sympathetic activation in DD and HFpEF

As with HFrEF, HFpEF is associated with neurohumoral activation (Chang et al 2013; Grimm & Brown 2010; Woo & Xiao 2012; Akhter et al 1997), Although the use of β-adrenergic blockers in HFpEF has never been demonstrated to produce beneficial effects on heart failure *per se*, whether sympathetic activation in HFpEF has beneficial effects through an enhanced myocardial lusitropy has never been described. This is of importance as HFpEF is most often a consequence of hypertension and frequently associated with coronary artery disease (Mohammed et al 2015; Desai et al 2013; Kamimura et al 2012) or atrial fibrillation (Hernandez

et al 2009; Reiken et al 2003). These conditions are well-recognised as benefitting from β-adrenergic receptor blockade. However, if sympathetic activation improves diastolic function in DD, the benefits of β-adrenergic receptor blockers in these conditions may be offset by hastening the progression of DD and hence HFpEF. As a consequence of this issue, as part of the present thesis I evaluated whether established animal models of hypertension are associated with clinically relevant measures of DD and whether through lusitropic effects, acute administration of the β-adrenergic receptor agonist, isoproterenol improves hypertensive DD. I selected animal models to study as maximal effects of β-adrenergic receptor agonists cannot be evaluated in humans due to the arrhythmic and myocardial ischaemic effects of β-adrenergic receptor stimulation. Moreover, the impact of β-adrenergic receptor agonists in humans with hypertensive DD would be confounded by the effects of antihypertensive therapy. What were the findings of this study and how does this extend our knowledge of LV DD?

I demonstrated that the LV of both Spontaneously Hypertensive Rats (SHR) and Dahl salt-sensitive (DSS) rats receiving additional NaCl, is characterized by a reduced LV relaxation (decreased e'), and increased filling pressures (increased E/e'). These diastolic abnormalities were noted despite preserved indexes of LV chamber (including ejection fraction) and myocardial (deformation or motion) systolic function. Importantly, through increases in ventricular ejection with consequent reductions in filling volumes, but not through lusitropic effects (e'), β-adrenergic receptor stimulation decreased filling pressures (E/e') in DSS rats. In contrast however, although adrenergic stimulation in SHR induced lusitropic effects (e'), and through increases in rotational velocity increased EF and reduced filling volumes, these changes failed to translate into decreases in filling pressures (E/e'). These findings have several implications.

Importantly, the non-invasive (echocardiographic) assessments of LV diastolic function performed in the present study (myocardial tissue Doppler combined with trans-mitral blood flow velocity) are presently incorporated in guidelines for the assessment of DD in humans (Nagueh et al 2016). It is now well recognized that hypertensive heart disease is often associated with LV diastolic dysfunction, including decreases in e' and increases in E/e', with little change in

systolic function (Tadic et al 2018). Moreover, these abnormalities may be all that is noted in the LV of patients with overt heart failure (Lee et al 2009; Zile et al 2004; Lewis et al 2017). These changes are also consistent with left shifts in LV pressure-dimension (chamber compliance) and stress-strain (myocardial compliance) relations, the gold-standard assessments of DD, previously reported to occur in SHR by our group (Norton et al 1997; Tsotetsi et al 2001). Similar alterations in echocardiographic assessments of DD have also been reported in SHR by other groups (Shah et al 2014). Although several interstitial changes in-part explain reductions in diastolic function in SHR, alterations in cardiomyocyte calcium release and sequestration are well-described in the heart of SHR (Shah et al 2014). Consequently, sympathetic activation during physical activity or when the transition to HFpEF occurs, could benefit myocardial relaxation and thus in hypertensive heart disease, maintain normal LV filling pressures whilst filling volumes increase. However, in the present study I demonstrated that despite abnormalities in myocardial relaxation in hypertensive rat models, β-adrenoreceptor activation of an LV with DD does not improve myocardial relaxation in some forms of DD (DSS rats), and despite promoting lusitropic effects in SHR, fails to reduce LV filling pressures. Thus, in the management of hypertensives or alternative patient groups (coronary artery disease or atrial fibrillation) with LV DD, the use of agents that block β-adrenoreceptors are unlikely to oppose any beneficial actions of sympathetic-induced lusitropic effects that may occur during physical activity, or at rest on LV filling pressures. Furthermore, it is unlikely that neurohumoral activation in HFpEF has benefits to DD and hence heart failure *per se*. Thus, blocking β-adrenoreceptors is unlikely to be harmful in DD or HFpEF.

In the present study I did not determine the exact reason why despite promoting lusitropic effects in SHR, β-adrenoreceptor activation failed to reduce LV early diastolic filling pressures in SHR, However, a previous study has demonstrated a reduced adrenergic-induced increase in LV suction forces in patients with DD (Ohara et al 2012). In this regard, decreases in β-adrenergic-induced early diastolic suction in patients with DD are attributed to reduced inertial acceleration between the mid-LV and LV apex (Ohara et al 2012). These suction forces

normally generate enhanced filling because of reductions in LV early diastolic pressures, but with reductions in DD are likely to contribute to increases in filling pressures.

Importantly, lusitropic effects (an increased e') were noted in SHR, but nor DSS rats in the present study. An increased heart rate contributes to the normal lusitropic response to β-adrenoreceptor stimulation in-part through the relaxation-frequency relation (Izawa et al 1997) and this may be retained in those with DD (Wachter et al 2009). As heart rate increased less in DSS rats after β-adrenergic receptor stimulation (despite the same increases in EF) as compared to SHR, it is possible that the reduced lusitropic effects in DSS rats could be attributed to the limited β-adrenoreceptor-induced chronotropic effects in DSS rats. This has important potential implications that could not be evaluated in the present study but should be considered. In this regard, in the transition from LV DD to HFpEF, it is possible that sympathetic stimulation is necessary to maintain high heart rates to induce lusitropic effects. However, this is unlikely to be beneficial to hypertensive heart failure as in SHR with DD β-adrenoreceptor-induced lusitropic effects failed to translate into decreased filling pressures.

Increased filling pressures in DD contribute to exercise intolerance (Paulus 2010). Indeed, exercise-induced pulmonary hypertension and an elevation of pulmonary capillary wedge pressures occurs in patients with exertional dyspnea and DD (Borlaug et al 2010). Importantly exercise activates the sympathetic nervous system and in a normal LV enhances myocardial relaxation, thus reducing filling pressures and accommodating greater filling volumes (thus allowing for a greater Frank-Starling effect). Although I did not assess the impact of adrenergic stimulation after enhancing filling volumes, such as occurs with exercise-induced increases in preload, the present study suggests that in hypertensive heart disease, adrenergic activation either fails to improve myocardial relaxation (DSS rats), or if it increases myocardial relaxation, this fails to translate into reduced filling pressures, despite reductions in filling volumes (SHR). The consequence could be that the LV does not accommodate a higher filling volume during exercise, thus reducing the Frank-Starling mechanism and contributing to exercise intolerance. The limited beneficial effect of β-adrenergic receptor activation on DD in hypertensive hearts suggests that the use of β-adrenoreceptor blocking agents in hypertension

will have no deleterious actions on DD during physical activity. Moreover, the limited beneficial effect of β-adrenergic receptor activation on DD in hypertensive hearts suggests that any sympathetic activation in the hypertensive heart with HFpEF will have no beneficial actions on DD even at rest. Thus, if β-adrenoreceptor blocking agents are required in DD or HFpEF, such as in patients with refractory hypertension or coronary artery disease or atrial fibrillation that these agents should not worsen the heart failure.

5.3 Left ventricular hypertrophy and remodelling and LV DD or HFpEF

A frequently employed guide to a high risk of heart failure is the presence of LV hypertrophy in hypertension. In this regard, there is extensive evidence to support this view (Woodiwiss and Norton 2015). In addition, the echocardiographic identification of concentric LV remodelling is an important guide to whether heart failure will occur as a consequence of LV DD. Importantly, LV hypertrophy is well-recognised as progressing to both DD and heart failure and concentric LV hypertrophy progresses to HFpEF (Drazner 2011). Thus, the focus of risk prediction for the transition to LV dysfunction and subsequently heart failure in hypertension has been on structural changes in the heart. This can be determined using simple electrocardiographic criteria or with less costly echocardiograms which are unable to perform tissue Doppler imaging. Nevertheless, the importance of LV hypertrophy as a cause pf DD was emphasised at the time of less contemporary approaches to assessing DD. In this regard, LV DD was noted to occur in up to 84% of hypertensive individuals with LV hypertrophy (Wachtell et al 2000). Conversely, it was estimated that only 11% to 20% of hypertensive patients have LV DD without exhibiting LV hypertrophy (Dini et al 2013; Phillips et al 1989). Although when identifying DD using more contemporary measures of LV diastolic function, DD is worse in patients with LV hypertrophy as compared to those without LV hypertrophy (Kattel et al 2016), no data have been provided indicating the sensitivity for DD detection using LVH or centric LV remodeling. Indeed, over the years evidence has accumulated to question the importance of

structural changes in the LV as a necessary prelude to the development of DD and hence HFpEF.

Importantly, animal models of DD without LVH are well described (Norton et al 1996) and in animal models of hypertensive LVH, antihypertensive agents that reduce pressures without modifying LV mass are equally as effective at preventing LV DD as agents that regress LV hypertrophy (Norton et al 1997). Moreover, many patients with HFpEF do not have LV hypertrophy (Lam et al 2007) despite the fact that hypertension is the dominant risk factor for this form of heart failure. Moreover, LV DD without LV hypertrophy is an early manifestation of hypertensive heart disease (Messerli et al 2017). Furthermore, a dissociation has been noted between ethnicity and LV mass versus DD in a recent large echocardiographic study (Shantsila et al 2018), and the limited contribution of concentric LV hypertrophy to DD in hypertensives recently described (Nazário Leão et al 2017). In addition, it is mainly those with LV hypertrophy who, in addition to a structural change in the LV, have biomarker evidence of increased loading conditions or myocardial damage produced presumably by increased loads, that progress to heart failure (Seliger et al 2015). Further, assigning those with LV hypertrophy to concentric versus eccentric subtypes only moderately differentiates participants at increased risk of heart failure with a preserved versus reduced ejection fraction (Ho et al 2013). Also in the ASCOT, the marked differences in treatment groups in improvements in LV diastolic function with antihypertensive therapy were unaffected by adjustments for LV mass index (Tapp et al 2010). Of importance is that several cellular changes induced by hypertension that are unrelated to the hypertrophic process determine LV diastolic function. Consequently, the value of measures of LV hypertrophy or the remodelling process as effective indices that herald the presence of LV DD is unclear. However, until the work performed in the present thesis, the exact contribution of LV hypertrophy and concentric LV remodelling to DD had not previously been evaluated. What has the present thesis demonstrated and how is this information useful for risk predicting for HFpEF in hypertension?

In a large community-based study with a high prevalence of untreated and uncontrolled hypertension I show that although both LV mass index (LVMI) and relative wall thickness (RWT)

are independently associated with several characteristic changes in LV diastolic function, they account for little of the relationship between blood pressure (BP) and LV diastolic function. Indeed, in product of coefficient mediation analysis, the contribution of either LVMI or RWT to relationships between BP and lateral wall e', E/e', E/A and e'/a' was minor at best and adjustments for LVMI or RWT failed to significantly modify relationships between BP and either indices of LV diastolic function or the presence of DD.

The present study suggests that BP effects on LV DD as assessed using more contemporary echocardiographic approaches cannot be accounted for by structural changes in the LV. Hence, to identify those at risk of HFpEF caused by hypertension, neither LVMI nor RWT will provide useful information. Although several preclinical studies have dissociated structural changes in the LV from BP effects in hypertension and demonstrated cellular alterations unrelated to the hypertrophic process as being causally related to DD (Norton et al 1997), is there clinical evidence to show similar effects? In this regard, circulating concentrations of substances that index myocardial fibrosis are well correlated with tissue Doppler indices of DD in hypertensive patients irrespective of LVM, and these indices of myocardial fibrosis may precede LV hypertrophy in the evolution of hypertensive heart disease (Muller-Brunotte et al 2007). Nevertheless, whether as recently suggested, the combination of LV hypertrophy and DD is a worse cardiac phenotype in hypertensive heart disease than either considered separately (Messerli et al 2017) requires further study. In short however, the results of the present study indicate that when identifying those at risk of DD, measures of LVM and RWT do not provide a complete clinical picture of the risk conferred by BP or alternative risk factors. Only contemporary measures of DD will adequately index the extent of DD produced by several risk factors including hypertension.

5.4 Obesity or its metabolic effects and DD or HFpEF

As indicated in several sections throughout the present thesis there is presently little evidence for proven treatment benefits for HFpEF (Borlaug & Redfield 2011; Borlaug & Paulus

2011; Pitt et al 2014). In this regard, it is possible that HFpEF may not be a single clinical entity and that the pathophysiological mechanisms responsible for DD may differ depending on the risk factors involved. If this is indeed the case, risk factor effects on the myocardium may vary and hence therapeutic approaches may differ in efficacy depending on the risk factor involved. As an example, although it is well recognized that the major risk factor for HFpEF is hypertension, irrespective of the degree of BP control, not all hypertensives progress to HFpEF. The question therefore arises as to which hypertensives are most likely to develop LV DD and hence possibly progress to HFpEF? In this regard, a common co-morbidity with hypertension is obesity, but the role of obesity or the associated metabolic disturbances alone, or in combination with hypertension, is uncertain. Although some prior studies conducted in select populations report on an equal or greater impact of obesity as compared to hypertension on left ventricular (LV) diastolic function (Rusco et al 2011; Çil et al 2012; Aljaroudi et al 2012), in studies conducted across the full adult age range in a community sample with a high proportion of obesity and hypertension, but a low proportion of participants who were receiving antihypertensive therapy, BP was noted to be the main determinant of DD (Millen et al 2014). Nevertheless, more recent evidence suggests that a more concentrically remodeled LV determines whether obesity-related insulin resistance (IR) is associated with LV diastolic function (Peterson et al 2016). As hypertension is a strong determinant of concentric LV remodeling, the possibility exists that even if obesity or associated IR have only a modest impact on LV diastolic function, that obesity or its metabolic consequences may determine the extent to which DD occurs in hypertension. As part of the present thesis I therefore evaluated whether adiposity indices or IR influence the extent to which DD occurs in hypertension in a community sample with a high prevalence of hypertension and obesity. In this regard I demonstrated that although indices of excess adiposity and IR were both independently associated with indices of LV diastolic function (e' and E/e'), IR, but not adiposity indices determined whether hypertension translated into LV DD. Indeed, whilst HOMA-IR was independently associated with lateral wall e' in both hypertensives and normotensives, HOMA-IR was independently associated with E/e' in hypertensives, but not in normotensives.

Consequently, HOMA-IR determined whether hypertensives developed LV DD as compared to normotensives. In contrast, adiposity indices were associated with indices of diastolic function less well in hypertensives as compared to normotensives and adiposity indices were consequently not independently associated with DD.

As highlighted in chapter 4, there are several potential implications of the findings of that study (Bamaiyi et al 2019). First, these data suggest that from a clinical perspective, hypertensives with IR may be particularly prone to the development of LV DD and thus possibly the progression to HFpEF. Importantly, in contrast to adiposity indices, which had less of an ability to determine the impact of hypertension on DD, the assessment of IR may better characterize hypertensives at risk of developing DD and hence HFpEF. However, longitudinal studies are required to assess this question. Second, the present data suggest that targeting IR as opposed to an excess adiposity *per se* with behavioral modification in hypertensives may have marked benefits to preventing the development of DD and hence HFpEF. In this regard, intervention studies are also required to assess this hypothesis. Third, the present study suggests that a combination of the mechanisms responsible for DD in hypertension and obesity may be particularly important in producing more severe forms of DD and hence HFpEF. Thus, developing animal models where LV DD occurs more readily because of an additive effect of hypertensive and obesity-associated changes in myocardial function may caste light on a particularly important pathophysiological mechanism for drug development.

5.5 Limitations

Although the limitations of the individual studies described in the present thesis have been fully discussed in the data chapters, in the following I will highlight some additional key points. As highlighted in the introductory chapter, there are several reasons why patients with LV DD may require β-adrenergic receptor blockers, but as also underscored whether this produces adverse effects on LV DD was unknown. Although in chapter 2 I demonstrated that β-adrenergic receptor stimulation has no obvious acute beneficial effect to LV DD, the possibility

that long-term blockade of β_2-adrenergic receptors may have deleterious effects was not evaluated. Indeed, as discussed in chapter 1, β_2-adrenergic receptors when activated, have beneficial effects. Although β-adrenergic receptor blockers are generally designed to target β_1-adrenergic receptors, they all have the potential for blocking β_2-adrenergic receptors. Thus, further studies are required to evaluate whether β-adrenergic receptor blockers worsen LV DD when employed chronically. Second, mediation analysis provides indirect evidence for the effect of LVM on LV DD. However, to provide direct evidence to determine the extent to which LVH causes DD would require the use of agents that regress LVH without modifying loading conditions. To the best of my knowledge this approach does not exist. Third, there is little evidence to show that hypertension and obesity target the same cellular systems to cause LV DD and hence whether a single approach can be developed to target the impact of both on LV DD is unknown. This question can only be addressed once animal models of LV DD caused by a combination of hypertension and obesity have been developed.

5.6 Conclusions

In conclusion, in the present thesis I provide further insights into several aspects of LV DD in hypertension. In this regard, I show that β-adrenergic receptor activation in animal models of hypertension with LV DD has no beneficial effects on indexes of LV filling pressures despite producing lusitropic effects in some circumstances, Second, I show that neither LVM nor the degree of concentric LV remodeling can explain the impact of BP on LV DD. Third, I show that although not accounting for the impact of hypertension on DD, insulin resistance increases the chances that hypertension is associated with LV DD. These data therefore suggest that β-adrenergic receptor blockers are unlikely to worsen LV DD when required for use in hypertensive heart disease or HFpEF. Moreover, these data indicate that the assessment of LVMI or LV remodeling is an insufficient measure of the impact of BP on cardiac diastolic function. In this regard, the clinical assessment of diastolic function is therefore an essential index complementing LV structural assessments in risk assessment for predicting future heart

failure. In addition, these data suggest that the assessment of IR may be a useful index of those hypertensives at risk of HFpEF and that targeting IR may be an important approach in hypertensives with LV DD.

APPENDICES

Appendix A

Ethical clearance certificates

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2016/03/08/A

APPLICANT: Mr B Adamu

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: Impact of habitual exercise training on central aortic pressures in spontaneously hypertensive rats (SHRs)

Number and Species

60 male SHR rats

Approval was given for the use of animals for the project described above at an AESC meeting held on 2016/03/29. This approval remains valid until 2018/04/10.

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: 15th April 2016

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: 14 April 2016

cc: Supervisor: Professor G Norton
Director: CAS

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STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2016/06/28/B

APPLICANT: Mr B Adamu

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: Impact of habitual exercise training on central aortic pressures in dahl salt-sensitive hypertensive rats

Number and Species

90 75-80g Male Dahl salt-sensitive hypertensive rats

Approval was given for the use of animals for the project described above at an AESC meeting held on 2016/06/28. This approval remains valid until 2018/07/06.

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:

None

Signed: _____ (Chairperson, AESC) Date: 8th July 2016

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: 13 JUL 2016

cc: Supervisor: Professor G Norton
Director: CAS

Works 2000/lain0015/AESCCert.wps

R14/49 Dr Bamaiyi Adamu et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170271

NAME: (Principal Investigator)	Dr Bamaiyi Adamu et al
DEPARTMENT:	School of Physiology Cardiovascular Pathophysiology and Genomics Research Unit Charlotte Maxeke Johannesburg Academic Hospital Hypertension Clinic
PROJECT TITLE:	Resistance Exercise-Induced Increases in Aortic Backward Waves and their Effect on Left Ventricular Function in Hypertension: Impact of Left Ventricular Hypertrophy
DATE CONSIDERED:	24/02/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Gavin Norton and Prof Angela Woodiwiss
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/04/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Prof Angela Woodiwiss and Prof Gavin Norton

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170401

NAME: (Principal Investigator)	Prof Angela Woodiwiss and Prof Gavin Norton
DEPARTMENT:	School of Physiology
PROJECT TITLE:	Gene Candidates as Determinants of Blood Pressure and Intermediary Phenotypes in the Pathogenesis of Hypertension in Black South Africans
DATE CONSIDERED:	26/04/2002 (Initial Approval)
DECISION:	Approved unconditionally
CONDITIONS:	Renewal for 5 Years Valid for the Period 01 April 2017 - 30 April 2022 Previously M1204108, M070469 and M020472
SUPERVISOR:	Prof Angela Woodiwiss
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/04/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in April and will therefore be due in the month of April each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Prof A/G Woodiwiss/Norton

CLEARANCE CERTIFICATE	**M1204108**
PROJECT	Gene Candidates as Determinants of Blood Pressure and Intermediary Phenotypes in Pathogenesis of Hypertension in Black South Africans (Previously M020472 and M070469)
INVESTIGATORS	Prof A/G Woodiwiss/Norton.
DEPARTMENT	School of Physiology
DATE CONSIDERED	Ad hoc
DECISION OF THE COMMITTEE*	Renewal Approved

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 2012/05/18 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof A Woodiwiss

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Woodiwiss/Norton

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER MO70469**
PROJECT	Gene Candidates As Determinants of Blood Pressure and Intermediary Phenotypes in Pathogenesis of Hypertension in Black S Africans
INVESTIGATORS	Profs A/G Woodiwiss/Norton
DEPARTMENT	School of Physiology
DATE CONSIDERED	07.05.09
DECISION OF THE COMMITTEE*	Approved unconditionally (refer M020472)

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.05.09 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Woodiwiss A Prof

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Woodiwiss/Norton et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-04-72

PROJECT Gene Candidates As Determinants of Blood Pressure And Intermediary Phenotypes In Pathogenesis of Hypertension In Black South Africans

INVESTIGATORS Prof's AJ/G et al Woodiwiss/Norton et al

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2007 -05- 09
JOHANNESBURG

DECISION OF THE COMMITTEE *

Approved unconditionally

This clearance is valid and within the next 5-year validity

DATE 02-05-14 **CHAIRMAN** .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof AJ Woodiwiss
Dept of School of Physiology, Wits Medical School

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B

“Turn-it-in” Plagiarism report

ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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3	Adamu J Bamaiyi, Angela J Woodiwiss, Vernice Peterson, Monica Gomes, Carlos D Libhaber, Pinhas Sareli, Gavin R Norton. "Insulin Resistance Influences the Impact of Hypertension on Left Ventricular Diastolic Dysfunction in a Community Sample", Clinical Cardiology, 2018 Publication	4%
4	"Heart Failure: From Research to Clinical Practice", Springer Nature, 2018 Publication	1%

5	"Biomarkers in Cardiovascular Disease", Springer Nature, 2016 Publication	1 %
6	Adamu J. Bamaiyi, Angela J. Woodiwiss, Vernice Peterson, Monica Gomes, Carlos D. Libhaber, Pinhas Sareli, Gavin R. Norton. "Insulin resistance influences the impact of hypertension on left ventricular diastolic dysfunction in a community sample", Clinical Cardiology, 2019 Publication	1 %
7	Peterson, Vernice, Gavin R. Norton, Andrew Raymond, Carlos D. Libhaber, Aletta M.E. Millen, Olebogeng H.I. Majane, Muzi J. Maseko, and Angela J. Woodiwiss. "Insulin resistance-associated decreases in left ventricular diastolic function are strongly modified by the extent of concentric remodeling in a community sample", International Journal of Cardiology, 2016. Publication	1 %
8	Adamu J. Bamaiyi, Gavin R. Norton, Vernice Peterson, Carlos D. Libhaber, Pinhas Sareli, Angela J. Woodiwiss. "Limited contribution of left ventricular mass and remodelling to the impact of blood pressure on diastolic function in a community sample", Journal of	1 %

	Hypertension, 2019 Publication	
9	"Heart Failure", Springer Nature, 2017 Publication	<1 %
10	Wilson Nadruz, Amil M. Shah, Scott D. Solomon. "Diastolic Dysfunction and Hypertension", Medical Clinics of North America, 2017 Publication	<1 %
11	Vernice R. Peterson, Gavin R. Norton, Carlos D. Libhaber, Muzi J. Maseko, Pinhas Sareli, Angela J. Woodiwiss. "Intrafamilial aggregation and heritability of tissue Doppler indexes of left ventricular diastolic function in a group of African descent", Journal of the American Society of Hypertension, 2016 Publication	<1 %
12	Dimitrios M. Konstantinou, Yiannis S. Chatzizisis, George D. Giannoglou. "Pathophysiology-based novel pharmacotherapy for heart failure with preserved ejection fraction", Pharmacology & Therapeutics, 2013 Publication	<1 %
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a community sample with prevalent obesity", International Journal of Cardiology, 2015.
Publication

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21 Gavin A. Lewis, Erik B. Schelbert, Simon G. Williams, Colin Cunnington, Fozia Ahmed, Theresa A. McDonagh, Christopher A. Miller. "Biological Phenotypes of Heart Failure With Preserved Ejection Fraction", Journal of the American College of Cardiology, 2017
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37	"Monday, 1 September 2008", European Heart Journal, 09/02/2008 Publication	<1%
38	Michael Kindermann, Jan-Christian Reil, Burkert Pieske, Dirk J. van Veldhuisen, Michael Böhm. "Heart Failure With Normal Left Ventricular Ejection Fraction: What is the Evidence?", Trends in Cardiovascular Medicine, 2008 Publication	<1%
39	M MOVSESIAN. "Altered cAMP-mediated signalling and its role in the pathogenesis of dilated cardiomyopathy", Cardiovascular Research, 06/2004	<1%

Publication

40	Millen, Aletta M.E., Carlos D. Libhaber, Olebogeng H.I. Majane, Elena Libhaber, Muzi J. Maseko, Angela J. Woodiwiss, and Gavin R. Norton. "Relative impact of blood pressure as compared to an excess adiposity on left ventricular diastolic dysfunction in a community sample with a high prevalence of obesity :", Journal of Hypertension, 2014. Publication	<1%

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