

https://dspace.org/

Left ventricular diastolic dysfunction in a community of African ancestry

Peterson, Vernice Roxanne

2017

http://hdl.handle.net/10539/23157

Peterson, Vernice Roxanne (2017) Left ventricular diastolic dysfunction in a community of African ancestry, University of the Witwatersrand, Johannesburg, <http://hdl.handle.net/10539/23157>

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

LEFT VENTRICULAR DIASTOLIC DYSFUNCTION IN A COMMUNITY OF AFRICAN ANCESTRY

Vernice Roxanne Peterson

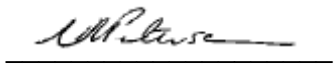
A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, South Africa

2017

DECLARATION

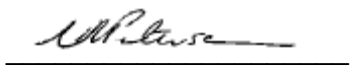
I, Vernice Roxanne Peterson, declare that the work included in this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. The work contained in this thesis has not been submitted before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



Vernice Roxanne Peterson

Signed on 16th day of MAY, 2017 in PARKTOWN

I certify that the studies included in this thesis have the approval of the Human Research Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics clearance number is M02-04-72 and renewed as M07-04-69 and M12-04-108.

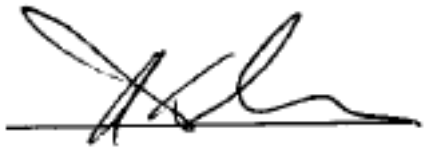


Vernice Roxanne Peterson

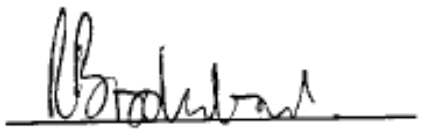
Signed on 16th day of MAY, 2017 in PARKTOWN



Prof G. Norton (Supervisor)



Prof A. Woodiwiss (Supervisor)



Prof R. Brooksbank (Supervisor)

I dedicate this thesis to my parents, husband and daughter. This journey has been filled with many of life's blessings.

ABSTRACT

Almost half of all cases of heart failure have a preserved ejection fraction. However, therapy targeting the mechanisms of this disorder has not improved outcomes. Left ventricular (LV) diastolic dysfunction is a characteristic feature of heart failure with a preserved ejection fraction. A more sound understanding of the mechanisms responsible for LV diastolic dysfunction produced by risk factors may lead to better approaches to preventing this syndrome.

Although obesity is thought to be a major risk factor for LV diastolic dysfunction, this does not occur in all obese individuals. In the present thesis I have demonstrated in 737 randomly recruited participants from a community sample of African ancestry, that the relationship between insulin resistance (homeostasis model) and LV diastolic function, as assessed from trans-mitral velocity (E/A) and tissue Doppler imaging of the lateral and septal walls of the LV (e' and E/e'), is markedly altered by the presence of a more concentrically remodelled LV (as indexed by LV relative wall thickness [RWT]). Importantly, insulin resistance was only associated with LV diastolic function or dysfunction in those with an RWT above a threshold value. In contrast no interactive effects on LV diastolic function between either blood pressure or age and RWT were noted. These data therefore suggest that obesity will only translate into LV diastolic dysfunction if it is associated with insulin resistance and a concentrically remodeled LV.

Although hypertension is thought to play an important role in contributing to LV diastolic dysfunction, the pulsatile hemodynamic change primarily responsible for this effect is uncertain. In 524 randomly selected individuals from a community sample I have demonstrated that independent of confounders including left ventricular mass and RWT, aortic backward wave pressure effects (as determined using wave separation analysis), antedate the impact of aortic stiffness (indexed by aortic pulse wave velocity) or the factors determined by aortic stiffness (the time of backward wave return or forward wave pressures) on LV filling pressures (E/e'). These data therefore suggest that to adequately prevent LV diastolic dysfunction, targeting aortic backward wave pressures may be required.

As conventional risk factors account for only a portion of the inter-individual variations in LV diastolic function, it is thought that the genetic factors may play a

significant role. In 694 randomly recruited participants of African ancestry belonging to nuclear families, I demonstrated that independent of conventional risk factors, heritability accounts for approximately 50% of the variation in LV RWT, an important LV structural determinant of LV diastolic function. Moreover, in 442 randomly recruited individuals of African ancestry belonging to nuclear families, I also demonstrated that heritability accounts for approximately 50% of the variation in the index of LV filling pressures, E/e', independent of LV mass or RWT remodeling and aortic function. These data provide strong evidence that genetic factors responsible for LV diastolic dysfunction and the structural determinants thereof should be sought.

In conclusion, the results provided in the present thesis have advanced our knowledge of possible pathophysiological mechanisms that play a role in the development of LV diastolic dysfunction and hence possibly heart failure with a preserved ejection fraction.

PUBLICATIONS

The following publications have arisen from this work:

1. Peterson V.R., Norton G.R., Redelinghuys M, Libhaber C.D., Maseko M.J., Majane O.H., Brooksbank R and Woodiwiss A.J. (2015) Intrafamilial Aggregation and Heritability of Left Ventricular Geometric Remodeling is Independent of Cardiac Mass in Families of African Ancestry, Am J Hypertens, 28, 657-663.
2. Peterson V.R., Woodiwiss A.J., Libhaber C.D., Raymond A, Sareli P and Norton G.R. (2016). Cardiac Diastolic Dysfunction is Associated with Aortic Wave Reflection, but not Stiffness in a Predominantly Young-to-Middle-Aged Community Sample, Am J Hypertens, 29, 1148-1157.
3. Peterson V.R., Norton G.R., Libhaber C.D., Maseko M.J., Sareli P and Woodiwiss A.J. (2016). Intrafamilial aggregation and heritability of tissue Doppler indexes of left ventricular diastolic function in a group of African descent, J Am Soc of Hypertens, 10, 517-526.
4. Peterson V.R., Norton G.R., Raymond A., Libhaber C.D., Millen A.M.E., Majane O.H.I., Maseko M.J. and Woodiwiss, A.J. (2016). Insulin-resistance-associated decreases in left ventricular diastolic function are strongly modified by the extent of concentric remodeling in a community sample, Int J of Cardiol, 220, 349-355.

PRESENTATIONS

The following presentations have arisen from this work.

Oral presentations:

Oral presentation at the Physiological Society of Southern Africa (PSSA) Conference, 15-19 September, Pretoria, SA, 2013. Presentation Title: Inheritance of Left Ventricular Geometry Independent of Left Ventricular Mass.

Oral presentation at the Physiological Society of Southern Africa (PSSA) Conference, 6-9 September, Parys, Free State, SA 2015. Presentation Title: Dominant Impact of Aortic Backward Waves versus Alternative Aortic Hemodynamic changes to Variations in Tissue Doppler indices of Left Ventricular Diastolic Dysfunction.

Oral presentation at the Stroke and Hypertension Congress, 19-21 August, Misty Hills, Muldersdrift, Johannesburg, SA, 2016. Presentation Title: Left Ventricular Diastolic Dysfunction is Associated with Aortic Backward Wave Pressure, but not Stiffness in a predominantly Young-to-Middle Aged Community Sample. (Awarded Gold first prize)

Oral presentation at the Faculty of Health Sciences Research Day. 1 September, Wits University, Medical Campus, Parktown, Johannesburg, SA, 2016. Presentation Title: Intra-familial Aggregation and Heritability of Tissue Doppler Indexes of Left Ventricular Diastolic Function of African Descent.

Poster presentations:

Presented research poster at the 2014 Annual Scientific meeting and Exposition of the American Society of Hypertension 16-20 May 2014. NYC, USA. Poster Title: The Intra-familial Aggregation and Heritability of Left Ventricular Geometric Remodelling is Independent of Cardiac Mass in Families of African Ancestry.

Presented research poster at the Stroke and Hypertension Congress, 22-24 August, Durban, SA, 2014. Poster Title: The Intra-familial Aggregation and Heritability of Left Ventricular Geometric Remodelling is Independent of Cardiac Mass in Families of African Ancestry.

Presented research poster at the Stroke and Hypertension Congress, 19-21 August, Misty Hills, Muldersdrift, Johannesburg, SA, 2016. Poster Title: Intra-familial

Aggregation and Heritability of Tissue Doppler Indexes of Left Ventricular Diastolic Function of African Descent. (Awarded Gold first prize)

Presented research poster at the Faculty of Health Sciences Research Day. 1 September, Wits University, Medical Campus, Parktown, Johannesburg, SA, 2016. Poster Title: Left Ventricular Diastolic Dysfunction is Associated with Aortic Backward Wave Pressure, but not Stiffness in a predominantly Young-to-Middle Aged Community Sample.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisors, Professor's Angela Woodiwiss, Gavin Norton and Richard Brooksbank for their mentorship, guidance and constant support throughout the duration of my PhD. The invaluable skills and knowledge that you have imparted through the years will never be forgotten and have added to my research etiquette, professionalism as well as my personal development. I would like to thank members of the Cardiovascular Pathophysiology and Genomics Research Unit for their assistance in data collection and the memorable moments shared in the lab and on conferences. To Samantha and Janine, your companionship and moral support proved invaluable throughout the PhD journey. I would also like to thank the National Research Foundation/DAAD Scholarship as well as the University of the Witwatersrand Postgraduate Scholarship and Faculty Research Committee Grant for financially supporting this project. To all my family, thank you for your constant support and encouragement and for allowing me to pursue my dreams. Lastly, to my bestie and husband, Riaaz, we have come along way! Thank you for your continuous support and love that have allowed me to "fly the flag" and achieve my goals.

TABLE OF CONTENTS

Page

DECLARATION i
DEDICATION ii
ABSTRACT iii
PUBLICATIONS AND PRESENTATIONS v
ACKNOWLEDGEMENTS viii
TABLE OF CONTENTS ix
LIST OF FIGURES xiv
LIST OF TABLES xvii
LIST OF ABBREVIATIONS xx
PREFACE xxiii

CHAPTER 1 – INTRODUCTION 1

1.1 Introduction 2
1.2 Heart failure 4
1.2.1 Current understanding of the pathophysiology of heart failure with preserved ejection fraction 5
1.2.2 What are the current therapeutic approaches to preventing and treating heart failure with preserved ejection fraction?..... 6
1.3 Cardiac diastolic function and outcomes 9
1.4 Measurement of and approaches to the classification of cardiac diastolic dysfunction 10
1.4.1 Non-invasive measurements of diastolic dysfunction 10
1.4.1.1 Mitral inflow 11
1.4.1.2 Tissue Doppler imaging 13
1.4.1.3 LA volume 13
1.4.2 Classification of diastolic dysfunction 14
1.5 Factors associated with the development of LV diastolic dysfunction 16
1.5.1 Obesity as a cause of LV diastolic dysfunction 16
1.5.1.1 Obesity as a risk factor for heart failure 17
1.5.1.2 Obesity as a risk factor for the development of LV diastolic dysfunction 18
1.5.1.3 Insulin resistance as a possible mechanism explaining

the independent relationship between obesity and LV diastolic dysfunction ……. 20
1.5.1.4 Could LV remodeling influence the impact of obesity or insulin resistance on LV diastolic function?……..…..... 24
1.5.2 Hypertension as a cause of LV diastolic dysfunction ……..... 25
1.5.2.1 Hypertension as a cause of heart failure and a reduced LV diastolic function ……..…… 26
1.5.2.2 Loading conditions responsible for LV diastolic dysfunction: the importance of aortic stiffness ……..……..27
1.5.2.3 Loading conditions responsible for LV diastolic dysfunction: the importance of aortic backward waves …… 28
1.5.3 Genetic factors may play a significant role in determining LV diastolic dysfunction ……..…… 32
1.5.3.1 Possible role of genetic factors as determinants of LV remodeling …….....33
1.5.3.2 Possible role of genetic factors as determinants of LV diastolic function beyond confounders …….. 35
1.6 Aims …….....37

CHAPTER 2 – Insulin Resistance-Associated Decreases in Left Ventricular Diastolic Function are Strongly Modified by the Extent of Concentric Remodelling in a Community Sample……..……..38

2.1 Abstract …….. 39
2.2 Introduction …….....40
2.3 Methods ……...41
2.3.1 Study group …….. 41
2.3.2 Clinical, demographic, blood and anthropometric measurements ……...41
2.3.3 Echocardiography ……42
2.3.4 Data analysis …… 44
2.4 Results ……46
2.4.1 Participant characteristics …….. 46
2.4.2 LV mass and remodelling are associated with LV diastolic function ……46
2.4.3 Metabolic factors and LV diastolic function …….. 46

2.5 Discussion 51

CHAPTER 3 – Cardiac Diastolic dysfunction is Associated with Aortic Wave Reflection, but not Stiffness in a Predominantly Young-to-Middle-Aged Community Sample..... 66

3.1 Abstract 67
3.2 Introduction 68
3.3 Methods 69
3.3.1 Study participants 69
3.3.2 Clinical, demographic, anthropometric and laboratory assessments 69
3.3.3 Office blood pressure 70
3.3.4 Aortic haemodynamics 70
3.3.5 Echocardiography 73
3.3.6 Data analysis 75
3.4 Results 75
3.4.1 Participant characteristics 75
3.4.2 Factors correlated with LV diastolic function 76
3.4.3 Independent associations between aortic haemodynamics and E/A 76
3.4.4 Independent associations between aortic haemodynamics and E/e' 76
3.4.5 Independent associations between aortic haemodynamics and moderate-to-severe diastolic dysfunction (E/e'≥12) 82
3.4.6 Associations between aortic haemodynamics and E/e' or moderate-to-severe diastolic dysfunction (E/e'≥12) independent of brachial pressures 82
3.5 Discussion 82

CHAPTER 4 - Intra-Familial Aggregation and Heritability of Left Ventricular Geometric Remodelling is Independent of Cardiac Mass in Families of African Ancestry 90

4.1 Abstract 91
4.2 Introduction 92

4.3 Methods ……..93
4.3.1 Study participants …… 93
4.3.2 Clinical, demographic, anthropometric and laboratory assessments ……..93
4.3.3 Office blood pressure ……..94
4.3.4 Aortic blood pressure ……..94
4.3.5 Echocardiography ……95
4.3.6 Data analysis ……95
4.4 Results ……96
4.4.1 Characteristics of participants ……96
4.4.2 LVM and LVMI are associated with RWT ……97
4.4.3 Heritability of LVM and LVMI ……97
4.4.4 Intra-familial aggregation of RWT ……97
4.4.5 Heritability estimates of RWT ……97
4.5 Discussion ……102

CHAPTER 5 - Intra-Familial Aggregation and Heritability of Tissue Doppler Indexes of Left Ventricular Diastolic Function in a Group of African Descent …….. 105

5.1 Abstract …… 106
5.2 Introduction ……....107
5.3 Methods ……...108
5.3.1 Study participants …… 108
5.3.2 Clinical, demographic, anthropometric and laboratory assessments ……..110
5.3.3 Office blood pressure ……..110
5.3.4 Aortic haemodynamics …….. 111
5.3.5 Echocardiography ……111
5.3.6 Data analysis ……112
5.4 Results ……119
5.4.1 Characteristics of participants ……119
5.4.2 LV remodelling and aortic function are associated with diastolic function ……...120
5.4.3 Intra-familial aggregation of indexes of diastolic function ……..120
5.4.4 Heritability estimates of indexes of diastolic function ……128

5.5 Discussion ……..128

CHAPTER 6 - Contextual Narrative and Conclusion ……..138

6.1 Introduction ……139
6.2 Left ventricular diastolic dysfunction as a cause of heart failure with a preserved ejection fraction …….. 140
6.3 Obesity and obesity-related insulin resistance and LV diastolic dysfunction? ……. 141
6.4 Blood Pressure: the effect of aortic backward wave pressures on LV diastolic function ……..… 143
6.5 Genetic effects on LV diastolic function and the determinants thereof?..... 146
6.5.1 Possible genetic effects on LV geometric remodelling ……...147
6.5.2 Possible genetic effects on LV diastolic function beyond geometric LV remodelling ……...149
6.6 Limitations ……..150
6.7 Conclusions ……151

Appendices…… 152
References …… 158

LIST OF FIGURES

Figure number **Page**

Chapter 1

1.1 Example of echocardiographic images of pulsed wave Doppler transmitral E wave and A wave velocities and TDI peak myocardial early and late diastolic velocity……..……..... 12

1.2 The contribution of aortic forward and aortic backward waves to aortic and radial (approximate of brachial) pulse waves…….…….……..... 30

1.3 Age effects on aortic and radial artery pressure waves…….…….. 31

Chapter 2

2.1. Echocardiographic M-mode image showing the measurement of left ventricular dimensions……..……..…...43

2.2 Echocardiographic image showing the measurement of left atrial area and long axis by planimetry in the apical four-chamber and two-chamber views at left ventricular end systole (maximum left atrial dimensions)….....45

2.3 Synergistic effect of left ventricular relative wall thickness and the homeostasis model of insulin resistance, or blood pressure on indexes of left ventricular diastolic function. ……..…..….57

2.4. Synergistic effect of left ventricular relative wall thickness and the homeostasis model of insulin resistance or blood pressure on the odds of left ventricular diastolic dysfunction…….59

2.5 Relations between blood pressure and left ventricular diastolic function in participants with a left ventricular relative wall thickness above and below the median for the sample……..……..……....60

2.6 Impact of synergistic effect of left ventricular relative wall thickness and the homeostasis model of insulin resistance on left ventricular diastolic function……61

Chapter 3

3.1 A SphygmoCor device coupled with an applanation tonometer used to determine aortic haemodynamics……71
3.2 Derivation of aortic functional parameters from the aortic pressure waveform using SphygmoCor software.....72
3.3 Aortic pulse wave velocity as determined from sequential pressure waveform measurements at carotid and femoral sites.....74
3.4. Impact of adjustments for aortic haemodynamic parameters on relations between central aortic pulse pressure and left ventricular early trans-mitral blood flow velocity/ velocity of early diastolic myocardial tissue lengthening at the level of the mitral annulus (lateral wall) or moderate-to-severe left ventricular diastolic dysfunction……..81

Chapter 4

4.1 Age- and sex-adjusted and multivariate adjusted intra-familial correlations of left ventricular relative wall thickness. ……..…100

Chapter 5

5.1. Flow chart summarising the derivation of the study sample. ……..109
5.2. Distribution of indexes of left ventricular diastolic function before and after logarithmic transformation. ..……...115
5.3 Multivariate adjusted intra-familial correlations of left ventricular diastolic function derived from trans-mitral velocity measurements. …… 124
5.4 Multivariate adjusted intra-familial correlations of left ventricular diastolic function derived from the average of lateral and septal wall tissue Doppler imaging…… 125
5.5 Multivariate adjusted intra-familial correlations (r values) of left ventricular diastolic function (lateral wall tissue Doppler imaging)……126
5.6 Multivariate adjusted intra-familial correlations of left ventricular diastolic function (septal wall tissue Doppler imaging)……..……127
5.7 Multivariate adjusted intra-familial correlations of left ventricular diastolic function derived from the average of lateral and septal wall tissue Doppler imaging with further adjustments for central aortic

systolic blood pressure or aortic backward wave pressure……129

LIST OF TABLES

Table number **Page**

Chapter 2

2.1. Characteristics of the study sample ……47

2.2. Multivariate adjusted relationships between indexes of adiposity or the homeostasis model of insulin resistance and left ventricular diastolic function in a community sample ……48

2.3. Multivariate adjusted relationships between indexes of adiposity or the homeostasis model of insulin resistance and left ventricular diastolic function in a community sample ……..…50

2.4. Multivariate adjusted associations between indexes of adiposity or the homeostasis model of insulin resistance and left ventricular (LV) diastolic dysfunction in the study group ……..…….52

2.5. Independent associations between adiposity index (or the homeostasis model of insulin resistance)-left ventricular relative wall thickness interactions and LV diastolic function in a community sample ……..……53

2.6. Independent associations between adiposity index (or the homeostasis model of insulin resistance)-left ventricular relative wall thickness interactions and LV diastolic function in a community sample ……..……55

2.7. Lack of independent associations between age or blood pressure-left ventricular relative wall thickness interactions and LV diastolic function in a community sample ……..……..…56

2.8. Strength (partial r) of relations between the homeostasis model of insulin resistance and septal e' in participants with a LV relative wall thickness above or below the median for the sample ……..…..58

2.9. Adjusted septal e' across tertiles of the homeostasis model of insulin resistance in participants with a LV relative wall thickness above or below the median for the sample ……..……....62

Chapter 3

3.1. Demographic, clinical, anthropometric and haemodynamic characteristics of the study sample with and without measures of

tissue Doppler indices of left ventricular diastolic function …….....77
3.2 Multivariate adjusted relationships between aortic haemodynamic parameters and left ventricular early-to-late (atrial) trans-mitral blood flow velocity (E/A) ……..……79
3.3. Multivariate adjusted relationships between aortic haemodynamic parameters and left ventricular early trans-mitral blood flow velocity / velocity of lateral wall myocardial tissue lengthening in early diastole at the mitral annulus (E/e') …….……80
3.4. Multivariate adjusted associations between aortic functional parameters and moderate-to-severe left ventricular diastolic dysfunction in the study group ……..….83
3.5. Brachial pulse pressure-adjusted associations between aortic functional parameters and left ventricular early trans-mitral blood flow velocity / velocity of the mean value of lateral and septal wall myocardial tissue lengthening in early diastole at the mitral annulus (E/e') or moderate-to-severe LV diastolic dysfunction (E/e'≥12) in the study group …….....84

Chapter 4

4.1. Characteristics of parents and offspring of the study sample ……..98
4.2. Unadjusted and multivariate adjusted heritability estimates of left ventricular mass and left ventricular mass index……..……...99
4.3. Unadjusted and multivariate adjusted heritability estimates of left ventricular relative wall thickness adjusted for left ventricular mass and LV mass index ……...101

Chapter 5

5.1. Distribution of left ventricular diastolic function parameters before and after transformation of data ……..…113
5.2. Stepwise regression models showing factors independently associated with trans-mitral E/A, E and A and myocardial tissue Doppler e'/a' (average of lateral and septal wall values) ……116
5.3. Stepwise regression models showing factors independently associated

with TDI (average of lateral and septal wall) measures of left ventricular diastolic function ……... 117
5.4. Stepwise regression models showing factors independently associated with TDI (lateral and septal wall given separately) measures of left ventricular diastolic function ……118
5.5 Characteristics of parents and offspring of the study sample …….....121
5.6 Characteristics of study sample with and without measures of tissue Doppler indices of left ventricular diastolic function ……....123
5.7. Multivariate adjusted heritability estimates of several indexes of left ventricular diastolic function derived from trans-mitral (blood flow) Doppler velocities ……130
5.8. Multivariate adjusted heritability estimates of several indexes of left ventricular diastolic function derived from the average of lateral and septal wall tissue Doppler measurements ……131
5.9. Multivariate adjusted heritability estimates of several indexes of left ventricular diastolic function derived from lateral wall tissue Doppler measurements ……...132
5.10 Multivariate adjusted heritability estimates of several indices of left ventricular diastolic function derived from septal wall tissue Doppler measurements ……133
5.11 Multivariate adjusted heritability estimates of left ventricular diastolic function derived from the average of lateral and septal wall tissue Doppler measurements with additional adjustments for central aortic systolic blood pressure or aortic backward wave pressures ……134

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
ACEI	angiotensin-converting enzyme inhibitors
Aldo-DHF	aldosterone receptor blockade in diastolic heart failure
AIx	aortic augmentation index
ARBs	angiotensin receptor blockers
ASE	American Society of Echocardiography
BB	beta blockers
BMI	body mass index
BP	blood pressure
CARDIA	coronary artery risk development in young adults
CCB	calcium channel blockers
CHARM	candesartan in heart failure: assessment of reduction in mortality and morbidity
CIBIS	cardiac insufficiency bisoprolol study
DBP	diastolic blood pressure
DD	diastolic dysfunction
DIG-Preserved	digitalis investigation group with preserved ejection fraction
DM	diabetes mellitus
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
EF	ejection fraction
E wave	trans-mitral blood flow velocity in the early period of left ventricular diastolic filling
Ft	time to the peak of the forward wave
HFpEF	heart failure with preserved ejection fraction

HFrEF	heart failure with reduced ejection fracton
I-PRESERVE	irbesartan in heart failure with preserved ejection fraction
kg	kilogram
kg/m^2	kg per $meter^2$
LA	left atrial
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 $height^{2.7}$
HbA1c	percentage glycated haemoglobin
HOMA-IR	homeostasis model of insulin resistance
MAP	mean arterial pressure
MDC	metoprolol in dilated cardiomyopathy
MMP	matrix metalloproteinase
OPTIMIZE-ACE	organized program to initiate lifesaving treatment in hospitalized heart failure patients receiving angiotensin-converting enzyme
OPTIMIZE-BB	organized program to initiate lifesaving treatment in hospitalized heart failure patients receiving beta blockers
Pa	augmented pressure
Pb	backward wave pressure
PEP-CHF	perindopril in elderly people with chronic heart failure
Pf	forward wave pressure
PIIINP	collagen III N-terminal propeptide
PRECISE	a prospective, multi-center evaluation of the accuracy of a novel continuous implanted glucose sensor
PP	pulse pressure
PPc	central aortic pulse pressure
PW	pulsed-wave

PWED	left ventricular posterior wall thickness at end diastole
PWES	left ventricular posterior wall thickness at end systole
PWV	pulse wave velocity
RM	reflected wave magnitude
RWT	relative wall thickness
Rt	time to wave reflection
SD	standard deviation
SEPED	left ventricular septal wall thickness at end diastole
SEPES	left ventricular septal wall thickness at end systole
SOLVD	studies of left ventricular dysfunction
TDI	tissue doppler imaging
TIMP	tissue inhibitor of matrix metalloproteinase
TOPCAT	treatment of preserved cardiac function heart failure with an aldosterone antagonist
WC	waist circumference

PREFACE

Globally the prevalence of cardiovascular risk factors linked to the development of heart failure has become a burden to healthcare systems. Previously heart failure with reduced ejection fraction was the most common form of heart failure. However, over the past few decades heart failure with a preserved ejection fraction (HFpEF) has been reported to account for half of all cases of heart failure. However, the results of clinical trials employing therapy targeting the mechanisms of HFpEF have been disappointing. In this regard, cardiac diastolic dysfunction characterizes HFpEF and can precede the development of overt heart failure. Although several risk factors for HFpEF are well recognized, including obesity and hypertension, the exact mechanisms through which these risk factors mediate cardiac diastolic dysfunction is still uncertain. Moreover, the extent to whuich genetic mechanisms play a role in contributing to cardiac diastolic dysfunction is unclear.

In the present thesis I set out to better identify the factors associated with obesity that contribute to cardiac diastolic dysfunction. Moreover, I further evaluated aspects of aortic function which contribute to pulsatile load and hence in-part explain the impact of increases in blood pressure on cardiac diastolic function. I also identified the intra-familial aggregation and heritability of aspects of cardiac diastolic function and the structural determinants thereof that had at the time of conducting the present thesis, been unclear. Overall the findings of the present thesis provide novel insights into the metabolic, haemodynanmic and genetic determinants of cardiac diastolic dysfunction and hence possibly a better understanding of the risk factors that lead to heart failure with a preserved ejection fraction.

In the present thesis, in chapter 1 I review the current understanding and controversies related to cardiac diastolic function, thus arguing in favor of conducting the studies described herein. In chapters 2-5 I describe each of the studies, their results and the implications thereof. These chapters are constructed as semi-independent chapters and each includes its own abstract, introduction, methods, results and discussion sections. The present thesis concludes with a chapter (chapter 6), which provides a summary of the findings reported; highlights the novelty of these findings and places them in context of our current understanding of the mechanisms responsible for cardiac diastolic dysfunction and heart failure with a preserved ejection fraction.

In support of this thesis, the data provided in chapter 2 has been published in the *International Journal of Cardiology* (Peterson[b] et al 2016); chapter 3 and 4 have

been published in the the *American Journal of Hypertension* (Peterson[c] et al 2016; Peterson et al 2015); and chapter 5 has been published in the *Journal of the American Society of Hypertension* (Peterson[a] et al 2016).

CHAPTER 1

INTRODUCTION

Current Understanding and Controversies Related to Left Ventricular Diastolic Function

1.1 Introduction

Cardiovascular disease is a major healthcare burden and a leading cause of death in low, middle and high-income countries (Bradshaw et al 2003; Bhatia et al 2006; Borlaug & Redfield 2011; Mozaffarian et al 2015). A number of factors increase the risk of developing cardiovascular disease. These factors include hypertension, obesity, diabetes mellitus, smoking, dyslipidaemia, a sedentary lifestyle and excess alcohol intake (Lee et al 2009; Mozaffarian et al 2015). Currently, in both American and European populations, hypertension, diabetes mellitus and obesity are the leading cause of cardiovascular related deaths (Nichols et al 2004; Mozaffarian et al 2015). Similarly, in South Africa, the prevalence of cardiovascular disease is increasing (Bradshaw et al 2003; Steyn et al 2006; Rayner 2010; Mozaffarian et al 2015) and this is also attributed to a rising prevalence of the cardiovascular risk factors hypertension, obesity and diabetes mellitus, a consequence of urbanisation, globalisation and marked changes in lifestyle (Steyn et al 2006; Rayner 2010; Maredza et al 2011).

The increasing global prevalence of certain cardiovascular risk factors has led not only to an increase in the development of heart failure (Kenchaiah et al 2002; Kane et al 2011; Burke et al 20 14; Nichols et al 2004), but also modified the type of heart failure that may develop. For a number of years, heart failure with a reduced ejection fraction (HFrEF), which is associated with systolic chamber dysfunction, was identified as the most common form of heart failure. In this regard, it was perceived that the lower the ejection fraction the less likely the patient was to survive (Federmann & Hess 1994; McMurray et al 2012). However, over the past few decades there has been an increase in the prevalence of heart failure with a preserved ejection fraction (HFpEF). Numerous studies have shown that heart failure with a preserved ejection fraction is accompanied by left ventricular (LV) diastolic dysfunction (DD), which is an abnormality of the LV during diastolic relaxation and filling periods (Aurigemma & Gaasch 2004). Importantly, heart failure with a preserved ejection fraction is commonly associated with hypertension, diabetes mellitus and obesity (Borlaug & Redfield 2011; Scantlebury & Borlaug 2011).

To date, most of the treatment strategies that have been implemented to improve the survival of patients with heart failure are based on targeting heart failure with a reduced ejection fraction (Paulus & van Ballegoij 2010; Borlaug & Redfield 2011). Data from large multicenter clinical trials show that current therapeutic approaches can reduce the risk of death or hospitalisation for heart failure in patients

with a reduced ejection fraction (Borlaug & Redfield 2011). However, data from clinical trials in patients with heart failure with a preserved ejection fraction show that none of the current therapeutic regimens make an impact on the prevention of death or hospitalisation for heart failure in patients with a preserved ejection fraction (Borlaug & Redfield 2011). In order to improve on the management of patients with heart failure with a preserved ejection fraction, a better understanding of the factors associated with LV diastolic dysfunction and its progression is therefore warranted. Moreover, as healthcare sectors worldwide are burdened by the high prevalence of cardiovascular disease (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 2014), it would be preferable to try and prevent heart failure with preserved ejection fraction by identifying individuals at risk of developing LV diastolic dysfunction and targeting the risk factors involved.

Major modifiable risk factors suggested to be associated with LV diastolic dysfunction include obesity and hypertension and the associated left ventricular hypertrophy (LVH) and remodelling that often occurs with these conditions. However, at present the relative independent contribution of obesity as a determinant of LV diastolic dysfunction is uncertain. In addition, although abnormalities of pulsatile haemodynamics and aortic function have frequently been described in hypertensive heart failure with a normal ejection fraction, the primary aortic functional change responsible for preclinical LV diastolic dysfunction is unclear. Moreover, ethnicity is reported to impact on the prevalence of LV diastolic dysfunction and ultimately heart failure with preserved ejection fraction. In this regard, the effectiveness of treatment strategies in heart failure with preserved ejection fraction may therefore also depend on the overall genetic variance of a population and a better understanding as to the mechanisms through which genetic factors linked either directly to LV diastolic dysfunction or to LV diastolic function through their impact on LVH and remodelling mediate their effects. Hence, in my thesis I further explored the possible independent role and relative importance of each of these risk factors (obesity, aortic haemodynamics, and inheritance) in determining LV diastolic dysfunction. To provide the contextual background to these questions addressed in my thesis, in the introduction to my thesis I will therefore briefly discuss the current classification of heart failure and the role of LV diastolic dysfunction as a determinant of heart failure with preserved ejection fraction. I will then review the literature to-date on the role of hypertension and obesity in the development of LV diastolic dysfunction. When discussing the role of obesity I will provide context as to the importance of insulin

resistance and when discussing the role of hypertension context as to the importance of the impact of aortic haemodynamic changes on LV diastolic function. Last, I will summarise the current literature on the role of genetic factors (inheritance) in determining LV remodelling and diastolic function.

1.2 Heart failure

Clinically, heart failure is described as a collection of signs and symptoms that arise from an increase in filling pressures in the heart (Borlaug & Redfield 2011; Yancy et al 2013). There are various classifications of the types of heart failure. One of the traditional categorisations of heart failure has been linked to cardiac systolic function as measured by LV ejection fraction (Hundely et al 2001; Lee et al 2009). According to this classification, there are two major types of heart failure, namely heart failure with reduced ejection fraction in which heart failure is accompanied by a decreased ejection fraction (EF<40%), and heart failure with preserved ejection fraction in which ejection fraction is normal. Heart failure with a reduced ejection fraction, which is also termed systolic heart failure as it is caused by chamber systolic dysfunction, is associated with coronary artery disease and cardiac dilatation (extensive eccentric LV remodelling), and is commonly seen in elderly males (Federmann & Hess 1994; Masoudi et al 2003; Bhatia et al 2006; Borlaug & Redfield 2011). Alternatively, heart failure with a preserved ejection fraction, which is also termed diastolic heart failure and is preceded by LV diastolic dysfunction, is associated with hypertension, obesity and LVH and more commonly occurs in elderly women (Borlaug & Redfield 2011).

The pathophysiology of heart failure with a reduced ejection fraction is essentially reasonably well understood and forms the basis of the current therapeutic approaches for heart failure. These approaches include the administration of agents that block the sympathetic nervous system (beta blockers, BB), or the renin-angiotensin-aldosterone system (angiotensin-converting enzyme inhibitors [ACEI], angiotensin receptor blockers [ARBs], and aldosterone receptor blockers). Importantly, data from large multicentre clinical trials such as CHARM-ALTERNATIVE, SOLVD, MDC, CIBIS, PRECISE, OPTIMIZE-BB, OPTIMIZE-ACE, RALES have shown that these current therapeutic approaches can reduce the risk of death or hospitalisation for heart failure in patients with heart failure with a reduced ejection fraction (Borlaug & Redfield 2011). Hence, survival in patients with systolic heart failure has improved over the years (Roger et al 2004).

The proportion of patients with heart failure with a preserved as opposed to reduced ejection fraction is increasing steadily. Indeed, each year the prevalence of diastolic heart failure increases by approximately 1% compared to systolic heart failure (Federmann & Hess 1994; Owan et al 2006; Fonarow et al 2007; Borlaug & Redfield 2011; Borlaug & Paulus 2011; Scantlebury & Borlaug 2011). The increased prevalence of heart failure with a preserved ejection fraction is possibly because of ageing populations and an increasing prevalence of obesity, and/or an increased physician recognition of the syndrome (Owan et al 2006). Generally there is a poor understanding of the pathophysiology of heart failure with a preserved ejection fraction, and hence there is a reliance on therapeutic approaches largely based upon the pathophysiology of heart failure with a reduced ejection fraction, which have failed to improve survival in patients with diastolic heart failure (Borlaug & Redfield 2011; Owan et al 2006). Bearing in mind the increased incidence of heart failure with a preserved ejection fraction and the poor survival rates in these patients, the topic of my thesis as previously mentioned, is to further elucidate the impact of risk factors associated with the development of LV diastolic dysfunction, which is thought to be the precursor to diastolic heart failure. Hence, in this chapter I will not further discuss systolic heart failure, but rather focus my discussion on diastolic heart failure. In this regard, what is currently known regarding the pathophysiology of diastolic dysfunction and heart failure with a preserved ejection fraction?

1.2.1 Current understanding of the pathophysiology of heart failure with a preserved ejection fraction

As previously indicated, data from large community-based studies have shown that patients diagnosed with heart failure with a preserved ejection fraction are more likely to be women and of an advanced age, are less likely to have coronary artery disease and more often present with underlying diseases such as obesity and hypertension (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 addition, in contrast to heart failure with a reduced ejection fraction which is associated with cardiac dilatation, concentric cardiac remodelling is commonly present in patients with heart failure with a preserved ejection fraction (Drazner et al 2004). It is therefore thought that age-, hypertension-, and/or obesity-related ventricular remodelling precedes the development of heart failure with a preserved ejection fraction (Borlaug & Redfield 2011; Scantlebury & Borlaug 2011).

Although the progression of a number of abnormalities such as loss of contractile reserve, chronotropic reserve, vasodilatory reserve and endothelial dysfunction (Borlaug et al 2006; Brubaker et al 2006; Borlaug[a] et al 2010; Borlaug[b] et al 2010) promote the transition from diastolic dysfunction to heart failure with a preserved ejection fraction, as mentioned in the aforementioned discussion, the essential abnormality of cardiac function in heart failure with a preserved ejection fraction is an abnormality of the diastolic period of the cardiac cycle. In this regard, the basic pathophysiology of diastolic heart failure is that cardiomyocyte resting tension (the tension during the diastolic period of the cardiac cycle) is increased (Borebely et al 2005; van Heerebeek et al 2006; van Heerebeek et al 2008). The higher cardiomyocyte resting tension may be produced by incomplete relaxation such as may occur when a decreased calcium reuptake by the sarcoplasmic reticulum occurs (van Heerebeek et al 2006); an increase in the stiffer isoform of the macromolecule titin (van Heerebeek et al 2006), or to alterations in the interstitium producing a stiffer myocardium. In this regard, increases in myocardial collagen (fibrosis) associated with an increased activity of the renin-angiotensin system and/or increases in the cross-linking of collagen (often associated with accumulation of advanced glycation end-products in diabetes mellitus) result in increased myocardial stiffness which impairs cardiac filling (Borlaug & Paulus 2011; Norton et al 1996; Kasner et al 2011; Lopez et al 2012; van Heerebeek et al 2006). Increases in the expression of the protein lysyl oxidase, which mediates collagen cross-linking, has been observed in association with increased collagen deposition and cross-linking in patients with heart failure with a preserved ejection fraction, even in the absence of diabetes mellitus (Kasner et al 2011; Lopez et al 2012). Based upon these pathophysiological changes, pharmacological agents that improve myocardial sarcoplasmic reticulum calcium re-uptake, decrease collagen deposition, or decrease collagen cross-linking, have been developed. However, only few of these agents have progressed from pre-clinical studies to clinical trials. The outcomes of these studies will be discussed in the section below which summarises the current therapeutic approaches to preventing and treating heart failure with a preserved ejection fraction.

1.2.2 *What are the current therapeutic approaches to preventing and treating heart failure with a preserved ejection fraction?*

Clinically, the treatment approaches to patients with heart failure with a preserved ejection fraction are largely based on an understanding the pathophysiology of systolic dysfunction and heart failure with a reduced ejection fraction (Federmann & Hess 1994; Yusuf et al 2003; Cleland et al 2006; Massie et al 2008; Hernandez et al 2009; Borlaug & Redfield 2011). However, data from large multicentre clinical trials (I-PRESERVE, CHARM-Preserved, PEP-CHF, DIG-Preserved, OPTIMIZE-BB, OPTIMIZE-ACE) have shown that none of the current therapeutic regimens (digitalis, ARBs, ACEIs and beta-blockers), reduce the rate of death or hospitalisation for heart failure in patients with heart failure with a preserved ejection fraction (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 outcomes of more novel agents (based upon the current understanding of the pathophysiological basis of heart failure with a preserved ejection fraction, as discussed in section 1.2.1 above) have been equally as disappointing. In this regard, therapies targeting fibrosis and related changes in ventricular and arterial stiffness, as well as vasoconstriction, have largely proved unsuccessful (Nanayakkara & Kaye 2015). Indeed, an advanced glycosylation end-product breaker (ALT-711) (Little et al 2005), failed to improve measurements of diastolic function (either early-to-late transmitral velocity or an index of ventricular filling pressure [E/e'], see section 1.3.2 for a description of these measurements). In addition, the aldosterone receptor antagonist, eplerenone (Deswal et al 2011) (which reduces the degree of myocardial fibrosis), showed no impact on standard measures of diastolic function (early-to-late transmitral velocity, see section 1.3.2) and only a modest (p=0.04) reduction in an index of ventricular filling pressure (E/e', see section 1.3.2). Moreover, despite evidence of an improved diastolic function with the aldosterone receptor antagonist, spironolactone in the Aldosterone Receptor Blockade in Diastolic Heart Failure (Aldo-DHF) study (Edelmann et al 2013), in a larger outcomes based study (TOPCAT), spironolactone treatment was not associated with an improved survival (Pitt et al 2014). Alternative antifibrotic approaches such as the use of the phosphodiesterase 5 inhibitor, sildenafil, have similarly reported no beneficial effects on clinical or cardiac structural measurements (Redfield et al 2013). The possible impact of soluble guanylate cyclase agonists, which potentially produce vasodilatation and

inhibit smooth muscle proliferation, is currently being assessed (Pieske et al 2014). Furthermore, the possible effects of other pharmacological approaches largely aimed at reducing vasoconstriction or promoting vasodilation such as endothelin receptor antagonism, iron supplementation, and the combination of hydralazine and isosorbide mononitrate are currently being investigated (Nanayakkara & Kaye 2015).

As current therapeutic approaches to the management of patients with heart failure with a preserved ejection fraction have proved inadequate (Borlaug & Redfield 2011), and the burden of disease 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 2014), the most likely approach that will effectively reduce morbidity and mortality related to this form of heart failure is to prevent the development of diastolic heart failure. In this regard, there may be better approaches to identifying those patients most at risk of developing heart failure with a preserved ejection fraction and targeting the fundamental problem. As various biomarkers of collagen turnover (matrix metalloproteinase [MMP]-2, MMP-8, tissue inhibitor of matrix metalloproteinase [TIMP]-4, collagen III N-terminal propetide [PIIINP]) are increased in patients with cardiac hypertrophy and heart failure with a preserved ejection fraction (Zile et al 2011), it has been suggested that combinations of these biomarkers could be used to identify patients with LV diastolic dysfunction who are likely to develop heart failure with a preserved ejection fraction. Although these biomarkers are unlikely to distinguish patients with diastolic heart failure from patients with systolic heart failure, as they are increased similarly in patients with either type of heart failure (de Denus et al 2012), there are presently no studies which have assessed whether these biomarkers of collagen turnover predict the development of heart failure with a preserved ejection fraction. Until such time as these studies provide evidence for the use of biomarkers of collagen turnover to predict the development of heart failure with a preserved ejection fraction, other approaches should be sought to identify those patients most at risk.

In summary the current understanding of the pathophysiology of heart failure with a preserved ejection fraction has not proved helpful in developing agents to target diastolic heart failure. Indeed, there is presently no treatment with proven benefit for heart failure with a normal ejection fraction (Borlaug & Paulus 2011; Borlaug & Redfield 2011; Pitt et al 2014). Hence, a better approach may be to improve on our understanding of the risk factors for LV diastolic dysfunction which is the precursor of heart failure with a preserved ejection fraction. Once we have better insight into how these risk factors mediate their adverse effects, targeting specific

aspects of these risk factors may assist in the prevention of LV diastolic dysfunction and hence the development of diastolic heart failure. As the current thesis is focused on aspects of LV diastolic function, for the remainder of this chapter I will discuss LV diastolic rather than systolic function.

1.3 Cardiac diastolic function and outcomes

What is the evidence in support of LV diastolic dysfunction being a precursor of cardiovascular events and heart failure with a preserved ejection fraction? In a large (approximately 36 000 patients) outcomes-based study LV diastolic dysfunction was shown to be an independent predictor of all-cause mortality (Halley et al 2011). These data confirmed earlier reports in a small follow-up study where LV diastolic dysfunction was shown to be associated with all-cause mortality independent of coronary artery disease or cancer (Achong et al 2009). Moreover, worsening of LV diastolic function is associated with a worse prognosis (AlJaroudi et al 2012), and LV diastolic dysfunction is a predictor of primary cardiovascular events (Desai et al 2012; Sharp et al 2010). There is therefore substantial evidence to support an association between LV diastolic dysfunction and cardiovascular or all-cause mortality. However, what is the evidence to support LV diastolic dysfunction as being the precursor to heart failure with a preserved ejection fraction?

There is now considerable evidence indicating that LV diastolic dysfunction can occur without the presence of heart failure (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 support of these studies, findings from our group performed in a South African population of African ancestry have also highlighted the relatively high prevalence of LV diastolic dysfunction in the absence of heart failure (Libhaber et al 2014; Millen et al 2014). Importantly, however, in support of earlier studies which demonstrated that in patients with a preserved ejection fraction, symptoms of congestive heart failure are associated with LV diastolic dysfunction (Senni et al 1998; Angeja & Grossman 2003), subclinical changes in diastolic function have been noted to precede the development of clinical heart failure (Bella et al 2002; Hogg et al 2003; Wan et al 2014; Wang et al 2003; Zile et al 2002), as well as to increase the risk of developing heart failure with a preserved ejection fraction (Bella et al 2002; Hogg et al 2003; Wang et al 2003; Zile et al 2002). Noteworthy is that a number of studies have now shown that LV diastolic dysfunction predicts the progression to heart failure with a preserved ejection fraction (Aurigemma et al 2001; Bella et al 2002; Kane et al 2011;

Lam et al 2011; Redfield et al 2003; Schillaci et al 2002). Also of importance is that the pathophysiological changes thought to determine heart failure with a preserved ejection fraction, such as increases in ventricular stiffness and reductions in relaxation, are associated with diastolic heart failure (Burke et al 2014; Mohammed et al 2012; Shah et al 2014; Westerman et al 2008; Zile et al 2004). Hence, there is substantial evidence to indicate that LV diastolic dysfunction is central to the pathophysiology and outcomes of heart failure with a normal ejection fraction. Before describing the evidence on the role of risk factors for LV diastolic dysfunction, it is nevertheless important to expand on the current approaches to assessing LV diastolic function and the classification of LV diastolic dysfunction.

1.4. Measurement of and approaches to the classification of cardiac diastolic dysfunction

The classification of cardiac diastolic dysfunction is based on measurements of function of the ventricular chamber during the diastolic period of the cardiac cycle including increases in filling pressures. As the studies in my thesis were conducted in a community-based sample only non-invasive assessments of diastolic function and filling pressures were performed. The physiological basis, merits and limitations of the non-invasive assessments performed in my studies will be discussed in the subsequent sections, following which the approaches to the classification of cardiac diastolic dysfunction will be addressed (see section 1.4.2).

1.4.1 Non-invasive measurements of diastolic dysfunction

The diastolic component of the cardiac cycle commences at the closure of the aortic and pulmonary valves and ends at the closure of the atrio-ventricular (mitral and tricuspid) valves. Diastole consists of 4 phases, namely isovolumic relaxation, rapid filling, diastasis and atrial contraction (Brutsaert et al 1993; Nagueh et al 2009). Left ventricular diastolic dysfunction is the result of impaired relaxation, reduced early diastolic suction and increased LV chamber stiffness, which result in an increase in cardiac filling pressures. Hence, in echocardiographic studies to determine the presence versus absence of LV diastolic dysfunction, LV diastolic function must be determined and LV filling pressures estimated. The most useful, feasible and reproducible echocardiographic measurements of LV diastolic function and estimates of LV filling pressures include traditional assessments of mitral inflow (trans-mitral

peak early [E] wave velocity, late diastolic or atrial filling [A] wave velocity, trans-mitral E/A ratio; and trans-mitral E wave velocity deceleration time) and more contemporary measurements of tissue Doppler imaging (pulsed-wave tissue Doppler imaging derived mitral annular early diastolic velocity [e'] and mitral E/e' ratio), as well as two-dimensional assessments of left atrial (LA) volume index (Nagueh et al 2016). Alternative variables, such as changes in mitral inflow with the Valsalva maneuver, are not feasible in all patients and need to be carefully standardised in order for accurate interpretations to be made (Nagueh et al 2016). In addition, the E-e' time interval is difficult to acquire and requires matching electrocardiographic R-R intervals (Nagueh et al 2016). Although isovolumic relaxation time (IVRT) is reported to be feasible and reproducible, it is in part affected by heart rate and arterial pressures which would impact on its interpretation (Nagueh et al 2016). Hence, in my thesis, I focused on the measurements of diastolic function and filling pressures which are both reported to be reliable and reproducible (Nagueh et al 2016), as well as to have a low degree of variability (Norton et al 2008) in our hands. These will be discussed below.

1.4.1.1 Mitral inflow

Mitral inflow measurements are obtained by pulsed-wave (PW) Doppler performed in the apical 4-chamber view of the heart. The primary measurements of mitral inflow include peak early (E wave) velocity, late diastolic or atrial filling (A wave) velocity, the ratio of E wave to A wave velocity (E/A), and deceleration time of early filling velocity (Figure 1.1). Measurements of mitral inflow velocity are used to non-invasively assess LV filling pressures (Appleton et al 1997). As mitral peak E wave velocity reflects the pressure gradient between the LA and LV during early diastole, it best relates to earlier LV diastolic pressures, such as mean pulmonary capillary wedge pressure and mean LV diastolic pressure. In comparison, mitral A wave velocity reflects the pressure gradient between the LA and LV during late diastole, and hence correlates best with LV end diastolic pressure (Nagueh et al 2016). The mitral A wave velocity is an index of LV compliance as well as contractile function of the LA; whereas mitral peak E wave velocity and E wave velocity deceleration time are indices of the rate of LV relaxation and LA pressures. In addition, the mitral E wave velocity is directly influenced by alterations in LV volumes and therefore LV volume preloads. Although a number of variables other than LV diastolic function and filling pressures (such as heart rate and rhythm, PR interval,

cardiac output, mitral annular size, and LA function) affect mitral inflow velocities, measurements of mitral inflow remain an integral component of the classification of diastolic dysfunction (see section 1.4.2 below).

1.4.1.2 Tissue Doppler imaging

Pulsed wave Tissue Doppler imaging (TDI) is a reproducible echocardiographic tool which is performed in the apical 4 chamber view to obtain quantitative assessments of peak myocardial early (e' or Ea) and late (a' or Aa) diastolic velocity at the mitral annulus (Waggoner & Bierig 2001) (Figure 1.1). These velocities can be measured from either the septal or the lateral mitral annulus; however septal measurement values are reported to be lower than lateral measurements (Isaaz et al 1993, Nagueh et al 2009). The current recommendation is that e' is expressed as the average of septal and lateral measurements (Nagueh et al 2009). Aa or a' represents active atrial contraction in late diastole (Thomas et al 2003). Similar to E wave velocity, e' reflects the rate of early diastolic myocardial relaxation (Nagueh et al 2001; Oki et al 1997). In normal individuals, e' is augmented by increases in the trans-mitral pressure gradient, as occurs with elevations in preload. However, in patients with an impaired relaxation, e' is decreased at rest and the response to increases in preload is reduced in comparison to normal individuals (Yu et al 2005). Therefore, in patients with impaired relaxation, because e' remains reduced (remains constant), whereas mitral E wave velocity increases in response to higher filling pressures, the ratio E/e' is increased (Ommen et al 2000). In comparison, as both e' and E wave velocity increase with increasing trans-mitral pressure gradients in normal individuals, E/e' is similar at rest and with exercise (usually <8). E/e' correlates well with mean LV filling pressure and pulmonary capillary wedge pressure (Sohn et al 1997, Nagueh et al 1997; Ommen et al 2000). If E/e' is ≥15 then the pulmonary capillary wedge pressure is reported to be ≥20 mm Hg, and if E/e' is <8 then the pulmonary capillary wedge pressure is reported to be normal. Moreover, the prognostic importance of E/e' has been highlighted by a number of studies which have reported increases in cardiac mortality in patients with heart failure who had E/e'≥15 (Dokainish et al 2005; Yamamoto et al 2003). Hence, the use of TDI to assess e' and a' and the subsequent calculation of E/e' has become an essential component of the echocardiographic evaluation of diastolic function (Sohn et al 1997, Nagueh et al 1997; Ommen et al 2000).

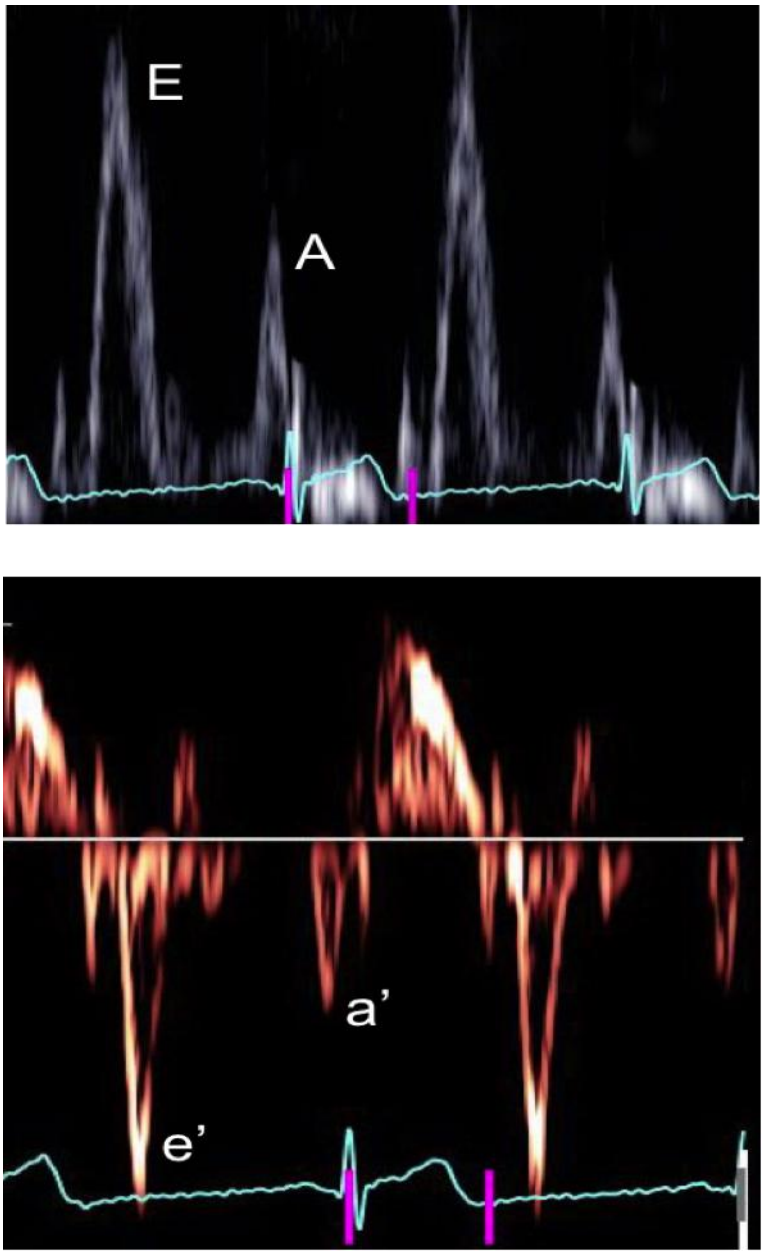


Figure 1.1 Example of echocardiographic images of pulsed wave Doppler transmitral E wave (E) and A wave (A) velocities (upper panel), and TDI peak myocardial early (e’) and late (a’) diastolic velocity at the mitral annulus(lower panel) used to assess diastolic function.

1.4.1.3 LA volume

Echocardiographic assessment of left atrial (LA) volume is most accurately performed from the apical 4-chamber and 2-chamber views (Lang et al 2015). Importantly, in order to correct for differences in body size so that comparisons to normal values can be made, LA volume is indexed to body surface area (Vasan et al 2000; Prichett et al 2003). Although increases in LA size can be caused by increases in filling volumes, they are most commonly related to increases in wall tension due to augmented filling pressures (Simek et al 1995; Appleton et al 1993). Importantly, the adverse cardiovascular outcomes associated with increased LA dimensions (Tsang et al 2002 & 2003; Kizer et al 2006) are more strongly related to increases in filling pressures than filling volumes. Left atrial enlargement is reported to be a marker of both the severity of diastolic dysfunction and the magnitude of LA pressure elevation (Kizer et al 2006; Simek et al 1995; Appleton et al 1993). An additional benefit of the assessment of LA volume is that unlike Doppler velocities which reflect filling pressures only at the time of measurement, LA volume reflects the cumulative effects of increases in filling pressures over time (Nagueh et al 2009; Kizer et al 2006; Simek et al 1995; Appleton et al 1993).

In large observational studies, an LA volume index ≥ 34 ml/m^2 is an independent predictor of death, heart failure, atrial fibrillation and ischaemic stroke (Abhayaratna[b] et al 2006). However, measurements of LA volume should not be interpreted in isolation, as alone they are not specific to diastolic dysfunction. A dilated LA is a feature of a number of other clinical entities including bradycardia and 4-chamber enlargement, anaemia and other high-output state, atrial flutter and fibrillation, and significant mitral valve disease in the absence of diastolic dysfunction (Nagueh et al 2009). In addition, an increased LA volume index is commonly reported in elite athletes in the absence of cardiovascular disease (Nagueh et al 2009). Hence, in the classification of diastolic function, measurements of LA volume index need to be assessed in conjunction with Doppler

parameters (see section 1.4.2 below).

1.4.2 Classification of diastolic dysfunction

One of the concerns about differentiating between normal and abnormal diastolic function is that normal aging is associated with slowing of LV relaxation.

Indeed, LV filling patterns in the elderly resemble those of younger patients (40 to 60 years) with mild diastolic dysfunction (Caballero et al 2015). However, in comparison to the mitral E/A ratio and e' velocity, the E/e' ratio appears to be less age dependent (Caballero et al 2015).

Another consideration in the classification of diastolic dysfunction is the development of a pseudonormal E wave to A wave velocity pattern. In normal individuals, the E wave is higher than the A wave, and this pattern reverses in early diastolic dysfunction. In advanced diastolic dysfunction, the E wave velocity again becomes higher than the A wave velocity resulting in a so-called pseudonormal pattern and hence the use of the mitral inflow patterns to diagnose diastolic dysfunction becomes problematic (Oh et al 2011). However, reductions in e' velocity precede reductions in E velocity by 10-15 years (Sohn et al 1997), and are reduced in subjects with early diastolic dysfunction. Hence, in the diagnosis of diastolic dysfunction, it is useful to combine measurements of mitral inflow and TDI annular velocities. Indeed, the utility of TDI assessments is evident from their inclusion in the EAE/ASE guidelines for the assessment of diastolic dysfunction (Nagueh et al 2009, Nagueh et al 2016).

According to the EAE/ASE guidelines diastolic dysfunction is defined primarily according to mitral inflow E/A, TDI septal e', TDI lateral e', and LA volume values (Nagueh et al 2009, Nagueh et al 2016). In accordance with these guidelines, in individuals with normal ejection fraction, the presence of LV diastolic dysfunction is identified by lateral e′ < 10 cm/s, septal e′ < 8 cm/s or left atrial volume ≥ 34 ml/m^2. Mild diastolic dysfunction is defined as E/A ≤ 0.80 (impaired relaxation) or E/e′ ≤ 8. Moderate diastolic dysfunction is defined as E/A from 0.80 to 1.5 or E/e′ from 9 to 12, and severe diastolic dysfunction is defined as E/A ≥ 2 or E/e′ ≥ 13. In addition, the change in E/A during the Valsalva maneuvre is used to grade mild (I) (Valsalva change in E/A<0.5) compared to moderate (II) and severe (IIII) (Valsalva change in E/A≥0.5) diastolic dysfunction. Although, the time difference between the duration of pulmonary venous flow and mitral inflow during atrial contraction (A wave) (Ar-A duration) and E wave velocity deceleration time were incorporated in the 2009 algorithm (Nagueh et al 2009), they were not included in the recently published updated algorithm (Nagueh et al 2016). According to the simplified 2016 algorithm, in persons with normal ejection fraction, diastolic function is normal if <50% of the following criteria are present: average E/e' > 14; septal e' < 7 cm/s or lateral e' <10 cm/s; LA volume index > 34 ml/m^2; tricuspid regurgitation velocity > 2.8 m/s. In persons with normal ejection fraction, diastolic dysfunction is present if >50% of the

following criteria are present: average E/e' > 14; septal e' < 7 cm/s or lateral e' <10 cm/s; LA volume index > 34 ml/m^2; tricuspid regurgitation velocity > 2.8 m/s.

1.5 Factors associated with the development of LV diastolic dysfunction

A number of factors are associated with the development of LV diastolic dysfunction. In this regard, the two major contributing factors are age and female gender (Borlaug & Redfield 2011). However, little is understood as to how age and female gender contribute toward LV diastolic dysfunction. Hence, these two factors are not currently potential modifiable targets in order to decrease the overall risk of developing heart failure with a preserved ejection fraction. I will therefore only discuss aspects of these risk factors and when doing so, this will only be done in a cursory manner. Among the number of modifiable risk factors which have been associated with LV diastolic dysfunction, obesity and associated metabolic abnormalities (diabetes mellitus, insulin resistance) as well as blood pressure or hypertension have been identified as the major determinants (Bhatia et al 2006; Bursi et al 2006; Desai et al 2012; Lee et al 2009; Masoudi et al 2003; Owan et al 2006; Redfield et al 2003). Importantly, increases in LV mass as well as a concentric LV geometry, which are often associated with these risk factors, have been shown to play a significant role (Chahal et al 2010; Lorell et al 1990). Nevertheless, obesity, blood pressure and associated changes in LV mass and geometry fail to account for all of the inter-individual variation in diastolic function. Hence, genetic factors may be responsible for some of the unexplained inter-individual variation in LV diastolic function. In the subsequent sections of the present chapter I will therefore discuss the current knowledge on the role of obesity, blood pressure (and the associated changes in LV mass and geometry) and the possible role of genetic factors in determining LV diastolic function. In so doing I will highlight the several areas of missing evidence that I subsequently pursued as part of my thesis to attempt to add to our understanding of these topics.

1.5.1 Obesity as a cause of LV diastolic dysfunction

In the following section I will discuss obesity as a possible cause of LV diastolic dysfunction. However, it is important in the context of the present thesis to provide a more overall approach to the relevance of this topic in the setting of obesity as a cause of heart failure. It is also important to underscore the mechanisms that

may explain the relationship between obesity and either heart failure or LV diastolic dysfunction. Hence, in the following sections I will deal with each of these topics in turn.

1.5.1.1 Obesity as a risk factor for heart failure

Over the past two decades, a nested case-control study and a number of prospective studies have demonstrated an independent relationship between the degree 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[a] et al 2005, Ingelsson[b] et al 2005, Nicklas et al 2006, Bahrami et al 2008, Spies et al 2009). This relationship has been demonstrated in the general population (He et al 2001, Kenchaiah et al 2002 Ingelsson[b] et al 2005, Bahrami et al 2008), in the middle-aged (Ingelsson[a] et al 2005), in the elderly (Chen et al 1999, Nicklas et al 2006), in men (Wilhelmsen et al 2001, Ingelsson[b] 2005), in women (He et al 2001), 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).

As compared to a referent body mass index (BMI) between 20 and 25 kg/m^2 (normal) a BMI of between 25 and 30 kg/m^2 (overweight) may increase the risk of heart failure by 39-49% (Bahrami et al 2008, Kenchaiah et al 2009) and a BMI greater than or equal to 30 kg/m^2 (obese) increases the risk of heart failure by 83-180% independent of conventional cardiovascular risk factors (Johansson et al 2001, Kenchaiah et al 2002, Bahrami et al 2008, Kenchaiah et al 2009). In one study, however, no increased risk for heart failure was noted in persons with a BMI of between 25 and 30 kg/m^2 (overweight) in a general practice setting (Johansson et al 2001). Moreover, in one study (Spies et al 2009), but not in other studies (Ingelsson[b] et al 2005, Nicklas et al 2006) the adipose tissue-heart failure relationship was better predicted by the use of indices of adiposity that reflect an accumulation of fat in central (abdominal) stores, such as waist circumference or waist-to-hip ratio, than indices of general adiposity, such as BMI. Depending on the study population and the study design, the independent risk for heart failure in people who are overweight or obese is quantitatively comparable with the impact of, for example, the presence of hypertension, or diabetes mellitus in these same studies.

Although the relationship between excess adiposity and the development of heart failure is modified by adjustments for conventional cardiovascular risk factors, including hypertension, diabetes mellitus and cholesterol concentrations as well as

with adjustments for coronary events, the impact of these adjustments is surprisingly modest. Indeed, considering overweight and obese persons overall, the percentage risk for heart failure is diminished by only 1-13% with these adjustments (He et al 2001, Ingelsson[a] et al 2005, Ingelsson[b] et al 2005, Nicklas et al 2006, Spies et al 2009) and by only 55% in obese individuals in a study in which obesity increased the risk for heart failure by 180% (Kenchaiah et al 2009). Thus, although there is no question that to prevent overweight/obesity-induced heart failure, targeting modifiable cardiovascular risk factors with lifestyle interventions and medication is an essential approach, the large residual risk for heart failure after adjustments for conventional cardiovascular risk factors indicates that this may not be the most appropriate solution. Better understanding of the mechanisms by which excess adiposity contributes toward heart failure may shed light on more effective therapeutic approaches other than weight loss *per se*.

1.5.1.2 Obesity as a risk factor for the development of LV diastolic dysfunction

The impact of obesity on the risk of heart failure may in part be explained by the association of obesity with LV diastolic dysfunction. Indeed, previous studies, first reported on by our group in a general community-based sample with a high prevalence of obesity (Libhaber et al 2009), have demonstrated that LV diastolic abnormalities are correlated with obesity and these studies were performed in study samples where generally most participants had a preserved ejection fraction (Tsioufis et al 2008; Libhaber et al 2009; Russo et al 2011, AlJaroudi et al 2012; Cil et al 2012). However, bearing in mind that obesity often occurs together with diabetes mellitus and hypertension, what is the evidence for an effect of obesity on diastolic function independent of these co-morbidities? In addition, obesity is a well-recognised cause of LVH and several studies have reported associations between obesity and an increased LV relative wall thickness (Millen et al 2014; Woodiwiss & Norton 2015). Moreover, as will be further discussed in section 1.5.1.4, increases in LV relative wall thickness are strongly correlated with changes in diastolic function (Hsuan et al 2010), and decreases in LV relative wall thickness subsequent to bariatric surgery are accompanied by increases in LV diastolic function (Hsuan et al 2010; Ippisch et al 2008; Luaces et al 2012). As obesity, diabetes mellitus and hypertension induce cardiac hypertrophy, and LVH as well as an increased relative wall thickness are determinants of diastolic function (as discussed in section 1.5.1.4

below), the evidence for an impact of obesity on LV diastolic function independent of increases in LV mass or relative wall thickness needs to be considered.

In 966 hypertensives, Tsioufis et al (2008) reported that waist circumference was independently associated with LV E/A, thus providing the first data from a large study sample that indices of obesity are independently and substantively associated with LV diastolic function. However, in that study (Tsioufis et al 2008) hypertensives were evaluated and it is uncertain whether the obesity effects could be dissociated from the blood pressure effects. Moreover, whether the relations between obesity and LV diastolic function in that study (Tsioufis et al 2008) were independent of LV mass and LV remodelling was unclear. However, Libhaber et al (2009) subsequently demonstrated in a randomly selected community sample with a low prevalence of antihypertensive therapy, that a significant proportion of LV E/A could be accounted for by increases in waist circumference and that this effect was independent of ambulatory and central aortic blood pressure and LV mass or LV relative wall thickness. However, in that study (Libhaber et al 2009), only E/A was assessed as a measure of LV diastolic function. In a subsequent larger community-based study conducted in 950 randomly recruited participants, an increased body mass index (BMI) was associated with LV diastolic dysfunction assessed from both trans-mitral and tissue Doppler indices of diastolic function, independent of LV mass, diabetes mellitus and hypertension (Russo et al 2011). However relative wall thickness was not assessed and blood pressure was not assessed as a continuous trait with diastolic and systolic blood pressure contributing differentially to different components of LV diastolic function (Libhaber et al 2014). In a subsequent large (21 666 patients) study, BMI was also associated with LV diastolic dysfunction assessed from both trans-mitral and tissue Doppler indices of diastolic function (AlJaroudi et al 2012). This relationship was independent of diabetes mellitus, blood pressure and LV mass; however, relative wall thickness was not assessed and again blood pressure was not assessed as a continuous trait with diastolic and systolic blood pressure contributing differentially to different components of LV diastolic function (Libhaber et al 2014). Moreover, in a large study of 2228 participants, BMI, hypertension and diabetes mellitus were all identified as independent predictors of LV diastolic dysfunction (Çil et al 2012). However, once again the possible impact of LV mass and relative wall thickness were not assessed and again blood pressure was not assessed as a continuous trait with diastolic and systolic blood pressure contributing differentially to different components of LV diastolic function (Libhaber et al 2014). Therefore, although several lines of evidence suggest that obesity is independently

associated with LV diastolic function, only one study (Libhaber et al 2009) showed that this was independent of both LV mass and LV remodeling, and many of these studies, again with the exception of Libhaber et al (2009), failed to appropriately adjust for blood pressure effects. Hence, it is possible that the relationship between adiposity indices and LV diastolic function in many of these studies were due to residual confounding of LV remodelling and blood pressure. In this regard, independent of blood pressure control during 4 years of anti-hypertensive therapy, an increase in BMI is associated with less of a decrease in LV mass (de Simone et al 2013). Moreover, more recent evidence from our group suggests that blood pressure effects on LV diastolic function at a community level are far more important than obesity effects on LV diastolic function (Millen et al 2014). Nevertheless, in order to identify cause versus effect relationships it is best to assess data from intervention studies. What is the evidence that weight loss influences LV diastolic function and is this independent of LV mass, LV remodelling and blood pressure effects?

The most effective method of losing weight (extent and sustainability) is bariatric surgery, an intervention that is usually only conducted in morbidly obese patients. In this regard, a number of intervention studies have assessed the impact of weight loss subsequent to bariatric surgery on LV diastolic function. Indeed, a systematic review and meta-analysis of 23 studies assessing the effects of bariatric surgery on cardiac structure and function in obese patients with preserved systolic function has fairly recently been published (Cuspidi et al 2014). This meta-analysis showed that improvements in LV diastolic function as well as reductions in LV mass and relative wall thickness were indeed a consequence of weight loss. Hence, it is possible that obesity is causally related to decreases in LV diastolic function as well as LVH and LV concentric remodelling. However, whether the improvements in LV diastolic function subsequent to weight loss were a consequence of the reductions in LV mass and LV relative wall thickness or independent of changes in LV mass and LV remodelling could not be determined from these studies. In this regard, a previous study has suggested an interactive effect between LV structure and diastolic function where improvements in LV E/A with a decrease in body weight were only observed in those patients with LVH (Syed et al 2009). Moreover, whether the improvements in LV diastolic function were independent of blood pressure or diabetes mellitus could not be assessed from the studies included in this meta-analysis (Cuspidi et al 2014). Is there a possible mechanism that could explain obesity effects on LV diastolic function independent of LVH, LV remodelling and blood pressure?

1.5.1.3 *Insulin resistance as a possible mechanism explaining the independent relationship between obesity and LV diastolic dysfunction*

Insulin resistance may occur as a consequence of excess adiposity and this effect is more closely associated with the extent of abdominal as opposed to general obesity (Kahn and Flier 2000). At a cellular level, insulin binds to its receptor on the surface of target cells, thereby causing tyrosine autophosphorylation and consequent intracellular signaling. These events culminate in cellular responses, such as the translocation of glucose transporters to the cell surface to allow glucose uptake for use or glycogen storage. In obesity, however, insulin signaling may be defective. Insulin-stimulated protein kinase activity of the insulin receptor, which mediates tyrosine autophosphorylation, is reduced in obese as compared to non-obese people, and it is further reduced in patients with obesity-induced type 2 diabetes mellitus (Caro et al 1989). However, euglycaemia is maintained in the initial stages of insulin-resistance through compensatory hyperinsulinaemia. Indeed, there is considerable evidence to suggest that a large proportion of obese individuals are insulin-resistant, but that compensatory hyperinsulinaemia maintains fasting blood glucose concentrations (Reaven 2003). Thus, insulin resistance goes undetected in these individuals.

Insulin resistance will undoubtedly promote adverse cardiac effects once type 2 diabetes mellitus develops and the effects of hyperglycaemia are noted. However, insulin resistance may also induce adverse cardiac changes prior to the development of diabetes mellitus and poor blood glucose control through a number of mechanisms. In this regard, insulin resistance is not specific to skeletal muscle, but also involves myocardial muscle tissue as well (Nikolaidis et al 2004, Ouwens et al 2005, Coort et al 2007) where it downregulates glucose uptake and hence precludes the energetic advantage provided by glucose versus free fatty acid oxidation (Nikolaidis et al 2004, Ouwens et al 2005). Moreover, insulin resistance is associated with an accumulation of intracellular triacylglycerol which promotes lipotoxicity (Coort et al 2007). Hence, through either a reduced efficiency of energy utilisation or through cell death (lipotoxicity), obesity could promote LV diastolic dysfunction through a reduced ability of the myocardium to relax.

Insulin resistance is often associated with leptin resistance and a reduced cardiac efficiency and an altered myocardial substrate metabolism may accompany obesity associated with leptin deficiency or leptin resistance (Buchanan et al 2005). These changes in myocardial substrate metabolism may contribute toward an

increased cardiomyocyte apoptosis in obesity models of leptin deficiency or leptin resistance (Barouch et al 2006). The cardiomyocyte apoptosis in leptin resistant or leptin deficient animals is thought to occur through a reduced cardioprotection mediated by leptin, possibly induced via a resultant increase in ectopic lipid overload in cardiac myocytes (lipoapoptosis) (Zhou et al 2000). Irrespective of the potential adverse myocardial changes that may be mediated by insulin resistance, an important question is to what extent does the current evidence suggest that obesity in humans may promote LV diastolic dysfunction through the presence of insulin resistance?

Evidence for a role for insulin resistance as a possible factor involved in promoting obesity-associated LV diastolic dysfunction has been conflicting. In this regard, in 33 hypertensives, although those with an impaired glucose tolerance had a reduced E/A, E/A was unrelated to the area under the insulin curve after an oral glucose load (Nagano et al 1994). In 50 hypertensives, insulin resistance, as assessed using the euglycaemic insulin clamp technique, was associated with E/A; however, whether this association survived adjustments for adiposity indices is uncertain (Lind et al 1995). In 27 otherwise healthy overweight and obese participants, 12 of whom had insulin resistance, insulin resistance was not associated with LV isovolumic relaxation time (an index of LV diastolic relaxation) after adjusting for body weight (Mureddu et al 1998). In 29 hypertensives men, insulin resistance, as assessed using the euglycaemic insulin clamp technique, was associated with LV isovolumic relaxation time independent of BMI (Galderisi et al 1997). In 26 hypertensives with an impaired glucose tolerance, 39 hypertensives with a normal glucose tolerance and 18 normotensives, insulin resistance, as determined from the insulin suppression test, was related to E/A independent of BMI (Watanabe et al 1999). In 89 hypertensives, insulin resistance, as assessed with the euglycaemic insulin clamp technique, was not independently associated with E/A and LV isovolumic relaxation time (Olsen et al 2003). In 109 obese and 33 normal weight participants, fasting insulin concentrations were inversely correlated with tissue Doppler indices of both systolic and diastolic myocardial but not chamber function (Wong et al 2004). In 29 participants with a normal glucose tolerance, 20 with impaired glucose tolerance and 70 with type 2 diabetes mellitus, insulin resistance, as identified from the Quantitative Insulin Sensitivity Check Index, was associated with LV diastolic dysfunction independent of BMI and waist-to-hip ratio (Bajraktari et al 2006). In 208 patients with normal ejection fraction, of whom 57 had type 2 diabetes mellitus, insulin resistance was independently associated with left

ventricular diastolic dysfunction in subjects without overt type 2 diabetes mellitus (Dinh et al, 2010). Although in 29 insulin resistant and 36 insulin sensitive women, diagnosed on the basis of the homeostasis model of insulin resistance (HOMA-IR), LV peak filling rate, an index of an abnormal diastolic function was reduced, it is uncertain whether these differences were independent of adiposity indices (Utz et al 2011). Similarly, in 42 prediabetic adults with a HOMA-IR above 2.7, as compared to 79 prediabetics with a HOMA-IR below 2.7, although E/A was reduced (Sliem et al 2011), whether these differences were independent of adiposity indices is uncertain. Furthermore, in 92 participants either with or without an impaired glucose tolerance or diabetes mellitus, HOMA-IR was not correlated with E/A (Wada et al 2010) and in 102 otherwise healthy individuals although visceral fat was associated with diastolic dysfunction, HOMA-IR was not (Wu et al 2012). In 18 lean and 25 overweight/obese people, although tissue Doppler indices of diastolic function were noted to be abnormal in overweight or obese individuals, HOMA-IR did not account for these differences (Lambert et al 2010). The results of the aforementioned studies conducted in small sample sizes of select clinical populations, may nevertheless reflect inadequate sample sizes and a selection bias. What is the evidence with respect to studies conducted with a large sample size in unselected populations?

In 2399 participants of the Jackson Heart Study, in women with a normal fasting blood glucose, HOMA-IR was related to a higher proportion with a lower EF after adjustments for BMI, but this only achieved marginal significance ($p=0.02$) (Fox et al 2011). In that study (Fox et al 2011), although diastolic function was assessed using trans-mitral velocity and tissue Doppler assessments, the results were not reported on. I therefore assume that no relationship was noted beyond indices of obesity. Moreover, in 1599 participants being evaluated for health checks, although increasing quartiles of HOMA-IR were associated with decreases in E/A and increases in E/e', whether these relationships survived adjustments for indices of excess adiposity was not reported on (Hwang et al 2012).

In a longitudinal study (CARDIA, Coronary Artery Risk Development in Young Adults) in which 1211 participants had measurements before and after 25 years, the relationship between the diagnosis of diabetes mellitus and diastolic function was assessed (Kishi et al 2016). In this study (Kishi et al 2016), both early (diagnosis before year 15) and late (diagnosis at or after year 15) onset of diabetes mellitus with poor blood glucose control (HbA1c>7%) was associated with an increase in E/e'. In addition, the groups with early and late onset of diabetes mellitus and poor blood glucose control had an increased risk of developing LV diastolic dysfunction (defined

as E/e'≥13) (OR [95% CI]: 3.27 [1.07 to 10.04] for early and 2.21 [1.03 to 4.75] for late). Although the relationship between onset of diabetes mellitus with poor blood glucose control and E/e' survived adjustments for waist-to-hip ratio, the risk of developing diastolic dysfunction was no longer significant (OR [95% CI]: 2.99 [0.96 to 9.37] for early and 2.01 [0.90 to 4.47] for late) (Kishi et al 2016). Moreover, in this study, moderate and high HOMA-IR in the absence of diabetes mellitus were associated with increased relative wall thickness independent of BMI category. However, the impact of HOMA-IR in the non-diabetes mellitus cohort on E/e' and diastolic dysfunction was not reported on in this study (Kishi et al 2016). Hence, I assume that no relationships between HOMA-IR and E/e' or diastolic dysfunction were observed independent of obesity.

Thus, in summary, the aforementioned studies were either characterised by small sample sizes in select clinical populations, thus raising the question of a selection bias confounding the results or type I or II statistical errors occurring, or positive relationships between HOMA-IR and diastolic function were not adjusted for indices of excess adiposity. Moreover, although in some large studies relationship between indices of insulin resistance and LV diastolic function were noted, it is unclear whether the relationship was independent of adiposity indices. Hence, not only is there uncertainty as to the role of obesity as an independent determinant of LV diastolic function, but also the role of insulin resistance. Hence, there is a need to better identify those factors which determine whether obesity or insulin resistance translate into LV diastolic dysfunction. In this regard, in the present thesis I propose that LV remodelling may influence the relationship between either obesity or insulin resistance and LV diastolic function. What is the evidence that LV remodelling may determine LV diastolic dysfunction and is there an indication that LV remodelling may influence whether obesity or insulin resistance influence LV diastolic function?

1.5.1.4 *Could LV remodelling influence the impact of obesity or insulin resistance on LV diastolic function?*

It is well known that LVH is a major risk factor for cardiovascular events (including heart failure), independent of conventional risk factors and coronary artery disease (Levy et al 1990; Taylor et al 2007; McMurray et al 2012; Yancy et al 2013). In addition, concentric LV remodelling (an increased relative wall thickness without an increase in LV mass) is associated with a worse prognosis in comparison to a normal LV geometry (Bluemke et al 2008; Koren et al 1991; Milani et al 2006;

Verdecchia et al 1991). Although LVH rather than concentric LV remodelling is known to predict incident heart failure (Bluemke et al 2008), concentric LV remodelling is associated with the development of LV diastolic dysfunction (Chahal et al 2010). In addition, concentric LVH (an increased relative wall thickness together with an increase in LV mass), rather than eccentric LVH (an decreased or normal relative wall thickness together with an increase in LV mass, is associated with greater increases in indices of LV filling pressures (Chahal et al 2010). Hence, the extent of concentric LV remodelling may determine whether progression from LVH to heart failure with a preserved rather than a reduced systolic chamber function (ejection fraction) occurs. In summary, there is considerable evidence to suggest that a more concentrically remodelled LV is independently associated with LV diastolic dysfunction and that this may determine whether LVH progresses to LV diastolic heart failure. As several studies have reported associations between obesity and an increased LV relative wall thickness (Millen et al 2014; Woodiwiss & Norton 2015), due consideration should be given to the possibility that the LV remodelling process in obesity modifies the impact of obesity or insulin resistance on LV diastolic function. In this regard, overweight individuals with insulin resistance are reported to have an increased relative wall thickness and a decreased diastolic function in comparison to overweight individuals without insulin resistance (Dahiya et al 2015). Importantly, although possible interactive effects between body weight and LVH on LV diastolic function have been reported (Syed et al 2009), to my knowledge no study has investigated the possible interactive effect of LV geometric remodelling on the association between metabolic disease and LV diastolic function. Hence, as part of the present thesis, *I aimed to evaluate whether the extent of LV concentric remodelling determines the impact of either obesity or insulin resistance on LV diastolic function*. These data have been published in the *International Journal of Cardiology* (Peterson[b] et al 2016) and are described in chapter 2 of the present thesis.

1.5.2 Hypertension as a cause of LV diastolic dysfunction

In the following section I will discuss hypertension as a possible cause of LV diastolic dysfunction. However, as with the topic of obesity and LV diastolic dysfunction it is important in the context of the present thesis to provide a more overall approach to the relevance of this topic from a perspective of hypertension as a cause of heart failure. In this regard, it is important to focus this discussion on

hypertension as a cause of heart failure with a preserved ejection fraction and a reduced LV diastolic function and the possible haemodynamic determinants through which hypertension may mediate these effects. Hence, in the following sections I will deal with each of these topics in turn.

1.5.2.1 Hypertension as a cause of heart failure and a reduced LV diastolic function

There is substantial evidence to support a role for hypertension as a major cause of heart failure. Indeed, hypertension is one of the most common comorbidities in heart failure (Levy et al 1996) and in primary care settings, approximately 48% of patients diagnosed with heart failure have hypertension (Cleland et al 2002). Moreover, in clinical trials of heart failure, hypertension is one of the four most frequently cited comorbidities (Krum and Gilbert 2003). The role of blood pressure in heart failure is highlighted by the ability of antihypertensive therapy to prevent the development of heart failure (Dahlof et al 1991, Kostis et al 1997, MRC Working Party Medical Research Council 1992). Hypertension may contribute to heart failure with either a reduced or preserved ejection fraction. Indeed, as previously highlighted, heart failure with a preserved ejection fraction is frequently associated with hypertension (Masoudi et al 2003, Redfield et al 2003, Owan et al 2006, Desai et al 2009, Kane et al 2011) and hypertension is a major co-morbidity associated with LV diastolic dysfunction (AlJaroudi et al 2012; Redfield et al 2003; Russo et al 2011; Sharp et al 2010). What is the evidence to support a role for hypertension as a major cause of LV diastolic dysfunction?

Several studies have shown relationships between systolic blood pressure (SBP) (Abhayaratna et al 2008; Chung et al 2010; Hsu et al 2010; Masugata et al 2005; Pavlopoulos et al 2008) or diastolic blood pressure (DBP) (AlJaroudi et al 2012; Hwang et al 2012; Tsioufis et al 2008; Libhaber et al 2014) and LV E/A. Importantly, in multivariate regression analysis the blood pressure most strongly related to E/A or LV diastolic dysfunction in some large studies is DBP rather than SBP (AlJaroudi et al 2012, Hwang et al 2012, Tsioufis et al 2008, Libhaber et al 2009) and more recently DBP has been demonstrated to be associated with E/A independent of SBP whilst SBP is not associated with E/A independent of DBP (Libhaber et al 2014). In contrast to what is noted with E/A, SBP, but not DBP, is more frequently associated with an index of LV filling pressures (E/e') (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, in one study both SBP

and DBP (Hwang et al 2012) were noted to be independently related to E/e'. Irrespective of the brachial blood pressure value that best associates with decreases in LV diastolic function in cross-sectional studies, importantly, antihypertensive therapy produces improvements in tissue Doppler measures of diastolic dysfunction and the blood pressure value which best associates with these improvements in diastolic function is SBP (Almuntaser et al 2009). Moreover, in cross-sectional analysis of a community-based study with a high prevalence of obesity, blood pressure contributed substantially more to LV diastolic dysfunction than did any other factor other than age (Millen et al 2014). Although the debate on the brachial blood pressure best associated with LV diastolic function is an important clinical debate, of more fundamental importance is the possibility that neither brachial SBP nor DBP are the best assessments to determine the impact of vascular load on LV diastolic function. What is the evidence to support a role of vascular haemodynamic factors that may not be best indexed by brachial blood pressure measurements as determinants of LV diastolic function?

1.5.2.2 Loading conditions responsible for LV diastolic dysfunction: The importance of aortic stiffness

Increased LV loading conditions in hypertension may be determined to a large extent by an interaction between pressures generated by ventricular stiffening in systole, (which may be enhanced in a concentrically remodelled LV), and the impedance to aortic flow generated by several factors, of which a stiffer than normal aorta is the most commonly cited. This interaction is frequently referred to as a "vascular–ventricular interaction" and may have a major role to play in determining LV diastolic dysfunction. This haemodynamic effect has been proposed to be a critical mechanism involved in the transition from compensated LVH to heart failure with a preserved ejection fraction (Borlaug et al 2007, Borlaug & Kass 2008). The increased LV systolic stiffness is thought to be mediated by the same mechanisms as the increased LV diastolic stiffness accompanying a heart with diastolic dysfunction (see section 1.2.1). The increased aortic stiffness is mediated by several factors of which the most important is age (Weber et al 2008; Desai et al 2009; Kang et al 2010; Scantlebury et al 2011), hence accounting for heart failure with a preserved ejection fraction presenting more frequently in the elderly. These age-induced changes in aortic stiffness then manifest as isolated systolic hypertension and hence hypertension, especially isolated systolic hypertension, is a common co-

morbidity in heart failure with a preserved ejection fraction (Masoudi et al 2003; Borlaug & Redfield 2011; Scantlebury & Borlaug 2011). However, the age-induced changes in aortic stiffness may be exacerbated by several additional factors, of which hypertension (increases in either DBP or SBP) is the most important cause. There is substantial evidence to support a role for aortic stiffness as an important determinant of LV diastolic dysfunction. In this regard, increases in large artery stiffness have frequently been associated with heart failure with a preserved ejection fraction (Mohammed et al 2012, Hundley et al 2001, Kawaguchi et al 2003, Kang et al 2010, Desai et al 2009, Kitzman et al 2013). Moreover, large artery stiffness has also often been shown to be associated with LV diastolic dysfunction even in patients without heart failure (Palmieri et al 2003; Yambe et al 2004; Tsioufis et al 2005; Mottram et al 2005; Masugata et al 2005; Borlaug et al 2007; Abhayaratna et al 2008; Weber et al 2008; Chung et al 2010; Borlaug 2011; Shim et al 2011; Xu et al 2011; Russo et al 2012; Coutinho et al 2013).

Although an increase in aortic stiffness is undisputedly an important cause of LV diastolic dysfunction and the progression from compensated LVH to heart failure with a preserved ejection fraction, aortic stiffness only begins to increase to a significant degree from an age of 50-60 years (Mitchell et al 2010, McEniery et al 2005, Hodson et al 2016), whereas community-based studies indicate that LV diastolic dysfunction can occur well before this age (Libhaber et al 2009, Libhaber et al 2014, Millen et al 2014). As LV diastolic dysfunction is determined by the adverse effects of LV load, another factor which should be considered as a possible important determinant of LV diastolic dysfunction is the load on the LV induced by increases in aortic backward (reflected) waves. In this regard, aortic backward waves begin to increase from early adulthood (McEniery et al 2005, Booysen et al 2015, Hodson et al 2016). What are aortic backward waves, what is the importance of aortic backward waves in contributing to LV load and do aortic backward waves contribute to LV diastolic dysfunction?

1.5.2.3 Loading conditions responsible for LV diastolic dysfunction: The importance of aortic backward waves

Pressure waves determined by ventricular ejection into the aorta (aortic forward waves) travel down arteries and encounter reflection points which may occur at innumerable sites in the arterial bed (Nichols et al 2011). These reflection points are generated by discontinuities in the arterial tree produced by branch points, changes in

wall structure and tapering of vessels. Increases in tapering of vessels may occur with vasoconstriction of either arterioles or of more proximal vessels. At these sites pressure waves are reflected back and return to the ascending aorta. Summation of reflected waves derived from multiple reflection points, results in a single backward wave returning to the aorta. The backward wave may return sufficiently early that the pressure generated by this wave (Pb) adds to the pressure generated by the forward wave (Pf) and hence Pb augments aortic SBP (Figure 1.2). With aging, because backward wave pressures (Pb) begin to increase from as early as 20-30 years of age, brachial SBP increases less than aortic SBP from early adult life to old age. This is because brachial SBP only detects the peak of the percussion wave which is driven by the forward wave pressure (Pf) (Figure 1.3). In contrast, aortic SBP increases more than brachial SBP as aortic SBP is determined by both Pf and Pb (Figure 1.3). With advanced age, Pb may be sufficiently large that peak brachial SBP reflects either Pf or Pb (Figure 1.3)(Nichols et al 2011). Hence, it is important to note that to adequately assess aortic backward wave pressure effects, aortic pressure measurements are required. Despite aortic backward wave pressures being lower than forward wave pressures (Figures 1.2 and 1.3), there is now significant evidence that aortic backward waves contribute more than forward waves to increases in aortic pulse pressure and LV mass across the adult lifespan (Booysen et al 2015). The relatively greater contribution of the aortic backward as compared to the forward wave pressures to variations in aortic pulse pressure and left ventricular mass applies to several other end-organ changes as well (Sibiya et al 2015) and translates into a greater contribution of aortic backward wave pressures as compared to forward wave pressures to cardiovascular outcomes (Zamani et al 2015). Hence, the question arises as to the role of aortic backward (reflected) wave pressures as possible determinants of LV diastolic function beyond aortic forward wave pressures. What is the evidence to support a role of aortic reflected wave function as a determinant of LV diastolic function and the development of heart failure with a preserved ejection fraction and is there clarity on the role of aortic backward waves in this regard?

Few studies have provided the evidence to suggest that increases in aortic backward (reflected) waves are associated with LV diastolic dysfunction (Weber et al 2006, Weber et al 2008, Ikonomidis et al 2008, Hashimoto et al 2006). Moreover, in one small study (n=188) a dominant role of aortic stiffness over aortic augmented pressures, an index of aortic backward wave function, in the identification of LV diastolic dysfunction was noted (Abhayaratna[a] et al 2006) and in another small study

(n=53) (Desai et al 2009) a relationship between aortic stiffness, but not aortic augmentation index and heart failure with a preserved ejection fraction was reported.

This raises the possibility that aortic backward waves may not play as significant a role in contributing toward LV diastolic dysfunction and ultimately heart failure with a preserved ejection fraction as do increases in aortic stiffness. From a preventative perspective however, whilst there is little evidence to support a therapeutic approach to modify age-related increases in aortic stiffness, there is significant evidence to suggest that aortic backward wave pressures may be targeted by conventional antihypertensive therapy (Agabiti-Rosei et al 2007). Importantly, as indicated in previous sections, marked age-related increases in backward wave pressures precede significant age-related increases in aortic stiffness by several decades (Booysen et al 2015). Moreover, increases in aortic backward wave pressures are a fundamental mechanism responsible for increases in aortic stiffness (Sibiya et al 2015) and hence may be the initial (primary) target in preventing the progression to LV diastolic dysfunction and consequently heart failure with a preserved ejection fraction.

However, it is uncertain whether the impact of backward wave pressures on LV diastolic dysfunction antedate the effects of aortic stiffness. In this regard, prior studies assessing relations between aortic and LV diastolic function have assessed patients with average ages of 60-77 years, (Mohammed et al 2012; Hundley et al 2001; Kawaguchi et al 2003; Weber et al 2006; Weber et al 2008; Fernandes et al 2008; Kang et al 2010; Desai et al 2009; Kitzman et al 2013), age ranges when large vessel stiffness is markedly increased (Mitchell et al 2010, Hodson et al 2016). Hence, none of these studies could address the question of whether the impact of backward wave pressures on LV diastolic dysfunction antedate the effects of aortic stiffness. Hence, as part of the present thesis, *I aimed to evaluate the relationship between several aortic functional parameters and LV diastolic function in a predominantly young-to-middle aged community-based sample with an average age where increases in aortic backward wave pressures are well established, but prior to an age when striking increases in aortic stiffness are noted*. These data have been published in the *American Journal of Hypertension* (Peterson[c] et al 2016) and are described in chapter 3 of the present thesis.

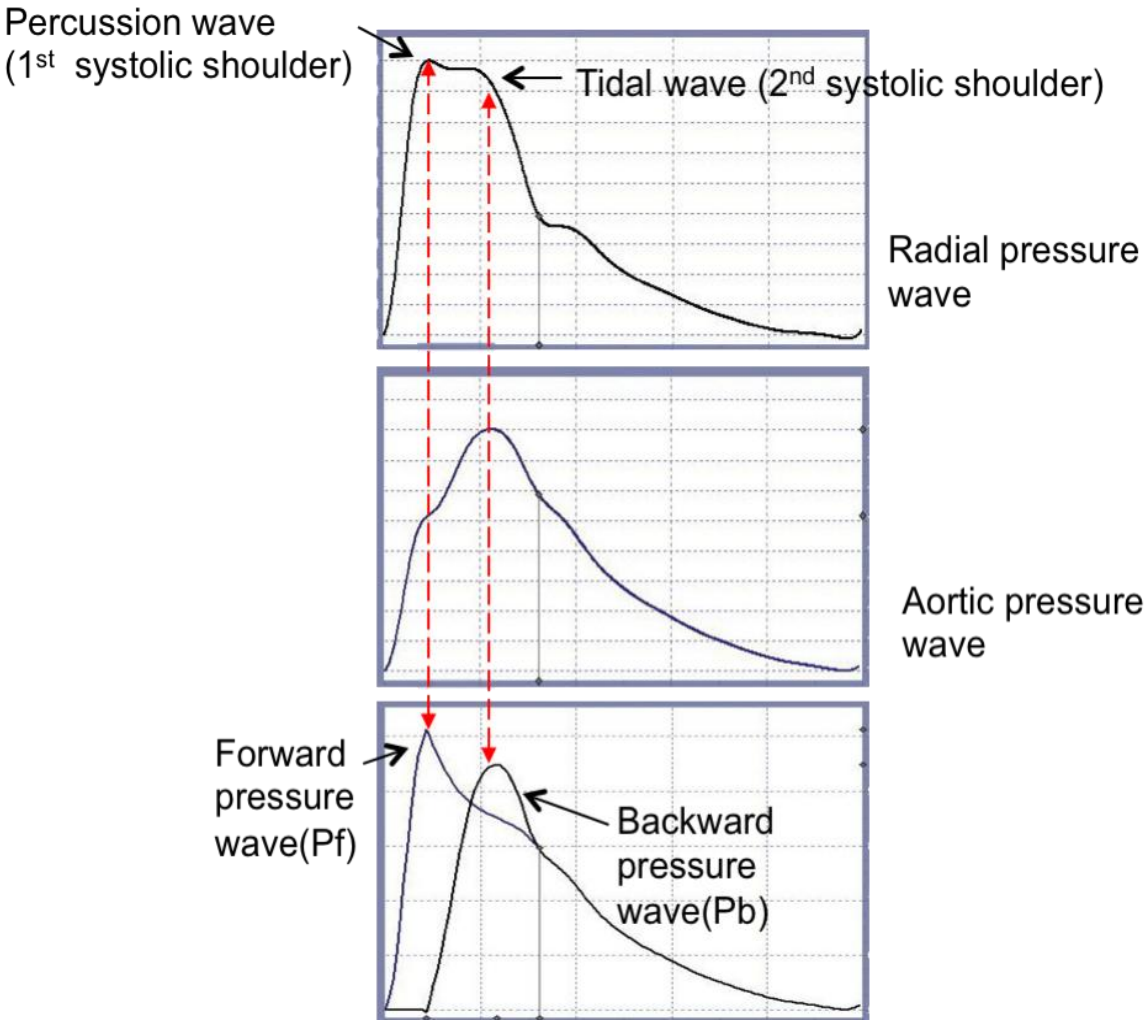


Figure 1.2. The contribution of aortic forward and aortic backward waves to aortic and radial (approximate of brachial) pulse waves. The dashed lines show temporal alignment of 1st and 2nd systolic shoulders. The figure shows actual data obtained from SphygmoCor recordings.

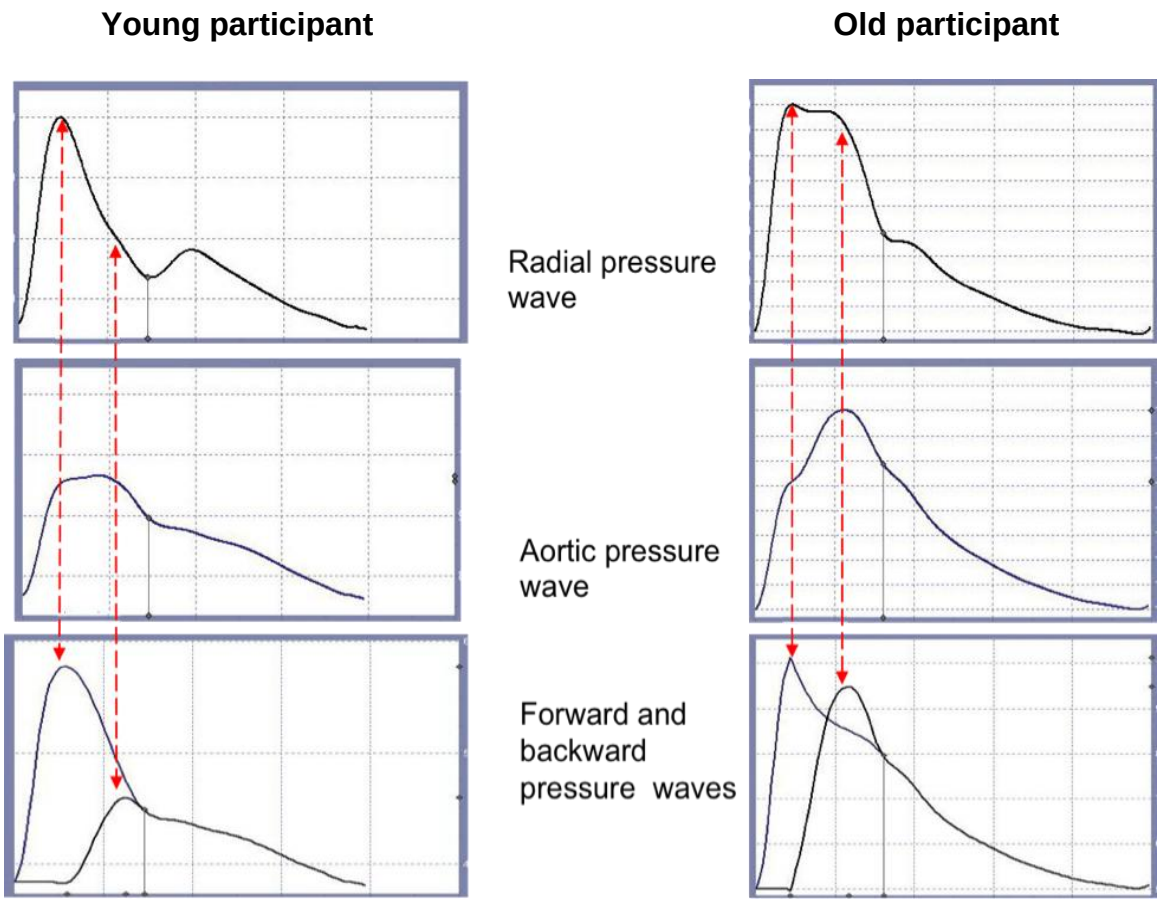


Figure 1.3 Age effects on aortic and radial artery pressure waves (which approximate brachial pressure waves). The figure shows changes in the combined effect of the aortic forward and aortic backward waves on pressure waveforms with age. The dashed lines show how the forward and backward pressure waves contribute to radial and aortic pressure waves in a young and an old participant. The figures show actual data obtained from SphygmoCor recordings.

1.5.3 Genetic factors may play a significant role in determining LV diastolic dysfunction

The well recognised demographic and clinical risk factors for heart failure with a preserved ejection fraction, which include age, female gender, obesity, hypertension and diabetes mellitus fail to account for all of the inter-individual variation in LV diastolic function (Bielin et al 1991; Fox et al 2010; Jin et al 2011; Kloch-Badelek et al 2014; Noh et al 2015; Swan et al 2003; Tang et al 2002). Moreover, ethnicity is reported to impact on the prevalence of LV diastolic dysfunction and ultimately heart failure with preserved ejection fraction (Bhatia et al 2006; Yancy et al 2006; Fonarow et al 2007; Lam et al 2007; Scantlebury & Borlaug 2011; Chirinos et al 2012; Nichols et al 2004; Mozaffarian et al 2015). Hence, independent of well recognised demographic and clinical risk factors for heart failure with a preserved ejection fraction, genetic factors may be responsible for some of the unexplained inter-individual variation in LV diastolic function. In this regard because of the impact of LV remodelling on LV diastolic function (see section 1.5.1.4), these genetic factors may contribute toward LV diastolic dysfunction through the effects of LV remodelling. Alternatively independent of LV structural changes, genetic factors may influence LV diastolic function through direct effects on cardiomyocyte function or through interstitial changes. In this regard, as will be discussed, both LVH and LV relative wall thickness as well as LV diastolic function have been shown to aggregate in families and to be inherited. However, at the time of conducting the present thesis there were several aspects of these findings which required further clarity. Before embarking on attempting to identify the genetic factors that could explain LV remodelling and LV diastolic function, a better understanding of these issues is required. In the following section I will therefore review the current understanding of the evidence for genetic factors contributing to LV remodelling and diastolic function, including the evidence on the intra-familial aggregation and heritability of LV remodelling and diastolic function. In doing so I will also highlight the outstanding issue on these topics and hence argue in favor of several of the studies conducted as part of the present thesis.

1.5.3.1 Possible role of genetic factors as determinants of LV remodelling.

The impact of age (Dannenberg et al 1989), sex (Aurigemma & Gaasch 1989), blood pressure (Devereux et al 1983), and obesity (Cuspidi et al 2014; Lauer

et al 1991; Woodiwiss & Norton 2015) on LV geometric remodelling has been well described. However, these factors only partly explain the inter-individual variation in LV mass and geometry in any population (Bella & Goring 2012). Furthermore, the prevalence of LVH and geometric remodelling is reported to differ amongst various population groups. In this regard, populations of African ancestry are reported to have a higher LV mass (Arnett et al 1994; Fox et al 2010) as well as a greater LV relative wall thickness (Arnett et al 1994; Devereux et al 1998; Tang et al 2002; Fox et al 2010) in comparison to other populations. Although various factors, including blood pressure levels and blood pressure control, could explain these ethnic differences, a greater LV wall thickness for the same level of clinic blood pressure has been reported in African as compared to white Americans (Devereux et al 1998; Tang et al 2002). Hence, it has been suggested that these differences in LV mass and relative wall thickness between population groups have a genetic basis. Indeed, LV mass (Arnett et al 2001; Assimes et al 2007; Bella et al 2004; Bella et al 2012; Bielen et al 1991; Fox et al 2010; Harshfield et al 1990; Kuznetsova et al 2003; Schunkert et al 1999; Tang et al 2002; Verhaaren et al 1991) and LV relative wall thickness have been shown to be inherited in various populations (Arnett et al 2001; Bella et al 2004; Fox et al 2010; Kuznetsova et al 2003). There is however uncertainty as to the role of genetic factors in determining LV relative wall thickness independent of LV mass. In this regard LV mass is a major determinant of LV wall thickness (Harshfield et al 1990; Verhaaren et al 1991; Bielen et al 1991; Schunkert et al 1999; Arnette et al 2001; Kuznetsova et al 2003; Bella et al 2004; Assimes et al 2007; Bella & Goring 2012). It is important to distinguish between these two factors for several reasons. First, an increased LV relative wall thickness, although not a predictor of heart failure *per se*, will determine whether LVH progresses to LV diastolic or LV systolic heart failure. Indeed, concentric LV remodeling is associated with the development of LV diastolic dysfunction (Chahal et al 2010). Second, an increased LV relative wall thickness may be determined not just by the extent of LVH, but by several other cellular factors (Badenhorst et al 2003). Hence, if LV relative wall thickness is inherited, and LV remodelling is a major determinant of LV diastolic function, then the genetic determinants of LV diastolic dysfunction are not only going to be regulated by those factors which influence the extent of hypertrophy of the heart, but rather those that determine the remodelling process itself. Is there evidence to either support or refute a role for LV relative wall thickness being inherited independent of LV mass?

Siblings of those with LVH have a greater risk of concentric LVH as compared to age- and sex-matched controls (Schunkert et al 1999). In that study (Schunkert et al 1999), the siblings of those with LVH had no greater risk of eccentric LVH than the age- and sex-matched controls. Hence, the possibility arises that the intrafamilial clustering of concentric LVH in that study (Schunkert et al 1999) was in-part driven by the LV remodelling process. However, whether these siblings had a greater risk of concentric LV remodelling was not reported. Hence, it is also possible that the increased risk of concentric LVH in that study (Schunkert et al 1999) may have been largely a consequence of LV mass effects. Furthermore, in the Framingham Heart Study although the risk of concentric LVH was markedly greater in related as compared to unrelated individuals, the risk of concentric LV remodelling was reported to be only modestly greater in related compared to unrelated individuals (Lam et al 2010). These data (Lam et al 2010) again point toward the possibility that the inheritance of LV mass explains the inheritance of LV concentric remodelling. Although, a number of previous studies report that LV relative wall thickness is inherited (Arnett et al 2001; Bella et al 2004; Fox et al 2010; Kuznetsova et al 2003), none of these previous studies have reported on the inheritance of LV relative wall thickness independent of LV mass. Hence, as part of the present thesis, I aimed to evaluate the intrafamilal aggregation and heritability of LV relative wall thickness independent of LV mass in a group of African ancestry. These data have been published in the *American Journal of Hypertension* (Peterson et al 2015) and are described in chapter 4 of the present thesis.

1.5.3.2 Possible role of genetic factors as determinants of LV diastolic function beyond confounders.

What is the evidence for a possible contribution of genetic factors to LV diastolic function and is there a need for further clarity on this issue? Several studies have demonstrated heritability or intra-familial aggregation of LV diastolic function identified from trans-mitral blood flow velocity measurements. In this regard, studies in twins have reported heritability estimates of 43% and 49% for the E and 26% and 36% for the A wave respectively (Bielen et al 1991; Swan et al 2003). Similarly, in black and white hypertensive sibships, it was estimated that 54% to 77% of the genetic variation in E and A waves, respectively, were due to shared genes (Tang et al 2002). Furthermore, in an African-American cohort of hypertensive siblings, heritability estimates of 37% and 45% for E and A waves respectively were reported

(Fox et al 2010). In family-based studies, heritability estimates ranging from 11% to 29% for the E wave, from 25% to 27% for the A wave (Jin et al 2011; Kloch-Badelek et al 2014), and of 25% for E/A (Noh et al 2015) have been reported.

Despite consistent evidence for the heritability of E and A waves across various populations, the genetic influence on contemporary measures employed to evaluate LV diastolic dysfunction, such as e' or E/e' (as discussed in section 1.3.2), is nevertheless unclear. In this regard, few studies have reported on the heritability of E/e'. Although a Korean twin and family study showed significant heritability (33%) for E/e' (Noh et al 2015), a study conducted in 52 European families failed to show significant heritability (Jin et al 2011). However, subsequent analysis in an expanded data set (316 European families) showed significant heritability (31%) (Kloch-Badelek et al 2014). Although the heritability of E/e' has been assessed in Caucasian and Asian groups, whether E/e' is inherited in groups of African ancestry has not been assessed. Hence, the inheritance of E/e' is controversial, and whether E/e' is inherited in groups of African ancestry is unknown.

Importantly, LV mass and LV remodelling which, as previously discussed in section 1.5.1.4, are well recognised determinants of LV diastolic dysfunction (Chahal et al 2010) are also inherited (see section 1.3.4.3 above and Peterson et al 2015). In addition, other factors which influence LV diastolic function such as blood pressure, aortic function, obesity and diabetes mellitus or insulin resistance (see sections 1.5.1 and 1.5.2 above) are also inherited (see section 1.5.3.). Hence, it is possible that the heritability of E/e' is by virtue of genetic influences on factors such as LV mass, LV relative wall thickness, brachial and aortic blood pressure, body mass, diabetes mellitus and insulin resistance. Is there sufficient evidence to indicate that the heritability of LV E/e' is independent of these potential confounders? In this regard, the heritability of E/e' has been shown to be independent of body mass index and brachial blood pressure (Kloch-Badelek et al 2014). In addition, Noh et al (2015) reported heritability of E/e' after adjusting for confounding factors including body mass index, brachial blood pressure and diabetes mellitus (fasting blood glucose and receiving medication for diabetes mellitus). However, these previous studies (Jin et al 2011; Kloch-Badelek et al 2014; Noh et al 2015) failed to determine whether E/e' is inherited independent of LV mass and relative wall thickness. Hence, whether significant heritability of E/e' occurs independent of LV remodelling is currently unknown. Moreover, although previous studies have reported on the inheritance of E/e' independent of brachial blood pressure (Kloch-Badelek et al 2014; Noh et al 2015), whether heritability of E/e'' is independent of aortic BP is unknown. In this

regard, central aortic blood pressure has been reported to show stronger heritability estimates than brachial blood pressure (Redelinghuys et al 2012). Hence, to address the aforementioned questions, as part of the present thesis, *I aimed to evaluate whether the intrafamilal aggregation and heritability of LV diastolic function is independent of LV mass, LV relative wall thickness and aortic blood pressure in a group of African ancestry.* These data have been published in the *Journal of the American Society of Hypertension* (Peterson[a] et al 2016) and are described in chapter 5 of the present thesis.

1.6 Aims

In summary, the aims of the present thesis are as follows:

1) To determine whether relations between adiposity indices or associated-insulin resistance and LV diastolic function are modified by LV mass or relative wall thickness and if so whether these interactive effects with LVH or remodelling are also noted for alternative causes of LV diastolic abnormalities such as increases in blood pressure or age. These data are described in chapter 2 of the present thesis and have been published in the *International Journal of Cardiology* (Peterson[b] et al 2016).

2) To determine whether the impact of aortic backward wave pressure on LV diastolic dysfunction antedates the effects of aortic stiffness in a predominantly young-to-middle–aged community-based sample with an average age where increases in aortic backward wave pressures are well established, but prior to an age when striking increases in aortic stiffness are noted. These data are described in chapter 3 of the present thesis and have been published in the *American Journal of Hypertension* (Peterson[c] et al 2016).

3) To determine whether LV relative wall thickness shows intra-familial aggregation and heritability independent of LV mass and additional confounders in a family-based study. These data are described in chapter 4 of the present thesis thesis and have been published in *American Journal of Hypertension* (Peterson et al 2015).

4) To determine whether the intra-familial aggregation and heritability of LV filling pressures (E/e') occur independent of LV remodelling and aortic function in a community of African ancestry. These data are described in chapter 5 of the present thesis and have been published in *Journal of the American Society of Hypertension* (Peterson[a] et al 2016).

CHAPTER 2

Insulin Resistance-Associated Decreases in Left Ventricular Diastolic Function are Strongly Modified by the Extent of Concentric Remodelling in a Community Sample.

The data in this chapter have been published in the International Journal of Cardiology:

Peterson V.R., Norton G.R., Raymond A., Libhaber C.D., Millen A.M.E., Majane O.H.I., Maseko M.J. and Woodiwiss, A.J. (2016). Insulin Resistance-Associated Decreases in Left Ventricular Diastolic Function are Strongly Modified by the Extent of Concentric Remodelling in a Community Sample, *International Journal of Cardiology*, 220, 349-355.

2.1 Abstract

Background. Whether excess adiposity, associated metabolic abnormalities or alternative risk factors for left ventricular (LV) diastolic function are modified rather than mediated by geometric LV remodelling, is uncertain.

Methods. Echocardiographic LV mass index (LVMI), relative wall thickness (RWT) and diastolic function (lateral and septal wall myocardial tissue lengthening at the level of the mitral annulus [e'] [n=430], ratio of early-to-late transmitral blood flow velocity (E/A), and E/e' [n=430]) were determined in 737 randomly recruited participants of a community-based study (43% obese).

Results. Independent of LVMI and confounders, indices of adiposity and the homeostasis model of insulin resistance (HOMA-IR) were independently associated with LV diastolic function ($p<0.05$). In addition, RWT was independently associated with LV diastolic function ($p<0.002$). Importantly, an independent interaction between HOMA-IR and RWT, but not between blood pressure or age and RWT, was related to LV diastolic function ($p<0.05$). This translated into an independent relationship between HOMA-IR and lateral e' (partial $r=-0.17$, $p<0.02$), septal e' (partial $r=-0.14$, $p=0.05$), E/A (partial $r=-0.17$, $p<0.005$) and E/e' (partial $r=0.19$, $p<0.01$) in those with RWT above, but a lack of relationship between HOMA-IR and LV diastolic function ($p>0.59$) in those with RWT below the median for the sample. Similarly, HOMA-IR was independently associated with LV diastolic dysfunction in those with RWT above ($p<0.05$) but not below ($p>0.19$) the median for the sample.

Conclusions. The relationship between insulin resistance, but not alternative risk factors and LV diastolic function is markedly modified by the presence of a more concentrically remodelled LV.

2.2 Introduction

Independent of conventional cardiovascular risk factors and myocardial infarction, obesity and associated insulin resistance predicts the development of heart failure (Kenchaiah et al 2002; Ingelsson et al 2005; Bahrami et al 2008). The impact of obesity or insulin resistance on the risk for heart failure may in-part be explained by abnormalities in left ventricular (LV) diastolic function. Indeed, LV diastolic abnormalities are correlated with obesity (Tsioufis et al 2008; Russo et al 2011; Çil et al 2012; AlJaroudi et al 2012) independent of haemodynamic confounders (Libhaber et al 2009); improved by bariatric surgery-induced weight loss (Cuspidi et al 2014); and predict the development of heart failure (Aurigemma et al 2001; Kane et al 2011; Lam et al 2011). However, the relative importance of obesity, as opposed to alternative co-morbidities as a determinant of LV diastolic dysfunction has recently been questioned (Millen et al 2014). Hence identifying clinical features that characterise those in whom weight loss may be of benefit in preventing the progression to heart failure is of importance.

Well-recognised determinants of a reduced LV diastolic function are LV hypertrophy (LVH) and concentric LV remodelling (as indexed by relative wall thickness [RWT]) (Chahal et al 2010). As obesity causes LVH and several studies have shown associations between obesity and an increased RWT (concentric LV remodelling) (Cuspidi et al 2014; Woodiwiss & Norton 2015), the question arises as to whether LVH and/or RWT modify the impact of obesity or associated metabolic alterations on diastolic LV function. In this regard, although numerous studies have reported an association between metabolic disease and diastolic dysfunction independent of LVH (Masaguta et al 2006; de las Feuntes et al 2007; Ayalon et al 2014); to our knowledge no study has investigated the possible interactive (synergistic) effect of LV remodelling on the association between metabolic disease or alternative risk factors and diastolic dysfunction. Importantly, LV remodelling may not mediate the effect of a risk factor, but may have a pronounced modifying influence on the impact of a risk factor on LV diastolic function. Indeed, as strong correlations between change in RWT and diastolic function (Hsuan et al 2010) and consistent decreases in RWT and increases in diastolic function (Hsuan et al 2010; Ippisch et al 2008; Luaces et al 2012) occur with bariatric surgery, it is possible that RWT if not mediating the impact of metabolic disease on diastolic function, may modify the impact of metabolic disease on diastolic function. This would suggest that targeting metabolic disease may only influence LV diastolic function in a more

concentrically remodelled LV. However, whether obesity- or insulin resistance-associated LV diastolic abnormalities are influenced by LV geometric remodelling is unknown. Hence, in the present study conducted in a relatively large community-based sample with a high prevalence of obesity-associated LVH (Woodiwiss et al 2008; Libhaber et al 2014) and increases in RWT (Woodiwiss et al 2008), I evaluated whether relations between adiposity indexes or associated-insulin resistance and LV diastolic function are modified by LV mass or RWT and if so whether these interactive effects with LVH or remodelling are also noted for alternative causes of LV diastolic abnormalities such as increases in blood pressure or age.

2.3 Methods

2.3.1 Study group

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 and M12-04-108). Participants gave informed, written consent. The study design has previously been described (Woodiwiss et al 2008; Libhaber et al 2014; Woodiwiss et al 2009). 737 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. Tissue Doppler measures of myocardial function were obtained in a sub-study conducted in 430 participants.

2.3.2 Clinical, demographic, blood and anthropometric measurements

A standardised questionnaire was administered to obtain demographic and clinical data (Woodiwiss et al 2008; Libhaber et al 2014; Woodiwiss et al 2009). 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 haemoglobin (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 hypoglycaemic agents or a glycated haemoglobin (Roche Diagnostics, Mannheim, Germany) value greater than 6.1%. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin [uU/ml] x glucose [mmol/l])/22.5.

Nurse-derived conventional blood pressure (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; Redelinghuys et al 2010). 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 >140/90 mm Hg in those not receiving medication.

2.3.3 Echocardiography

Echocardiographic measurements were performed as previously described (Millen et al 2014; Libhaber et al 2014) by two experienced observers (AJW and CDL) using a Sonosite M-Turbo ultrasound (SonoSite® Inc., Bothell, WA, USA) device 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 (LV) dimensions were determined using two-dimensional directed M-mode echocardiography in the short axis view (Figure 2.1) and these recordings were analysed according to the American Society of Echocardiography convention (Sahn et al 1978). The LV dimensions were measured only when appropriate visualisation 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) (LVM = 0.8 x [1.04 (LV end diastolic diameter + LV end diastolic septal wall thickness + LV end diastolic posterior wall thickness)^3 – (LV end diastolic diameter)^3] + 0.6g) and indexed (LVMI) to height$^{1.7}$(at the request of reviewers, I used height$^{1.7}$ and not height$^{2.7}$ as was used in subsequent chapters).

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

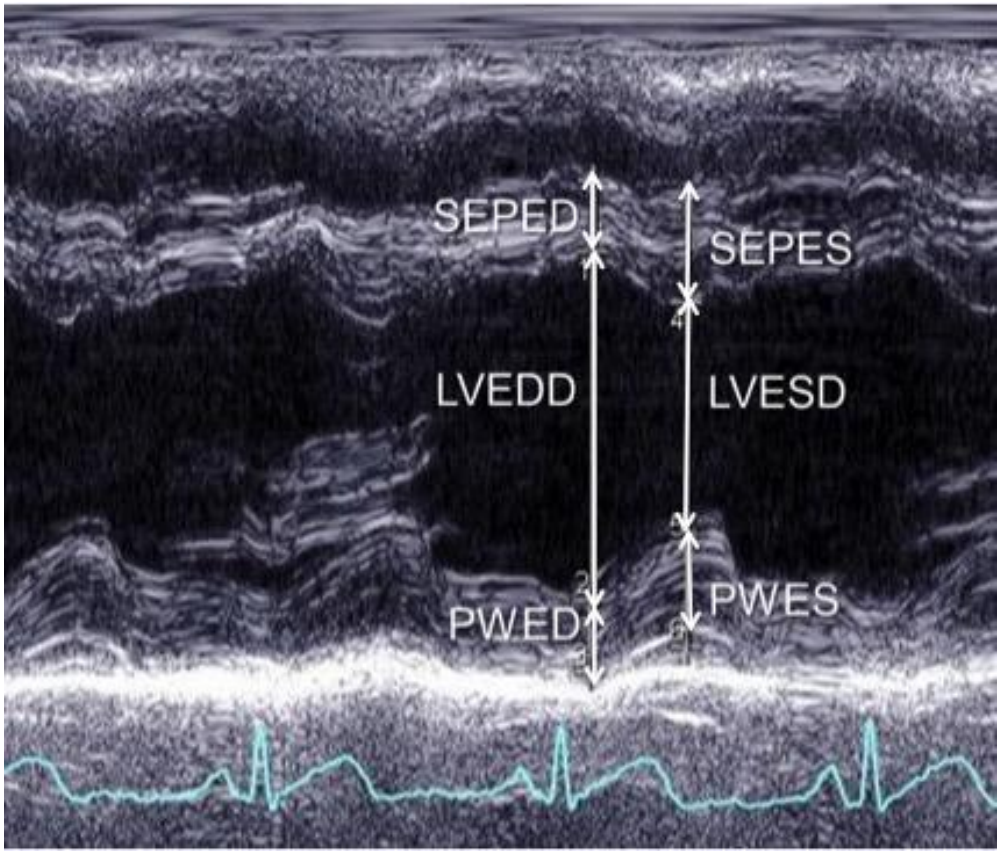


Figure 2.1 Echocardiographic M-mode image showing the measurement of left ventricular dimensions. LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; PWED, left ventricular posterior wall thickness at end diastole; PWES, left ventricular posterior wall thickness at end systole; SEPED, left ventricular septal wall thickness at end diastole; SEPES, left ventricular septal wall thickness at end systole.

as left atrial volumes (Nagueh et al 2009). 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) and late (atrial-A) 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') and late (atrial) (a') diastole were measured and the mean of septal and lateral wall values calculated. 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) (Figure 2.2). The presence of left ventricular diastolic dysfunction (DD) was identified by lateral e'<10 cm/s, septal e'<8 cm/s or left atrial volume ≥34ml/m^2. Mild DD was defined as E/A≤0.80 (impaired relaxation) or E/e'≤8. Moderate-to-severe DD was defined as E/A≥0.80 or E/e'≥9.

2.3.4 Data analysis

Database management and statistical analyses were performed with SAS software, version 9.3 (SAS Institute Inc., Cary, North Carolina, USA). Data from individuals were averaged and expressed as mean±SD. To improve on distribution of data, HOMA-IR, lateral e', septal e', E/A, E/e' and left atrial volume 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. As we have recently demonstrated that systolic BP (SBP) is more closely related to E/e' and diastolic BP (DBP) to E/A (Libhaber et al 2014), SBP was employed in models with lateral e', septal e' and E/e' and DBP in models with E/A. Relationships (partial r values) were compared with z-statistics.

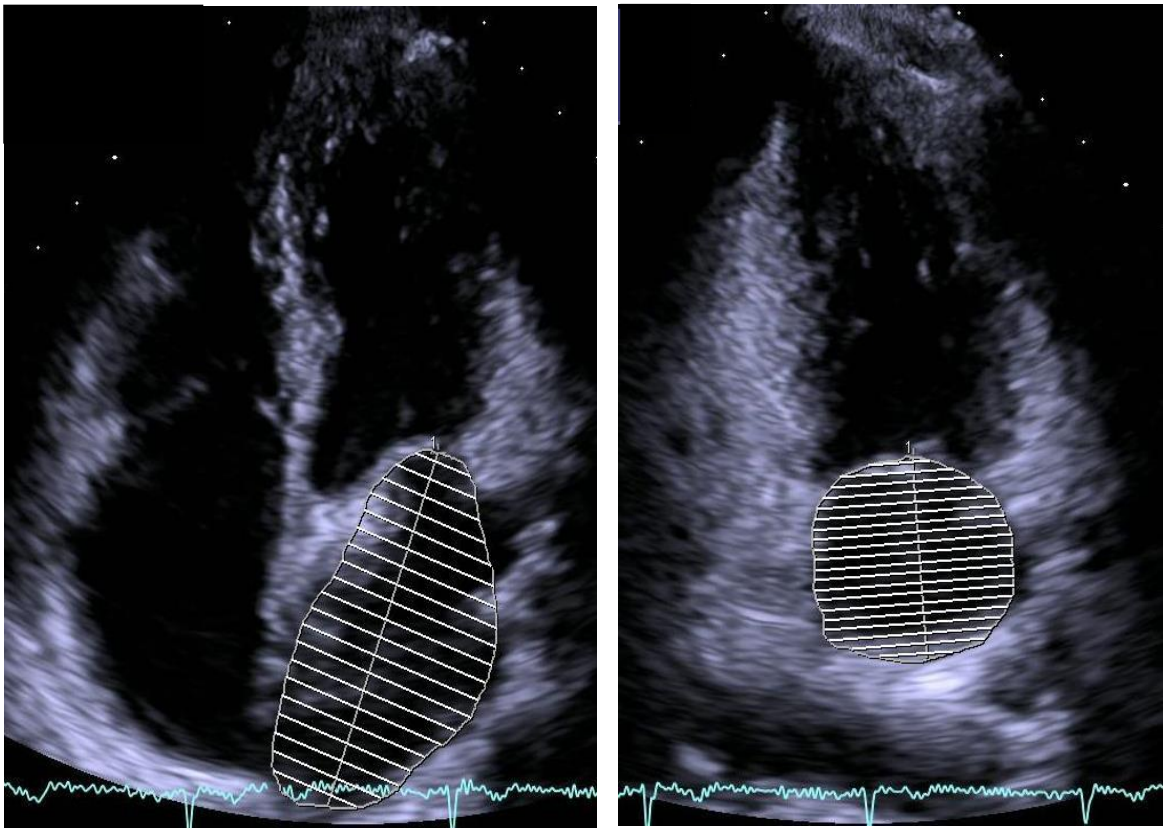


Figure 2.2 Echocardiographic images showing the measurement of left atrial area and long axis lenght by planimetry in the apical four-chamber (left image) and two-chamber (right image) views at left ventricular end systole (maximum left atrial dimensions). These measurements were used to calculate left atrial volume indexed to body surface area using the area-length method.

2.4 Results

2.4.1 Participant characteristics

Table 2.1 gives the demographic and clinical characteristics of the participants. More women than men participated in the study and a high proportion of participants were overweight, obese or morbidly obese and had central obesity. In participants with TDI measurements, 13.5% of participants had mild DD, and 16.5% had moderate-to-severe DD. No participants had an ejection fraction<40% and 3.1% had an ejection fraction<50%. Of the sample 47% had LV hypertrophy (LVMI>80 $g/m^{1.7}$ for men and >60 $g/m^{1.7}$ for women) and 26.3% had concentric LV remodelling (RWT>0.42). 42.5% of participants with normal diastolic function had RWT greater than the median and 31.0% and 33.8% of participants with mild or moderate-to-severe DD respectively had RWT<median.

2.4.2 LV mass and remodelling are associated with LV diastolic function

With adjustments for age, sex, diastolic BP, regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%), and pulse rate both LVMI (partial r=-0.08, 95% CI=-0.15 to -0.01, p<0.05) and RWT (partial r=-0.12, 95% CI=-0.19 to -0.05, p<0.005) were associated with log E/A. Moreover, with adjustments for the same confounders except pulse rate and with systolic rather than diastolic BP in the model, RWT (partial r=0.14, 95% CI=0.04 to 0.23, p<0.01) but not LVMI (partial r=0.07, 95% CI=-0.03 to 0.16, p=0.17) was associated with E/e'. Similarly, RWT (lateral e': partial r=-0.14, 95% CI=-0.24 to -0.05, p=0.005; septal e': partial r=-0.18, 95% CI=-0.27 to -0.09, p=0.0001) but not LVMI (lateral e': partial r=-0.09, 95% CI=-0.18 to 0.01, p=0.07; septal e': partial r=-0.08, 95% CI=-0.17 to 0.01, p=0.10) was associated with lateral e' and septal e'.

2.4.3 Metabolic factors and LV diastolic function

With adjustments for confounders, BMI, waist circumference and HOMA-IR were all independently associated with E/A, E/e', lateral e' (Table 2.2) and septal e' (Table 2.3). However, with further adjustments for either LVMI or RWT, only relations between waist circumference or HOMA-IR and E/A or E/e' persisted (Table 2.2). With

Table 2.1. Characteristics of the study sample.

Sample number (% female)	737 (64.0)
Age (years)	44.8±18.0
Body mass index (kg/m^2)	29.6±7.8
% Overweight/obese/morbidly obese	23.6/19.9/24.3
Waist circumference (cm)	91.3±16.6
% Abnormal waist circumference	45.8
Regular tobacco (% subjects)	14.5
Regular alcohol (% subjects)	19.5
% Diabetes mellitus or an HbA_{1c}>6.1%	26.6
% Hypertensive	45.9
% Treated hypertension	25.1
HOMA-IR	3.30±4.85
Brachial SBP/DBP (mm Hg)	130±22/84±12
E/A	1.27±0.48
E/e' (n=455)	7.81±4.38
Lateral e' (cm/s) (n=455)	10.9±3.7
Septal e' (cm/s) (n=455)	9.6±3.4
Left atrial volume index (ml/m^2) (n=455)	19.7±6.6
Left ventricular mass index (g/m$^{1.7}$)	68.3±23.9
LV relative wall thickness	0.39±0.08

HbA_{1c}, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic BP; HOMA-IR, homeostasis model of insulin resistance; E/A, ratio of transmitral early-to-atrial (late) blood flow velocity; E/e', E/velocity of the mean value of lateral and septal wall myocardial tissue lengthening in early diastole at the mitral annulus; LV, left ventricle.

Table 2.2. Multivariate adjusted relationships between indexes of adiposity or the homeostasis model of insulin resistance (HOMA-IR) and left ventricular diastolic function in a community sample.

	Adjustments	Partial r (95% CI)	p value
<u>Waist circumference versus</u>			
Log E/A (n=737)	*	-0.10 (-0.17 to -0.02)	<0.05
	* + LVMI	-0.08 (-0.16 to -0.01)	<0.05
	* + RWT	-0.09 (-0.17 to -0.02)	<0.05
Log E/e' (n=430)	*	0.13 (0.03 to 0.23)	<0.01
	* + LVMI	0.12 (0.02 to 0.22)	<0.02
	* + RWT	0.12 (0.02 to 0.22)	<0.02
Log lateral e' (n=430)	*	-0.14 (-0.23 to -0.04)	<0.01
	* + LVMI	-0.12 (-0.22 to -0.03)	<0.02
	* + RWT	-0.13 (-0.23 to -0.03)	<0.02
<u>Body mass index versus</u>			
Log E/A (n=737)	*	-0.07 (-0.15 to -0.001)	<0.05
	* + LVMI	-0.05 (-0.13 to 0.02)	=0.15
	* + RWT	-0.07 (-0.14 to 0.000)	=0.05
Log E/e' (n=430)	*	0.10 (0.003 to 0.19)	<0.05
	* + LVMI	0.08 (-0.01 to 0.18)	=0.09
	* + RWT	0.09 (-0.01 to 0.18)	=0.07
Log lateral e' (n=430)	*	-0.13 (-0.23 to -0.04)	<0.01
	* + LVMI	-0.11 (-0.21 to -0.02)	<0.02
	* + RWT	-0.12 (-0.21 to -0.03)	<0.02
<u>Log HOMA-IR versus</u>			
Log E/A (n=737)	*	-0.11 (-0.18 to -0.04)	<0.005
	* + LVMI	-0.10 (-0.17 to -0.03)	<0.01
	* + RWT	-0.10 (-0.17 to -0.03)	<0.01
Log E/e' (n=430)	*	0.12 (0.02 to 0.21)	<0.02
	* + LVMI	0.11 (0.01 to 0.20)	<0.05
	* + RWT	0.11 (0.01 to 0.20)	<0.05
Log lateral e' (n=430)	*	-0.12 (-0.21 to -0.02)	<0.02
	* + LVMI	-0.11 (-0.20 to -0.01)	<0.05

* + RWT	-0.10 (-0.20 to -0.01)	<0.05

E/A, ratio of transmitral early-to-atrial (late) blood flow velocity; E/e', E/velocity of the mean value of lateral and septal wall myocardial tissue lengthening in early diastole at the mitral annulus; LVMI, left ventricular mass index; RWT, relative wall thickness. *Adjustments are for age, sex, diastolic blood pressure (E/A) or systolic blood pressure (E/e' and lateral e'), regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%), pulse rate (E/A) and LVMI or RWT as indicated.

Table 2.3. Multivariate adjusted relationships between indexes of adiposity or the homeostasis model of insulin resistance (HOMA-IR) and left ventricular diastolic function in a community sample.

	Adjustments	Partial r (95% CI)	p value
Waist circumference versus			
Log septal e' (n=430)	*	-0.18 (-0.27 to -0.08)	<0.0005
	* + LVMI	-0.16 (-0.26 to -0.06)	=0.001
	* + RWT	-0.17 (-0.26 to -0.07)	<0.001
Log LA vol (n=430)	*	0.05 (-0.05 to 0.15)	=0.28
	* + LVMI	0.01 (-0.09 to 0.11)	=0.81
	* + RWT	0.05 (-0.05 to 0.15)	=0.33
Body mass index versus			
Log septal e' (n=430)	*	-0.17 (-0.26 to -0.07)	=0.0005
	* + LVMI	-0.14 (-0.23 to -0.05)	<0.005
	* + RWT	-0.15 (-0.25 to -0.06)	<0.005
Log LA vol (n=430)	*	0.07 (-0.03 to 0.16)	=0.15
	* + LVMI	0.02 (-0.08 to 0.11)	=0.73
	* + RWT	0.06 (-0.03 to 0.16)	=0.19
Log HOMA-IR versus			
Log septal e' (n=430)	*	-0.11 (-0.20 to -0.02)	<0.05
	* + LVMI	-0.10 (-0.20 to -0.01)	<0.05
	* + RWT	-0.10 (-0.19 to -0.01)	<0.05
Log LA vol (n=430)	*	0.05 (-0.05 to 0.15)	=0.30
	* + LVMI	0.03 (-0.06 to 0.13)	=0.49
	* + RWT	0.05 (-0.05 to 0.14)	=0.36

LVMI, left ventricular mass index; RWT, relative wall thickness; e', velocity of the septal wall myocardial tissue lengthening in early diastole at the mitral annulus; LA vol, left atrial volume. *Adjustments are for age, sex, systolic blood pressure, regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%), and L VMI or RWT as indicated.

further adjustments for either LVMI or RWT, relations between waist circumference, BMI or HOMA-IR and lateral e' (Table 2.2) or septal e' (Table 2.3) persisted. In contrast, neither indexes of obesity nor HOMA-IR were independently associated with E/A<0.80, moderate-to-severe DD or LA volume (Table 2.3 and Table 2.4). HOMA-IR but not indexes of obesity were independently associated with E/A<0.80 (Table 2.4). Independent of the individual terms and additional confounders, interactions between HOMA-IR and RWT, but not LVMI (data not shown) were independently associated with lateral e', septal e', E/A, E/e' and mild or moderate-to-severe DD (Tables 2.5 and 2.6). Interactions between HOMA-IR and RWT were not independently associated with left atrial volume (Table 2.6). No significant interactions between indexes of excess adiposity and RWT were independently associated with indexes of LV diastolic function (Table 2.5 and Table 2.6). Moreover, independent of the individual terms and additional confounders, interactions between neither age nor blood pressure and RWT, were independently associated with lateral e', septal e', E/A, E/e' (Table 2.7), and mild or moderate-to-severe DD (data not shown). The interactions between HOMA-IR and RWT translated into independent relationships between HOMA-IR and lateral e', septal e', E/A and E/e' (Figure 2.3 and Table 2.8), and between HOMA-IR and the odds of E/A<0.80 or moderate-to-severe DD (Figure 2.4) only in those with a RWT above the median for the sample. In contrast, similar independent relationships were noted between age or blood pressure and E/A, E/e', lateral e' or septal e' (Figures 2.3 and 2.5) and between BP or age and the odds of E/A<0.80 or moderate-to-severe DD (Figure 2.4) in those with a RWT above the median for the sample compared to those with RWT below the median. Notably lateral e', septal e', E/A and E/e' showed stepwise increases across tertiles of HOMA-IR only in those participants with a RWT above the median for the sample (Figure 2.6 and Table 2.9). Only in those with the highest tertile of HOMA-IR was diastolic function significantly different between those with and those without RWT values above versus below the median for the sample (Figure 2.6 and Table 2.9).

2.5 Discussion

The main findings of the present study are as follows: In a large, community-based sample with a high prevalence of obesity and LV DD, an interaction between

Table 2.4. Multivariate adjusted associations between indexes of adiposity or the homeostasis model of insulin resistance (HOMA-IR) and left ventricular (LV) diastolic dysfunction (DD) in the study group.

	Odds ratio* (95% CI)	Wald X^2	p value
Associations with E/A<0.80			
Waist circumference	1.014 (0.994 to 1.034)	1.920	=0.17
Body mass index	1.017 (0.977 to 1.058)	0.666	=0.41
Log HOMA-IR	1.967 (1.112 to 3.480)	1.054	=0.02
Associations with moderate-to-severe DD (Lateral e'<10, Septal e'<8, or LA volume≥34, and E/e'≥13 and E/A≥0.80)			
Waist circumference	1.014 (0.992 to 1.037)	1.54	=0.21
Body mass index	1.029 (0.983 to 1.077)	1.53	=0.22
Log HOMA-IR	1.462 (0.769 to 2.783)	1.34	=0.25

E/A, ratio of transmitral early-to-atrial (late) blood flow velocity; E/e', E/velocity of the mean value of lateral and septal wall myocardial tissue lengthening in early diastole at the mitral annulus. *Included in the models were age, sex, diastolic blood pressure (decreased E/A) or systolic blood pressure (moderate-to-severe DD), regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%), pulse rate (for E/A) and left ventricular mass index.

Table 2.5. Independent associations between adiposity index (or the homeostasis model of insulin resistance [HOMA-IR])-left ventricular (LV) relative wall thickness (RWT) interactions and LV diastolic function in a community sample.

	Adjustments		
		Partial r (95% CI)	p value
Log E/A vs			
Waist circumference*RWT interaction	*	0.07 (-0.01 to 0.14)	=0.09
Body mass index*RWT interaction	*	0.07 (0.00 to 0.14)	=0.05
Log HOMA-IR*RWT interaction	*	-0.09 (-0.17 to -0.02)	<0.02
Log E/e' vs			
Waist circumference*RWT interaction	*	0.04 (-0.06 to 0.14)	=0.39
Body mass index*RWT interaction	*	-0.01 (-0.10 to 0.09)	=0.89
Log HOMA-IR*RWT interaction	*	0.11 (0.02 to 0.21)	<0.02
Log lateral e' vs			
Waist circumference*RWT interaction	*	0.03 (-0.07 to 0.13)	=0.56
Body mass index*RWT interaction	*	0.08 (-0.02 to 0.17)	=0.12
Log HOMA-IR*RWT interaction	*	-0.11 (-0.24 to -0.05)	<0.005

		Odds ratio* (95% CI)	Wald X^2	p value
E/A<0.80 vs				
Waist circumference*RWT interaction	*	1.045 (0.844 to 1.293)	0.16	=0.69
Body mass index*RWT interaction	*	1.052 (0.706 to 1.567)	0.06	=0.80
Log HOMA-IR*RWT interaction	*	7.305 (1.821 to 29.305)	7.87	<0.005
Moderate-to-severe DD (Lateral e'<10, Septal e'<8, LA volume≥34, E/e'≥13, E/A≥0.80) vs				
Waist circumference*RWT interaction	*	0.879 (0.696 to 1.110)	1.17	=0.28
Body mass index*RWT interaction	*	0.668 (0.412 to 1.084)	2.66	=0.10
Log HOMA-IR*RWT interaction	*	5.829 (1.296 to 26.223)	6.09	=0.014

E/A, ratio of transmitral early-to-atrial (late) blood flow velocity; E/e', E/velocity of the mean value of lateral and septal wall myocardial tissue lengthening in early diastole at the mitral annulus. *Adjustments are for age, sex, diastolic blood pressure (E/A) or systolic blood pressure (E/e' and lateral e'), regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%), and pulse rate (E/A).

Table 2.6. Independent associations between adiposity index (or the homeostasis model of insulin resistance [HOMA-IR])-left ventricular (LV) relative wall thickness (RWT) interactions and LV diastolic function in a community sample.

	Adjustments	Partial r (95% CI)	p value
Log septal e' vs			
Waist circumference*RWT interaction	*	0.02 (-0.07 to 0.12)	=0.63
Body mass index*RWT interaction	*	0.05 (-0.05 to 0.14)	=0.32
Log HOMA-IR*RWT interaction	*	-0.10 (-0.20 to -0.01)	<0.05
Log LA volume vs			
Waist circumference*RWT interaction	*	-0.03 (-0.13 to 0.07)	=0.57
Body mass index*RWT interaction	*	-0.04 (-0.10 to 0.06)	=0.43
Log HOMA-IR*RWT interaction	*	-0.06 (-0.15 to 0.04)	=0.24

e', velocity of the septal wall myocardial tissue lengthening in early diastole at the mitral annulus; LA, left atrial. *Adjustments are for age, sex, systolic blood pressure, regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%).

Table 2.7. Lack of independent associations between age or blood pressure-left ventricular (LV) relative wall thickness (RWT) interactions and LV diastolic function in a community sample.

	Adjustments	Partial r (95% CI)	p value
Log E/A vs			
Age*RWT interaction	*	0.04 (-0.04 to 0.11)	=0.31
DBP*RWT interaction	*	0.05 (-0.02 to 0.12)	=0.18
Log E/e' vs			
Age*RWT interaction	*	0.07 (-0.02 to 0.16)	=0.15
SBP*RWT interaction	*	0.03 (-0.06 to 0.13)	=0.49
Log lateral e' vs			
Age*RWT interaction	*	-0.03 (-0.13 to 0.06)	=0.51
SBP*RWT interaction	*	-0.04 (-0.13 to 0.06)	=0.44
Log septal e' vs			
Age*RWT interaction	*	-0.07 (-0.17 to 0.02)	=0.13
SBP*RWT interaction	*	-0.06 (-0.16 to 0.03)	=0.19

e', velocity of the septal wall myocardial tissue lengthening in early diastole at the mitral annulus; LA, left atrial. *Adjustments are for age, sex, diastolic blood pressure (E/A), systolic blood pressure (E/e', lateral e' and septal e'), regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%) and pulse rate (E/A).

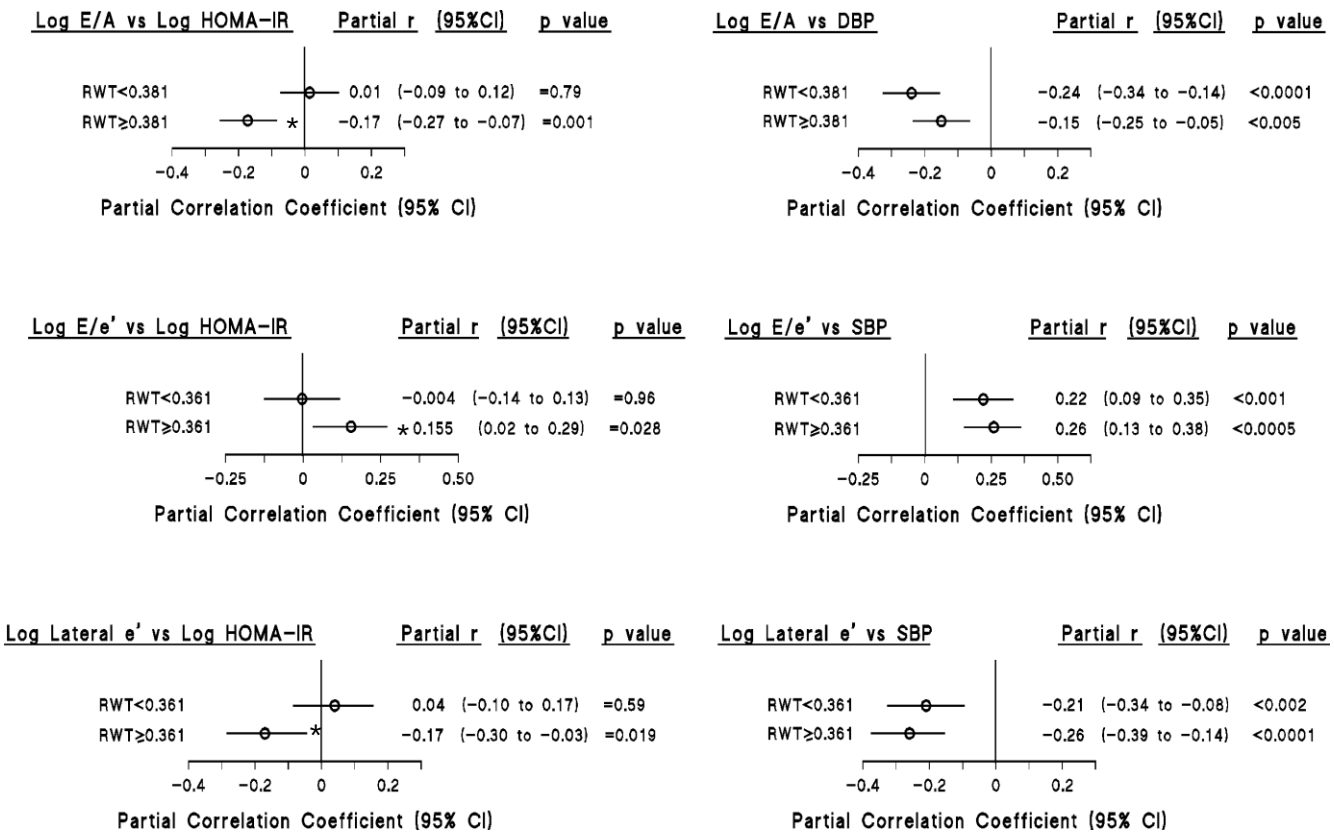


Figure 2.3. Synergistic effect of left ventricular (LV) relative wall thickness (RWT) and the homeostasis model of insulin resistance (HOMA-IR) (left panels), or blood pressure (BP) (right panels) on indexes of left ventricular (LV) diastolic function. Figures show significant relations (partial r) between HOMA-IR and LV diastolic function in participants with a RWT above, but not below the median for the sample, whilst relations between BP and LV diastolic function are similar in participants with a RWT above and below the median for the sample. See table 2.1 for abbreviations. Adjustments are for age, sex, diastolic blood pressure (HOMA-IR vs E/A), systolic blood pressure (HOMA-IR vs E/e' and lateral e'), pulse rate (E/A), regular smoking, regular alcohol consumption, diabetes mellitus or an HbA1c>6.1%, treatment for hypertension, and left ventricular mass index. *p<0.05 versus the partial r value of the HOMA-IR vs E/A, E/e', or lateral e' relationship in those with a RWT< median for the sample.

Table 2.8. Strength (partial r) of relations between the homeostasis model of insulin resistance (HOMA-IR) and septal e' in participants with a LV relative wall thickness (RWT) above or below the median for the sample.

	Adjustments	Partial r (95% CI)	p value
		RWT<0.361	
Log HOMA-IR versus			
Log septal e' (n=215)	*	-0.03 (-0.17 to 0.11)	=0.66
		RWT≥0.361	
Log HOMA-IR versus			
Log septal e' (n=215)	*	-0.14 (-0.27 to -0.01)	=0.05

e', velocity of the septal wall myocardial tissue lengthening in early diastole at the mitral annulus; LA vol, left atrial volume. *Adjustments are for age, sex, systolic blood pressure, regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%) and left ventricular mass index.

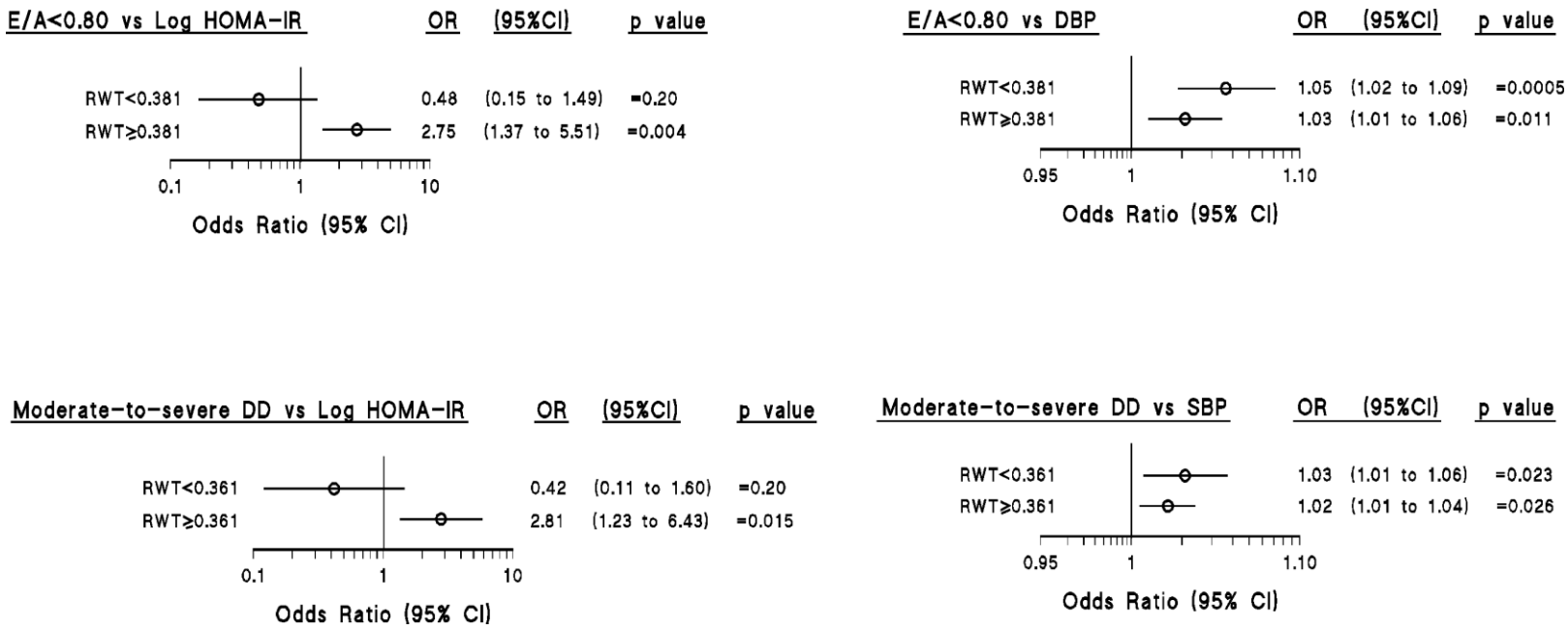


Figure 2.4. Synergistic effect of left ventricular (LV) relative wall thickness (RWT) and the homeostasis model of insulin resistance (HOMA-IR) (left panels), or blood pressure (BP) (right panels) on the odds of LV diastolic dysfunction (DD) (E/A<0.80 or lateral e'<10, septal e'<8, LA volume≥34, E/e'≥13, E/A≥0.80). Figures show an impact of HOMA-IR on DD in participants with a RWT above, but not below the median for the sample, but a similar impact of BP on DD in participants with a RWT above and below the median for the sample. See table 2.1 for abbreviations. Adjustments are as given in figure.2.3.

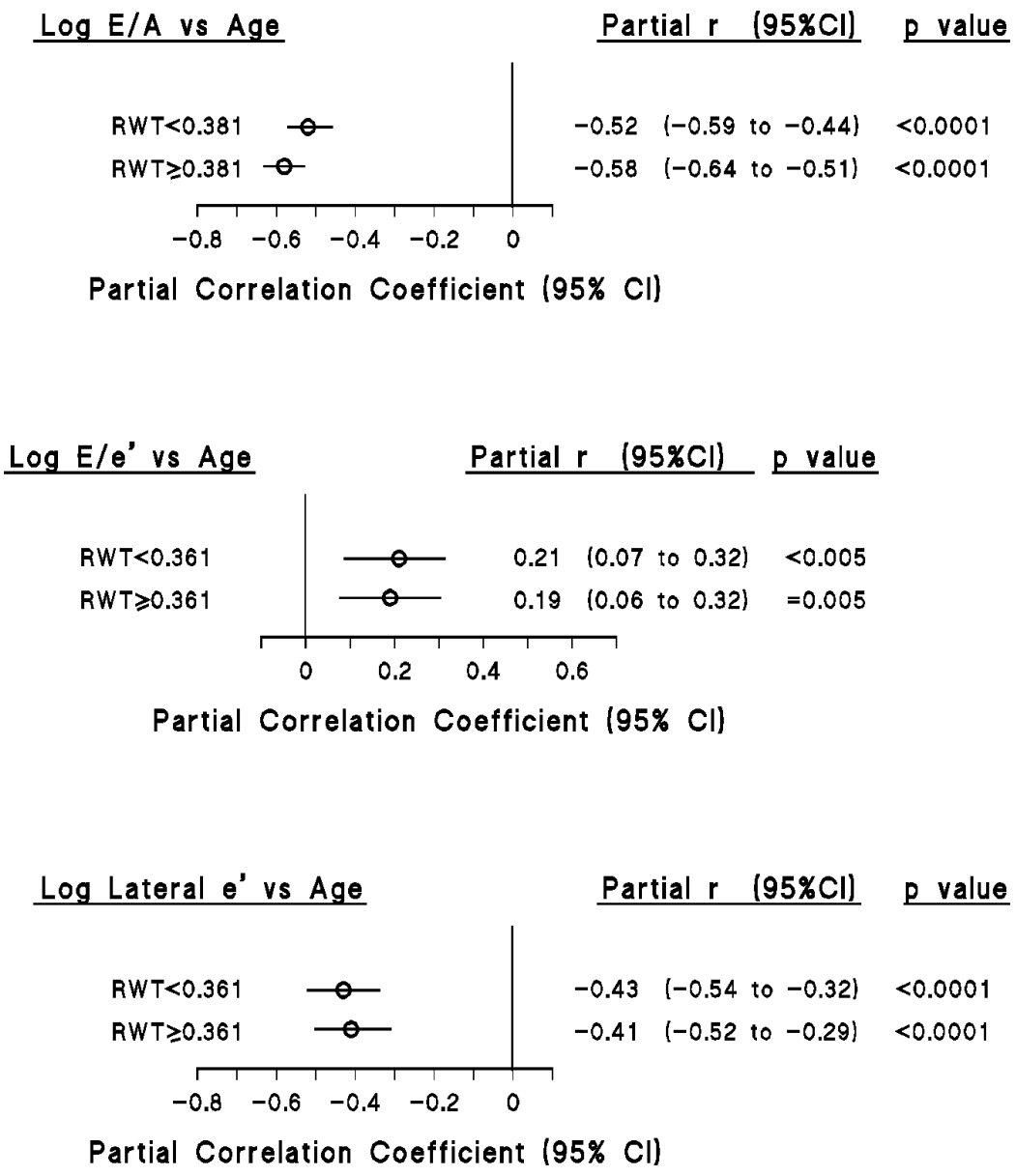


Figure 2.5. Relations (partial r) between blood pressure (BP) and left ventricular (LV) diastolic function in participants with a LV relative wall thickness (RWT) above and below the median for the sample. See table 2.1 for abbreviations. Adjustments are for age, sex, pulse rate (E/A), regular smoking, regular alcohol consumption, diabetes mellitus or an HbA1c>6.1%, treatment for hypertension, and left ventricular mass index.

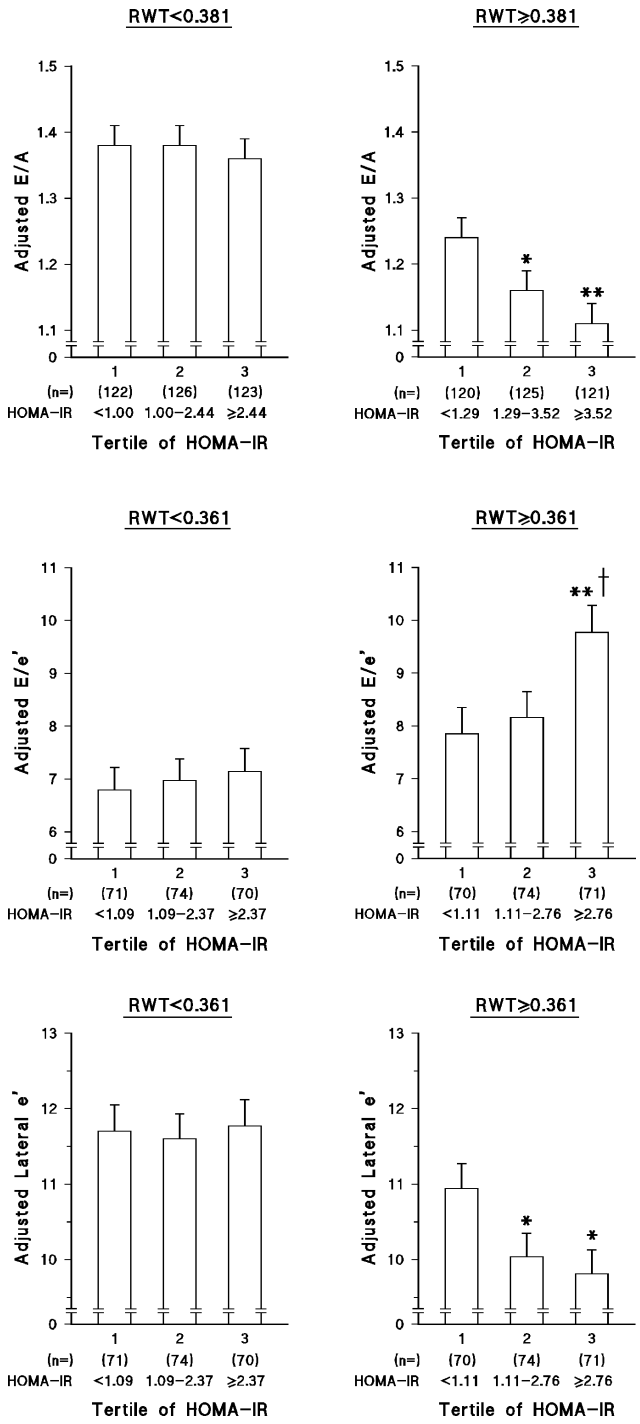


Figure 2.6 Impact of synergistic effect of left ventricular (LV) relative wall thickness (RWT) and the homeostasis model of insulin resistance (HOMA-IR) on LV diastolic function. Figures show differences in LV diastolic function across tertiles of HOMA-IR in participants with a RWT above, but not below the median for the sample. See table 2.1 for abbreviations. Adjustments are as given in figure.2.3. *p<0.05, **p<0.005 versus tertile 1, †p<0.05 versus tertile 2.

Table 2.9. Adjusted septal e' across tertiles of the homeostasis model of insulin resistance (HOMA-IR) in participants with a LV relative wall thickness (RWT) above or below the median for the sample.

		Tertiles of HOMA-IR	
	1	2	3
		RWT<0.361	
HOMA-IR	<1.09	1.09 – 2.37	≥2.37
n=	71	74	70
Adjusted septal e'	10.27±0.32	10.71±0.31	10.18±0.32
		RWT≥0.361	
HOMA-IR	<1.11	1.11 – 2.76	≥2.76
n=	70	74	71
Adjusted septal e'	9.54±0.32	9.19±0.30	8.58±0.31*

e', velocity of the septal wall myocardial tissue lengthening in early diastole at the mitral annulus. Adjustments are for age, sex, systolic blood pressure, regular tobacco use, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an abnormal blood glucose control (HbA_{1c}>6.1%) and left ventricular mass index. *p<0.05 versus tertile 1.

HOMA-IR and RWT, but not other risk factors (namely age, blood pressure, waist circumference and BMI), was independently associated with lateral e', septal e', E/A, E/e' and mild or moderate-to-severe DD. This interaction translated into an association between HOMA-IR and diastolic function or dysfunction and stepwise decreases in E/A, lateral e' and septal e' and increases in E/e', only in those with a RWT above the median for the sample. In those with a RWT below the median for the sample, HOMA-IR was not independently associated with indices of LV diastolic function or with the prevalence of LV DD. In contrast, blood pressure and age, the major risk factors for diastolic dysfunction were equally as well associated with diastolic function or dysfunction in those with a RWT greater than as compared to less than the median for the sample.

Although several large cross-sectional and intervention (bariatric surgery) studies have demonstrated independent associations between adiposity indices and abnormalities of LV diastolic function (Tsioufis et al 2008; Russo et al 2011; Çil et al 2011; AlJaroudi et al 2012; Libhaber et al 2009; Cuspidi et al 2014), the relative importance of obesity as compared to alternative risk factors has recently been highlighted as being modest at best in some populations (Millen et al 2014). Indeed, in the present study neither indices of excess adiposity, nor of insulin resistance were independently associated with the presence of moderate to severe LV DD. As weight loss programs seldom achieve target body weight or result in sustained decreases in body weight (Latner et al 2002; Anderson et al 2001), there is a need to identify those in whom weight loss is most likely to prevent the progression to heart failure. In this regard, the present study suggests that the presence of a more concentric LV (RWT>0.361 or the median of the sample) characterises those in whom insulin resistance is likely to translate into either mild or moderate-to-severe DD. Importantly, in contrast to the lack of impact of HOMA-IR on moderate-to-severe DD in the whole group, in those with a RWT >median for the sample, HOMA-IR was independently associated with both mild and moderate-to-severe DD. Moreover, the strength of the relations between HOMA-IR and indices of LV diastolic function in those with a RWT above the median of the sample was similar to that of the strength of the relation between BP and LV diastolic function. Hence, the present study suggests that those at risk of progressing to obesity-associated DD, and hence possibly heart failure with a preserved ejection fraction, are most likely to be insulin resistant individuals with a more concentric LV.

Although several studies have demonstrated independent relations between adiposity indices and abnormalities of LV diastolic function (Russo et al 2011; Çil et

al 2011; AlJaroudi et al 2012; Libhaber et al 2009; Millen et al 2014), few of these studies (Libhaber et al 2009; Millen et al 2014) adjusted for BP as a continuous trait or employed diastolic BP when adjusting relations with E/A and systolic BP when adjusting relations with E/e', as recently recommended (Libhaber et al 2014). Indeed, a number of these studies identified independent relations between obesity and DD using only the presence of hypertension as an adjustor (Russo et al 2011; Çil et al 2011; AlJaroudi et al 2012), and hence did not fully account for the confounding effect of BP as a co-morbidity. Thus, although these studies (Russo et al 2011; Çil et al 2011; AlJaroudi et al 2012) demonstrated strong independent relations between BMI and LV DD, the strength of these relations is likely to be attributed to BP effects. Indeed, as noted in the present study, with appropriate BP adjustments, the impact of an excess adiposity or the associated insulin resistance on LV DD in unselected individuals may be modest at best (Millen et al 2014). Nevertheless, in the present study we provide evidence to suggest that in those with a more concentrically remodelled LV, insulin resistance, a well-recognised metabolic abnormality in obesity, is indeed independently associated with both mild and moderate-to-severe DD. In those without concentric remodelling, neither obesity nor the associated insulin resistance impact on LV diastolic function. Thus, it is possible that attempts to modify insulin resistance are most likely to have benefits to the development of DD in those individuals with a more concentrically remodelled LV.

An important caveat of the present study is that the present results do not suggest that obesity or insulin resistance mediate LV DD through concentric LV remodelling. Indeed, independent relations between adiposity indices or HOMA-IR and LV diastolic functional parameters were unaffected by adjustments for RWT. Rather, the interactive effect of LV remodelling with HOMA-IR should be viewed as RWT providing a modifying influence on the impact of insulin resistance. In this regard RWT is likely to influence diastolic function (Chahal et al 2010) and hence generate the conditions where insulin resistance-induced metabolic effects on diastolic function translate into relaxation abnormalities and raised LV filling pressures. Whether therapeutic approaches that increase insulin resistance translate into an improved diastolic function and hence a reduced risk for heart failure mainly in those with a more concentrically remodelled LV requires further study.

The limitations of the present study are as follows: First, this is a cross-sectional study and hence we cannot draw conclusions regarding cause and effect. Whether the development of insulin resistance influences the development of DD in those with concentric rather than eccentric LV remodelling requires further study.

Second, we failed to measure several alternative indices of diastolic function previously recommended (Nagueh et al 2009) and which have now been incorporated into guidelines. However, many of these measurements showed a low degree of reproducibility and a high degree of inter-observer variability in our hands and absolute world-wide consensus on which measures are required to best identify DD has not been reached. Third, we were not statistically powered to perform sex-specific analysis and hence it is possible that as more women than men volunteered for the study, that the results may relate mainly to women.

In conclusion, in a relatively large community-based sample with a high prevalence of obesity, we show that independent of confounders, although a higher RWT does not mediate the impact of insulin resistance on LV diastolic function, a more concentrically remodelled LV modifies the impact of insulin resistance on LV diastolic function. These data suggest that from a clinical perspective, those with a more concentrically remodelled LV may be particularly prone to insulin resistance-associated LV DD and thus possibly the progression to heart failure with a preserved ejection fraction. Future studies will determine whether targeting the degree of insulin resistance in obese individuals is more likely to be beneficial in those with a more concentrically remodelled LV.

CHAPTER 3

Cardiac Diastolic dysfunction is Associated with Aortic Wave Reflection, but not Stiffness in a Predominantly Young-to-Middle-Aged Community Sample

The data in this chapter have been published in the American Journal of Hypertension:

Peterson, V.R., Woodiwiss, A.J., Libhaber, C.D., Raymond, A., Sareli, P. and Norton, G.R. (2016). Cardiac Diastolic Dysfunction is Associated with Aortic Wave Reflection, but not Stiffness in a Predominantly Young-to-Middle-Aged Community Sample. *American Journal of Hypertension*, 29, 1148-1157.

3.1 Abstract

Background. Whether the impact of backward wave pressures on left ventricular (LV) diastolic dysfunction (DD) antedates the effects of aortic stiffness is uncertain. We compared the relative contribution of various aortic haemodynamic parameters to preclinical DD in a predominantly young-to-middle aged community-based sample.

Methods. In 524 randomly selected participants of African ancestry (mean age=46.8±18.4 years) we assessed central aortic pulse pressure (PPc), forward wave pressure (Pf), backward wave pressure (Pb), augmented pressure (Pa), the time to wave reflection (Rt) and aortic pulse wave velocity (PWV) using applanation tonometry (SphygmoCor software). LV mass index (LVMI), early to late trans-mitral velocity (E/A) and E/velocity of myocardial tissue lengthening (E/e') were determined using echocardiography.

Results. Independent of age, sex, mean arterial pressure, body mass index, diabetes mellitus or and HbA_{1c}>6.1%, regular smoking, regular alcohol intake, treatment for hypertension, pulse rate and LVMI; PPc ($p<0.002$), Pb ($p<0.0005$), Pa ($p<0.002$), and Pf ($p<0.02$), but not Rt or PWV were independently associated with E/e' (but not with E/A). With adjustments for confounders, PPc ($p<0.005$), Pb ($p<0.002$) and Pa ($p<0.001$), but not Pf, Rt or PWV were independently associated with E/e' ≥12 (moderate-to-severe DD, n=69). The independent relations between PPc and E/e' or moderate-to-severe DD were not affected by adjustments for PWV, Pf or Rt, but were abolished with adjustments for Pb.

Conclusions. In a predominantly young-to-middle aged community sample, the impact of backward wave pressures on LV DD antedates the effects of aortic stiffness, the time to wave reflection or forward wave pressures.

3.2 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 no treatment with proven benefit for heart failure with a normal ejection fraction (Borlaug & Redfield 2011; Borlaug & Paulus 2011; Pitt et al., 2014) and hence understanding the pathophysiological mechanisms responsible may shed light on potential therapeutic targets. In this regard, 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). Although abnormalities of pulsatile haemodynamics and aortic function have frequently been described in hypertensive heart failure with a normal ejection fraction (Mohammed et al 2012; Hundley et al 2001; Kawaguchi et al 2003; Weber et al 2006; Weber et al 2008; Borlaug et al 2007; Fernandes et al 2008; Kang et al 2010; Desai et al 2009; Kitzman et al 2013) the primary aortic functional change responsible for pre-clinical left ventricular (LV) DD is unclear.

Several studies have identified associations between aortic stiffness or reflected waves and aspects of LV diastolic function in those without heart failure (Kawaguchi et al 2003, Weber et al 2006; Weber et al 2008; Borlaug et al 2007). However, whilst there is little evidence to support a therapeutic approach to modify age-related increases in aortic stiffness, there is significant evidence to suggest that aortic backward wave pressures may be targeted by conventional therapy (Agabiti-Rosei et al 2007). Importantly, marked age-related increases in backward wave pressures precede significant age-related increases in aortic stiffness by several decades (Booysen et al 2015). Moreover, increases in aortic backward wave pressures are a fundamental mechanism responsible for increases in aortic stiffness (Sibiya et al 2015), and hence may be the initial (primary) target in preventing the progression to LV DD and consequently heart failure with a preserved ejection fraction. However, it is uncertain whether the impact of backward wave pressures on LV DD antedates the effects of aortic stiffness. In this regard, prior studies assessing relations between aortic and LV diastolic function have assessed patients with average ages of 60-77 years (Mohammed et al 2012; Hundley et al 2001; Kawaguchi et al 2003; Weber et al 2006; Weber et al 2008; Fernandes et al 2008; Kang et al

2010; Desai et al 2009; Kitzman et al 2013), age ranges when large vessel stiffness is markedly increased (Booysen et al 2015). Hence, none of these studies could address the question of whether the impact of backward wave pressures on LV DD antedate the effects of aortic stiffness. In the present study we therefore evaluated the relationship between several aortic functional parameters and LV diastolic function in a predominantly young-to-middle aged community-based sample with an average age where increases in aortic backward wave pressures are well established, but prior to an age when striking increases in aortic stiffness are noted (Booysen et al 2015).

3.3 Methods

3.3.1 Study participants.

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The present study design has previously been described (Booysen et al 2015). Briefly, families of black African descent (Nguni and Sotho chiefdoms) with siblings older than 16 years of age were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. Of 832 participants with echocardiography, in a sub-study 524 participants had trans-mitral velocity measurements, myocardial tissue Doppler imaging (TDI) and all aortic function measurements. None of these participants had evidence of significant valve abnormalities assessed using 2-dimensional and colour Doppler imaging. None of the participants had previously had a myocardial infarction (only three had a history of ischaemic heart disease), and there were no cases of atrial fibrillation.

3.3.2 Clinical, demographic, anthropometric and laboratory assessments.

A standardised questionnaire was administered to obtain demographic and clinical data (Booysen et al 2015). Height and weight 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. Standard laboratory blood tests of renal function, liver function, blood glucose, haematological

parameters, and percentage glycated haemoglobin (HbA_{1c}) were performed. Diabetes mellitus (DM) or abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA_{1c} value greater than 6.1%.

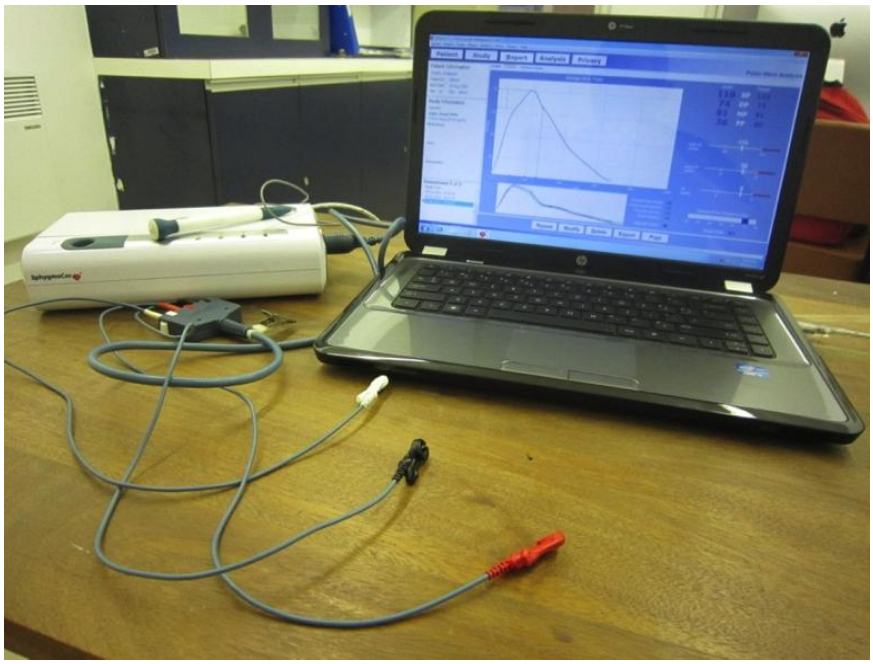
3.3.3 Office blood pressure.

High quality office BP measurements were obtained by a trained nurse-technician using a standard mercury sphygmomanometer (Booysen et al 2015) according to guidelines. The nurse was of the same ethnic origins (black African) as the participants and had previously lived in SOWETO. Korotkov phases I and V were employed to identify systolic and diastolic BP respectively and care was taken to avoid auscultatory gaps. Office BP was measured 5 times consecutively using appropriately sized cuffs after the subjects had rested for 5-10 minutes in the sitting position. The mean of all 5 office BP measurements was used in the analysis.

3.3.4 Aortic haemodynamics.

Aortic haemodynamic parameters were assessed from pulse wave and wave separation analysis conducted using techniques previously described (Booysen et al 2015) (Figures 3.1 and 3.2). After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm) pulse were recorded by applanation tonometry during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryder, New South Wales, Australia). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded. All measurements were made by a single experienced trained technician unaware of the clinical history of the participants. To obtain central aortic PP (PPc), the pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. From an inbuilt validated generalised transfer function an aortic waveform was generated from which PPc was derived. Aortic backward (Pb) and forward (Pf) wave pressures were determined using SphygmoCor software which separates the aortic waveform using a triangular flow wave (Figure 3.2). Reflected wave magnitude (RM) was determined as Pb/Pf x 100. Aortic augmented pressure (Pa) was determined

A



B



Figure 3.1 A SphygmoCor device coupled with an applanation tonometer (panel A) which was used to determine aortic haemodynamics. Panel B illustrates the applanation tonometer used to detect the pressure waveforms.

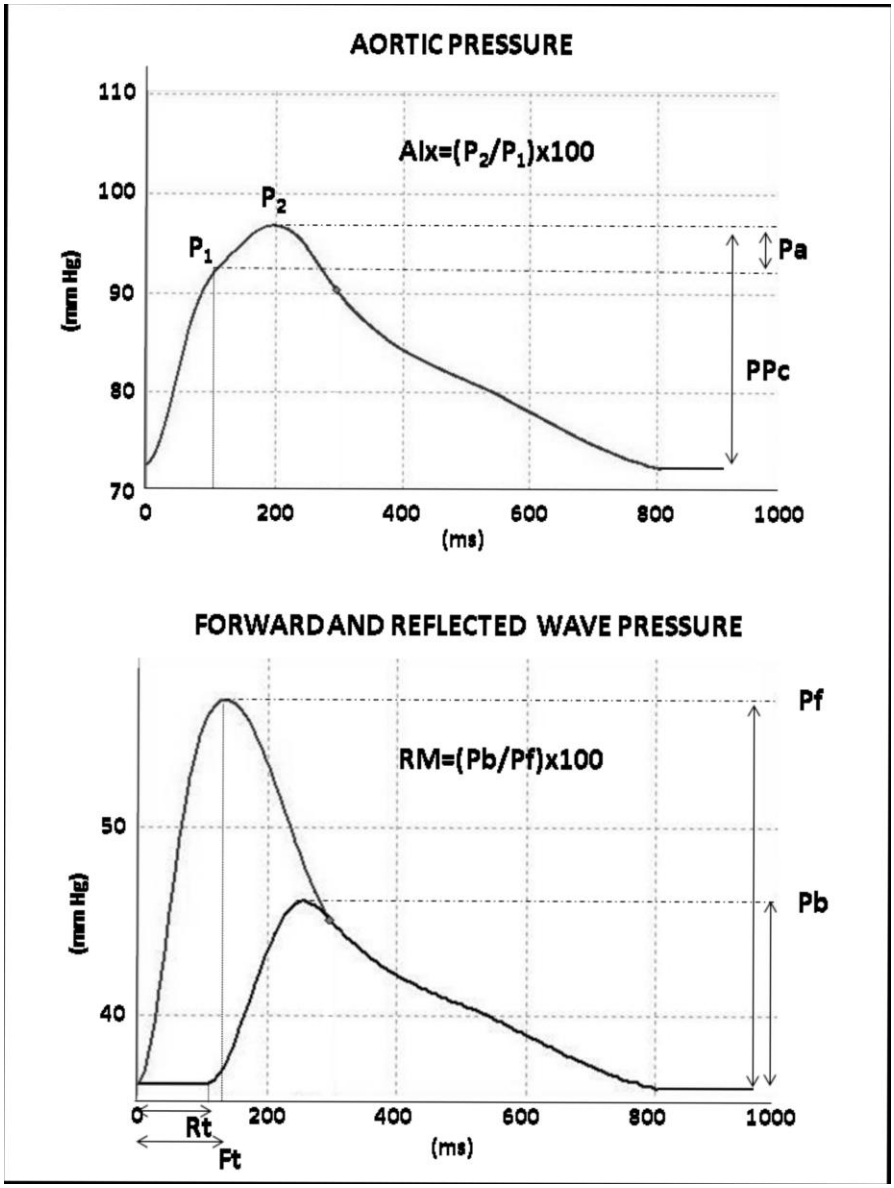


Figure 3.2 Derivation of aortic functional parameters from the aortic pressure waveform using SphygmoCor software. The aortic pressure wave was separated into the forward and backward waves assuming the aortic flow wave to have a triangular shape. Pa, augmented pressure; PPc, central aortic pulse pressure; AIx, augmentation index; Pf, forward wave pressure; Pb, backward (reflected) wave pressure; RM, reflection magnitude; Ft, time to peak forward wave; Rt, reflected wave time; P_1, first systolic peak; P_2, second systolic peak.

using SphygmoCor software and identified as the difference between aortic systolic BP and the first systolic peak of the aortic pulse wave (Figure 3.2). To avoid obtaining negative aortic augmentation index (AIx) values in young participants, AIx was determined as the pressure at the second systolic peak of the aortic pulse wave/the pressure at the first systolic peak of the aortic pulse wave expressed as a percentage. The time to the peak of the forward wave (Ft) and the time to wave reflection (Rt) were also determined from SphygmoCor software (Figure 3.2).

Aortic PWV was determined from sequential waveform measurements at carotid and femoral sites using applanation tonometry and SphygmoCor software (Figure 3.3). The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Aortic PWV was calculated as the ratio of the distance to the transit time (m/sec).

3.3.5 Echocardiography.

Echocardiographic measurements were performed using a Sonosite M-Turbo ultrasound (SonoSite® Inc., Bothell, WA, USA) device with the patient in the partial left decubitus position as previously described (Libhaber et al 2014; Millen et al 2014; Norton et al 2008; Woodiwiss et al 2009) (see chapter 2, section 2.3.3). Left ventricular mass (LVM) was indexed (LVMI) to height$^{2.7}$. An LVMI>51 g/m$^{2.7}$ was considered as LV hypertrophy (LVH). Left ventricular end diastolic and systolic volumes were determined from M-mode images using the Teichholz method. Left ventricular ejection fraction (EF) was calculated as [(LV end diastolic volume-LV end systolic volume)/ LV end diastolic volume] x 100.

Left ventricular diastolic function was assessed from a pulsed wave Doppler examination of the mitral inflow at rest and using TDI (Quiñones et al 2002). 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 blood flow velocity measurements were obtained during the early (E) and late (atrial-A) period of left ventricular diastolic inflow and expressed as the E/A ratio. To perform TDI, the velocity of myocardial tissue lengthening at the level of the mitral annulus

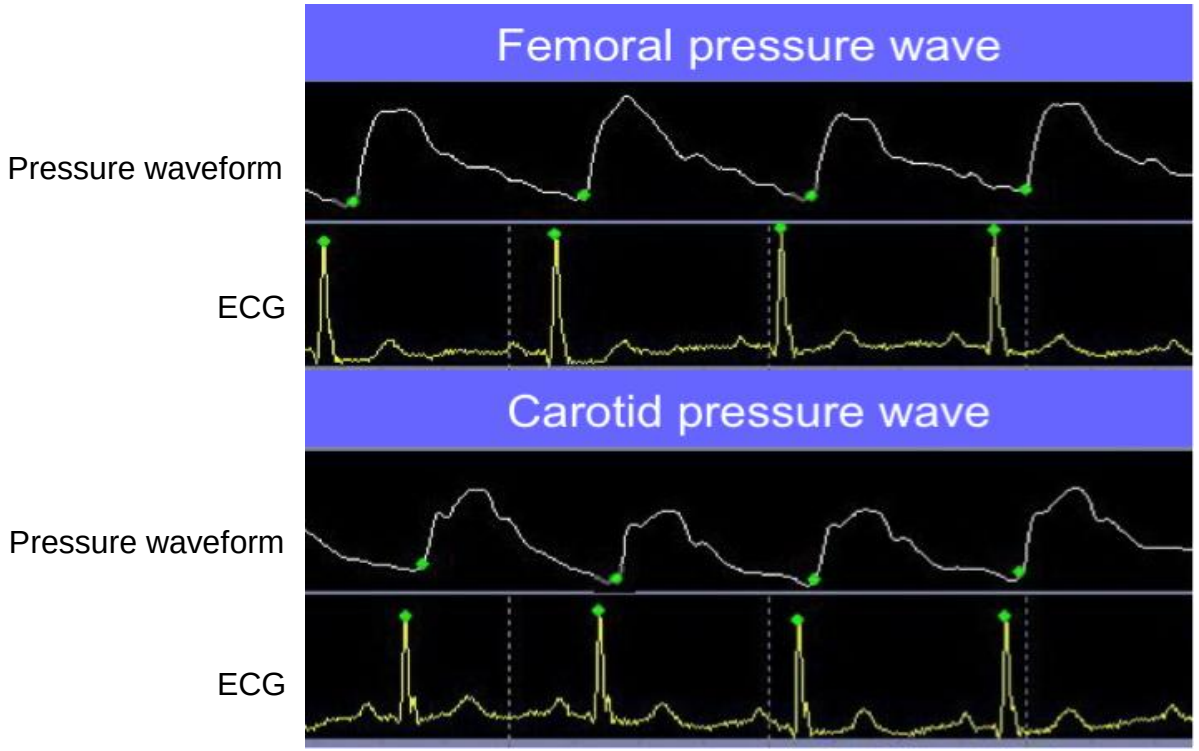


Figure 3.3 Aortic pulse wave velocity was determined from sequential pressure waveform measurements at carotid and femoral sites. The R-wave in a simultaneously recorded electrocardiogram (ECG) was used as a fiducial point to determine the time delay in the waves between the carotid and femoral sites (green dots on both ECG and pressure waveform).

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') and late (atrial) (a') diastole were measured. Data were expressed as the E/lateral wall e' ratio (an index of LV filling pressures). Left ventricular diastolic dysfunction (DD) was evaluated as mild (impaired relaxation when E/A≤0.75), moderate (pseudonormal, where E/A>0.75<1.5, and E/e'≥12) and severe (where E/A≥1.5 and E/e'≥12). A threshold of 12 for E/e' was identified from the upper 97.5 percentile (11.7) of E/e' identified in 140 normotensive, non-obese, non-diabetic participants who did not smoke or consume alcohol and who had an estimated glomerular filtration rate>60 ml/min per 1.73m^2, an LVMI<51 g/$m^{2.7}$, and no cardiovascular disease.

3.3.6 Data analysis.

Database management and statistical analyses were performed with SAS software, version 9.4 (The SAS Institute Inc., Cary, North Carolina, USA). Data are expressed as mean±SD or proportions. Indexes of diastolic function (E/A and E/e') were logarithmically transformed to improve on the distribution. Relationships were evaluated from univariate or multivariate linear (continuous analysis) or logistic (discrete analysis) regression analysis. To determine probability values, further adjustments for non-independence of family members was performed using non-linear regression analysis (mixed procedure as defined in the SAS package).

3.4 Results

3.4.1 Participant characteristics

When assessing the characteristics of the total sample with and without TDI measures, 48.3% of the participants with TDI had uncontrolled hypertension and 45.2% of these participants were obese (Table 3.1). Other than a modestly higher age, and proportion who smoked, and a modestly lower PPc, Pb, PWV and LVMI, no differences were noted between participants with and without TDI. In the group with TDI, 12.6% of participants had mild DD, 9.4% moderate DD, and 1.5% severe DD. No participants had an EF<40% and 5.7% had an EF<50%. LVH was more prevalent in those with a decreased E/A (≤7.5) (28.8%) or increased E/e' (≥12)(27.5%) as compared to those with either a normal E/A or E/e' (15.1% or 15.2%, p<0.02 for

both). 81.2% of participants were receiving low-dose hydrochlorothiazide, 22.4% calcium channel blockers, 24.8% angiotensin-converting enzyme inhibitors, 0.6% beta-adrenergic receptor blockers, and 9.1% other anti-hypertensive agents (Table 3.1). 57.5% of participants were receiving monotherapy (88.3% diuretics), and 33.3% were receiving combined therapy (50.9% diuretic plus angiotensin-converting enzyme inhibitor; 30.2% diuretic plus calcium channel blocker; 18.9% diuretic plus others). 9.2% of participants were receiving triple or more therapy.

3.4.2 Factors correlated with LV diastolic function

On univariate analysis, age, female sex, treatment for hypertension, hypertension, diabetes mellitus, body mass index, PP (brachial and aortic), diastolic BP (DBP), mean arterial pressure (MAP), Pf, Pb, RM, aortic PWV, Rt, Ft and LVMI were associated with E/A, E/e' and either mild or moderate-to-severe DD ($p<0.005$).

3.4.3 Independent associations between aortic haemodynamics and E/A.

Independent of MAP, and various confounders, only Rt showed modest independent associations with E/A (Table 3.2). Similar findings were noted irrespective of whether LVMI was or was not included as an adjustor in multivariate models (Table 3.2).

3.4.4 Independent associations between aortic haemodynamics and E/e'.

Independent of MAP, and various confounders, and either before or after adjustments for LVMI, PPc, Pb, Pa and Pf, but not PWV, RM, AIx, Ft or Rt were independently associated with E/e' (Table 3.3). In support of a role for aortic backward wave pressures, but not aortic stiffness, forward wave pressures or the timing of forward and backward wave pressures accounting for the PPc-E/e' relations, whilst adjustments for Pb and Pa abolished relations between PPc and E/e', adjustments for Pf, aortic PWV, Ft or Rt failed to significantly modify relations between PPc and E/e' (Figure 3.4, upper panel).

Table 3.1. Demographic, clinical, anthropometric and haemodynamic characteristics of the study sample with and without measures of tissue Doppler indices (TDI) of left ventricular diastolic function.

	With TDI	Without TDI
Sample number (% female)	524 (65.6)	402 (65.2)
Age (years)	46.8±18.4(16.2 to 87.6)*	44.3±18.0 (16.2 to 84.1)
Body mass index (kg/m^2)	29.6±7.5	29.9±8.0
% overweight/obese	23.7/45.2	22.9/45.0
Regular tobacco (% subjects)	16.6	11.9
Regular alcohol (% subjects)	18.5	21.1
% diabetes mellitus or HbA1c>6.1%	22.1	25.6
% hypertensive	48.3	46.0
% treated for hypertension	31.9	24.4
% diuretics/CCBs/ACEs/others	81.2/22.4/24.8/9.7	82.6/17.2/24.5/11.3
Brachial SBP/DBP (mm Hg)	128±22/83±12	130±23/84±13
Mean arterial pressure (mm Hg)	99±15	101±17
Central aortic PP (mm Hg)	35±15	37±14
Backward wave pressure (Pb) (mm Hg)	17±8	18±8
Forward wave pressure (Pf) (mm Hg)	24±10	25±8
Reflected wave magnitude (RM) (%)	71±21	73±22
Augmented pressure (Pa) (mm Hg)	10±7	11±8
Augmentation index (Alx)	142±25	142±26
Time to peak forward wave (Ft) (msecs)	126±36	129±38
Reflected wave time (Rt) (msecs)	118±14	117±14
Aortic pulse wave velocity (PWV) (m/sec)	5.68±2.41*	6.80±2.98
LVM index (g/m$^{2.7}$)	39.1±15.1*	44.7±13.8
LV ejection fraction (%)	67.2±9.6	67.1±8.4
E/A	1.31±0.56	1.25±0.49
E/e’	7.64±4.08	7.99±4.38

Data are shown as mean±SD (range) or proportions. CCB, calcium channel blockers; ACEIs, angiotensin-converting enzyme inhibitors; SBP, systolic blood pressure; DBP, diastolic BP; PP, pulse pressure; LVM, left ventricular mass; E/A, ratio of transmitral

early-to-atrial (late) blood flow velocity; E/e', E/velocity of lateral wall myocardial tissue lengthening in early diastole at the mitral annulus. *p<0.05 versus without TDI.

Table 3.2 Multivariate adjusted relationships between aortic haemodynamic parameters and left ventricular (LV) early-to-late (atrial) trans-mitral blood flow velocity (E/A)(n=524).

Log E/A vs	Partial r (95% CI)	p value	Partial r (95% CI)	p value
Adjustments→	*		* + LVMI	
PPc	0.06 (-0.02 to 0.15)	=0.16	0.07 (-0.02 to 0.16)	=0.12
Pb	0.04 (-0.04 to 0.13)	=0.32	0.05 (-0.04 to 0.14)	=0.25
Pf	0.05 (-0.04 to 0.14)	=0.24	0.06 (-0.03 to 0.14)	=0.21
Pa	0.02 (-0.07 to 0.10)	=0.70	0.02 (-0.07 to 0.11)	=0.65
RM	-0.06 (-0.14 to 0.03)	=0.19	-0.06 (-0.14 to 0.03)	=0.21
AIx	-0.05 (-0.13 to 0.04)	=0.28	-0.05 (-0.14 to 0.04)	=0.25
PWV	-0.07 (-0.16 to 0.01)	=0.10	-0.07 (-0.16 to 0.02)	=0.11
Ft	-0.08 (-0.17 to 0.01)	=0.07	-0.08 (-0.17 to 0.01)	=0.06
Rt	0.10 (0.02 to 0.19)	<0.05	0.10 (0.01 to 0.19)	<0.05

For abbreviations see Figure 3.2 and Table 3.1. *Adjustments are for age, sex, mean arterial pressure, body mass index, diabetes mellitus or and HbA1c>6.1%, regular smoking, regular alcohol intake, treatment for hypertension, pulse rate and LV mass index as indicated.

Table 3.3. Multivariate adjusted relationships between aortic haemodynamic parameters and left ventricular (LV) early trans-mitral blood flow velocity / velocity of lateral wall myocardial tissue lengthening in early diastole at the mitral annulus (E/e')(n=524).

Log E/e' vs Adjustments→	Partial r† (95% CI) *	Standardised β-coefficient±SEM‡ *	p value	Partial r† (95% CI) * + LVMI	Standardised β-coefficient±SEM‡ * + LVMI	p value
PPc	0.15 (0.06 to 0.23)	0.20±0.06	<0.001	0.14 (0.05 to 0.22)	0.16±0.05	<0.002
Pb	0.17 (0.08 to 0.25)	0.22±0.06	<0.0005	0.16 (0.07 to 0.24)	0.21±0.06	<0.0005
Pf	0.11 (0.02 to 0.19)	0.11±0.04	<0.02	0.10 (0.02 to 0.19)	0.10±0.05	<0.02
Pa	0.14 (0.06 to 0.23)	0.21±0.06	<0.002	0.14 (0.05 to 0.22)	0.20±0.06	<0.002
RM	0.05 (-0.04 to 0.13)	0.06±0.05	=0.26	0.05 (-0.04 to 0.13)	0.06±0.05	=0.28
AIx	0.02 (-0.07 to 0.10)	0.02±0.05	=0.68	0.02 (-0.07 to 0.11)	0.03±0.05	=0.62
PWV	0.05 (-0.04 to 0.14)	0.06±0.05	=0.24	0.05 (-0.04 to 0.14)	0.06±0.05	=0.27
Ft	0.05 (-0.04 to 0.13)	0.04±0.04	=0.30	0.05 (-0.04 to 0.13)	0.04±0.04	=0.31
Rt	-0.03 (-0.11 to 0.06)	-0.03±0.04	=0.51	-0.03 (-0.11 to 0.06)	-0.03±0.04	=0.54

For abbreviations see Figure 3.2 and Table 3.1. *Adjustments are for age, sex, mean arterial pressure, body mass index, diabetes mellitus or and HbA1c>6.1%, regular smoking, regular alcohol intake, treatment for hypertension, pulse rate and LV mass index as indicated.†represents the strength of the relationship. ‡represents the slope of the relationship.

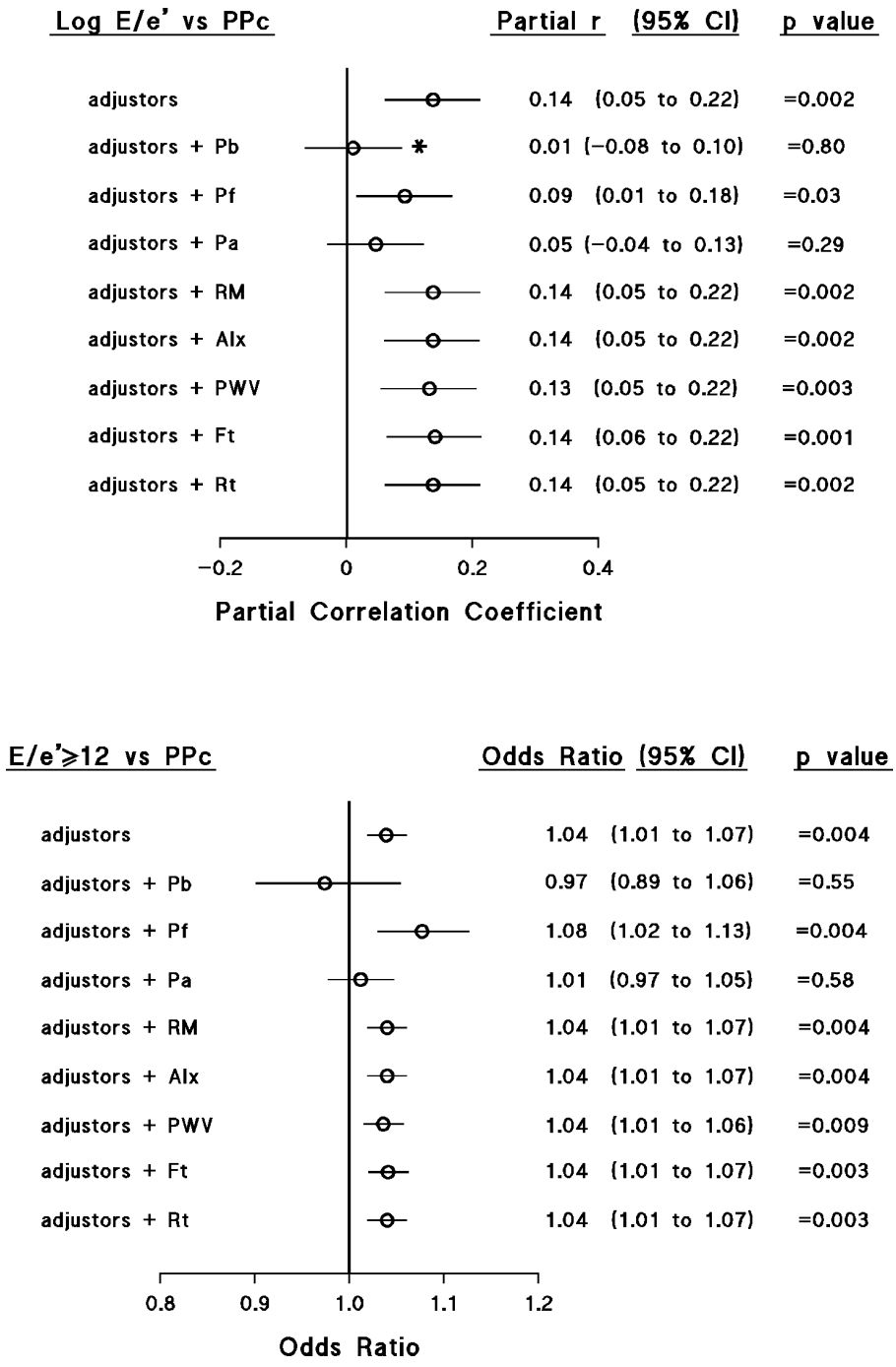


Figure 3.4. Impact of adjustments for aortic haemodynamic parameters on relations between central aortic pulse pressure (PPc) and left ventricular (LV) early trans-mitral blood flow velocity/ velocity of early diastolic myocardial tissue lengthening at the level of the mitral annulus (lateral wall) (E/e')(upper panel) or moderate-to-severe LV diastolic dysfunction (LV DD)(lower panel). See table 3.1 for further abbreviations. *p<0.05 versus partial r of PPc-E/e' relations without adjustments for aortic haemodynamic variables.

3.4.5 Independent associations between aortic haemodynamics and moderate-to-severe diastolic dysfunction (E/e'≥12).

Independent of MAP, and various confounders, and either before or after adjustments for LVMI, PPc, Pb, Pa and RM, but not Pf, PWV, AIx, Ft or Rt were independently associated with moderate-to-severe DD (Table 3.4). Again in support of a role for aortic backward wave pressures, but not aortic stiffness, forward wave pressures or the timing or forward or backward wave pressures accounting for relations between PPc and LV DD, whilst adjustments for Pb and Pa abolished relations between PPc and LV DD, adjustments for Pf, aortic PWV, Ft or Rt failed to significantly modify relations between PPc and LV DD (Figure 3.4, lower panel).

3.4.6 Associations between aortic haemodynamics and E/e' or moderate-to-severe diastolic dysfunction (E/e'≥12) independent of brachial pressures.

Independent of brachial PP and additional confounders, the aortic haemodynamic variables independently associated with E/e' and/or a moderate-to severe DD included PPc, Pb, Pa and RM (Table 3.5). In contrast, neither Pf, aortic PWV nor Ft and Rt were associated with E/e' or moderate-to-severe LV DD independent of brachial PP (Table 3.5).

3.5 Discussion

The main findings of the present study are as follows: In a predominantly young-to-middle-aged community sample with a high prevalence of moderate-to-severe pre-clinical LV DD (10.9%), aortic backward wave pressures (Pb derived from wave separation analysis and Pa) consistently showed independent relations with E/e', an index of filling pressures, and the presence of LV DD (moderate-to-severe), whilst neither forward wave pressures, aortic stiffness (aortic PWV), nor the timing of the forward or backward waves were consistently associated with E/e' and moderate to severe LV DD. Moreover, aortic backward wave pressures, but not aortic forward wave pressures, aortic stiffness or the timing of forward and backward wave pressures accounted for independent relations between aortic PP and increases in E/e' or moderate-to-severe LV DD. Several aortic haemodynamic changes which account for aortic pulsatility, including increases in aortic stiffness and backward wave pressures have been described in heart failure with a preserved ejection fraction (Mohammed et al 2012; Hundley et al 2001; Kawaguchi et al 2003; Weber et al 2006; Weber et al 2008; Borlaug et al 2007; Fernandes et al 2008; Kang et al 2010; Desai et

Table 3.4. Multivariate adjusted associations between aortic functional parameters and moderate-to-severe left ventricular (LV) diastolic dysfunction (69 of 524 participants with an E/e'≥12) in the study group.

E/e'≥12 vs Adjustments→	Odds ratio (95% CI) *	Wald X^2 *	p value	Odds ratio (95% CI) * + LVMI	Wald X^2 * + LVMI	p value
PPc	1.039 (1.013 to 1.067)	8.49	<0.005	1.039 (1.013 to 1.067)	8.46	<0.005
Pb	1.084 (1.032 to 1.139)	10.26	<0.002	1.085 (1.032 to 1.140)	10.2	<0.002
Pf	1.020 (0.993 to 1.048)	2.1	=0.14	1.020 (0.993 to 1.048)	2.1	=0.14
Pa	1.104 (1.041 to 1.171)	10.97	<0.001	1.104 (1.041 to 1.171)	10.96	<0.001
RM	1.020 (1.002 to 1.037)	5.01	<0.05	1.020 (1.002 to 1.037)	4.98	<0.05
AIx	1.008 (0.994 to 1.022)	1.31	=0.25	1.008 (0.994 to 1.022)	1.33	=0.25
PWV	1.115 (0.985 to 1.263)	2.97	=0.09	1.115 (0.985 to 1.263)	2.95	=0.09
Ft	1.005 (0.998 to 1.012)	2.04	=0.15	1.005 (0.998 to 1.012)	2.01	=0.16
Rt	0.987 (0.969 to 1.007)	1.64	=0.20	0.988 (0.969 to 1.007)	1.62	=0.20

For abbreviations see Figure 3.2 and Table 3.1. *Adjustments are for age, sex, mean arterial pressure, body mass index, diabetes mellitus or and HbA1c>6.1%, regular smoking, regular alcohol intake, treatment for hypertension (in all participants), pulse rate and LV mass index as indicated.

Table 3.5. Brachial pulse pressure-adjusted associations between aortic functional parameters and left ventricular (LV) early trans-mitral blood flow velocity / velocity of the mean value of lateral and septal wall myocardial tissue lengthening in early diastole at the mitral annulus (E/e') or moderate-to-severe LV diastolic dysfunction (E/e'≥12) in the study group (n=524).

	Partial r (95% CI)*†	Standardised β-coefficient±SEM*‡	p value
Log E/e' vs			
PPc	0.09 (0.00 to 0.17)	0.30±0.15	=0.05
Pb	0.10 (0.01 to 0.18)	0.24±0.11	<0.05
Pf	-0.004 (-0.09 to 0.08)	-0.01±0.07	=0.93
Pa	0.09 (0.001 to 0.17)	0.15±0.08	<0.05
RM	0.07 (-0.02 to 0.16)	0.08±0.05	=0.11
AIx	0.04 (-0.04 to 0.13)	0.05±0.05	=0.34
PWV	0.02 (-0.07 to 0.11)	0.02±0.05	=0.66
Ft	0.06 (-0.03 to 0.14)	0.05±0.04	=0.19
Rt	-0.04 (-0.13 to 0.05)	-0.04±0.04	=0.38
	Odds ratio*	Wald X^2	p value
E/e'≥12 vs	(95% CI)		
PPc	1.091 (1.007 to 1.182)	4.54	<0.05
Pb	1.128 (1.024 to 1.242)	5.95	<0.05
Pf	0.965 (0.900 to 1.034)	1.03	=0.31
Pa	1.095 (1.017 to 1.179)	5.77	<0.05
RM	1.023 (1.006 to 1.041)	6.84	<0.01
AIx	1.011 (0.997 to 1.025)	2.24	=0.13
PWV	1.079 (0.948 to 1.227)	1.33	=0.25
Ft	1.006 (0.999 to 1.013)	2.71	=0.10
Rt	0.986 (0.968 to 1.005)	2.04	=0.15

For abbreviations see Figure 3.2 and Table 3.1. *Adjustments are for age, sex, brachial pulse pressure, mean arterial pressure, body mass index, diabetes mellitus or and HbA1c>6.1%, regular smoking, regular alcohol intake, treatment for

hypertension, pulse rate and LV mass index. †represents the strength of the relationship. ‡represents the slope of the relationship.

al 2009; Kitzman et al 2013), and in pre-clinical DD (Kawaguchi et al 2003; Weber et al 2006; Weber et al 2008; Borlaug et al 2007).

However, all of these studies (Mohammed et al 2012; Hundley et al 2001; Kawaguchi et al 2003; Weber et al 2006; Weber et al 2008; Fernandes et al 2008; Kang et al 2010; Desai et al 2009; Kitzman et al 2013) were conducted in participants of an average age of 60-77 years where striking increases in aortic stiffness occur (Booysen et al 2015). In contrast, the present study was conducted in a community sample with an average age (46.8 years) where marked increases in aortic stiffness have not as yet occurred, but where increases in aortic backward waves are well established (Booysen et al 2015). Importantly, only three prior studies employed study sample sizes larger than the present study (Mohammed et al 2012; Fernandes et al 2008; Kang et al 2010) and these studies assessed large vessel stiffness, but not backward wave function, thus precluding a comparison of the contribution of these two aortic functional changes to LV diastolic function. As I show that aortic backward wave pressures, but not aortic stiffness are associated with LV DD, the present study adds to prior studies (Mohammed et al 2012; Hundley et al 2001; Kawaguchi et al 2003; Weber et al 2006; Weber et al 2008; Fernandes et al 2008; Kang et al 2010; Desai et al 2009; Kitzman et al 2013) by suggesting that the impact of backward wave pressures on LV DD antedates the effects of aortic stiffness or increases in forward wave pressures. The present results therefore suggest an important approach to prevent the development of heart failure associated with a normal ejection fraction. In this regard, prospective studies evaluating the impact of targeting aortic backward wave pressures rather than simply brachial BP on the development of heart failure with a normal ejection fraction are required.

In the present study I was unable to show relations between aortic backward wave changes and E/A, an index of myocardial relaxation, independent of age or steady-state pressures (MAP). This is in apparent contrast to two smaller (n=143 to 336) studies conducted in clinical samples (Weber et al 2008; Ikonomidis et al 2008), but is consistent with an alternative small (n=106) study (Tsioufis et al 2005). There are several possible explanations for these discrepancies including the use of a select group of hypertensive patients (Ikonomidis et al 2008), or high risk patients referred for catheterisation (Weber et al 2008), whom are more likely to have LV relaxation abnormalities than that noted in a largely healthy community-based sample (present study), or in newly diagnosed hypertensives (Tsioufis et al 2005). However, the most likely reason for discrepant data is that diastolic is more important

than systolic BP in associations with E/A, whilst systolic BP (or PP) is more important than diastolic BP in associations with E/e' (Libhaber et al 2014). Hence, the impact of aortic function (which influences systolic BP) on E/A will depend on the confounding effects of diastolic BP in the sample studied. Importantly, increases in E/e', although not a 'gold-standard' measure of diastolic function, is the LV diastolic functional change most likely to progress to heart failure with a preserved ejection fraction (Kane et al 2011).

The results of the present study should be interpreted with caution. The present results do not suggest that aortic stiffness does not contribute to pre-clinical DD. Indeed, prior studies have repeatedly demonstrated relations between large vessel stiffness and indexes of DD (Mohammed et al 2012; Hundley et al 2001; Kawaguchi et al 2003; Weber et al 2006; Weber et al 2008; Fernandes et al 2008; Kang et al 2010; Desai et al 2009; Kitzman et al 2013; Tsioufis et al 2005; Abhayaratna[a] et al 2006). In this regard, one of the three prior studies that were larger than the present study assessed carotid distensibility (Fernandes et al 2008) rather than aortic function and there is dispute as to whether carotid stiffness predicts outcomes or adequately reflects changes in aortic stiffness. In addition, one of the three prior studies that were larger than the present study assessed brachial-ankle stiffness (Kang et al 2010), which incorporates a stiffness assessment of medium-sized vessels (which contain little elastic tissue) as well as the aorta. Nevertheless, in one small study (n=188) a dominant role of aortic stiffness over aortic augmented pressures in the identification of DD was noted (Abhayaratna[a] et al 2006), and in another small study (n=53) (Desai et al 2009) a relationship between aortic stiffness, but not aortic augmentation index and heart failure with a preserved ejection fraction was reported. Thus large vessel stiffness should be seen as a critical pre-requisite to the development of DD. The present study nevertheless suggests that prior to an age when large vessel stiffness contributes toward DD, increases in aortic backward waves are a major contributing factor to DD. As age-related increases in backward wave pressures largely account for pulsatile haemodynamic effects on aortic PWV (Sibiya et al 2015), targeting backward wave pressures early in life may prevent increases in aortic stiffness and consequently further attenuate the transition to heart failure with a preserved ejection fraction.

Several studies have demonstrated relations between reflected waves as identified from augmentation index (AIx) and cardiac diastolic function (Weber et al 2006; Weber et al 2008; Ikonomidis et al 2008; Hashimoto et al 2006). However, there is some question as to whether the impact of reflected waves should be

assessed using AIx or Pa or whether reflected wave function should be derived from wave separation analysis and consequently Pb or RM. In this regard, whilst AIx may not predict heart failure, RM may show a marked ability to predict heart failure (Chirinos et al 2012). Consistent with this notion, in the present study, although aortic reflected wave pressures derived with (Pb) or without (Pa) wave separation analysis showed independent relations with LV DD, RM but not AIx was independently related to LV DD.

The limitations of the present study include the cross-sectional nature of the study design and hence conclusions regarding cause and effect cannot be drawn. Second, more women volunteered for the present study than men, and sex differences may exist in the pathophysiology of LV DD (Scantlebury et al 2011). In this regard, I was not statistically powered to evaluate the impact of aortic functional parameters in sex-specific groups. Hence, the present results may reflect a dominant effect in women. Third, the assumptions intrinsic to the use of the 'triangulation method' of aortic wave separation may not be ideal (Kips et al 2009). However, in 392 participants of the present sample with aortic velocity measurements, we were able to show a correlation (r^2) between Pb derived from the 'triangulation method' versus 'actual aortic flow' methods of wave separation of 0.82. Hence, at least in the present study, the use of the 'triangulation method' is unlikely to have significantly affected our results. Fourth, in the present study, calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries (Picone et al 2015). Hence, aortic pressures are likely to have been underestimated using the current approach. Last, several measures of LV structure and function, not evaluated in the present study, may be employed to identify DD, but world-wide consensus as to the exact measures required to best identify DD has not been reached. In this regard, in our hands we are unable to reproducibly assess DD during the Valsalva manoeuvre, or pulmonary venous velocity. Hence, we may have underestimated the extent of DD.

In conclusion, in the present study I show that in a community sample of predominantly young-to-middle aged participants, increases in backward (reflected) wave pressures, but not aortic stiffness or forward wave pressures account for aortic pulsatile pressure effects on LV diastolic dysfunction. Hence, the impact of backward wave pressures on LV diastolic dysfunction antedates the effects of aortic stiffness. Therefore, in the transition to pre-clinical DD and possibly therefore to heart failure with a preserved ejection fraction, aortic backward wave pressures rather than aortic stiffness could be the primary aortic haemodynamic disturbance that should be

targeted and this approach should be initiated in the young-to-middle aged. These data, in conjunction with several alternative studies showing relations of backward waves with end organ damage, regression of LVMI and cardiovascular outcomes (Booysen et al 2015; Hashimoto et al 2008; Hughes 2014) provide further support for the need to target aortic backward waves with antihypertensive therapy.

CHAPTER 4

Intra-Familial Aggregation and Heritability of Left Ventricular Geometric Remodelling is Independent of Cardiac Mass in Families of African Ancestry.

The data in this chapter have been published in the Journal of American Society of Hypertension:

Peterson V.R., Norton G.R., Redelinghuys M, Libhaber C.D., Maseko M.J., Majane O.H., Brooksbank R and Woodiwiss A.J. (2015). Intra-Familial Aggregation and Heritability of Left Ventricular Geometric Remodelling is Independent of Cardiac Mass in Families of African Ancestry. *American Journal of Hypertension* 28 (5): 657-663.

4.1 Abstract

Background. Whether left ventricular r (LV) geometric remodelling, as indexed by relative wall thickness (RWT), aggregates in families and is inherited independent of LV mass (LVM) and additional confounders is uncertain.

Methods. I determined whether RWT as assessed from two-dimensional targeted M-mode echocardiography shows intra-familial aggregation and heritability independent of LVM in 181 nuclear families (73 spouse pairs, 403 parent-child pairs and 177 sibling-sibling pairs) with 16 families including three generations from an urban developing community of black Africans. Intra-familial aggregation and heritability estimates (SAGE software) were assessed independent of confounders, including central aortic systolic blood pressure (SBPc)(radial applanation tonometry and SphygmoCor software).

Results. Independent of confounders including SBPc, LV RWT was correlated in parent-child ($r=0.32$, $p<0.0001$) and sibling-sibling ($r=0.29$, $p<0.0001$), but not in spouse ($r=0.11$, $p=0.33$) pairs. The relationships between parent-child ($r=0.28$, $p<0.0001$) and sibling-sibling ($r=0.24$, $p<0.001$) pairs persisted with further adjustments for LVM, or LVM indexed to height$^{2.7}$ (LVMI). Similarly, independent of confounders, LV RWT showed significant heritability ($h^2\pm SEM=0.56\pm0.09$, $p<0.0001$) and this persisted with further adjustments for LVM ($h^2\pm SEM=0.48\pm0.09$, $p<0.0001$) or LVMI ($h^2\pm SEM=0.49\pm0.09$, $p<0.0001$).

Conclusions. In a group of African ancestry, independent of LVM, LV geometric remodelling shows significant intra-familial aggregation and heritability. Genetic factors may in-part determine the LV geometric remodelling process independent of the extent of cardiac hypertrophy.

4.2 Introduction

As acknowledged by all guidelines, left ventricular hypertrophy (LVH) is a major risk factor for cardiovascular events independent of conventional risk factors and coronary artery disease. In addition, as compared to a normal LV geometry, concentric LV remodelling (an increased relative wall thickness [RWT] without an increase in LV mass [LVM]) is associated with a worse prognosis (Verdecchia et al 1995; Koren et al 1991; Bluemke et al 2008; Milani et al 2006). Although LVM rather than concentric LV remodeling predicts incident heart failure (Bluemke et al 2008), concentric LV remodelling without LVH is associated with the development of diastolic dysfunction, and concentric rather than eccentric LVH is associated with greater increases in indices of LV filling pressures (Chahal et al 2010). Thus, the extent of concentric LV remodelling may determine whether progression from LVH to heart failure with a preserved rather than reduced systolic chamber function occurs. The factors that determine LV remodelling are therefore of considerable interest. Although the impact of age, sex, blood pressure and obesity on LV geometric remodelling have been well described, there is nevertheless uncertainty as to the role of genetic factors independent of LVM, a major determinant of LV wall thickness, and a change which itself is well recognised as being inherited (Harshfield et al 1990; Verhaaren et al 1991; Bielen et al 1991; Schunkert et al 1999; Arnette et al 2001; Kuznetsova et al 2003; Bella et al 2004; Assimes et al 2007; Bella & Goring 2012).

As compared to age-, and sex-matched controls, siblings of those with LVH have a greater risk of concentric, but not eccentric LVH (Schunkert et al 1999). However, in that study (Schunkert et al 1999), whether siblings were also at risk for concentric LV remodelling is uncertain and hence LVM may have made a major contribution to the inheritance of concentric LVH. Moreover, in the Framingham Heart Study, the risk for concentric LV remodelling was only modestly increased in related as compared to unrelated individuals, whereas the risk for concentric LVH was markedly augmented (Lam et al 2010). Hence, again, LVM may have been the major determinant of the inheritance of LV remodelling. Although alternative studies indicate that RWT is indeed inherited (Arnett et al 2001; Kuznetsova et al 2003; Bella et al 2004; Fox et al 2010), none of these studies reported on the inheritance of RWT independent of LVM. To address the aforementioned uncertainty as to the extent to which genetic factors contribute toward concentric LV remodelling beyond LVM, in the present study we aimed to evaluate the intra-familial aggregation and heritability of RWT independent of LVM and additional confounders. We hypothesised that RWT

would show intra-familial aggregation and heritability independent of LVM and additional confounders.

4.3 Methods

4.3.1 Study participants

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. Briefly families of black African descent (Nguni and Sotho chiefdoms) with siblings older than 16 years of age were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa using the population census figures of 2001. Family members were invited to take part in the study if at least one or two offspring and one or both parents were available for examination. Eight hundred and twenty-nine participants (91%) consented to an echocardiographic procedure and had no evidence of significant valve abnormalities assessed using 2-dimensional and colour Doppler imaging. None of the participants had previously had a myocardial infarction (only three had a history of ischaemic heart disease), and there were no cases of atrial fibrillation. High quality echocardiograms could be obtained in 694 participants. The high prevalence of obesity limited the quality of echocardiograms obtained in 135 participants of whom 91.5% were female (BMI=39.4±5.9 kg/m^2). Six hundred and seventy-five participants from 181 nuclear families with 16 families including three generations with complete familial pairing were therefore available for the present analysis.

4.3.2 Clinical, demographic, anthropometric and laboratory assessments

A standardised questionnaire was administered to obtain demographic and clinical data (Norton et al 2008; Woodiwiss et al 2009; Redelinghuys et al 2010; Maseko et al 2013). Regular alcohol consumption was defined as at least five glasses of beer per week or 1 bottle of wine per week or ½ bottle of spirits per week. Height and weight 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. Standard laboratory blood tests of renal function,

liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA_{1C}) were performed. Diabetes mellitus (DM) or abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA_{1C} value greater than 6.1%. (Bennett & Guo 2007). Participants' blood groups (ABO and Rhesus) were evaluated to confirm Mendelian segregation. Mendelian inconsistencies were identified if blood groups of family members were incompatible with relationships between family members representing first, second or third generations.

4.3.3 Office blood pressure

High quality office BP measurements were obtained by a trained nurse-technician who measured brachial artery systolic and diastolic BP to the nearest 2 mm Hg using a standard mercury sphygmomanometer, according to the recommendations of the European Society of Hypertension (O'Brien et al 2003) and the American Heart Association (Pickering et al 2005). The nurse was of the same ethnic origins (black African) as the participants and had previously lived in SOWETO. Korotkov phases I and V were employed to identify systolic and diastolic BP respectively and care was taken to avoid auscultatory gaps. Office BP was measured 5 times consecutively using appropriately sized cuffs (a standard sized cuff was used, but if upper arm circumference exceeded 31cm then a larger cuff was used) after the subjects had rested for 5-10 minutes in the sitting position in a quiet room away from onlookers. The mean of all 5 office BP measurements was used in the analysis.

4.3.4 Aortic blood pressure

To determine central aortic systolic BP (SBPc), pulse wave analysis was conducted using techniques previously described (Redelinghuys et al 2010) (see chapter 3, section 3.3.4). To determine SBPc, the pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. From an inbuilt validated generalised transfer function an aortic waveform was generated from which SBPc was derived.

4.3.5 *Echocardiography*

Two-dimensional targeted M-mode echocardiography was employed to determine short axis dimension measurements as previously described (Norton et al 2008; Woodiwss et al 2009; Maseko et al 2013) and analysed according to the American Society of Echocardiography convention (Sahn et al 1978) (see chapter 2, section 2.3.3). The intra- and inter-observer variability have previously been described (Maseko et al 2013). Left ventricular hypertrophy was identified as an LVMI>51 g/$m^{2.7}$ (Nunez et al 2005). Left ventricular RWT was calculated as (LV end diastolic septal [anterior] + posterior wall thickness)/LV end diastolic diameter. An RWT of >0.45 was considered as increased.

4.3.6 Data analysis

Database management and statistical analyses were performed with SAS software, version 9.1 (The SAS Institute Inc., Cary, North Carolina, USA). Data from individual subjects were expressed as mean±SD. For intra-familial aggregation analysis, intra-familial correlations were determined from the PROC GENMOD procedure of the SAS package to determine concordance between family members. Using this approach, pairs of relatives are treated as clusters and the working correlation matrix is defined as unstructured (Kuznetsova et al 2003). Separate correlations were determined between father-mother pairs, parent-child pairs and sibling pairs. Hence, the same individual can be part of more than one pair. These data were confirmed using the FCOR procedure in Statistical Analysis for Genetic Epidemiology (S.A.G.E) software (version 6.3) (Department of Epidemiology and Statistics, Case Western Reserve, University of Cleveland, Ohio). Age- and sex-adjusted and multivariate adjusted (including adjustments for SBPc or brachial office systolic BP) correlation coefficients were determined in 73 father-mother, 403 parent-child and 177 sibling-sibling pairs. Based upon multivariate stepwise association with at least two LV structural traits (LVM, LVMI or LV RWT) the adjustors included in regression models were age, age^2, sex, aortic systolic BP (or brachial office systolic BP), body weight, body height (except for LVMI), regular smoking, regular alcohol intake, treatment for hypertension, treatment for diabetes mellitus or a glycated haemoglobin >6.1% and pulse rate. Adjustments were made for aortic systolic BP in the primary analyses as RWT is more strongly related to central aortic than brachial blood pressure (Roman et al 2010).

Heritability (h^2) of LV parameters was estimated using Statistical Analysis for Genetic Epidemiology (S.A.G.E) software (version 6.3) (Department of Epidemiology and Statistics, Case Western Reserve, University of Cleveland, Ohio) using the Marker-Trait Associations in Pedigree Data (ASSOC) programme. The ASSOC programme estimates heritability by maximum likelihood, assuming a generalization of multivariate normality, correlation structures (Elston et al 1992) and regression models (George & Elston 1987) previously described. The ASSOC programme uses a linear regression model, in which the total residual variance is partitioned into the sum of an additive polygenic component and a subject-specific random component. Heritability is the polygenic component divided by the total residual variance. The additive polygenic component of the variance of a trait is calculated from the pedigree data in which relationship pairs are defined. Hence, an individual can be part of more than one pair.

For the heritability estimates, linear regression models were determined either with adjustments for age and sex only (age- and sex-adjusted) or with (adjusted) the inclusion of the potential confounders namely age, age^2, sex, aortic systolic BP (or brachial office systolic BP), body weight, body height (except for LVMI), regular smoking, regular alcohol intake, treatment for hypertension, treatment for diabetes mellitus or a glycated haemoglobin >6.1% and pulse rate. Additional adjustments were made for LVM, and LVMI in separate models.

4.4 Results

4.4.1 Characteristics of participants

The number of offspring (2nd and 3rd generations) per family (n=181) amounted to one in 63 families, two in 84 families (7 of these families included grandchildren), three in 20 families, and more than three in 14 families (which included 4 families with 4 offspring, 1 family with 5 offspring, and 9 families with grandchildren and half-siblings). Pairs were defined based upon relationships within families. Consequently, an individual can be part of more than one pair. There were 73 father-mother pairs, 403 parent-child pairs (46 father-son, 70 father-daughter, 104 mother-son, and 183 mother-daughter pairs), and 177 sibling-sibling pairs (24 son-son, 84 daughter-daughter, and 69 daughter-son pairs). No cases of Mendelian inconsistency were noted. More women than men participated in the study. The characteristics of the parents and siblings are given in Table 4.1. Importantly, 43% of

participants were hypertensive, 25% of participants were receiving antihypertensive medication and 35% of participants had uncontrolled hypertension (those that were not receiving anti-hypertensive medication plus those that were receiving anti-hypertensive therapy but whose blood pressure was not controlled). 67% of participants had a normal LVMI and RWT, 11% had a normal LVMI, but an increased RWT (concentric LV remodelling), 6% had an increased LVMI and RWT (concentric hypertrophy), and 16% had an increased LVMI, but a normal RWT (eccentric hypertrophy). The prevalence of concentric or eccentric LVH in siblings was low (Table 4.1). The general characteristics of participants not included in the study were similar to the characteristics of the participants evaluated (data not shown).

4.4.2 LVM and LVMI are associated with RWT

Both LVM ($r=0.30$, $p<0.0001$) and LVMI ($r=0.33$, $p<0.0001$) were correlated with RWT.

4.4.3 Heritability of LVM and LVMI

With adjustments for confounders LVM and LVMI were inherited (Table 4.2).

4.4.4 Intra-familial aggregation of RWT

With adjustments for confounders, the correlation coefficients of parent-sibling and sibling-sibling pairs were significant for RWT and these relationships persisted with further adjustments for either LVM or LVMI (Figure 4.1). With adjustments for confounders, no significant correlations were noted for RWT between spouse pairs (Figure 4.1).

4.4.5 Heritability estimates of RWT

With adjustments for confounders, as well as with further adjustments for either LVM or LVMI, RWT showed heritability (Table 4.3).

Table 4.1. Characteristics of parents and offspring of the study sample.

	Parents (n=320*)	Offspring (n=369*)
Sex (% females)	68	63
Age (years)	58±11	30±11
Body mass index (kg/m^2)	33±7	27±7
% Regular smoking	11	17
% Regular alcohol intake	17	23
% with DM or HbA1C>6.1%	43	11
% with hypertension	71	19
% treated for hypertension	47	6
Office SBP/DBP (mm Hg)	140±23/88±13	119±16/80±12
Central aortic SBP (mm Hg)	132±23	110±18
Left ventricular mass (LVM)(g)	172±57	139±40
LVM index (g/m$^{2.7}$)	48±16	38±10
LV relative wall thickness	0.43±0.09	0.37±0.07
LV mean wall thickness (cm)	0.96±0.21	0.83±0.17
LV end diastolic diameter (cm)	4.80±0.59	4.70±0.52
% concentric LVH	11	1
% eccentric LVH	25	8
% concentric LV remodelling	15	7

DM, participants receiving medication for diabetes mellitus; HbA1C, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic BP; LVH, left ventricular hypertrophy. *14 participants are included as both offspring and parents as they are parents of third generation offspring.

Table 4.2. Unadjusted and multivariate adjusted heritability estimates (h^2) of left ventricular mass (LVM) and left ventricular mass index (LVMI).

	Adjustments*	h^2±SEM	p value
LVM	Age- and sex-adjusted	0.52±0.08	<0.0001
	Adjustors including aortic SBP	0.49±0.09	<0.0001
	Adjustors including brachial SBP	0.48±0.09	<0.0001
LVMI	Age- and sex-adjusted	0.47±0.09	<0.0001
	Adjustors including aortic SBP	0.46±0.09	<0.0001
	Adjustors including brachial SBP	0.48±0.09	<0.0001

*Adjustments are for age, age^2, sex, aortic systolic blood pressure (SBP) or brachial SBP, body weight, body height (except for left ventricular mass index), regular smoking, regular alcohol intake, treatment for hypertension, treatment for diabetes mellitus or a glycated haemoglobin >6.1%, and pulse rate.

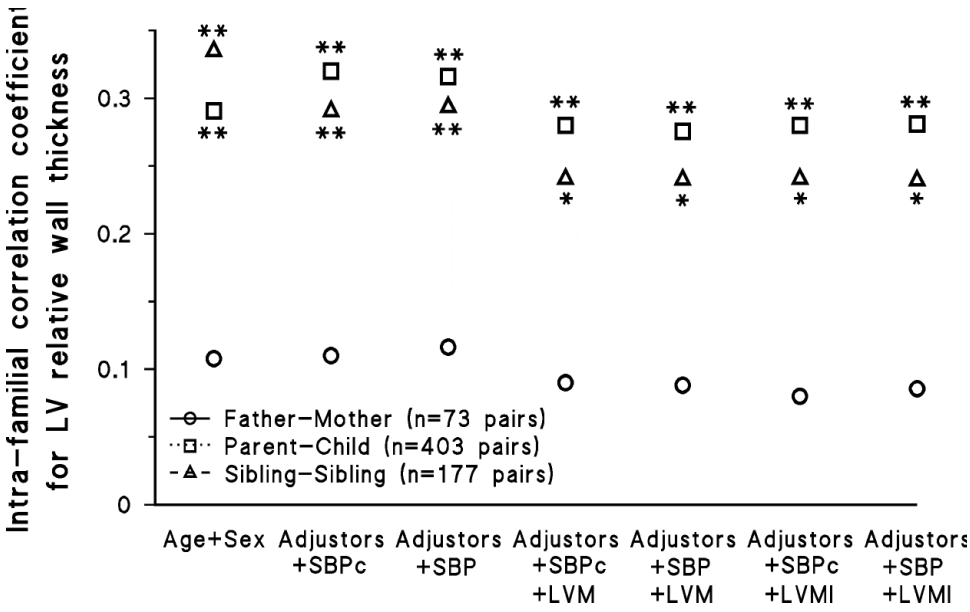


Figure 4.1 Age- and sex-adjusted and multivariate adjusted intra-familial correlations (r-values) of left ventricular (LV) relative wall thickness. LVM, left ventricular mass; LVMI, left ventricular mass indexed to height$^{2.7}$; *p<0.001, **p<0.0001 for significance of correlation coefficient. Adjustments are for age, age^2, sex, aortic systolic blood pressure (SBPc) or brachial systolic blood pressure (SBP), body weight, body height (except when adjusting for LVMI), regular smoking, regular alcohol intake, treatment for hypertension, treatment for diabetes mellitus or a glycated haemoglobin>6.1%, and pulse rate.

Table 4.3. Unadjusted and multivariate adjusted heritability estimates (h^2) of left ventricular (LV) relative wall thickness (RWT) adjusted for left ventricular mass (LVM) and LV mass index (LVMI).

	Adjustments*	h^2±SEM	p value
LV RWT	Age- and sex-adjusted	0.54±0.09	<0.0001
	Adjustors including aortic SBP	0.56±0.09	<0.0001
	Adjustors including brachial SBP	0.55±0.09	<0.0001
	Adjustors including aortic SBP + LVM	0.48±0.09	<0.0001
	Adjustors including brachial SBP + LVM	0.48±0.09	<0.0001
	Adjustors including aortic SBP + LVMI	0.49±0.09	<0.0001
	Adjustors including brachial SBP + LVMI	0.48±0.09	<0.0001

*Adjustments are for age, age^2, sex, aortic systolic blood pressure (SBP) or brachial SBP, body weight, body height, regular smoking, regular alcohol intake, treatment for hypertension, treatment for diabetes mellitus or a glycated haemoglobin >6.1%, and pulse rate.

4.5 Discussion

In a relatively large family-based study, we show that RWT, an index of the extent of concentric LV geometric remodelling, demonstrated intra-familial aggregation and heritability (h^2±SEM: 0.56±0.09). Importantly, the intra-familial aggregation and heritability of RWT were independent of LVM and LVMI as well as other confounders (h^2±SEM: 0.48±0.09).

Although a number of prior studies have reported heritability or intra-familial aggregation of LVM or LVMI (Harshfield et al 1990; Verhaaren et al 1991; Bielen et al 1991; Schunkert et al 1999; Arnett et al 2001; Kuznetsova et al 2003; Bella et al 2004; Assimes et al 2007; Bella & Goring 2012), few studies have assessed the intra-familial aggregation or heritability of LV RWT (Schunkert et al 1999; Arnett et al 2001; Bella et al 2004; Bella & Goring 2012; Fox et al 2010). Only two of these five prior studies reported heritability values (h^2±SEM: 0.17±0.08 (Bella et al 2004); 0.25 (Fox et al 2010)) and in another prior study, heritability could be estimated from twice the sibling correlations (ranged from 0.08 to 0.24 (Arnett et al 2001)). In none of these studies (Arnett et al 2001; Bella et al 2004; Fox et al 2010) was the heritability of RWT identified to be independent of LVM or LVMI. In our study without adjustments for LVMI we obtained heritability estimates for RWT of 0.56±0.09. The higher heritability values obtained in our study compared to two of these previous studies (Arnett et al 2001; Bella et al 2004) could be due to the characteristics of the populations assessed. Indeed, the mean RWT in the current study was considerably greater (0.48 in the parents and 0.37 in the offspring) than in two of the prior studies (0.35 in both) (Arnett et al 2001; Bella et al 2004). In one of these previous studies (Arnett et al 2001) the majority of the participants were hypertensives receiving antihypertensive medication, which would have influenced LV structural values.

Although three prior studies (Arnett et al 2001; Bella et al 2004; Lam et al 2010), have demonstrated that RWT is inherited (h^2±SEM: 0.17±0.08 (Bella et al 2004); 0.25 (Lam et al 2010); 2xsibling correlation ranged from 0.08 to 0.24 (Arnett et al 2001) and aggregates in families (Arnett et al 2001; Bella et al 2004), in these studies no adjustments for LVM or LVMI were made and hence these effects can be attributed to the well-known influence of hypertrophy on concentric LV geometric remodelling. Indeed, in the present study both LVM and LVMI were strongly associated with RWT. One prior study has reported on familial aggregation of concentric, but not eccentric LVH (Schunkert et al 1999). In contrast however, familial aggregation was noted for both concentric and eccentric LVH in the

Framingham study (Lam et al 2010). Nevertheless, in the Framingham study concentric LV remodelling (without LVH) also showed intra-familial aggregation (Lam et al 2010), an effect that suggests that concentric LV geometric remodelling may be inherited independent of LVM. However, in that (Lam et al 2010) no adjustments for LVM or alternative structural determinants of RWT were reported on. The present study extends the findings of this prior study (Lam et al 2010) and shows that RWT indeed aggregates in related individuals in families and shows heritability independent of LVM (h^2±SEM: 0.48±0.09) and LVMI (h^2±SEM: 0.49±0.09). The present and this prior study (Lam et al 2010) together therefore provide a degree of confidence that concentric LV remodelling shows heritability independent of hypertrophy or alternative LV structural parameters.

In the present study, independent of confounders, heritability estimates (h^2±SEM) for RWT were 0.56±0.09 before adjustments for confounders and LVM or LVMI. These RWT heritability estimates (h^2±SEM) are stronger than those previously reported on in American Indians (0.17±0.08) (Bella et al 2004), and in largely treated (70%) hypertensive African Americans (0.25) (Fox et al 2010) without adjustments for LVM or LVMI. At present there are no data RWT heritability estimates in Caucasians. The greater heritability estimates reported on in the present study compared to previous studies, is possibly because we studied randomly recruited South African families of black African ancestry with a low prevalence (25%) of treatment for hypertension, and it is well recognised that groups of African descent have a greater RWT than other ethnic groups, a difference that could be attributed to stronger genetic effects on LV geometric remodelling. However, sibling correlations for RWT have been reported to be greater in European as compared to African-Americans (Arnett et al 2001). Nevertheless, in that study (Arnett et al 2001) only hypertensive siblings were evaluated and hence intra-familial correlations for LV structure may have been confounded by the effects of BP or antihypertensive treatment. Importantly, in the Multi-Ethnic Study of Atherosclerosis, compared to non-Hispanic whites, non-Hispanic blacks had an increased risk for concentric remodelling with an odds ratio of 1.4 (Rodriguez et al 2010).

An important consideration of the present study is that the intra-familial aggregation of RWT was independent of LVM or LVMI. Thus, although LVM and LVMI showed heritability, the heritability of LV hypertrophy does not appear to determine the intra-familial aggregation and heritability of the geometric LV remodelling process. Hence, the genes that determine LV hypertrophy should not be seen as necessarily the same genes that influence the overall LV geometric

remodelling process. This has important implications for genetic studies as it is possible that the molecular mechanisms that determine cardiomyocyte cell growth (hypertrophy) are unable to account for a significant portion of the LV remodelling process.

The limitations of the present study require consideration. Due to the young age of the siblings studied, a low prevalence of LVH was noted in sibling pairs. Consequently, few siblings had either concentric or eccentric LVH. Therefore, we could not assess the intra-familial aggregation and heritability of either concentric or eccentric LVH, considered as discrete traits, independent of LVM or LVMI. Second, we assessed the intra-familial aggregation and heritability of RWT in one ethnic group only. Whether similar effects are noted in alternative ethnic groups requires further study. Third, bearing in mind the high prevalence of cardiovascular risk factors such as obesity, hypertension, DM or abnormal blood glucose control and LVH in the parents, it is possible that the heritability of RWT tracks the heritability of these risk factors. However, this is unlikely given that the adjusted intra-familial aggregation coefficients were similar between parent-child pairs and sibling pairs despite the higher prevalence of cardiovascular risk factors in the parents compared to the children. Moreover, in the present study on multivariate analyses, the heritability of RWT was independent of these cardiovascular risk factors.

In conclusion, in the present study I demonstrate that RWT, an index of concentric LV geometric remodelling, aggregates in families and shows heritability, independent of LVM and LVMI. Thus, a considerable proportion of the genetic factors that determine LV geometric remodelling occur independent of those genes that influence cardiomyocyte hypertrophy. As LV geometric remodelling predicts outcomes independent of LVH (Verdecchia et al 1995; Koren et al 1991; Bleumke et al 2008; Milani et al 2006) and may influence whether LVH progresses to heart failure with a preserved or reduced ejection fraction (Chahal et al 2010), discovering the genes associated with RWT independent of LVH may cast further light on the genetic mechanisms responsible for adverse cardiovascular outcomes and the progression to heart failure.

CHAPTER 5

Intra-Familial Aggregation and Heritability of Tissue Doppler Indexes of Left Ventricular Diastolic Function in a Group of African Descent.

The data in this chapter have been published in the Journal of American Society of Hypertension:

Peterson V.R., Norton G.R., Libhaber C.D., Maseko M.J., Sareli P and Woodiwiss A.J. (2016). Intra-Familial Aggregation and Heritability of Tissue Doppler Indexes of Left Ventricular Diastolic Function in a Group of African Descent. *Journal of American Society of Hypertension* 10 (6): 517-526.

5.1 Abstract

Background. Although several indexes of left ventricular (LV) diastolic function show heritability, the genetic influence on the tissue Doppler index, E/e' (early transmitral velocity/velocity of myocardial tissue lengthening), an index of LV filling pressures in those of black African descent is currently unknown. Further, whether any genetic influences on E/e' are through an impact of LV remodelling or aortic function is unknown.

Methods. Intra-familial aggregation and heritability (SAGE software) of E/e' (echocardiography) were assessed in 129 nuclear families (29 spouse pairs, 216 parent-child pairs and 113 sibling-sibling pairs) from an urban developing community of black Africans, independent of LV mass index (LVMI), LV relative wall thickness (RWT), central aortic systolic pressure (SBPc) and backward wave pressures (Pb) (applanation tonometry, SphygmoCor software).

Results. Independent of confounders including LVMI and RWT, E/e' was correlated in parent-child ($r=0.23$, $p<0.001$) and sibling-sibling ($r=0.29$, $p<0.005$), but not in spouse ($r=0.13$, $p=0.51$) pairs. The relationships between parent-child ($r=0.22$ $p<0.001$) and sibling-sibling ($r=0.29$, $p<0.005$) pairs persisted with adjustments for SBPc. The relationships between parent-child ($r=0.22$, $p<0.001$) and sibling-sibling ($r=0.26$, $p<0.01$) pairs also persisted with adjustments for Pb. Independent of confounders including LVMI and RWT, E/e' showed significant heritability ($h^2 \pm SEM=0.51\pm0.11$, $p<0.0001$) which similarly persisted with adjustments for SBPc ($h^2 \pm SEM=0.50\pm0.11$, $p<0.0001$) and Pb ($h^2 \pm SEM=0.49\pm0.11$, $p<0.0001$).

Conclusions. In a group of African ancestry, independent of LV remodelling and aortic function, E/e', shows significant intra-familial aggregation and robust heritability. Hence, genetic factors may play an important role in determining moderate-to-severe LV diastolic dysfunction independent of cardiac remodelling or aortic function in groups of black African ancestry.

5.2 Introduction

The prevalence of heart failure with a preserved ejection fraction may soon exceed that of a reduced ejection fraction (Borlaug & Redfield 2011). The outcomes of heart failure with a preserved ejection fraction are as poor as in heart failure with a reduced ejection fraction (Lee et al 2009; Owan et al 2006; Bhatia et al 2006), yet there is presently no treatment with proven benefit for heart failure with a preserved ejection fraction (Borlaug & Redfield 2011; Borlaug & Paulus 2011; Pitt et al 2014). Understanding the pathophysiological mechanisms responsible for heart failure with a preserved ejection fraction may reveal potential therapeutic targets. Cardiac diastolic dysfunction is central to the pathophysiology and outcomes of heart failure with a preserved ejection fraction (Zile et al 2004; Westermann et al 2008; Burke et al 2014; Mohammed et al 2012; Shah et al 2014). However, the factors that determine cardiac diastolic function have not been completely elucidated.

Several studies have demonstrated heritability or intra-familial aggregation of various aspects of left ventricular (LV) diastolic function identified from trans-mitral blood flow velocity measurements (Bielin et al 1991; Tang et al 2002; Swan et al 2003; Fox et al 2010; Jin et al 2011; Noh et al 2015; Kloch-Badelek et al 2014). However, contemporary evaluation of LV diastolic dysfunction also includes E/velocity of myocardial tissue lengthening at the mitral annulus (Ea or e') (E/e'), an index of filling pressures employed to identify moderate-to-severe diastolic dysfunction and which is independently associated with the development of heart failure with a preserved ejection fraction (Kane et al 2011; Lam et al 2011). The genetic influence on E/e' across ethnic groups is nevertheless unclear. Whilst a Korean study showed significant heritability for E/e' (Noh et al 2015), a study conducted in Europeans failed to do so (Jin et al 2011), but subsequent analysis in an expanded data set showed significant heritability (Kloch-Badelek et al 2014). Whether E/e' is inherited in groups of African ancestry and the extent to which this occurs is unknown. Moreover, whether significant heritability of E/e' occurs independent of LV remodelling, which is also inherited and a well-recognised determinant of diastolic dysfunction, or of aortic function, which is similarly inherited and a strong determinant of diastolic dysfunction (Weber et al 2006; Weber et al 2008; Borlaug et al 2007; Ferander et al 2008), is unknown. Hence, in the present study I aimed to assess whether intra-familial aggregation and heritability of E/e' occurs independent of LV remodelling and aortic function in a community of African ancestry.

5.3 Methods

5.3.1 Study participants

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The present study design has previously been described (Peterson et al 2015; Woodiwiss et al 2009; Redelinghuys et al 2010; Libhaber et al 2014; Millen et al 2014). Briefly families of black African descent (Nguni and Sotho chiefdoms) with siblings older than 16 years of age were randomly recruited (based on population census figures from 2001) from the South West Township (SOWETO) of Johannesburg, South Africa. Eight hundred and twenty-nine participants (91% consent rate) consented to an echocardiographic procedure and had no evidence of significant valve abnormalities assessed using 2-dimensional and colour Doppler imaging. None of the participants had previously had a myocardial infarction (only three had a history of ischaemic heart disease), and there were no cases of atrial fibrillation. High quality echocardiograms with complete familial pairing could be obtained in 694 participants and in a sub-study 442 participants from 129 nuclear families with 12 families including three generations with complete familial pairing had myocardial tissue Doppler imaging (see figure 5.1 for derivation of the study sample).

5.3.2 Clinical, demographic, anthropometric and laboratory assessments

A standardised questionnaire was administered to obtain demographic and clinical data (Peterson et al 2015; Woodiwiss et al 2009; Redelinghuys et al 2010; Libhaber et al 2014; Millen et al 2014). Regular alcohol consumption was defined as at least five glasses of beer per week or 1 bottle of wine per week or ½ bottle of spirits per week. Height and weight 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. Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA_{1C}) were performed. Diabetes mellitus (DM)

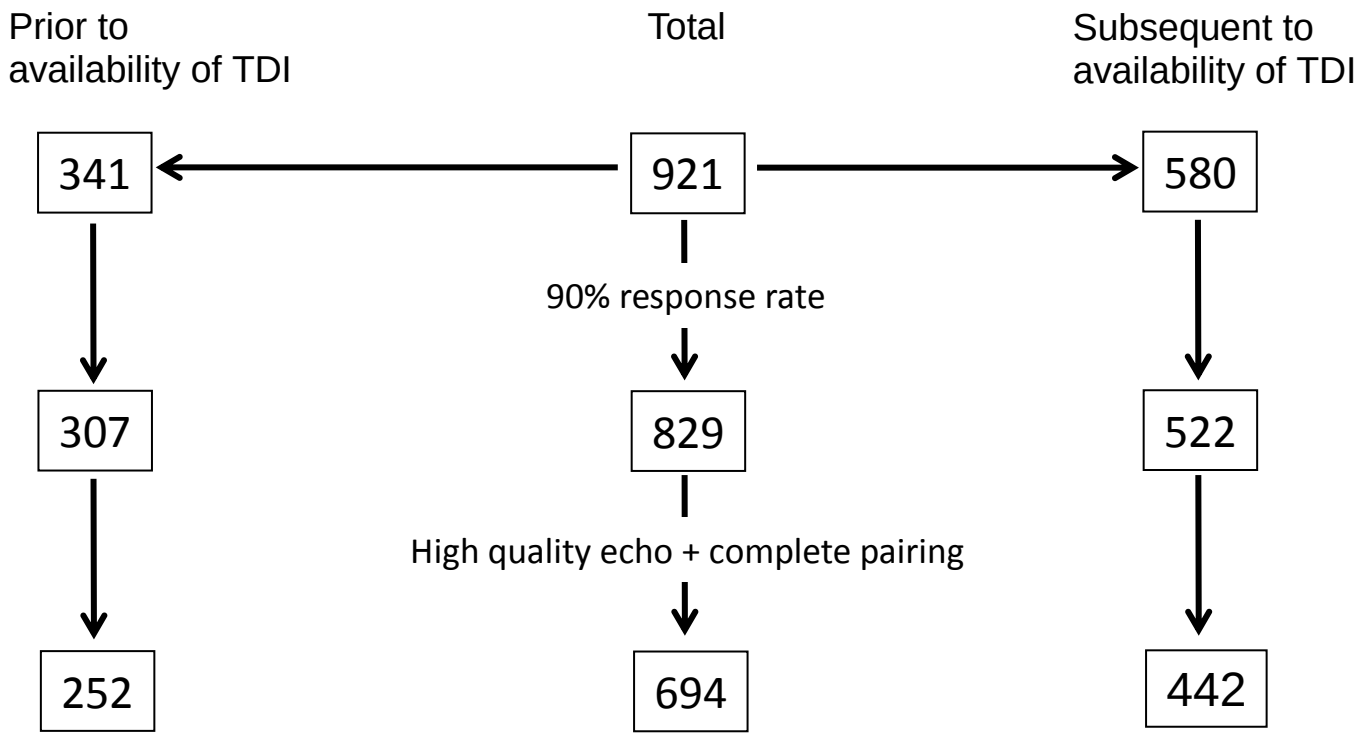


Figure 5.1. Flow chart summarising the derivation of the study sample. Complete pairing refers to sample with at least either 2 parents and one sibling or one parent and 2 siblings. 341 participants were approached to participate in the study prior to the availability of tissue Doppler imaging (TDI) and 580 participants were approached to participate in the study after the availability of TDI. Echo, echocardiography.

or abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA_{1C} value greater than 6.1%. Menopause was confirmed with measurements of follicle stimulating hormone concentrations. Participants' blood groups (ABO and Rhesus) were evaluated to confirm Mendelian segregation. Mendelian inconsistencies were identified if blood groups of family members were incompatible with relationships between family members representing first, second or third generations.

5.3.2 Clinical, demographic, anthropometric and laboratory assessments

A standardised questionnaire was administered to obtain demographic and clinical data (Peterson et al 2015; Woodiwiss et al 2009; Redelinghuys et al 2010; Libhaber et al 2014; Millen et al 2014). Regular alcohol consumption was defined as at least five glasses of beer per week or 1 bottle of wine per week or ½ bottle of spirits per week. Height and weight 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. Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA_{1C}) were performed. Diabetes mellitus (DM) or abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA_{1C} value greater than 6.1%. Menopause was confirmed with measurements of follicle stimulating hormone concentrations. Participants' blood groups (ABO and Rhesus) were evaluated to confirm Mendelian segregation. Mendelian inconsistencies were identified if blood groups of family members were incompatible with relationships between family members representing first, second or third generations.

5.3.3. Office blood pressure

High quality office BP measurements were obtained by a trained nurse-technician using a standard mercury sphygmomanometer (Woodiwiss et al 2009). The nurse was of the same ethnic origins (black African) as the participants and had previously lived in SOWETO. Korotkov phases I and V were employed to identify systolic and diastolic BP respectively and care was taken to avoid auscultatory gaps. Office BP was measured 5 times consecutively using appropriately sized cuffs after

the subjects had rested for 5-10 minutes in the sitting position. The mean of all 5 office BP measurements was used in the analysis.

5.3.4 Aortic haemodynamics

To determine central aortic systolic BP (SBPc) and backward wave pressures (Pb), pulse wave analysis was conducted using techniques previously described (Peterson et al 2015; Woodiwiss et al 2009) (see chapter 3, section 3.3.4).To determine SBPc, the pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. From an inbuilt validated generalised transfer function an aortic waveform was generated from which SBPc was derived. Aortic backward wave pressures (Pb) were determined using SphygmoCor software which separates the aortic waveform assuming a triangular flow wave.

5.3.5 Echocardiography

Echocardiographic measurements were performed using a Sonosite M-Turbo ultrasound (SonoSite® Inc., Bothell, WA, USA) device with the patient in the partial left decubitus position as previously described (Peterson et al 2015; Libhaber et al 2014; Millen et al 2014) (see chapter 2, section 2.3.3). Left ventricular mass (LVM) was determined using a standard formula (Devereux et al 1986) (see chapter 2, section 2.3.3). Left ventricular RWT was calculated as (LV end diastolic septal [anterior] + posterior wall thickness)/LV end diastolic diameter. An LVMI>51 $g/m^{2.7}$ was considered as LV hypertrophy (LVH) and an RWT of >0.45 was considered as concentric remodelling (if LVMI was normal) or hypertrophy (if LVMI was increased). Left ventricular end diastolic and systolic volumes were determined from M-mode images using the Teichholz method. Left ventricular ejection fraction (EF) was calculated as [(LV end diastolic volume-LV end systolic volume)/ LV end diastolic volume] x 100.

Left ventricular diastolic function was assessed from a pulsed wave Doppler examination of the mitral inflow at rest and using tissue Doppler imaging (TDI) (Quiñones et al 2002) (see chapter 3, section 3.3.5). Data were expressed as e'/a' and the E/e' ratio (an index of LV filling pressures). Left ventricular diastolic dysfunction (DD) was evaluated as mild (impaired relaxation when E/A≤0.75),

moderate (pseudonormal, where E/A>0.75<1.5, and E/e'≥10) and severe (where E/A≥1.5 and E/e'≥10) (Redfield et al 2003).

5.3.6 Data analysis

Database management and statistical analyses were performed with SAS software, version 9.3 (The SAS Institute Inc., Cary, North Carolina, USA). Data from individual subjects were expressed as mean±SD. Indexes of diastolic function were logarithmically transformed to improve on the distribution (Table 5.1 and Figure 5.2). For intra-familial aggregation analysis, intra-familial correlations were determined from the PROC GENMOD procedure of the SAS package to determine concordance between family members. Using this approach, pairs of relatives are treated as clusters and the working correlation matrix is defined as unstructured (Kuznetsova et al 2003). Separate correlations were determined between father-mother pairs, parent-child pairs and sibling pairs. Hence, the same individual can be part of more than one pair. These data were confirmed using the FCOR procedure in Statistical Analysis for Genetic Epidemiology (S.A.G.E) software (version 6.3) (Department of Epidemiology and Statistics, Case Western Reserve, University of Cleveland, Ohio). Multivariate adjusted correlation coefficients were determined in 29 spouse pairs, 216 parent-child pairs and 113 sibling-sibling pairs. The adjustors included in regression models were based upon multivariate stepwise associations with measures of diastolic function (Tables 5.2 to 5.4). Stepwise regression analysis was performed with age, sex, body mass index, heart rate, systolic BP (for TDI indexes), diastolic BP (for trans-mitral velocity measurements), regular smoking, regular alcohol intake, treatment for hypertension and diabetes mellitus or an HbA1c>6.1% included in the models. Probability values in stepwise models were considered significant at a 5% level ($p \leq 0.05$). If factors were independently associated with several indexes of diastolic function they were also included as potential confounders for additional indexes when assessing intra-familial correlations. Hence, for E/A, E, A and e'/a', age, sex, body mass index, heart rate and diastolic blood pressure were included as covariates and for E/e', e' and a', age, sex, body mass index, heart rate and systolic blood pressure were included as covariates. Additional adjustments were made for LVMI, RWT, SBPc, and/or Pb where appropriate.

Heritability (h2) of LV diastolic parameters was estimated using Statistical Analysis for Genetic Epidemiology (S.A.G.E) software (version 6.3) (Department of

Table 5.1. Distribution of left ventricular diastolic function parameters before and after transformation of data.

Parameter	Skewness	Kurtosis	Shapiro-Wilk
Non-transformed			
Trans-mitral E/A	0.849	0.682	0.954
Trans-mitral E	1.184	1.750	0.923
Trans-mitral A	0.884	0.536	0.945
Average e’	0.254	-0.568	0.986
Average a’	0.932	1.050	0.949
Average e’/a’	0.788	0.379	0.948
Average E/e’	1.210	1.038	0.893
Lateral wall e’	0.240	-0.580	0.986
Lateral wall a’	0.808	0.341	0.952
Lateral wall e’/a’	0.887	0.535	0.939
Lateral wall E/e’	1.317	1.479	0.884
Septal wall e’	0.374	-0.188	0.984
Septal wall a’	1.095	1.909	0.940
Septal wall e’/a’	0.989	1.220	0.935
Septal wall E/e’	1.306	1.565	0.888
Logarithm of:			
Trans-mitral E/A	-0.154	-0.228	0.996
Trans-mitral E	0.124	-0.101	0.996
Trans-mitral A	-0.090	-0.360	0.995
Log average e’	-0.460	-0.229	0.995
Log average a’	0.163	-0.278	0.996
Log average e’/a’	-0.205	-0.728	0.984
Log average E/e’	0.290	-0.456	0.985
Log lateral wall e’	-0.503	-0.232	0.976
Log lateral wall a’	0.025	-0.461	0.994
Log lateral wall e’/a’	-0.202	-0.651	0.986
Log lateral wall E/e’	0.316	-0.376	0.986
Log septal wall e’	-0.701	1.371	0.970
Log septal wall a’	0.129	0.129	0.997
Log septal wall e’/a’	-0.196	-0.542	0.987

Log septal wall E/e’	0.274	-0.440	0.987

E and A, early and late (atrial) trans-mitral blood flow velocity; e’ and a’, velocity of myocardial tissue lengthening in early (e’) and late (atrial-a’) diastole.

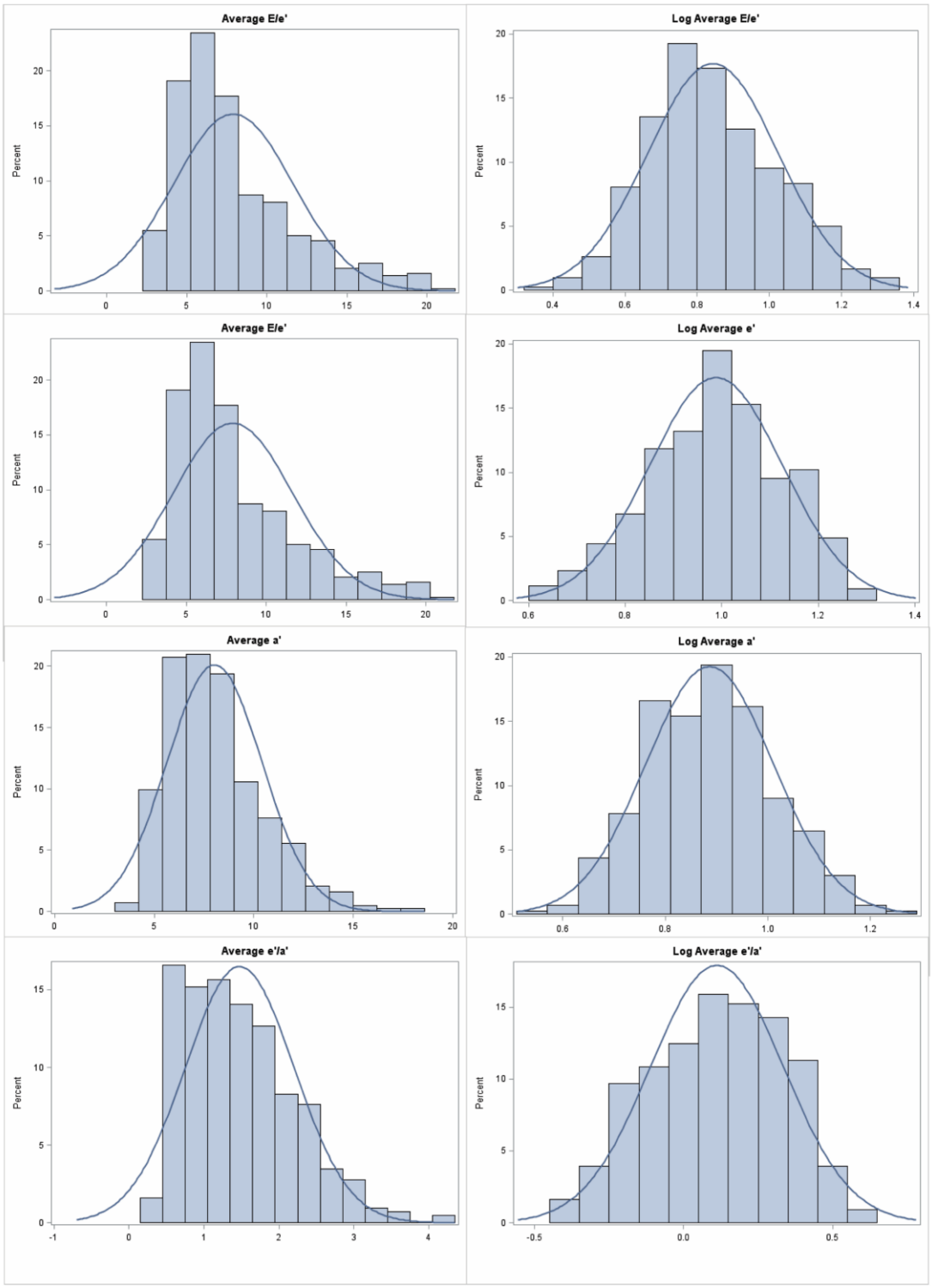


Figure 5.2. Distribution of indexes of left ventricular diastolic function before and after logarithmic transformation. E and A, early and late (atrial) trans-mitral blood flow velocity; e' and a', velocity of myocardial tissue lengthening in early (e') and late (atrial-a') diastole, table 5.1 for characteristics of the distribution curves as well as table 5.1 for improved characteristics with logarithmically transformed indexes.

Table 5.2. Stepwise regression models showing factors independently associated with trans-mitral E/A, E and A and myocardial tissue Doppler e'/a' (average of lateral and septal wall values).

Factor	Log trans-mitral E/A		Log trans-mitral E		Log trans-mitral A		Log e'/a'	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value
Age	-0.488	<0.0001	-0.050	<0.0001	0.214	<0.0001	-0.576	<0.0001
Female	-	-	0.014	<0.02	0.024	<0.0005	0.007	<0.005
Body mass index	-0.004	<0.05	-	-	-	-	-0.026	<0.0001
Heart rate	-0.043	<0.0001	-0.017	=0.005	-	-	-0.014	<0.0001
DBP	-0.012	<0.001	-	-	0.008	<0.05	-0.015	<0.0001
Model r^2	0.550	<0.0001	0.092	<0.0001	0.254	<0.0001	0.648	<0.0001

DBP, diastolic BP; E and A, early and late (atrial) trans-mitral blood flow velocity; e' and a', velocity of myocardial tissue lengthening in early (e') and late (atrial-a') diastole. Factors that did not reach significance in the models include regular smoking, regular alcohol intake, treatment for hypertension and diabetes mellitus or an HbA1c>6.1%.

Table 5.3. Stepwise regression models showing factors independently associated with TDI (average of lateral and septal wall) measures of left ventricular diastolic function.

Factor	Log E/e'		Log e'		Log a'	
	r^2	p value	r^2	p value	r^2	p value
Age	0.189	<0.0001	-0.491	<0.0001	0.253	<0.0001
Female	0.023	<0.0005	-	-	-0.010	<0.05
Body mass index	0.008	<0.05	-0.029	<0.0001	0.011	<0.01
Heart rate	-	-	-0.006	<0.05	0.020	<0.001
SBP	0.029	<0.001	-0.024	<0.0001	-	-
Model r^2	0.253	<0.0001	0.553	<0.0001	0.300	<0.0001

DBP, diastolic BP; E and A, early and late (atrial) trans-mitral blood flow velocity; e' and a', velocity of myocardial tissue lengthening in early (e') and late (atrial-a') diastole. Factors that did not reach significance in the models include regular smoking, regular alcohol intake, treatment for hypertension and diabetes mellitus or an HbA1c>6.1%.

Table 5.4. Stepwise regression models showing factors independently associated with TDI (lateral and septal wall given separately) measures of left ventricular diastolic function.

	Lateral wall						Septal wall					
Factor	Log E/e'		Log e'		Log a'		Log E/e'		Log e'		Log a'	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value
Age	0.160	<0.0001	-0.460	<0.0001	0.214	<0.0001	0.183	<0.0001	-0.414	<0.0001	0.180	<0.0001
Female	0.030	<0.0001	0.014	<0.05	0.024	<0.0005	-	-	-	-	-0.009	<0.0001
Body mass index	0.007	=0.05	-	-	-	-	0.020	=0.001	-0.017	<0.0005	0.013	<0.01
Heart rate	-	-	-0.017	=0.005	-	-	-	-	-	-	0.029	<0.0001
SBP	0.036	<0.0001	0.008	=0.05	0.019	<0.001	0.018	<0.005	-0.009	<0.01	-	-
Model r^2	0.236	<0.0001	0.501	<0.0001	0.265	<0.0001	0.228	<0.0001	0.444	<0.0001	0.235	<0.0001

DBP, diastolic BP; E and A, early and late (atrial) trans-mitral blood flow velocity; e' and a', velocity of myocardial tissue lengthening in early (e') and late (atrial-a') diastole. Factors that did not reach significance in the models include regular smoking, regular alcohol intake, treatment for hypertension and diabetes mellitus or an HbA1c>6.1%.

Epidemiology and Statistics, Case Western Reserve, University of Cleveland, Ohio) using the Marker-Trait Associations in Pedigree Data (ASSOC) programme. The ASSOC programme estimates heritability by maximum likelihood, assuming a generalisation of multivariate normality, correlation structures (Elston et al 1992) and regression models (George & Elston 1987) previously described. The ASSOC programme uses a linear regression model, in which the total residual variance is partitioned into the sum of an additive polygenic component and a subject-specific random component. Heritability is the polygenic component divided by the total residual variance. The additive polygenic component of the variance of a trait is calculated from the pedigree data in which relationship pairs are defined. Hence, an individual can be part of more than one pair. For the heritability estimates, linear regression models were determined with the inclusion of potential confounders identified as being independently associated with measures of diastolic function in stepwise regression analysis (Tables 5.2 to 5.4). If factors were independently associated with several indexes of diastolic function they were also included as potential confounders for additional indexes when assessing heritability estimates. Hence, for E/A, E, A and e'/a', age, sex, body mass index, heart rate and diastolic blood pressure were included as covariates and for E/e', e' and a', age, sex, body mass index, heart rate and systolic blood pressure were included as covariates. Additional adjustments were made for LVMI, RWT, SBPc, and/or Pb where appropriate

5.4 Results

5.4.1 Characteristics of participants

The number of offspring (2nd and 3rd generations) per family (n=129) amounted to one in 44 families, two in 54 families (5 of these families included grandchildren), three in 19 families, and more than three in 12 families (which included 4 families with 4 offspring, 1 family with 5 offspring, and 7 families with grandchildren and half-siblings). Pairs were defined based upon relationships within families. Consequently, an individual can be part of more than one pair. There were 29 father-mother pairs, 216 parent-child pairs (22 father-son, 33 father-daughter, 58 mother-son, and 103 mother-daughter pairs), and 113 sibling-sibling pairs (18 son-son, 50 daughter-daughter, and 45 daughter-son pairs). No cases of Mendelian inconsistency were noted. The characteristics of the parents and siblings are given in

Table 5.5. More women than men participated in the study. Importantly, 47.1% of participants were hypertensive, 27.4% of participants were receiving antihypertensive medication and 34.4% of participants had uncontrolled hypertension (those that were not receiving anti-hypertensive medication plus those that were receiving anti-hypertensive therapy but whose blood pressure was not controlled). Other than a slightly higher E/A, and a modestly lower E and A, no differences were noted between participants with and without TDI (Table 5.6). In the group with TDI, 12.2% of participants had mild DD, 16.5% moderate DD, and 3.8% severe DD. The prevalence of DD in the siblings was low (Table 5.5).

5.4.2 LV remodelling and aortic function are associated with diastolic function.

LVM indexed to height$^{1.7}$ (r=-0.28, p<0.0001) or height$^{2.7}$ (r=-0.31, p<0.0001), RWT (r=-0.28, p<0.0001), SBPc (r=-0.38, p<0.0001), and Pb (r=-0.34, p<0.0001) were correlated with E/A. Indexes of LV remodelling were similarly correlated with E and A (data not given). LVM indexed to height$^{1.7}$ (r=-0.38, p<0.0001) or height$^{2.7}$ (r=-0.40, p<0.0001), RWT (r=-0.33, p<0.0001), SBPc (r=-0.46, p<0.0001), and Pb (r=-0.42, p<0.0001) were also correlated with e'/a'. Similarly, LVM indexed to height$^{1.7}$ (r=0.25, p<0.0001) or height$^{2.7}$ (r=0.29, p<0.0001), RWT (r=0.27, p<0.0001), SBPc (r=0.36, p<0.0001), and Pb (r=0.40, p<0.0001) were correlated with E/e'. Indexes of LV remodelling were likewise correlated with e' and a' (data not given).

In multivariate models LVMI and RWT retained independent relations with E/e' (p<0.05), and e' (p<0.05) but not E/A (p>0.15), E (p>0.06), A (p>0.06), or a' (p>0.42) and RWT (p<0.02), but not LVMI (p=0.36) retained independent relations with e'/a'. Moreover, in multivariate models with mean arterial pressure included in the models, SBPc (p<0.005), and Pb (p<0.0005) retained independent relations with E/e'. However, no independent relations between SBPc or Pb and e' (p>0.49), E/A (p>0.09), E (p>0.09), A (p>0.08), e'/a' (p>0.09), or a' (p>0.26) were noted.

5.4.3 *Intra-familial aggregation of indexes of diastolic function*

With adjustments for confounders, the correlation coefficients of parent-sibling and/or sibling-sibling pairs were significant for E, and A (Figure 5.3), and E/e' and a' derived from the average of lateral and septal wall measurements (Figure 5.4), lateral wall E/e', e' and a' (Figure 5.5) and septal wall E/e' and a' (Figure 5.6). In contrast, no significant father-mother correlations were noted (Figures 5.3 to 5.6)

Table 5.5 Characteristics of parents and offspring of the study sample.

	Parents (n=193*)	Offspring (n=262*)
Sex (% females)	70	63
Age (years)	61±11	34±12
Body weight (kg)	82±18	73±20
Body height (m)	1.59±0.08	1.62±0.09
Body mass index (kg/m^2)	32±7	28±8
% Regular smoking	13	17
% Regular alcohol intake	11	21
% with DM or HbA1C>6.1%	43	15
% with hypertension	75	27
% treated for hypertension	53	8
Heart rate (bts/min)	66±11	66±11
Office SBP/DBP (mm Hg)	139±23/86±13	121±17/81±13
Central aortic SBP (mm Hg)	129±23	111±20
Aortic backward wave (mm Hg)	21±8	13±5
LVM index (g/m$^{2.7}$)	46±18	34±11
Relative wall thickness (RWT)	0.39±0.08	0.35±0.07
Trans-mitral E/A†	0.94 (0.76 to 1.19)	1.46 (1.18 to 1.81)
Trans-mitral E†	66.0 (49.5 to 87.4)	74.7 (60.3 to 95.5)
Trans-mitral A†	72.9 (53.8 to 91.9)	51.7 (39.5 to 66.8)
Average e'†	7.8 (6.5 to 9.7)	12.0 (9.8 to 14.1)
Average a'†	8.7 (7.5 to 10.6)	7.0 (5.9 to 8.2)
Average e'/a'†	0.87 (0.67 to 1.23)	1.72 (1.31 to2.34)
Average E/e'†	8.41 (6.19 to 11.67)	6.12 (4.75 to 7.87)
Lateral wall e'†	8.5 (6.8 to 10.7)	12.8 (10.4 to 15.1)
Lateral wall a'†	9.2 (7.3 to 11.2)	7.0 (5.7 to 8.6)
Lateral wall e'/a'†	0.91 (0.68 to 1.30)	1.76 (1.36 to 2.31)
Lateral wall E/e'†	7.76 (5.71 to 10.92)	5.77 (4.30 to 7.52)
Septal wall e'†	7.1 (5.7 to 9.0)	11.3 (9.0 to 13.4)
Septal wall a'†	8.3 (6.8 to 9.9)	6.9 (5.7 to 8.2)
Septal wall e'/a'†	0.80 (0.64 to 1.18)	1.61 (1.22 to 2.09)
Septal wall E/e'†	9.05 (6.82 to 13.03)	6.61 (5.00 to 8.24)
% E/A<0.75	22	5

% E/e'≥10	38	15
% LVMI>51 g/$m^{2.7}$	35	5

DM, participants receiving medication for diabetes mellitus; HbA_{1C}, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic BP; LVMI, left ventricular mass index; E and A, early and late (atrial) trans-mitral blood flow velocity; e' and a', velocity of myocardial tissue lengthening in early (e') and late (atrial-a') diastole. *Thirteen participants are included as both offspring and parents as they are parents of third generation offspring. †values are median and interquartile range.

Table 5.6 Characteristics of study sample with and without measures of tissue Doppler indices (TDI) of left ventricular diastolic function.

	With TDI	Without TDI
Sample number (% females)	442 (65.2)	252 (65.2)
Age (years)	45.3±18.1	43.6±18.0
Body weight (kg)	77±20	76±19
Body height (m)	1.61±0.08	1.62±0.09
Body mass index (kg/m^2)	29.7±7.9	29.2±7.4
% Regular smoking	15.4	12.3
% Regular alcohol intake	17.4	22.1
% with DM or HbA_{1C}>6.1%	26.5	25.6
% with hypertension	47.1	45.3
% treated for hypertension	27.4	22.8
Heart rate (bts/min)	66±11	66±11
Office SBP/DBP (mm Hg)	128±22/83±13	131±22/84±12
Central aortic SBP (mm Hg)	119±23	122±23
Aortic backward wave (mm Hg)	16±8	17±8
Trans-mitral E/A†	1.23 (0.89 to 1.62)*	1.17 (0.86 to 1.55)
Trans-mitral E†	72.5 (55.5 to 91.3)*	78.1 (64.7 to 91.2)
Trans-mitral A†	58.2 (44.5 to 80.4)*	66.8 (57.5 to 78.1)

DM, participants receiving medication for diabetes mellitus; HbA_{1C}, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic BP. *p<0.05 versus without TDI. † values are median and interquartile range.

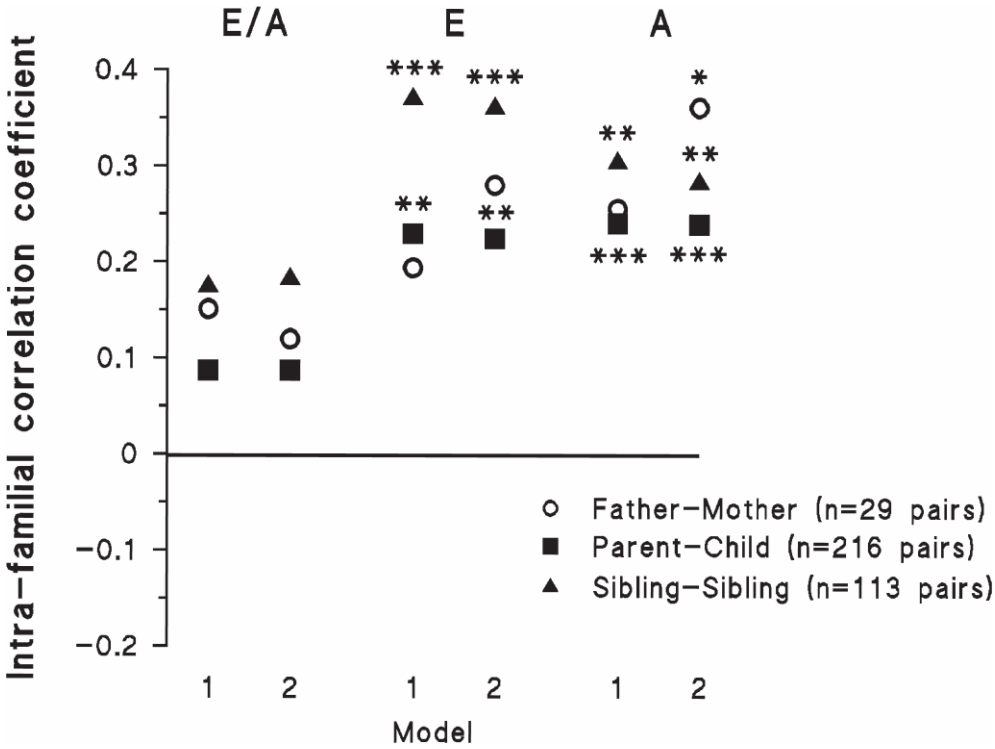


Figure 5.3 Multivariate adjusted intra-familial correlations (r values) of left ventricular diastolic function derived from trans-mitral velocity measurements. Model 1 is adjusted for confounders indicated in the footnote to table 5.6. Model 2 includes additional adjustments for left ventricular mass index and relative wall thickness. See table 5.5 for abbreviations. *p<0.05, **p<0.005, ***p<0.0005 for significance of correlation coefficient.

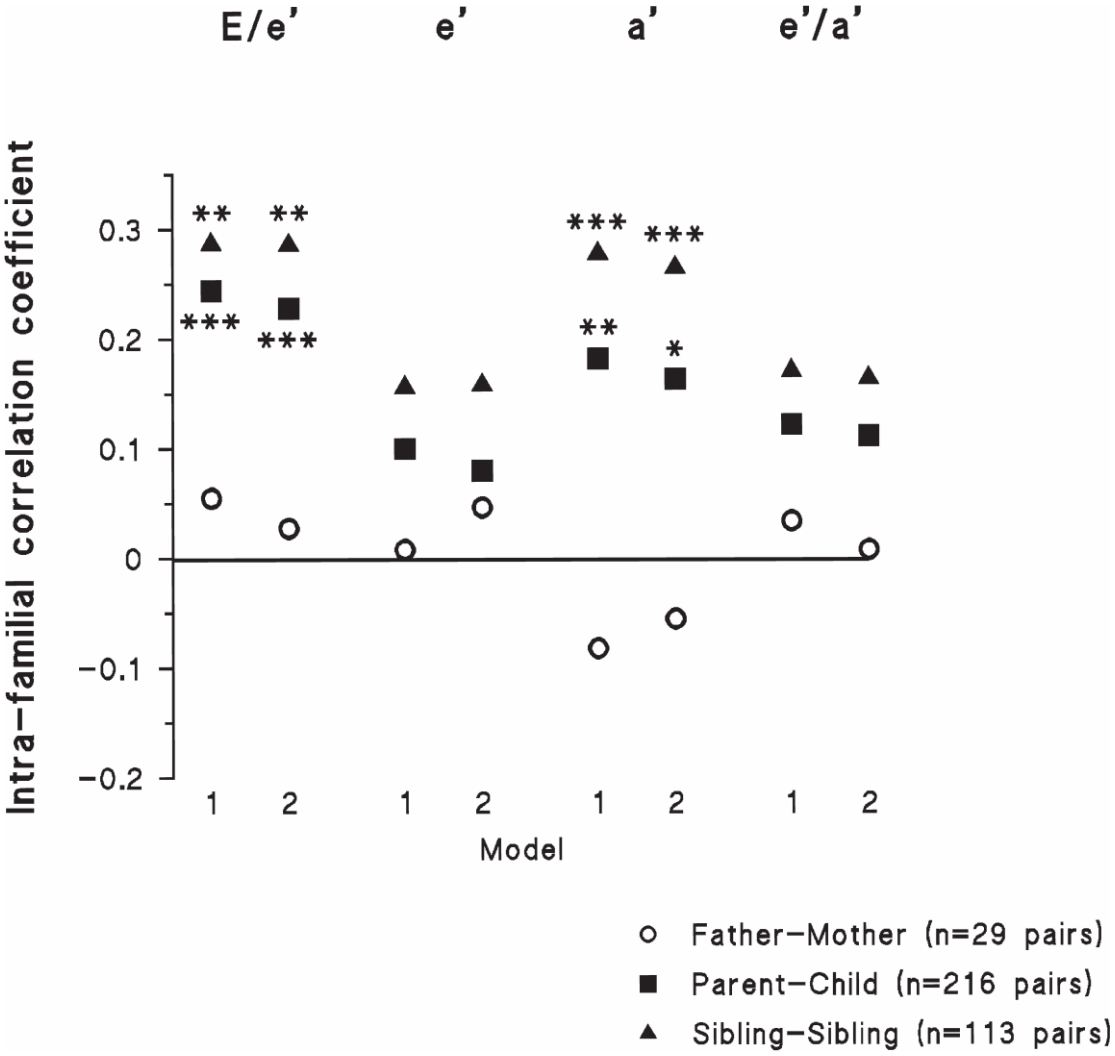


Figure 5.4 Multivariate adjusted intra-familial correlations (r values) of left ventricular diastolic function derived from the average of lateral and septal wall tissue Doppler imaging. Model 1 is adjusted for confounders indicated in the footnote to table 5.6. Model 2 includes additional adjustments for left ventricular mass index and relative wall thickness. See table 5.5 for abbreviations. *p<0.05, **p<0.005, ***p<0.0005 for significance of correlation coefficient.

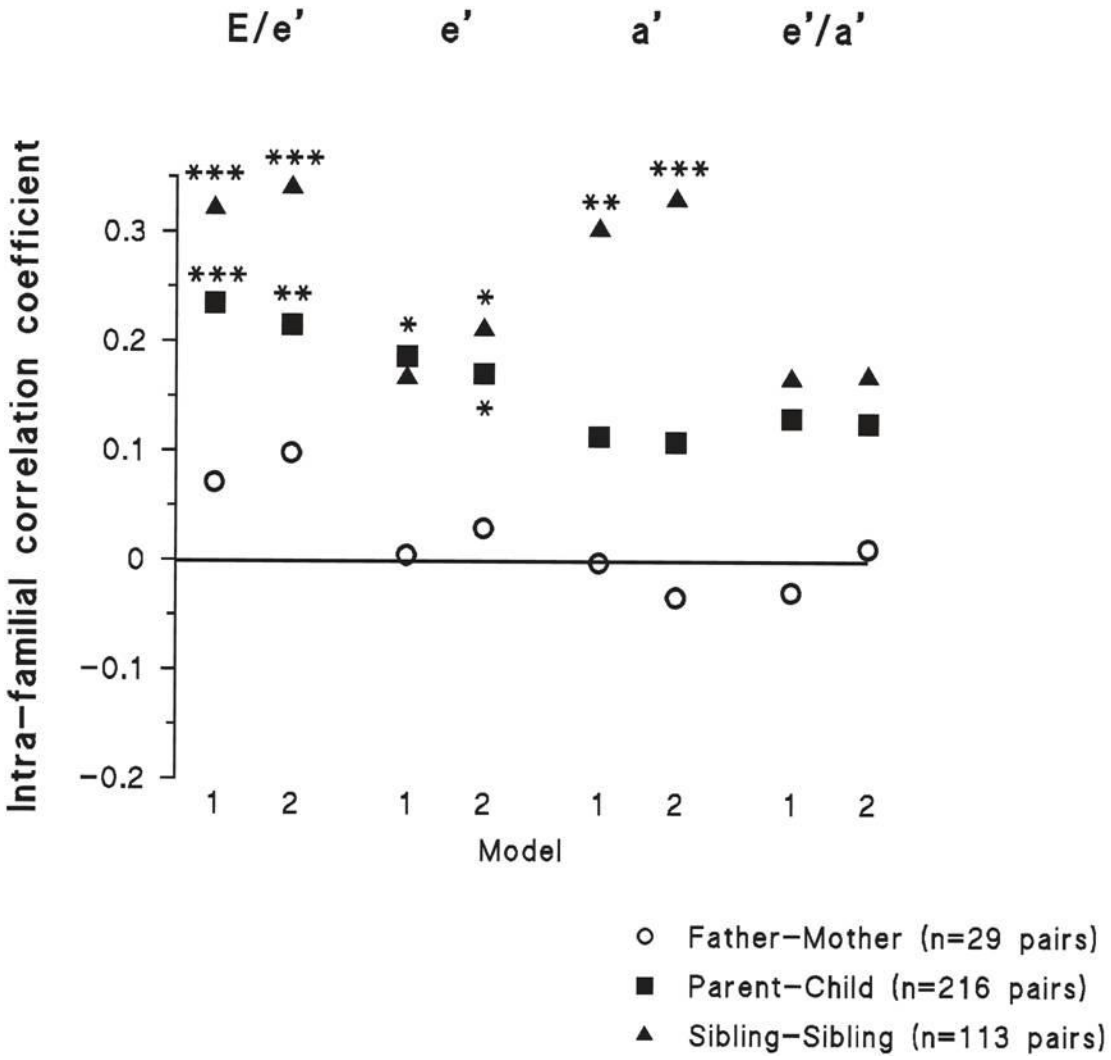


Figure 5.5 Multivariate adjusted intra-familial correlations (r values) of left ventricular diastolic function (lateral wall tissue Doppler imaging). Model 1 is adjusted for confounders indicated in the footnote to table 5.6. Model 2 includes additional adjustments for left ventricular mass index and relative wall thickness. See table 1 for abbreviations. *p<0.05, **p<0.005, ***p<0.0005 for significance of correlation coefficient.

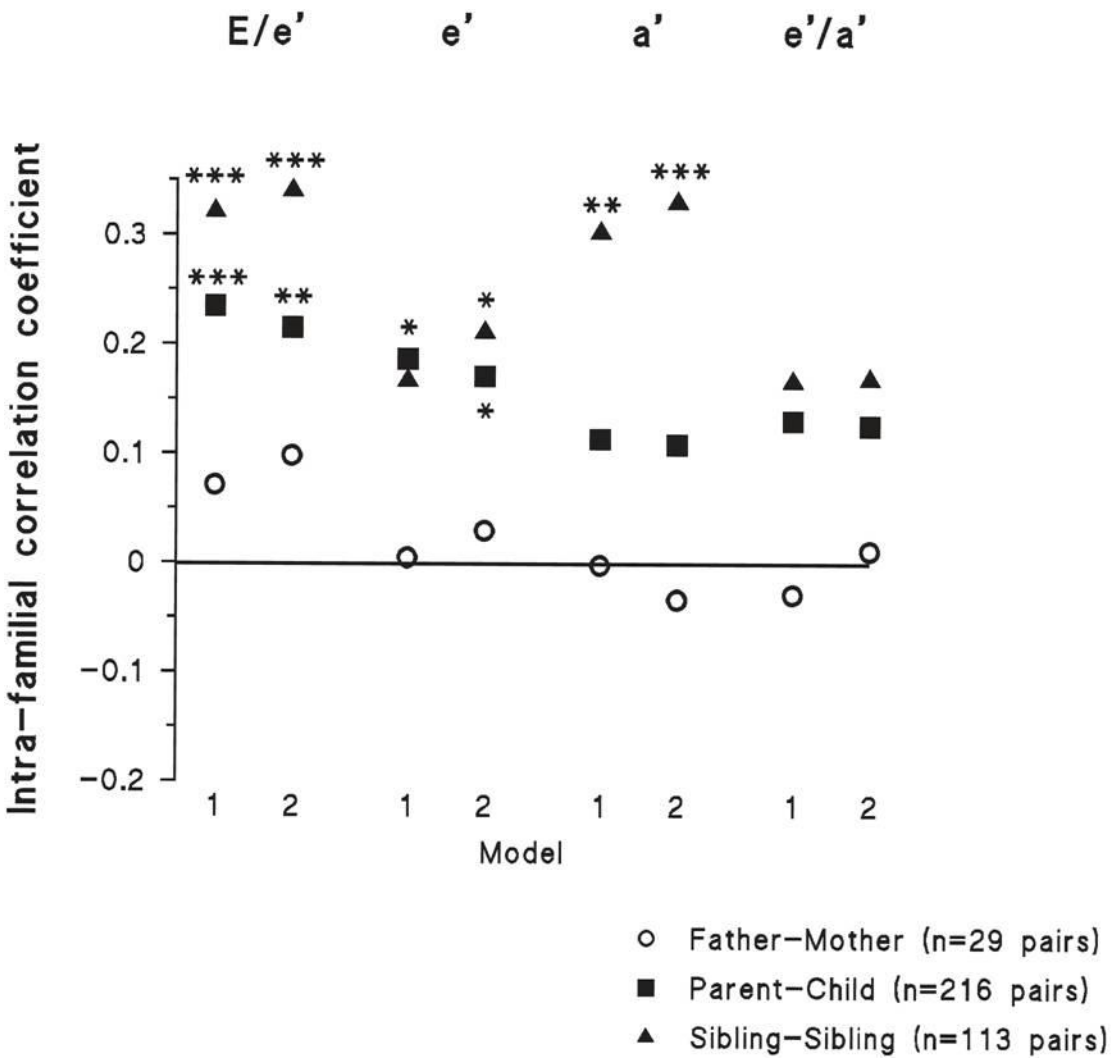


Figure 5.6 Multivariate adjusted intra-familial correlations (r values) of left ventricular diastolic function (septal wall tissue Doppler imaging). Model 1 is adjusted for confounders indicated in the footnote to table 5.6. Model 2 includes additional adjustments for left ventricular mass index and relative wall thickness. See table 5.5 for abbreviations. *p<0.05, **p<0.005, ***p<0.0005 for significance of correlation coefficient.

except for A after adjustments for indexes of LV remodelling (Figure 5.3). The significant parent-sibling and sibling-sibling correlations were retained with further adjustments for LVMI and RWT (Figures 5.3 to 5.6) and for SBPc or Pb in place of brachial SBP (Figure 5.7). Similar intra-familial correlations were noted with adjustments for LVMI or RWT in separate models (data not shown).

5.4.4 Heritability estimates of indexes of diastolic function

With adjustments for confounders (Tables 5.7, 5.8, 5.9 and 5.10), as well as with further adjustments for LVMI and RWT (Tables 5.7 to 5.10) and additional adjustments for SBPc or Pb where appropriate (Table 5.11), indexes of LV diastolic function showed significant heritability. Importantly however, while E/A showed only weak heritability estimates (trend effects) (Table 5.7), the average of septal and lateral wall (Table 5.8), lateral wall (Table 5.9) and septal wall (Table 5.10) E/e' showed strong heritability estimates. Also in contrast to strong heritability estimates for E/e' and a', the average of septal and lateral wall e' and e'/a' (Table 5.8), and lateral wall e' and e'/a' (Table 5.9) showed intermediate heritability estimates and septal wall e' and e/a' (Table 5.10) showed weak or insignificant heritability estimates. Similar heritability estimates were noted with adjustments for LVMI or RWT in separate models (data not shown).

5.5 Discussion

The main findings of the present study are that in a relatively large family-based study conducted in a group of African ancestry, E/e', an index of LV filling pressures which when increased, is indicative of moderate-to-severe diastolic dysfunction, demonstrated strong intra-familial aggregation and heritability (h^2±SEM for the average of lateral and septal wall E/e'=0.52±0.11, $p<0.0001$). Importantly, a novel finding of this study is that the intra-familial aggregation and heritability of E/e' was independent of LVMI, geometric remodelling as indexed by RWT, and aortic function (SBPc and aortic backward wave pressures).

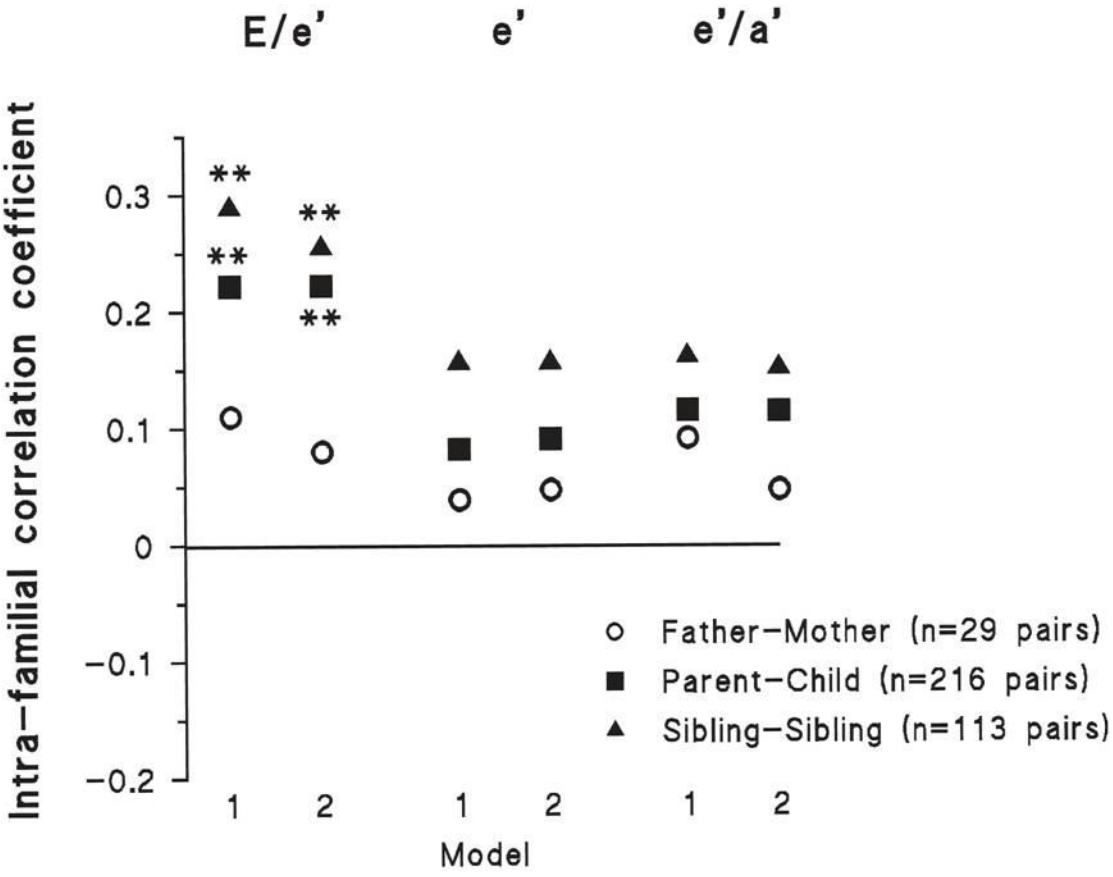


Figure 5.7 Multivariate adjusted intra-familial correlations (r values) of left ventricular diastolic function derived from the average of lateral and septal wall tissue Doppler imaging with further adjustments for central aortic systolic blood pressure (model 1) or aortic backward wave pressure (model 2). All models are adjusted for confounders indicated in the footnote to table 5.6 as well as left ventricular mass index and relative wall thickness. See table 5.5 for abbreviations. **p<0.005 for significance of correlation coefficient.

Table 5.7. indexes of left ventricular diastolic function derived from trans-mitral (blood flow) Doppler velocities.

	Adjustments	h^2±SEM	p-value	Genetic variance±SEM	p value	Total variance ±SEM	p-value	Proportion attributed to covariates (r^2)
Log Trans-mitral E/A	*	0.29±0.12	<0.01	0.004±0.002	<0.02	0.014±0.001	<0.0001	0.53
Log Trans-mitral E/A	* +LVMI+RWT	0.28±0.12	<0.02	0.004±0.002	<0.02	0.014±0.001	<0.0001	0.53
Log Trans-mitral E	*	049±0.11	<0.0001	0.012±0.003	<0.0001	0.024±0.002	<0.0001	0.11
Log Trans-mitral E	* +LVMI+RWT	0.49±0.11	<0.0001	0.012±0.003	<0.0001	0.023±0.002	<0.0001	0.13
Log Trans-mitral A	*	0.52±0.11	<0.0001	0.014±0.003	<0.0001	0.026±0.002	<0.0001	0.19
Log Trans-mitral A	* +LVMI+RWT	0.50±0.11	<0.0001	0.013±0.003	<0.0001	0.026±0.002	<0.0001	0.22

See table 5.5 for abbreviations. *Additional adjustments include age, sex, diastolic blood pressure, body mass index and heart rate (see Table 5.2 for justification of the inclusion of these adjustors).

Table 5.8. Multivariate adjusted heritability estimates of several indexes of left ventricular diastolic function derived from the average of lateral and septal wall tissue Doppler measurements.

Adjustments	h^2±SEM	p-value	Genetic variance ±SEM	p value	Total variance ±SEM	p-value	Proportion attributed to covariates (r^2)
Log mean E/e' *	0.52±0.11	<0.0001	0.015±0.004	<0.0001	0.029±0.002	<0.0001	0.19
Log mean E/e' * +LVMI+RWT	0.51±0.11	<0.0001	0.015±0.004	<0.0001	0.029±0.002	<0.0001	0.22
Log mean e' *	0.30±0.14	<0.05	0.003±0.002	<0.05	0.010±0.001	<0.0001	0.54
Log mean e' * +LVMI+RWT	0.29±0.14	<0.05	0.003±0.002	<0.05	0.010±0.007	<0.0001	0.56
Log mean a' *	0.60±0.11	<0.0001	0.007±0.002	<0.0001	0.011±0.001	<0.0001	0.25
Log mean a' * +LVMI+RWT	0.60±0.11	<0.0001	0.007±0.002	<0.0001	0.011±0.001	<0.0001	0.26
Log mean e'/a' *	0.35±0.13	<0.005	0.007±0.003	<0.005	0.018±0.001	<0.0001	0.59
Log mean e'/a' * +LVMI+RWT	0.31±0.13	<0.01	0.005±0.002	<0.02	0.018±0.001	<0.0001	0.60

See table 5.5 for abbreviations. * Additional adjustments include age, sex, brachial systolic blood pressure (except e'/a' where diastolic blood pressure was employed), body mass index, and heart rate (see Tables 5.2 to 5.4 for justification of the inclusion of these adjustors).

Table 5.9. Multivariate adjusted heritability estimates of several indexes of left ventricular diastolic function derived from lateral wall tissue Doppler measurements.

Adjustments	h^2±SEM	p-value	Genetic variance ±SEM	p value	Total variance ±SEM	p-value	Proportion attributed to covariates (r^2)
Log lateral wall E/e' *	0.48±0.11	<0.0001	0.015±0.004	<0.0001	0.032±0.002	<0.0001	0.17
Log lateral wall E/e' * +LVMI+RWT	0.48±0.11	<0.0001	0.015±0.004	<0.0001	0.031±0.002	<0.0001	0.20
Log lateral wall e' *	0.37±0.13	<0.005	0.005±0.002	<0.005	0.014±0.001	<0.0001	0.47
Log lateral wall e' * +LVMI+RWT	0.38±0.13	<0.005	0.005±0.002	<0.005	0.013±0.001	<0.0001	0.48
Log lateral wall a' *	0.41±0.12	<0.0005	0.007±0.002	<0.001	0.017±0.001	<0.0001	0.21
Log lateral wall a' * +LVMI+RWT	0.40±0.12	<0.0005	0.007±0.002	<0.001	0.017±0.001	<0.0001	0.22
Log lateral wall e'/a' *	0.33±0.13	<0.005	0.008±0.003	<0.01	0.024±0.002	<0.0001	0.54
Log lateral wall e'/a' * +LVMI+RWT	0.31±0.13	<0.01	0.007±0.003	<0.005	0.024±0.002	<0.0001	0.54

See table 5.5 for abbreviations. * Additional adjustments include age, sex, brachial systolic blood pressure (except e'/a' where diastolic blood pressure was employed), body mass index, and heart rate (see Tables 5.2 to 5.4 for justification of the inclusion of these adjustors).

Table 5.10 Multivariate adjusted heritability estimates of several indices of left ventricular diastolic function derived from septal wall tissue Doppler measurements.

Adjustments	h^2±SEM	p-value	Genetic variance ±SEM	p value	Total variance ±SEM	p-value	Proportion attributed to covariates (r^2)
Log septal wall E/e' *	0.55±0.11	<0.0001	0.018±0.004	<0.0001	0.033±0.002	<0.0001	0.18
Log septal wall E/e' * +LVMI+RWT	0.54±0.11	<0.0001	0.017±0.004	<0.0001	0.032±0.002	<0.0001	0.20
Log septal wall e' *	0.22±0.13	<0.05	0.004±0.002	=0.05	0.016±0.001	<0.0001	0.45
Log septal wall e' * +LVMI+RWT	0.19±0.13	=0.07	0.003±0.002	=0.08	0.016±0.001	<0.0001	0.46
Log septal wall a' *	0.45±0.13	<0.0005	0.006±0.002	<0.001	0.013±0.001	<0.0001	0.20
Log septal wall a' * +LVMI+RWT	0.43±0.13	<0.0005	0.006±0.002	<0.001	0.013±0.001	<0.0001	0.21
Log septal wall e'/a' *	0.12±0.12	=0.16	0.003±0.003	=0.16	0.025±0.002	<0.0001	0.52
Log septal wall e'/a' * +LVMI+RWT	0.10±0.12	=0.21	0.002±0.003	=0.21	0.024±0.002	<0.0001	0.52

See table 5.5 for abbreviations. * Additional adjustments include age, sex, brachial systolic blood pressure (except e'/a' where diastolic blood pressure was employed), body mass index, and heart rate (see Tables 5.2 to 5.4 for justification of the inclusion of these adjustors).

Table 5.11 Multivariate adjusted heritability estimates of left ventricular diastolic function derived from the average of lateral and septal wall tissue Doppler measurements with additional adjustments for central aortic systolic blood pressure (SBPc) or aortic backward wave pressures (Pb).

	Adjustments	h^2±SEM	p-value	Genetic variance±SEM	p value	Total variance ±SEM	p-value	Proportion attributed to covariates (r^2)
Log mean E/e'	* +SBPc	0.50±0.11	<0.0001	0.014±0.004	<0.0001	0.029±0.002	<0.0001	0.22
Log mean E/e'	* +Pb	0.49±0.11	<0.0001	0.014±0.004	<0.0001	0.029±0.002	<0.0001	0.22
Log mean e'	*+ SBPc	0.28±0.14	<0.05	0.003±0.002	<0.05	0.010±0.001	<0.0001	0.56
Log mean e'	* +Pb	0.30±0.14	<0.05	0.003±0.002	<0.05	0.0011±0.001	<0.0001	0.54
Log mean a'	*+ SBPc	0.60±0.11	<0.0001	0.007±0.002	<0.0001	0.011±0.001	<0.0001	0.26
Log mean a'	*+Pb	0.60±0.11	<0.0001	0.007±0.002	<0.0001	0.011±0.001	<0.0001	0.26
Log mean e'/a'	* +SBPc	0.33±0.13	<0.01	0.006±0.003	<0.01	0.018±0.001	<0.0001	0.60
Log mean e'/a'	* +Pb	0.33±0.13	<0.01	0.006±0.003	<0.01	0.018±0.001	<0.0001	0.59

See table 5.5 for abbreviations and *table 5.8 for additional adjustments with brachial systolic or diastolic blood pressure being replaced with the blood pressure value as indicated. Left ventricular mass index and relative wall thickness were also included as confounders.

As noted in the present study, significant heritability of E/e' has been described in Koreans (Noh et al 2015), and in Europeans (Kloch-Badelek et al 2014). Hence, the present findings corroborate the notion that E/e' is in-part genetically determined and that this effect extends across ethnic groups. However, the present findings suggest that the genetic contribution to E/e' may be markedly higher in groups of black African descent, as compared to other ethnic groups. In this regard, we show heritability estimates for E/e' (h^2±SEM=0.52±0.11, p<0.0001) which are markedly greater than that noted in Europeans (h^2=0.30, p<0.0001) (Kloch-Badelek et al 2014) or Koreans (h^2±SEM=0.33±0.05, p<0.05) (Noh et al 2015). This is despite similar heritability estimates for the index of relaxation, E/A in the present study (h^2±SEM=0.29±0.12, p=0.01) as compared to other ethnic groups (h^2±SEM, Europeans=0.30, p<0.0001, Koreans=0.25±0.04, p<0.05) (Noh et al 2015; Kloch-Badelek et al 2014). Moreover, this is also despite lower heritability estimates for the preload-independent index of relaxation e', in the present study (h^2±SEM=0.30±0.14, p<0.0001) as compared to other ethnic groups (h^2±SEM, Europeans=0.40, p<0.0001, Koreans=0.41±0.05, p<0.05) (Noh et al 2015; Kloch-Badelek et al 2014). These results therefore suggest that as compared to other ethnic groups, groups of black African ancestry may be predisposed to adverse genetic effects on LV filling pressures. Whether this translates into a greater chance of developing heart failure with a preserved ejection fraction requires further study.

An important aspect of the present study not previously addressed (Jin et al 2011; Noh et al 2015; Kloch-Badelek et al 2014) is whether genetic influences on E/e' are through an impact of LV remodelling or aortic function. In this regard, LVMI, RWT and aortic function are now well recognised as being inherited, a finding previously reported in the present community sample (Peterson et al 2015; Redelinghuys et al 2012). As LVMI, RWT and aortic function are also associated with LV diastolic function, the possibility arises that the heritability of E/e' is by virtue of genetic influences on LVMI, RWT or aortic function. This question has important implications as LV remodelling and LV loading conditions can be modified by conventional therapy, whereas there is currently no known therapy that directly influences diastolic function. In the present study we show significant intra-familial correlations and heritability for E/e' after adjustments for LVMI, RWT and aortic function. Thus, the present results suggest that the genetic effects responsible for E/e' are unlikely to be attributed to the impact of LVMI, RWT or aortic function. The present findings therefore provide the first evidence that the genetic determinants of E/e' are likely to produce direct effects on LV diastolic function. The present results therefore support

genetic studies being conducted to assess potential molecular targets responsible for increases in LV filling pressures and that these studies may be particularly important in groups of African ancestry.

In the present study, as compared to E/e', we report on significant, but weaker heritability estimates for E/A, an index of diastolic function determined by LV relaxation, by filling pressures and several other factors (including heart rate which was adjusted for, P-R interval and the diameter of the mitral annulus, which were not adjusted for). Although these data support the notion that genetic factors also contribute to LV relaxation with similar effects across ethnic groups, E/A may not be the best index of LV relaxation to detect intra-familial aggregation and heritability. Indeed lateral wall e' (h^2±SEM=0.37±0.12, p<0.0005),(but not septal wall e'), a preload-independent index of LV relaxation, showed stronger heritability estimates than E/A (h^2±SEM=0.29±0.12, p=0.01). However, the dependence of E/A on preload may not be the only explanation for the relatively low heritability estimates for E/A in the present study, as the heritability of lateral wall e'/a', which may also be less dependent on preload than E/A (Sohn et al 1997), similarly showed lower heritability estimates (h^2±SEM=0.31±0.13, p<0.005) than E/e'. The heritability estimates for e'/a' in the present study are indeed lower than those reported in Europeans (h^2=0.40, p<0.0001) where heritability estimates for E/A (h^2=0.30, p<0.0001) were lower than for e'/a' (Kloch-Badelek et al 2014).

In addition to a markedly greater heritability of E/e' noted in present as compared to prior studies conducted in groups of European or Asian ancestry (Jin et al 2011; Noh et al 2015; Kloch-Badelek et al 2014), we also show a significantly greater heritability of E and A considered separately (h^2±SEM=0.49±0.11 and 0.52±0.11, p<0.0001) than in other ethnic groups (h^2=0.11 to 0.29) (Noh et al 2015; Kloch-Badelek et al 2014). However, the physiological and clinical significance of E and A assessed independent of each other, is unclear and several factors could explain the differences between studies including differences in preload. The limitations of the present study are first because of the young age of the siblings studied, a low prevalence of DD was noted in sibling pairs. Therefore, we could not assess the intra-familial aggregation and heritability of DD considered as a discrete trait. In this regard, a comprehensive assessment of a number of variables is required to appraise diastolic function and analysis of variables independent of each other may give an incomplete picture of the genetic determinants of diastolic function.

Second, we assessed the intra-familial aggregation and heritability of E/e' in one ethnic group only. Although our data are consistent with that obtained in Koreans (Noh et al 2015), and Europeans (Kloch-Badelek et al 2014), whether E/e' shows intra-familial aggregation and heritability independent of LVMI, RWT or aortic function in other ethnic groups nevertheless requires further study. Moreover, TDI measurements were only available in around two-thirds of the sample studied and this may have limited our ability to show intra-familial aggregation or heritability.

In conclusion, in the present study we demonstrate that in a group of African ancestry that E/e', an index of LV filling pressures, and a measure employed to identify moderate-to-severe LV diastolic dysfunction thus predicting the onset of heart failure with a preserved ejection fraction (Kane et al 2011; Lam et al 2011), aggregates in families and shows robust heritability, independent of LVMI, geometric LV remodelling and aortic function. Discovering the genes associated with E/e', particularly in groups of African ancestry may cast further light on the genetic mechanisms responsible for the progression to heart failure with a preserved ejection fraction.

CHAPTER 6

Contextual Narrative and Conclusion

6.1 Introduction

In the present thesis, in chapters 2 to 5 I provide detailed discussion of the findings of my research. In the present chapter I will nonetheless provide a contextual narrative for chapters 2 to 5 and discuss the general limitations as well as possible clinical implications of my research. The increasing prevalence of cardiovascular risk factors in both developed and developing countries is contributing toward higher rates of heart failure worldwide (Bradshaw et al 2003; Bhatia et al 2006; Borlaug & Redfield 2011; WHO, 2015). Heart failure is a condition that from the time of diagnosis, was thought to subsequently result in survival rates that are comparable to those of malignancies with the worst possible outcomes (Cowie et al 2000, Stewart et al 2001). Because of the appalling outcomes in patients with heart failure, and the marked burden to health care systems and patients alike produced by heart failure, over the past three-to-four decades there has been a considerable number of studies that have been performed to attempt to improve that situation. In this regard there is now substantial evidence to support the use of neurohumoral blockers, biventricular pacing, assist devices, valve replacements, coronary artery bypass grafts, stents and several other interventions in improving survival rates in patients with heart failure. Although more modern and highly technical approaches to heart failure have subsequently considerably improved outcomes in heart failure, the prognosis from the time of diagnosis is nonetheless still poor. This is particularly notable for heart failure with a preserved or normal ejection fraction.

As indicated in chapter 1 of the present thesis, each year the prevalence of heart failure with a preserved ejection fraction or diastolic heart failure increases by approximately 1% compared to heart failure with a reduced ejection fraction or systolic heart failure (Federmann & Hess 1994; Owan et al 2006; Fonarow et al 2007; Borlaug & Redfield 2011; Borlaug & Paulus 2011; Scantlebury & Borlaug 2011). The increased prevalence of heart failure with a preserved ejection fraction is possibly because more persons are surviving other disorders to old age and ageing is the most important risk factor for heart failure with a preserved ejection fraction. In addition the prevalence rates of obesity are increasing world-wide and as discussed in chapters 1 and 2 of the present thesis obesity, in specific circumstances, is also an important risk factor for heart failure. Furthermore, there may be increased physician recognition of the syndrome of heart failure with a preserved ejection fraction (Owan et al 2006). Importantly, heart failure with a preserved or normal ejection fraction carries just as poor a prognosis as heart failure with a reduced ejection fraction

(Senni et al 1998; Owan et al 2006; Paulus & van Ballegoij 2010; Borlaug & Paulus 2011; Borlaug & Redfield 2011; Li et al 2013; McMurray et al 2012; Burke et al 2014; Pitt et al 2014). However, as reviewed in chapter 1 of the present thesis, despite the increasing prevalence of heart failure with a preserved ejection fraction in populations worldwide, heart failure treatment strategies as applied to patients with this form of heart failure have generally been shown to be unsuccessful and novel approaches targeting the pathophysiological mechanisms thought to be responsible for this form of heart failure have failed (Borlaug & Paulus 2011; Borlaug & Redfield 2011; Pitt et al 2014). Although there are ongoing studies attempting to improve outcomes in patients with heart failure with a preserved ejection fraction, in the present thesis I have argued that it is possible that we could do better at targeting those at risk and hence preventing this form of heart failure. How have the findings of the present thesis extended our knowledge of how best to identify those at risk of heart failure with a preserved ejection fraction and do these findings suggest better preventative approaches?

6.2 Left ventricular diastolic dysfunction as a cause of heart failure with a preserved ejection fraction

The presence of preclinical LV diastolic dysfunction is known to predict the progression to heart failure with preserved ejection fraction (Wan et al 2014). The obvious question is therefore what are the currently recognised risk factors for LV diastolic dysfunction which may be targeted early and hence prevent the progression to heart failure with a preserved ejection fraction? In both European and American populations, the risk factors known to predict the development of heart failure with preserved ejection fraction include hypertension, diabetes mellitus, and obesity (Kenchaiah et al 2002; Bahrami et al 2008; Lee et al 2009; Mozaffarian et al 2015). Although additional risk factors such as age and female gender are often even more important in mediating the development of heart failure with a preserved ejection fraction, there are no obvious approaches that can be employed to modify these risk factors. All of these conventional risk factors (age, female gender, hypertension, diabetes mellitus and obesity) are independently associated with LV diastolic dysfunction which may present with or without heart failure (Aurigemma et al 2001; Kane et al 2011; Lam et al 2011). In association with these risk factors, there is frequently LVH and/or an increased LV relative wall thickness, an index of concentric LV remodelling, which may also contribute toward the development of LV diastolic

dysfunction and ultimately heart failure with a preserved ejection fraction (Chahal et al 2010). As the presence of LV diastolic dysfunction predicts the development of heart failure with preserved ejection fraction (Wan et al 2014), it therefore may be important to better understand exactly how modifiable risk factors determine LV diastolic dysfunction. In this regard, it is not expected that every hypertensive, diabetic or obese patient will develop LV diastolic dysfunction and heart failure with a preserved ejection fraction. In other words not every hypertensive, diabetic or obese individual should be screened for LV diastolic dysfunction (the cost and resources required would be too great). Waiting for symptoms of heart failure to develop before screening for LV diastolic dysfunction is counter intuitive to an appropriate preventative strategy. However, in those with the known risk factors for LV diastolic dysfunction and hence heart failure with a preserved ejection fraction, additional measures may be employed to identify those most at risk. What has the present thesis identified with respect to the possibility of better identifying those at risk of LV diastolic dysfunction and hence possibly heart failure with a preserved ejection fraction?

6.3 Obesity and obesity-related insulin resistance and LV diastolic dysfunction?

As highlighted in chapters 1 and 2 of the present thesis, obesity and obesity-related insulin resistance are risk factors for the development of heart failure (Kenchaiah et al 2002; Ingelsson et al 2005; Bahrami et al 2008) and the presence of LV diastolic dysfunction (Bajraktari et al 2006; Libhaber et al 2009; Tsioufis et al 2008; Dinh et al 2010; Russo et al 2011; AlJaroudi et al 2012; Çil et al 2012; Cuspidi et al 2014). The obvious solution to this problem is to institute weight loss programs in obese individuals. However, weight reduction programs seldom result in obese individuals reaching target body weights (Latner et al 2002, Anderson et al 2001) and often result in an inability to maintain decreases in body weight (Jordan et al 2012, Hedayati et al 2011, Aucott et al 2009, Weiss et al 2007, The Trials of Hypertension Prevention Collaborative Research Group 1997). In this regard adherence to lifestyle changes decrease from as early as 6 months after the initiation of weight reduction programs (Elmer et al 2006). Thus, those obese individuals most at risk for LV diastolic dysfunction should be identified and in these individuals, if weight reduction programs do not achieve the desired goals, it may be necessary to introduce alternative approaches to prevent the transition from overweight and obesity to heart

failure. Do we have sufficient evidence to suggest that it is only in specific obese individuals that the risk for LV diastolic dysfunction exists and is there evidence to suggest alternative preventative strategies?

As reviewed in chapter 1 of the present thesis (section 1.5.1.3), there is some evidence for a role of insulin resistance as a mediator of obesity-associated LV diastolic dysfunction. However, as pointed out in this section of the thesis, this evidence is controversial. In chapter 2, I argue that because obesity is associated with increased LVH and concentric LV remodelling (Cuspidi et al 2014; Woodiwiss & Norton 2015) and that these structural changes in the LV are also associated with LV diastolic dysfunction, that the impact of obesity or insulin resistance on LV diastolic dysfunction may be in-part determined by the extent of the LV remodelling process. In chapter 2 of the present thesis, I have shown in 737 participants from a randomly recruited community-based study of African ancestry with a high prevalence of obesity and LV diastolic dysfunction, that in those with a more concentrically remodelled LV, insulin resistance, as indexed by HOMA-IR, is strongly and independently associated with several indices of LV diastolic function. In contrast however, in those with a less concentrically remodelled LV neither adiposity indices nor HOMA-IR were independently associated with LV diastolic function. Importantly, LV remodelling failed to modify age or blood pressure-LV diastolic function relations. How do these results change our current understanding of obesity effects on LV diastolic function?

Although several cross-sectional and intervention studies have demonstrated independent associations between adiposity indices and abnormalities of LV diastolic dysfunction (Tsiousfis et al 2008; Libhaber et al 2009; Russo et al 2011; Çil et al 2012; AlJaroudi et al 2012; Cuspidi et al 2014), our group have recently reported that even in a community with a high prevalence of obesity, this effect is modest at best as compared to the impact of blood pressure and age (Millen et al 2015). Similarly, in the present study, I was unable to show anything but a modest relation between indices of excess adiposity or insulin resistance and LV diastolic function when considered in the sample as a whole. Importantly, in this community sample there was no independent association between indices of adiposity, or insulin resistance and the presence of moderate to severe LV diastolic dysfunction. Hence, these data suggest that neither obesity nor insulin resistance, when considered in isolation are likely to make as significant a contribution to LV diastolic dysfunction at a community level as other risk factors such as age or blood pressure. However, the present findings also suggest that obesity effects on LV diastolic function are likely to be as

important as blood pressure or other risk factor effects if a patient's LV has a more concentric geometry and if insulin resistance exists. These data therefore provide a possible explanation as to why studies exploring relations between insulin resistance and LV diastolic function have produced controversial results (see section 1.5.1.3). In this regard, it is unknown whether relations between indices of insulin resistance and LV diastolic function in these studies (see section 1.5.1.3) were assessed in participants with a more concentrically remodelled LV. Moreover, the present findings suggest that to best identify those overweight or obese individuals who are likely to develop LV diastolic dysfunction and hence possibly heart failure with a preserved ejection fraction, the degree of insulin resistance should be defined and if insulin resistant, then the degree of LV remodelling should be determined. If these patients have a more concentrically remodelled LV, then intense weight loss programs should be instituted in these individuals, whilst those without insulin resistance or who have a less concentrically remodelled LV may not require as intensive weight loss programs or at least may not require repeated echocardiograms to monitor for LV diastolic dysfunction. Moreover, the present study suggests that if weight loss programs are unsuccessful, targeting the degree of insulin resistance may be an option to prevent the development of LV diastolic dysfunction. Further studies are required to investigate whether therapeutic strategies designed to increase insulin resistance improve LV diastolic function and possibly heart failure with a preserved ejection fraction in individuals who have obesity related concentric LV remodelling and insulin resistance.

6.4 Blood Pressure: the effect of aortic backward wave pressures on LV diastolic function.

As reviewed in chapter 1 (section 1.5.2) and chapter 3, although hypertension is a well-recognised risk factor for the development of heart failure with a preserved ejection fraction and a reduced LV diastolic function, the fundamental haemodynamic change responsible for these afterload-induced effects on the heart are at present not entirely clear. In this regard, hypertension may be associated with increases in various loading conditions, including a resistance afterload which increases diastolic blood pressure (and hence load at the end of isovolumic contraction), and an increased pulsatile afterload induced by increases in the aortic forward wave pressure (produced by and increased stroke volume and aortic stiffness and hence load at the end of the ejection period) and an increased pulsatile afterload induced by

increases in the aortic backward wave pressure (produced by wave reflections and hence load at the peak of systole). The debate as to the relative contribution of each of these afterloads to chronic changes in LV diastolic function is ongoing. Although it is most likely that all three forms of loading conditions contribute to myocardial damage and hence diastolic LV function, the timing of these effects is far less well understood. In other words, at what age do these loading conditions contribute to LV diastolic dysfunction? The most commonly held misconception is that a resistance afterload is more important in mediating myocardial damage and dysfunction in young-to-middle-aged persons, whereas pulsatile afterload only contributes in the elderly. In this regard, although this approach applies well when considering brachial blood pressure effects, it is not the case for aortic blood pressure. Indeed, from young adulthood, aortic backward waves begin to increase (Booysen et al 2015) and these increases impact more on aortic than on brachial pulse pressure (see section 1.5.2.3 for the explanation). Only from 50-60 years of age, when aortic stiffness begins to dramatically increase do increases in the forward wave pressure contribute to any substantial extent to age-related increases in aortic pulsatile load. Hence, while aortic pulsatile afterload increases from youth, brachial pulse pressure increases to a lesser extent. Hence, damage to the LV mediated by pulsatile afterload effects may begin to occur from young adulthood and these effects may not be adequately indexed by brachial blood pressure measurements.

As argued in chapter 3, although several studies have identified associations between aortic stiffness (which influences aortic forward wave magnitude) or reflected waves and aspects of LV diastolic function in those without heart failure (Kawaguchi et al 2003, Weber et al 2006; Weber et al 2008; Borlaug et al 2007), the relative role of aortic stiffness versus aortic reflected waves at the time of these publications was unclear. As marked age-related increases in backward wave pressures precede significant age-related increases in aortic stiffness by several decades (Booysen et al 2015) and increases in aortic backward wave pressures are a fundamental mechanism responsible for increases in aortic stiffness (Sibiya et al 2015), aortic backward waves may be the initial (primary) target in preventing the progression to LV diastolic dysfunction and consequently heart failure with a preserved ejection fraction. However, whether the impact of backward wave pressures on LV diastolic dysfunction antedates the effects of aortic stiffness was until the present thesis, unclear. In this regard, prior studies assessing relations between aortic and LV diastolic function evaluated patients with average ages of 60-77 years (Mohammed et al 2012; Hundley et al 2001; Kawaguchi et al 2003; Weber

et al 2006; Weber et al 2008; Fernandes et al 2008; Kang et al 2010; Desai et al 2009; Kitzman et al 2013), age ranges when large vessel stiffness is already markedly increased (Booysen et al 2015). In the present thesis I therefore determined whether the impact of aortic backward wave pressure on LV diastolic dysfunction antedates the effects of aortic stiffness in a predominantly young-to-middle–aged community-based sample with an average age where increases in aortic backward wave pressures are well established, but prior to an age when striking increases in aortic stiffness are noted.

In chapter 3, I have shown in 524 participants from a randomly recruited community-based study of African ancestry with an age in the predominantly young-to-middle-aged range, and with a high prevalence of moderate-to-severe pre-clinical LV diastolic dysfunction (10.9%), aortic backward wave pressures (Pb derived from wave separation analysis and Pa) consistently showed independent relations with E/e', an index of filling pressures, and the presence of moderate-to-severe LV diastolic dysfunction; whilst neither forward wave pressures, aortic stiffness (aortic PWV), nor the timing of the forward or backward waves were consistently associated with E/e' and moderate to severe LV diastolic dysfunction. Moreover, aortic backward wave pressures, but not aortic forward wave pressures, aortic stiffness or the timing of forward and backward wave pressures accounted for independent relations between aortic pulse pressure and increases in E/e' or moderate-to-severe LV diastolic dysfunction. There are several implications to these findings that warrant consideration.

To predict the risk of developing LV diastolic dysfunction and hence the possibly heart failure with a preserved ejection fraction, blood pressure is measured at the brachial artery. However, brachial blood pressure may not adequately index the pulsatile afterload that determines LV diastolic dysfunction. In this regard, the results of chapter 3 suggest that the aortic backward wave, which appears as a second and lower systolic shoulder, rather than as the peak systolic pressure at the peripheral pulse, is the main component of pulsatile load which contributes toward LV diastolic dysfunction over a young-to-middle age range. Hence, in the management of blood pressure, it is possible that targeting the aortic backward wave or aortic systolic pressure may produce a more robust effect on preventing the transition to heart failure with a preserved ejection fraction. In this regard, whilst there is little evidence to support a therapeutic approach to modify age-related increases in aortic stiffness, there is significant evidence to suggest that aortic backward wave pressures may be targeted by conventional antihypertensive therapy (Agabiti-Rosei

et al 2007). However, this may only be adequately achieved if the aortic backward wave pressure or aortic systolic pressure is measured. In this regard commercially available devices to assess aortic pressures are often no more expensive than a well validated oscillometric device to measure brachial blood pressure and these devices are now easy to use taking only 5-10 minutes to acquire all of the data. The present thesis therefore provides sufficient evidence to support a longitudinal study being conducted where antihypertensive therapy is titrated against aortic backward wave pressures (or aortic systolic pressures) as well as brachial blood pressure versus just brachial blood pressure alone and the effect on LV diastolic function and possibly the development of heart failure with a preserved ejection fraction assessed.

6.5 Genetic effects on LV diastolic function and the determinants thereof?

When assessing the risk for the development of LV diastolic dysfunction and ultimately the progression to heart failure with a preserved ejection fraction, it is important to be able to account for all rather than a fraction of the risk. As highlighted in chapter 1 of the present thesis (section 1.5), the well-recognised demographic and clinical risk factors for heart failure with a preserved ejection fraction, which include age, female gender, obesity, hypertension and diabetes mellitus, fail to account for all of the inter-individual variation in LV diastolic function (Bielin et al 1991; Fox et al 2010; Jin et al 2011; Kloch-Badelek et al 2014; Noh et al 2015; Swan et al 2003; Tang et al 2002). However, ethnicity is reported to impact on the prevalence of LV diastolic dysfunction and ultimately heart failure with preserved ejection fraction (Bhatia et al 2006; Yancy et al 2006; Fonarow et al 2007; Lam et al 2007; Scantlebury & Borlaug 2011; Chirinos et al 2012; Nichols et al 2014; Mozaffarian et al 2015). Although a significant proportion of these ethic effects may be attributed to differences in well-recognised risk factors for LV diastolic dysfunction, other possibilities must be considered. Hence, independent of well-recognised demographic and clinical risk factors for heart failure with a preserved ejection fraction, it has been proposed that the inter-individual variation in LV diastolic function may be explained to some extent by genetic factors that influence either the LV remodelling process, and hence indirectly affect LV diastolic function (and hence whether heart failure with a preserved ejection fraction occurs), or LV diastolic function through direct effects. In this regard, as reviewed in chapter 1, section 1.5.3.1, of the present thesis several studies have demonstrated that LV relative wall thickness, which is associated with LV diastolic dysfunction and as demonstrated in

chapter 2, is a strong modifier of the impact of insulin resistance on LV diastolic function, is inherited (Arnett et al 2001; Bella et al 2004; Fox et al 2010; Kuznetsova et al 2003). Moreover, as reviewed in chapter 1, section 1.5.3.2, of the present thesis, several studies have demonstrated heritability or intra-familial aggregation of LV diastolic function identified from trans-mitral blood flow velocity measurements (Bielen et al 1991; Swan et al 2003, Tang et al 2002, Fox et al 2010, Jin et al 2011; Kloch-Badelek et al 2014). Furthermore, some studies have demonstrated significant heritability of tissue Doppler indices of LV diastolic function (Noh et al 2015, Kloch-Badelek et al 2014). It may therefore be argued that the next step in the process of identifying the genetic determinants of LV diastolic function is to perform high throughput multi-loci gene screening using currently available sophisticated genetic approaches. However, in the present thesis I have argued that the genetic determinants of LV diastolic function or the LV structural determinants thereof are not entirely clear. What have the studies conducted in the present thesis therefore added to our understanding of the possible inheritance of LV diastolic function or the LV structural determinants thereof?

6.5.1 Possible genetic effects on LV geometric remodelling

There is considerable uncertainty as to the role of genetic factors in determining LV relative wall thickness independent of LV mass. In this regard LV mass is a major determinant of LV wall thickness and hence it may be argued that the same genetic factors that determine LV mass will also determine the geometric remodelling process. However, an increased LV relative wall thickness may be determined not just by the extent of LVH, but by several other cellular factors (Badenhorst et al 2003). Hence, if LV relative wall thickness is inherited, and LV remodelling is a major determinant of LV diastolic function, then the genetic determinants of LV diastolic dysfunction are not only going to be regulated by those factors which influence only the extent of hypertrophy of the heart, but rather those that determine the remodelling process itself. Is there some evidence prior to the study conducted in the present thesis to suggest that the LV geometric remodelling process is inherited independent of LV mass?

Siblings of those with LVH have a greater risk of concentric LVH as compared to age- and sex-matched controls (Schunkert et al 1999). In that study (Schunkert et al 1999), the siblings of those with LVH had no greater risk of eccentric LVH than the age- and sex-matched controls. Hence, from that study (Schunkert et al 1999) the

possibility arose that the intrafamilial clustering of concentric LVH was in-part driven by the LV remodelling process. However, whether these siblings had a greater risk of concentric LV remodelling was not reported. Hence, it is also possible that the increased risk of concentric LVH in that study (Schunkert et al 1999) may have been largely a consequence of LV mass effects. In the Framingham Heart Study although the risk of concentric LVH was markedly greater in related as compared to unrelated individuals; the risk of concentric LV remodelling was reported to be only modestly greater in related compared to unrelated individuals (Lam et al 2010). These data (Lam et al 2010) therefore point toward the possibility that the inheritance of LV mass explains the inheritance of concentric LVH. Importantly, in all of the studies which have reported on intrafamilial clustering or the inheritance of LV relative wall thickness (Arnett et al 2001; Bella et al 2004; Fox et al 2010; Kuznetsova et al 2003), none have adjusted for LV mass. In the present thesis as described in chapter 4, I nevertheless demonstrate that LV relative wall thickness shows strong intrafamilial aggregation and heritability beyond LV mass and several confounding variables. Indeed, independent of confounders, I showed that 56% of the variation in relative wall thickness was heritable (h^2±SEM=0.56±0.09, p<0.0001) and this persisted with further adjustments for LV mass (h^2±SEM=0.48±0.09, p<0.0001). There are several important implications of these findings.

As highlighted in chapter 2, geometric LV remodelling, as indexed by LV relative wall thickness, markedly influences whether insulin resistance impacts on LV diastolic function. The more concentric the LV geometry the greater the chance that LV diastolic dysfunction will occur. Moreover, whilst concentric LV remodelling has little influence on the chances of heart failure occurring, whether LVH progresses to heart failure with a preserved or reduced ejection fraction is strongly determined by the LV geometric remodelling process. As this LV remodelling process shows marked heritability, there may be an equally strong genetic basis, and hence identifying the genetic determinants thereof could enhance our ability to identify those who are at risk of developing heart failure with a preserved ejection fraction and may identify novel molecular mechanisms responsible for heart failure with a preserved ejection fraction. In this regard, as the intrafamilial aggregation and heritability of LV relative wall thickness occurred independent of LV mass, the present thesis suggests that genetic factors that influence the LV remodelling process beyond hypertrophy need to be sought. Hence, the results of the findings reported in chapter 4 contribute to forming the foundation of future genetic studies on this topic.

6.5.2 *Possible genetic effects on LV diastolic function beyond geometric LV remodelling*

Despite consistent evidence for the heritability of E and A waves across various populations, the genetic influence on contemporary measures employed to evaluate LV diastolic dysfunction, such as e' or E/e' (as discussed in section 1.4.1), is nevertheless unclear. This is an important issue as E/e' is an index of LV filling pressures, and a measure employed to identify moderate-to-severe LV diastolic dysfunction thus predicting the onset of heart failure with a preserved ejection fraction (Kane et al 2011; Lam et al 2011). In this regard, prior studies that have reported on the heritability of E/e' have provided conflicting data, with a Korean study showing significant heritability (33%) for E/e' (Noh et al 2015), but an alternative study conducted in European families failed to show significant heritability (Jin et al 2011). However, subsequent analysis in an expanded data set showed significant heritability (31%) (Kloch-Badelek et al 2014). However, these previous studies (Jin et al 2011; Kloch-Badelek et al 2014; Noh et al 2015) failed to determine whether E/e' is inherited independent of LV mass and relative wall thickness. Hence, until the time of the work performed in the present thesis, whether significant heritability of E/e' occurred independent of LV remodeling was unknown. Moreover, although previous studies have reported on the inheritance of E/e' independent of brachial blood pressure (Kloch-Badelek et al 2014; Noh et al 2015), whether heritability of E/e' was independent of aortic BP was also unknown. In this regard, central aortic blood pressure has been reported to show stronger heritability estimates than brachial blood pressure (Redelinghuys et al 2012). Therefore, how has the work performed in the present thesis added to our understanding of the possible genetic basis of LV diastolic function?

In chapter 5 of the present thesis, I report on heritability estimates for E/e' (h^2±SEM=0.52±0.11, p<0.0001) which are markedly greater than that noted in Europeans (h^2=0.30, p<0.0001) (Kloch-Badelek et al 2014) or Koreans (h^2±SEM=0.33±0.05, p<0.05) (Noh et al 2015). This is despite similar heritability estimates for the index of relaxation, E/A in the present study (h^2±SEM=0.29±0.12, p=0.01) as compared to other ethnic groups (h^2±SEM, Europeans=0.30, p<0.0001, Koreans=0.25±0.04, p<0.05) (Noh et al 2015; Kloch-Badelek et al 2014). Moreover, this is also despite lower heritability estimates for the preload-independent index of relaxation e', in the present study (h^2±SEM=0.30±0.14, p<0.0001) as compared to other ethnic groups (h^2±SEM, Europeans=0.40, p<0.0001, Koreans=0.41±0.05,

p<0.05) (Noh et al 2015; Kloch-Badelek et al 2014). In addition, these heritability estimates and the intrafamilial correlations of E/e' were independent of LV mass or the degree of concentric LV remodelling. These results therefore suggest that as compared to other ethnic groups, groups of black African ancestry may be predisposed to the adverse genetic effects on LV filling pressures and that in this ethnic group these effects are even beyond the effect of LV remodelling. Clearly, these data provide strong evidence in favor of identifying the genetic factors which contribute toward filling pressures in the LV in this ethnic group and assessing whether these genetic factors determine the progression to heart failure with a preserved ejection fraction. Hence, the results of the findings reported in chapter 5 also contribute to forming the foundation of future genetic studies on this topic.

6.6 Limitations

Throughout the present thesis I have highlighted the potential limitations of the studies conducted. However, there are some limitations that are worthy of emphasis. In the definition of diastolic dysfunction, I did not include changes in mitral inflow with the Valsalva maneuver as this measurement is not feasible in all patients and needs to be carefully standardised in order for accurate interpretations to be made (Nagueh et al 2016). Indeed, this measurement is not incorporated in the updated diagnostic algorithm for diastolic dysfunction (Nagueh et al 2016). In addition, this measurement is not used to define the presence versus absence of diastolic dysfunction, but only to assist together with E/A and average E/e', in the separation of mild diastolic dysfunction from moderate-to-severe diastolic dysfunction. In the definition of diastolic dysfunction, I also did not include the time difference between the duration of pulmonary venous flow and mitral inflow during atrial contraction (A wave) (Ar-A duration), and E wave velocity deceleration time; however these measurements were also not included in the updated diagnostic algorithm for diastolic dysfunction (Nagueh et al 2016). Moreover, I did include tricuspid regurgitation velocities and hence may have missed some participants with LV diastolic dysfunction. Importantly however, potential participants were excluded if significant valve abnormalities were present. Lastly, as the data presented in chapter 3 of this thesis were analysed prior to the publication of the 2016 algorithm (Nagueh et al 2016), LA volume assessments were not included in the definition of diastolic dysfunction, and the thresholds for septal e', E/e' and E/A were according to the 2009 algorithm (Nageuh et al 2009). In chapter 5 of the present thesis, a major

limitation of the intra-familial aggregation and heritability analysis was the young age of the siblings that were recruited for this study. Indeed, because of the low prevalence of diastolic dysfunction in younger individuals, I could not assess the intra-familial aggregation and heritability of diastolic dysfunction as a discrete trait. This could influence the conclusions drawn regarding the genetic determinants of diastolic dysfunction.

6.7 Conclusions

In conclusion, the findings from the present thesis provide further evidence to potentially assist in the identification of individuals at risk of LV diastolic dysfunction and in addition, add to our understanding of the pathophysiological mechanisms that may play an important role in the development of LV diastolic dysfunction. In this regard I show that the relationship between insulin resistance and not alternative risk factors and LV diastolic function is significantly altered by the presence of an increase in the degree of LV concentricity. Second, I show that the impact of backward wave pressures on LV diastolic dysfunction precedes the effects of aortic stiffness, the time to wave reflection or forward wave pressures. Third, I show significant intra-familial aggregation and strong heritability in LV filling pressures as indexed by E/e', independent of LV remodelling and aortic function as well as the geometric LV remodelling process that makes a strong contribution to the development of LV diastolic dysfunction. The findings of the present thesis argue in favour of a need to identify the genetic basis of LV diastolic function and for conducting several prospective longitudinal or intervention studies guided by genetic markers, measures of insulin resistance and aortic backward wave or aortic systolic pressures in attempting to prevent the progression to heart failure with a preserved ejection fraction.

APPENDICES

Appendix A

Ethical clearance certificates

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 Wits 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

Works2\lain0015\HumEth97.wdb\M 02-04-72
==========

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

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)

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...

Appendix B

"Turn-it-in" Plagiarism report

ORIGINALITY REPORT

11%	7%	11%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Witwatersrand Student Paper	5%
2	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	2%
3	academic.oup.com Internet Source	2%
4	departamentos.cardiol.br Internet Source	1%
5	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%
6	www.asecho.org Internet Source	1%
7	Diastolic Heart Failure, 2008. Publication	1%
8	physreports.physiology.org Internet Source	1%

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 1%
EXCLUDE BIBLIOGRAPHY	ON		

References

Abhayaratna[a] W.P., Barnes M.E., O'Rourke M.F., Gersh B.J., Miyasaka Y., Bailey K.R., Tsang T.S. (2006). Relation of arterial stiffness to left ventricular diastolic function and cardiovascular risk prediction in patients > or =65 years of age, Am J Cardiol, 98, 1387-1392.

Abhayaratna W.P., Fatema K., Barnes M.E., Seward J.B., Gersh B.J., Bailey K.R., Casaclang-Verzosa G., and Tang T.S. (2008). Left atrial reservoir function as a potent marker for first atrial fibrillation or flutter in persons > or = 65 years of age, Am J Cardiol, 101, 1626-1629.

Abhayaratna[b] W.P., Seward J.B., Appleton C.P., Douglas P.S., Oh J.K., Tajik A.J., and Tsang T.S. (2006). Left atrial size: physiologic determinants and clinical applications, J Am Coll Cardiol, 47, 2357-2363.

Achong N., Wahi S., Marwick T.H. (2009). Evolution and outcome of diastolic dysfunction, Heart, 95, 813-818.

Agabiti-Rosei E., Mulesan M.L., Salvetti M. (2007). New approaches to the assessment of left ventricular hypertrophy, Ther Adv Cardiovasc Dis, 1, 119-128.

AlJaroudi W., Halley C., Houghtaling P., Agarwal S., Menon V., Rodriguez L., Grimm R.A., Thomas J.D., and Jaber W.A. (2012). Impact of body mass index on diastolic function in patients with normal left ventricular ejection fraction, Nutr Diabetes, 2, e39.

Almuntaser I., Mahmud A., Brown A., Murphy R., King G., Crean P., and Feely J. (2009). Blood pressure control determines improvement in diastolic dysfunction in early hypertension, Am J Hypertens, 22, 1227-1231.

Anderson J.C, Konz E.C., Frederich R.C., Wood C.L. (2001). Long-term weight-loss maintenance: a meta-analysis of US studies, Am. J. Clin. Nutr, 74, 579-584.

Angeja B.G., and Grossman W. (2003). Evaluation and management of diastolic heart failure, Circulation, 107, 659-663.

Appelton C.P., Jensen J.L., Hatle L.K., and Oh J.K. (1997). Doppler evaluation of left and right ventricular diastolic function: a technical guide for obtaining optimal flow velocity recordings, J Am Soc Echocardiogr, 10, 271-292.

Appelton C.P., Galloway J.M., Gonzalez M.S., Gaballa M., Basnight M.A. (1993). Estimation of left ventricular filling pressures using two-dimensional and Doppler echocardiography in adult patients with cardiac disease. Additional value of analyzing left atrial size, left atrial ejection fraction and the difference

in duration of pulmonary venous and mitral flow velocity at atrial contraction, J Am Coll Cardiol, 22, 1972-1982.

Arnett D.K., Rautaharju P., Crow R., Folsom A.R., Ekelund L.G., Hutchinson R., Tyroler H.A., and Heiss G. (1994). Black-white differences in electrocardiographic left ventricular mass and its association with blood pressure (the ARIC study) Atherosclerosis Risk in Communities, Am J Cardiol, 74, 247-252.

Arnett D.K., Hong Y., Bella J.N., Oberman A., Kitzman D.W., Hopkins P.N., Rao D.C., and Devereux R.B. (2001). Sibling correlation of left ventricular mass and geometry in hypertensive African Americans and whites; the HyperGEN study. Hypertension Genetic Epidemiology Network, Am J Hypertens, 14, 1226-1230.

Assimes T.L., Narasimhan B., Seto T.B., Yoon S., Curb J.D., Olshen R.A., and Quertermous T. (2007). Heritability of left ventricular mass in Japanese families living in Hawaii: the SAPPHIRe Study, J Hypertens, 25, 985-992.

Aurigemma G.P., and Gaasch W.H. (2004). Clinical practice. Diastolic heart failure, N Engl J Med, 35, 1097-1105.

Aurigemma G.P., Gottdiener J.S., Shemanski L., Gardin J., and Kitzman D. (2001) Predictive value of systolic and diastolic function for incident congestive heart failure in the elderly: the Cardiovascular Health Study, J Am Coll Cardiol, 37, 1042-1073.

Ayalon N., Gopal D.M., Mooney D.M., Simonetti J.S., Grossman J.R., Dwivedi A., Donohue C., Perez A.J., Downing J., Gokce N., Miller E.J., Liang C.S., Apovian C.M., Colucci W.S., and Ho J.E. (2014). Preclinical left ventricular diastolic dysfunction in metabolic syndrome, Am J Cardiol, 114, 838-842.

Badenhorst D., Veliotes D., Maseko M., Tsotetsi O.J., Brooksbank R., Naidoo A., Woodiwiss A.J., Norton G.R. (2003). Beta- adrenergic activation initiates chamber dilatation in concentric hypertrophy, Hypertension, 41, 449-504.

Bahrami H., Bluemke D.A., Kronmal R., Bertoni A.G., Lloyd-Jones D.M., Shahar E., Szklo M and Lima J.A.C. (2008). Novel metabolic risk factors for incident heart failure and their relationship with obesity: the MESA (Multi-Ethnic Study of Atherosclerosis) study, J Am Coll Cardiol, 51, 1775-1783.

Bajraktari G., Koltai M.S., Ademaj F., Rexhepaj N., Qirko S., Ndrepepa G., Elezi S. (2006). Relationship between insulin resistance and left ventricular diastolic dysfunction in patients with impaired glucose tolerance and type 2 diabetes, Int J Cardiol, 110, 206-211.

Barouch L.A., Gao D., Chen L., Miller K.L., Xu W., Phan A.C., Kittleson M.M., Minhas K.M., Berkowitz D.E., Wei C., and Hare J.M. (2006). Cardiac myocyte apoptosis is associated with increased DNA damage and decreased survival in murine models of obesity, Circ Res, 98, 119-124.

Bella J.N. and Goring H.H. (2012). Genetic epidemiology of left ventricular hypertrophy, Am J Cardiaovasc Dis, 2, 267-278.

Bella J.N., MacCluer J.W., Roman M.J., Almasy L., North K.E., Best L.G., Lee E.T., Fabsitz R.R., Howard B.V., and Devereux R.B. (2004). Heritability of left ventricular dimensions and mass in American Indians: The Strong Heart Study, J Hypertens, 22, 281-286.

Bella J.N., Palmieri V., Roman M.J., Liu J.E., Welty T.K., Elisa T., Lee E.T., Fabsitz R.R., Howard B.V., and Devereux R.B. (2002). Mitral ratio of peak early to late diastolic filling velocity as a predictor of mortality in middle-aged and elderly adults: the Strong Heart Study, Circulation, 105, 1928-1933.

Bennett C.M., Guo M., and Dharmage S.C. (2007). HbA1c as a screening tool for the detection of type 2 diabetes: a systematic review, Diabet Med, 24, 333-343.

Bhatia R.S., Tu J.V., Lee D.S., Austin P.C., Fang J., Haouzi A., Gong Y., and Liu P.P. (2006). Outcomes of heart failure with preserved ejection fraction in a population-based study, N Engl J Med, 355, 260-269.

Bielin E., Fagard R., and Amery A. (1991). The inheritance of left ventricular structure and function assessed by imaging and Doppler echocardiography, Am Heart J, 121, 1743-1749.

Bluemke D.A., Kronmal R.A., Lima J.A., Liu K., Olson J., Burke G.L., and Folsom A.R. (2008). The relationship of left ventricular mass and geometry to incident cardiovascular events: the MESA (Multi-Ethnic Study of Atherosclerosis) study, J Am Coll Cardiol, 52, 2148-2155.

Booysen H.L., Woodiwiss A.J., Sibiya M.J., Hodson B., Raymond A., Libhaber E., Sareli P., and Norton G.R. (2015). Indexes of aortic pressure augmentation markedly underestimate the contribution of reflected waves toward variations in aortic pressure and left ventricular mass, Hypertension, 65, 540-546.

Borbely A., van der Velden J., Papp Z., Bronzwaer J.G., Edes I., Stienen G.J., Paulus W.J. (2005). Cardiomyocyte stiffness in diastolic heart failure. Circulation, 111, 774-781.

Borlaug B.A. (2011). Sex, load, and relaxation: are women more susceptible to load-dependent diastolic dysfunction? J Am Coll Cardiol, 57, 1234-1236.

Borlaug B.A., and Kass D.A. (2008). Ventricular-vascular interaction in heart failure, Heart Fail Clin, 4, 23-36.

Borlaug B.A., Melenovsky V, Redfield M.A., Kessier K, Chang H.J., Abraham T.P., and Kass D.A. (2007). Impact of arterial load and loading sequence on left ventricular tissue velocities in humans, J Am Coll Cardiol, 50, 1570-1577.

Borlaug B.A., Melenovsky V., Russell S.D., Kessler K., Pacak K., Becker L.C., Kass D.A. (2006). Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction, Circulation, 114, 2138-2147.

Borlaug[b] B.A., Nishimura R.A., Sorajja P., Lam C.S., Redfield M.M. (2010). Exercise hemodynamics enhance diagnosis of early heart failure with preserved ejection fraction, Circ Heart Fail, 3, 588-595.

Borlaug[a] B.A., Olson T.P., Lam C.S., Flood K.S., Lerman A., Johnson B.D., Redfield M.M. (2010). Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction, J Am Coll Cardiol, 56, 845-854.

Borlaug B.A., and Paulus W.J. (2011). Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment, Eur Heart J, 32, 670-679.

Borlaug B.A., Redfield M.M. (2011). Diastolic and systolic heart failure are distinct phenotypes within the heart failure spectrum, Circulation, 123, 2006-2014.

Bradshaw D., Groenewald P., Laubscher R., Nannan N., Nojilana B., Norman R., Pieterse D., Schneider M., Bourne D.E., Timaeus I.M., Dorrington R., and Johnson L. (2003). Initial burden of disease estimates for South Africa 2000, S Afr Med J, 93, 682-688.

Brubaker P.H., Joo K.C., Stewart K.P., Fray B., Moore B., Kitzman D.W. (2006). Chronotropic incompetence and its contribution to exercise intolerance in older heart failure patients, J Cardiopulm Rehabil, 26, 86-89.

Brutsaert D.L., Sys S.U., and Gillebert T.C. (1993). Diastolic failure: pathophysiology and therapeutic implications, J Am Coll cardiol, 22, 318-325

Buchanan J., Mazumder P.K., Hu P., Chakrabarti G., Roberts M.W., Yun U.J., Cooksey R.C., Litwin S.E., and Abel E.D. (2005). Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity, Endocrinology, 146, 5341-5349.

Burke M.A., Katz D.H., Beussink L, Selvaraj S, Gupta D.K., Fox J., Chakrabarti S., Sauer A.J., Rich J.D., Freed B.H., and Shah S.J. (2014). Prognostic

importance of pathophysiologic markers in patients with heart failure and preserved ejection fraction, Circ Heart Fail, 7, 288-299.

Bursi F., Weston S.A., Redfield M.M., Jacobsen S.J., Pakhomov S., Nkomo V.T., Meverden R.A., and Roger V.L. (2006). Systolic and diastolic heart failure in the community, JAMA, 296, 2209-2216.

Caballero L., Kou S., Dulgheru R., Gonjilashvili N., Athanassoupoulos G.D., Barone D., Baroni M., Cardim N., Gomez de Diego J.J., Oliva M.J., Hagendorff A., Hristova K., Lopez T., Magne J., Martinez C., de la Morena G., Popescu B.A., Penicka M., Ozyigit T., Carbonero J.D.R., Salustri A., Van De Veire N., Von Bardeleben R.S., Vinereanu D., Voigt J-U., Zamorano J.L., Bernard A., Donal E., Lang R.M., Badano L.P., and Lancellotti P. (2015). Echocardiographic reference ranges for normal cardiac Doppler data: results from the NORRE Study, Eur Heart J, doi:10.1093/ehjci/jev083.

Caro J.F., Dohm L.G., Pories W.J., and Sinha M.K. (1989). Cellular alterations in liver, skeletal muscle, and adipose tissue responsible for insulin resistance in obesity and type II diabetes, Diabetes Metab Rev, 5, 665-689.

Chahal N.S., Lim T.K., Jain P., Chambers J.C., Kooner J.S., Senior R. (2010). New insights into the relationship of left ventricular geometry and left ventricular mass with cardiac function: a population study of hypertensive subjects, Eur Heart J, 31, 588-594.

Chen Y.T., Vaccarino V., Williams C.S., Butler J., Berkman L.F., Krumholz H.M. (1999). Risk factors for heart failure in the elderly: a prospective community based study, Am J Med, 106, 605-612.

Chirinos, J. A., Kips, J. G., Jacobs, D. R., Brumback, L., Duprez, D. A., Kronmal, R., Kronmal, R., Bluemke, D.A., Townsend, R.R., Vermeersch, S. & Segers, P. (2012). Arterial wave reflections and incident cardiovascular events and heart failure: MESA (Multi-ethnic Study of Atherosclerosis), J Am Coll Cardiol, 60, 2170-2177.

Chung C.M., Chu C.M., Chang S.T., Cheng H.W., Yang T.Y., Wan P.C., Pan K.L., Lin Y.S., and Hsu J.T. (2010). Quantification of aortic stiffness to predict the degree of left ventricular diastolic function, Am J Med Sci, 340, 468-473.

Çil H., Bulur S., Türker Y., et al., MELEN Investigators. (2012). Impact of body mass index on left ventricular diastolic dysfunction, Echocardiography 29, 647-651.

Cleland J.G., Cohen-Solal A., Aguilar J.C., Dietz R., Eastaugh J., Follath F., Freemantle N., Gavazzi A., van Gilst W.H., Hobbs F.D., Korewicki J., Madeira H.C., Preda I., Swedeberg G., Widimsky J, IMPROVEMENT of Heart Failure

Programme Committees and Investigators. Improvement programme in evaluation and management, Study Group on Diagnosis of the Working Group on Heart Failure of the European Society of Cardiology. (2002). Management of heart failure in primary care (the IMPROVEMENT of Heart Failure Programme): an international survey, Lancet, 360, 1631-1639.

Cleland J.G., Tendera M., Adamus J., Freemantle N., Polonski L., Taylor J., PEP-CHF Investigators. (2006). The perindopril in elderly people with chronic heart failure (PEP-CHF) study, Eur Heart J, 27, 2338-2345.

Coort S.L., Bonen A., van der Vusse G.J., Glatz J.F., and Luiken J.J. (2007). Cardiac substrate uptake and metabolism in obesity and type-2 diabetes: role of sacolemmal substrate transporters, Mol Cell Biochem, 299, 5-18

Coutinho T., Borlaug B.A., Pellikka P.A., Turner S.T., and Kullo I.J. (2013). Sex differences in arterial stiffness and ventricular-arterial interactions, J Am Coll Cardiol, 61, 96-103.

Cuspidi C., Rescaldini M., Tadic M., Sala C., and Grassi G. (2014). Effect of bariatric surgery on cardiac structure and function: A systematic review and meta-analysis, Am J Hypertens, 27, 146-156.

Dahiya R., Shultz S.P., Dahiya A., Fu J., Flatley C., Duncan D., Cardianl J., Kostner K.M., Byrne N.M., Hills A.P., Harris M., Conwell L.S., and Leong G.M. (2015). Relation of reduced preclinical left ventricular diastolic function and cardiac remodeling in overweight youth to insulin resistance and inflammation, Am J Cardiol, 115, 1222-1228.

Dahlof B Lindholm L.H., Hansson L., Schersten B., Ekbom T., and Wester P.O. (1991). Morbidity and mortality in the Swedish trial in old patients with hypertension (STOP-Hypertension), Lancet, 338, 1281-1285.

Dannenberg A.L., Levy D., and Garrison R.J. (1989). Impact of age on echocardiographic left ventricular mass in a healthy population (the Framingham Study). Am J Cardiol, 64, 1066-1068.

de Denus S., Lavoie J., Ducharme A., O'Meara E., Racine N., Sirois M.G., Neagoe P.E., Zhu L., Rouleau J.L., and White M. (2012). Differences in biomarkers in patients with heart failure with a reduced vs a preserved left ventricular ejection fraction, Can J Cardiol, 28, 62-68.

de las Feuntes L., Brown A.L., Mathews S.J., Waggoner A.D., Soto P.F., Gropler R.J., Davila-Roman V.G. (2007). Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass, Eur Heart J, 28, 553-559.

Desai A.S., Mitchell G.F., Fang J.C., Creager M.A. (2009). Central aortic stiffness is increased in patients with heart failure and preserved ejection fraction, J Card Fail, 15, 658-664.

De Simone G., Devereux R.B., Izzo R., Girfoglio D., Lee E.T., Howard B.V., and Roman M.J. (2013). Lack of reduction of left ventricular mass in treated hypertension: the strong heart study, J Am Heart Assoc, 2, e000144 (doi: 10.1161/JAHA.113.000144)

Deswal A., Richardson P., Bozkurt B., and Mann D.L. (2011). Results of the randomized aldosterone anatagonism in heart failure with preserved ejection fraction trial (RAAM-PEF), J Card Fail, 17, 634-642.

Devereux R.B., Alonso D.R., Lutas E.M., Gottlieb G.J., Campo E., Sachs I., and Reichek N. Echocardiograph assessment of left ventricular hypertrophy: comparison to necropsy findings, Am J Cardiol, 57, 450-458.

Devereux R.B., de Simone G., Pickering T.G., Borer J.S., and Kligfield P. (1998). Relation of left ventricular midwall function to cardiovascular risk factors and arterial structure and function, Hypertension, 31, 929-936.

Devereux R.B., Pickering T.G., Harshfield G.A., Kleinert H.D., Denby L., Clark L., Pregibon D., Jason M., Kleiner B., Borer J.S., and Laragh J.H. (1983). Left ventricular hypertrophy in patients with hypertension: importance of blood pressure response to regularly recurring stress, Circulation, 68, 470-476.

Dinh W., Lankisch M., Nickl W., Scheyer D., Scheffold T., Kramer F., Klein R.M, Coll Barroso M., and Füth R. (2010). Insulin resistance and glyc emic abnormalities are associated with deterioration of left ventricular diastolic function. A cross-sectional study, Cardiovasc Diabetol, 9, 63.

Dokainish H., Zoghbi W.A., Lakkis N.M., Ambriz E., Patel R., Quiñones M.A., and Nagueh S.F. (2005). Incremental predictive power of B-type natriuretic peptide and tissue Doppler echocardiography in the prognosis of patients with congestive heart failure, J Am Coll Cardiol, 45, 1223-1226.

Drazner M.H., Rame J.E., Marino E.K., Gottdiener J.S., Kitzman D.W., Gardin J.M., Manolio T.A., Dries D.L., Siscovick D.S. (2004). Increased left ventricular mass is a risk factor for the development of a depressed left ventricular ejection fraction within five years: the Cardiovascular Health Study, J Am Coll Cardiol, 43, 2207-2215.

Edelmann F., Wachter R., Schmidt A.G., Kraigher-Krainer E., Colantonio C., Kamke W., Duvinage A., Stahrenberg R., Durstewitz K., Loffler M., Dungen H.D., Tschope C., Herrmann-Lingen C., Halle M., Hasenfuss G., Gelbrich G.,

Pieske B., Aldo-DHF Investigators. (2013). Effect of spironolactone on diastolic function and exercise capacity in patients with heart failure with preserve ejection fraction: the Aldo-DHF randomized controlled trial, JAMA, 309, 781-791.

Elston R.C., George V.T., and Severtson F. (1992). The Elston-Stewart algorithm for continuous genotypes and environmental factors, Hum Hered, 42, 16-27.

Federmann M., and Hess O.M. (1994). Differentiation between systolic and diastolic dysfunction, Eur Heart J, 15 Suppl D, 2-6.

Fernandes V.R.S., Polak J.E., Cheng S., Rosen B.D., Carvalho B., Nasir K., McClelland R., Hundley G., Pearson G., O'Leary D.H., Bluemke D.A., and Lima J.A. (2008). Arterial stiffness is associated with regional ventricular systolic and diastolic dysfunction: The Multi-Ethnic Study of Atherosclerosis, Arterioscler Thromb Vasc Biol, 28, 194-201.

Fischer M., Baessler A., Hense H.W., Hengstenberg C., Muscholl M., Holmer S., Doring A., Broeckel U., Riegger G., and Schunkert H. (2003). Prevalence of left ventricular diastolic dysfunction in the community: results from a Doppler echocardiographic-based survey of a population sample, Eur Heart J, 24, 320 -328.

Fonarow G.C., Stough W.G., Abraham W.T., Albert N.M., Gheorghiade M., Greenberg B.H., O'Connor C.M., Sun J-L., Yancy C.W., Young J.B., for the OPTIMIZE-HF Investigators and hospitals. (2007). Characteristics, treatments, and outcomes of patients with preserved systolic function hospitalized for heart failure. A report from the OPTIMIZE-HF registry, J Am Coll Cardiol, 50, 768-777.

Fox E.R., Klos K.L., Penman A.D., Blair G.J., Blossom B.D., Arnett D., Devereux R., Samdarshi T., Boerwinkle E., Mosley T.H. (2010). Heritability and genetic linkage of left ventricular mass, systolic and diastolic function in hypertensive African Americans (from the GENOA study), Am J Hypertens, 23, 870-875.

Galderisi M., Paolisso G., Tagliamonte M.R., Alfieri A., Petrocelli A., de Divitiis M., Varricchio M., and de Divitiis O. (1997). Is insulin action a determinant of left ventricular relaxation in uncomplicated essential hypertension? J Hypertens, 15, 745-50.

George V.T., Elston R.C. (1987). Testing the association between polymorphic markers and quantitative traits in pedigrees, Genet Epidemiol, 4, 193-201.

Halley C.M., Houghtaling P.L., Khalil M.K., Thomas J.D., Jaber W.A. (2011). Mortality rate in patients with diastolic dysfunction and normal systolic function, Arch Intern Med 171, 1082-1087.

Hashimoto, J., Watabe, D., Hatanaka, R., Hanasawa, T., Metoki, H., Asayama, K., Ohkubo, T., Totsune, K., & Imai, Y. (2006). Enhanced Radial Late Systolic Pressure Augmentation in Hypertensive Patients With Left Ventricular Hypertrophy*, Am J Hypertens, 19, 27-32.

Harshfield G.A., Grim C.E., Hwang C., Savage D.D., and Anderson S.J. (1990). Genetic and environmental influences on echocardiographically determined left ventricular mass in black twins, Am J Hypertens, 3, 538-543.

He J., Ogden L.G., Bazzano L.A., Vuppturi S., Loria C., Whelton P.K. (2001). Risk factors for congestive heart failure in US men and women:NHANES I epidemiologic follow-up study, Arch Intern Med, 161, 996-1002.

Hernandez A.F., Hammill B.G., O'Connor C.M., Schulman K.A., Curtis L.H., and Fonarow G.C. (2009). Clinical effectiveness of beta-blockers in heart failure. Findings from the OPTIMIZE-HF (Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure) registry, J Am Coll Cardiol, 53, 184-192.

Hodson B., Norton G.R., Booysen H.L., Sibiya M.J., Raymond A., Maseko M.J., Majane O.H., Libhaber E., Sareli P., and Woodiwiss A.J. (2016). Brachial Pressure Control Fails to Account for Most Distending Pressure-Independent, Age-Related Aortic Hemodynamic Changes in Adults, Am J Hypertens, 29, 605-613.

Hogg K., Swedberg K., McMurray J. (2004). Heart failure with preserved left ventricular systolic function: epidemiology, clinical characteristics and prognosis, J Am Coll Cardiol, 43, 317-327.

Hsu P-C., Lin T-H., Lee C-S., Lee H-C., Chu C-Y., Su H-M., Voon W-C., Lai W-T., Sheu S-H. (2010). Mismatch between arterial stiffness increase and left ventricular diastolic dysfunction, Heart Vessels, 25, 485-492.

Hsuan C-F., Huang C-K., Lin J-W., Lin L-C., Lee T-L., Tai C-M., Yin W-H., Tseng W-K., Hsu K-L., and Wu C-C. (2010). The effect of surgical weight reduction on left ventricular structure and function in severe obesity, Obesity, 18, 1188-1193.

Hundley W.G., Kitzman D.W., Morgan T.M., Hamilton C.A., Darty S.N., Stewart K.P., Herrington D.M., Link K.M., and Little W.C. (2001). Cardiac cycle dependent changes in aortic area and distensibility are reduced in older patients with

isolated diastolic heart failure and correlate with exercise intolerance, J Am Coll Cardiol, 38, 796-802.

Hwang Y-C., Jee JH., Kang M., Rhee E-J., Sung J., and Lee M-K. (2012). Metabolic syndrome and insulin resistance are associated with abnormal left ventricular diastolic function and structure independent of blood pressure and fasting blood glucose level, Int J Cardiol, 159, 107-111.

Ikonomidis I., Tzortzis S., Papaioannou T., Protogerou A, Stamatelopoulos K., Papamichael C., Zakopoulos N., Lekakis J. (2008). Incremental value of arterial wave reflections in the determination of left ventricular diastolic dysfunction in untreated patients with essential hypertension, J Hum Hypertens, 22, 687-698.

Ingelsson[a] E., Sundstrom J., Arnlov J., Zethelius B., and Lind L. (2005). Insulin resistance and risk of congestive heart failure, JAMA, 294, 334-341.

Ingelsson[b] E., Arnlov J., Sundstrom J., and Lind L. (2005). Inflammation, as measured by the erythrocyte sedimentation rate, is an independent predictor for the development of heart failure, J Am Coll Cardiol, 45, 1802-1806.

Ippisch H.M., Inge T.H., Daniels S.R., Wan B., Khoury P.R., Witt S.A., Glascock B.J., Garcia V.F., and Kimball T.R. (2008). Reversibility of cardiac abnormalities in morbidly obese adolescents, J Am Coll Cardiol, 51, 1342-1348.

Isaaz K., Munoz del Romeral L., Lee E., Schiller N.B. (1993). Quantitation of the motion of the cardiac base in normal subjects by Doppler echocardiography, J Am Soc Echocardiogr, 6, 166-176.

Johansson S., Wallander M.A., Ruigomez A., and Garcia Rodriguez L.A. (2001). Incidence of newly diagnosed heart failure in UK general practice, Eur J Heart Fail, 3, 225-231.

Jin Y., Kuznetsova T., Bochud M., Richart T., Thijs L., Cusi D., Fagard R., and Staessen J.A. (2011). Heritability of left ventricular structure and function in Caucasian families, Eur J Echocardiogr, 12, 326-332.

Kahn B.B., and Flier J.S. (2000). Obesity and insulin resistance, J Clin Invest, 106, 473-481.

Kane G.C., Karon B.L., Mahoney D.W., Redfield M.M., Roger V.L., Burnett J.C., Jacobsen S.J., and Rodeheffer R.J. (2011). Progression of left ventricular diastolic dysfunction and risk of heart failure, JAMA, 306, 856-863.

Kang S., Fan H-M., Li J., Fan L-Y., Miao A-Y., Bao Y., Wu L-Z., Zhu Y., Zhang D-F., and Liu Z-M. (2010). Relationship of arterial stiffness and early mild diastolic

heart failure in general middle and aged population, Eur Heart J, 31, 2799-2807.

Kasner M., Westermann D., Lopez B., Gaub R., Escher F., Kuhl U., Schultheiss H.P., and Tschope C. (2011). Diastolic tissue Doppler indexes correlate with the degree of collagen expression and cross-linkage in heart failure and normal ejection fraction, JACC, 57, 977-985.

Kawaguchi M., Hay I., Fetics B., Kass D.A. (2003). Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations, Circulation, 107, 714-720.

Kenchaiah S., Evans J.C., Levy D., Wilson P.W.F., Benjamin E.J., Larson S.D., Kannel W.B., and Vasan R.S. (2002). Obesity and the risk of heart failure. New Engl J Med, 347, 305-313.

Kenchaiah S., Sesso H.D., and Gaziano M.J. (2009). Body mass index and vigorous physical activity and the risk of heart failure among men, Circulation, 119, 44-52.

Kishi S., Gidding S.S., Reis J.P., Colangelo L.A., Venkatesh B.A., Armstrong A.C., Isogawa A., Lewis C.E., Wu C., Jacobs D.R., Liu K., Lima J.A. (2016). Association of insulin resistance and glycemic metabolic abnormalities with LV structure and function in middle age. The CARDIA study, J Am Coll Cardiol Img, doi: 10.1016/j.jcmg.2016.02.033.

Kitzman D.W., Herrington D.M., Brubaker P.H., Moore J.B., Eggebeen J., Haykowsky M.J. (2013). Carotid arterial stiffness and its relationship to exercise intolerance in older patients with heart failure and preserved ejection fraction, Hypertension, 61, 112-119.

Kizer J.R., Bella J.N., Palmieri V., Liu J.E., Best L.G., Roman M.J., Devereux R.B. (2006). Left atrial diameter as an independent predictor of first clinical cardiovascular events in middle-aged and elderly adults: the Strong Heart Study (SHS), Am Heart J, 151, 412-418.

Kloch-Badelek M., Knez J., Tikhonoff V., Thijs L., Sakiewicz W., Ryabikov A., Stolarz-Skrzypek K., Jin Y., Malyutina S., Casiqlia E., Narkiewicz K., Czarnecka D., Kawecka-Jaszcz K., Staessen J.A., Kuznetsova T., European Project on Genes in Hypertension (EPOGH) Investigators. (2014). Heritability and other determinants of left ventricular diastolic function in the family-based population study, J Hypertens, 32, 1854-1861.

Koren M.J., Devereux R.B., Casale P.N., Savage D.D., and Laragh J.H. (1991). Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension, Ann Intern Med, 114, 345-352.

Kostis J.B., Davis B.R., Cutler J., Grimm R.H., Berge K..G., Cohen J.D., Lacy C.R., Perry H.M., Blaufox M.D., Wassertheil-Smoller S., Black H.R., Schron E., Berkson D.M., Curb J.D., Smith W.M., McDonald R., Applegate W.B. (1997). Prevention of heart failure by antihypertensive drug treatment in older persons with isolated systolic hypertension. SHEP Cooperative Research Group, JAMA, 278, 212-216.

Krum H., and Gilbert R.E. (2003). Demographics and concomitant disorders in heart failure, Lancet, 362, 147-158.

Kuznetsova T., Staessen J.A., Olszanecka A., Ryabikov A., Stolarz K., Malyutina S., Fagard R., Kawecka-Jaszck K., Nikitin Y, on behalf of the European Project on Genes in Hypertension (EPOGH) Investigators. (2003). Maternal and paternal influences of left ventricular mass of offspring, Hypertension, 41, 69-74.

Lam C.S., Liu X., Yang Q., Larson M.G., Pencina M.J., Aragam J., Redfield M.M., Benjamin E.J., and Vasan R.S. (2010). Familial aggregation of left ventricular geometry and association with parental heart failure. The Framingham Heart Study, Circ Cardiovasc Genet, 3, 492-498.

Lam C.S.P., Lyass A., Kraiger-Krainer E., Massaro J.M., Lee D.S., Ho J.E., Levy D., Redfield M.M., Pieske B.M., Benjamin E.J., and Vasan R.S. (2011). Cardiac dysfunction and non-cardiac dysfunction as precursors of heart failure with reduced and preserved ejection fraction in the community, Circulation, 124, 24-30.

Lam C.S.P., Roger V.L., Rodeheffer R.J., Bursi F., Borlaug B.A., Ommen S.R., Kass D.A., Redfield M.M. (2007). Cardiac Structure and Ventricular-Vascular Function in Persons With Heart Failure and Preserved Ejection Fraction From Olmsted County, Minnesota, Circulation, 115, 1982-1990.

Lambert E., Sari C.I., Dawood T., Nguyen J., McGrane M., Eikelis N., Chopra R., Wong C., Chatzivlastou K., Head G., Straznicky N., Esler M., Schlaich M., and Lambert G. (2010). Sympathetic nervous system activity is associated with obesity-induced subclinical organ damage in young adults, Hypertension, 56, 351-358.

Lang R.M., Badano L.P., Mor-Avi V., Afilalo J., Armstrong A., Ernande L., Flachskampf F.A., Foster E., Goldstein S.A., Kuznetsova T., Lancellotti P.,

Muraru D., Picard M.H., Rietzschel E.R., Rudski L., Spencer K.T., Tsang W., Voigt J.U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging, J Am Soc Echocrdiogr, 28, 1-39.e14, doi: 10.1016/j.echo.2014.10.003.

Latner J., Wilson G., Stunkard A., and Jackson M. (2002). Self-help and long-term behaviour therapy for obesity, Behav Res Ther, 40, 805-812.

Lauer M.S., Anderson K.M., Kannel W.B., and Levy D. (1991). The impact of obesity on left ventricular mass and geometry. The Framingham Heart Study, JAMA, 266, 231-236.

Lee D.S., Gona P., Vasan R.S., Larson M.G., Benjamin E.J., Wang T.J.,Tu J.V., and Levy D. (2009). Relation of disease pathogenesis and risk factors to heart failure with preserved or reduced ejection fraction: insights from the Framingham heart study of the National Heart, Lung and Blood Institute, Circulation, 119, 3070-3077.

Levy D., Garrison R.J., Savage D.D., Kannel W.B., and Castelli W.P. (1990). Prognostic implications of echocardiographic measurement of left ventricular mass in the Framingham Heart Study, N Engl J Med, 322, 1561-1566.

Li J., Becher P.M., Blankenberg S., and Westermann D. (2013). Current Treatment of Heart Failure with Preserved Ejection Fraction : Should We Add Life to the Remaining Years or Add Years to the Remaining Life ? Cardiol Res Pract, 2013, 130724.

Libhaber C.D., Norton G.R., Majane O.H., Libhaber E., Essop M.R., Brooksbank R., Maseko M., and Woodiwiss A.J. (2009). Contribution of central and general adiposity to abnormal left ventricular diastolic function in a community sample with a high prevalence of obesity, Am J Cardiol, 104, 1527-1533.

Libhaber C.D., Woodiwiss A.J., Booysen H.L., Maseko M.J., Majane O.H.I., Sareli P., and Norton G.R. (2014). Differential relationships of systolic and diastolic blood pressure with components of left ventricular diastolic dysfunction, J. Hypertens, 32, 912-920.

Lind L., Andersson P.E., Andren B., Hanni A., and Lithell H.O. (1995). Left ventricular hypertrophy in hypertension is associated with the insulin resistance metabolic syndrome, J Hypertens, 13, 433-438

Little W.C., Zile M.R., Kitzman D.W., Hundley W.G., O'Brien T.X., Degroof R.C. (2005). The effect of alagebrium chloride (ALT-711), a novel glucose cross-

link breaker, in the treatment of elderly patients with diastolic heart failure, J Card Fail, 11, 191-195.

Lopez B., Querejeta R., Gonzalez A., Larman M and Diez J. (2012). Collagen cross-linking but not collagen amount associates with elevated filling pressures in hypertensive patients with stage C heart failure: potential role of lysyl oxidase, Hypertension, 60, 677-683.

Lorell B.H., Apstein C.S., Weinberg E.O., Cunningham M.J. (1990). Diastolic function in left ventricular hypertrophy: clinical and experimental relationships. Eur Heart J, 11, 54-64.

Luaces M., Chacofeiro V., Garcia-Munoz-Najar A., Medina M., González N., Cancer E., Rodriguez-Robles A., Cánovas G., and Antequera-Pérez A. (2012). Anatomical and functional alterations of the heart in morbid obesity, changes after bariatric surgery, Rev Esp Cardiol, 65, 14-21.

Maredza M., Bertram M.Y., and Tolliman S.M. (2011). Disease burden of stroke in rural South Africa: an estimate of incidence, mortality and disability adjusted life years, BMC Neurol, 15, 54, doi: 10.1186/s12883-015-0311-7

Masaguta H., Senda S., Goda F., Yoshihara Y., Yoshikawa K., Fujita N., Daikuhara H., Nakamura H., Taoka T., and Kohno M. (2006). Left ventricular diastolic dysfunction as assessed by echocardiography in metabolic syndrome, Hypertens Res, 29, 897-903.

Maseko M.J., Woodiwiss A.J., Majane O.H., Libhaber C.D., Brooksbank R., and Norton G.R. (2013). Isolated increases in in-office pressure account for a significant proportion of nurse-derived blood pressure-target organ relations, J Hypertens, 31, 1379-1386.

Masoudi F.A., Havranek E.P., Smith G., Fish R.H., Steiner J.F., Ordin D.L., Krumholz H.M. (2003). Gender, age, and heart failure with preserved left ventricular systolic function, J Am Coll Cardiol, 41, 217– 223.

Massie B.M., Carson P.E., McMurray J.J., Komajda M., McKelvie R., Zile M.R., Anderson S., Donovan M., Iverson E., Staiger C., and Ptaszynska A., for the I-PRESERVE Investigators. (2008). Irbesartan in Patients with Heart Failure and Preserved Ejection Fraction, N Engl J Med, 359, 2456-2467.

Masugata H., Senda S., Yoshikawa K., Yoshihara Y., Daikuhara H., Ayada Y., Matsushita H., Nakamura H., Taoka T., Kohno M. (2005). Relationships between echocardiographic findings, pulse wave velocity, and carotid atherosclerosis in type 2 diabetic patients, Hypertens Res, 28, 965-971.

McEniery C. M., Hall I. R., Qasem A., Wilkinson I. B., and Cockcroft J. R. (2005). Normal Vascular Aging: Differential Effects on Wave Reflection and Aortic Pulse Wave Velocity The Anglo-Cardiff Collaborative Trial (ACCT), J Am Coll Cardiol, 46, 1753-1760.

McMurray J.J., Adamopoulous S., Anker S.D., Auricchio A., Böhm M., Dickstein K., Falk V., Filippatos G., Fonseca C., Gomez-Sanchez M.A., Jaarsma T., Køber L., Lip G.Y., Maggioni A.P., Parkhomenko A., Pieske B.M., Popescu B.A., Rønnevik P.K., Rutten F.H., Schwitter J., Seferovic P., Stephinska J., Trindada P.T., Voors A.A., Zannad F., Zeiher A.; ESC Committee for Practice Guidelines. (2012). ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC, Eur Heart J, 33, 1787-1847.

Milani R.V., Lavie C.J., Mehra M.R., Ventura H.O., Kurtz J.D., and Messerli F.H. (2006). Left ventricular geometry and survival in patients with normal left ventricular ejection fraction, Am J Cardiol, 97, 959-963.

Millen A.M.E., Libhaber C.D., Majane O.H.I., Libhaber E., Maseko M.J., Woodiwiss A.J. and Norton G.R. (2014). 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, J Hypertens, 32; 2457-2464.

Mitchell G. F., Hwang S. J., Vasan R. S., Larson M. G., Pencina M. J., Hamburg N. M., Vita J.A., Levy D., & Benjamin E. J. (2010). Arterial stiffness and cardiovascular events the Framingham Heart Study, Circulation, 121, 505-511.

Mohammed S.F., Borlaug B.A., Roger V.L., Mirzoyev S.A., Rodeheffer R.J., Chirinos J.A., and Redfield M.M. (2012). Comorbidity and ventricular and vascular structure and function in heart failure with preserved ejection fraction. A community-based study, Circ Heart Fail, 5, 710-719.

Mottram P.M., Haluska B.A., Leano R., Carlier S., Case C., and Marwick T.H. (2005). Relation of arterial stiffness to diastolic dysfunction in hypertensive heart disease, Heart, 91, 1551-1556.

Mozaffarian D., Benjamin E.J., Go A.S., Arnett D.K., Blaha M.J., Cushman M., de Ferranti S., Després J.P., Fullerton H.J., Howard V.J., Huffman M.D., Judd S.E., Kissela B.M., Lackland D.T., Lichtman J.H., Lisabeth L.D., Liu S., Mackey R.H., Matcher D.B., McGuire D.K., Mohler E.R., Moy C.S., Muntner

P., Mussolino M.E., Nasir K., Neumar R.W., Nichol G., Palaniappan L., Pandey D.K., Reeves M.J., Rodriguez C.J., Sorlie P.D., Stein J., Towfighi A., Turan T.N., Virani S.S., Willey J.Z., Woo D., Yeh R.W., Turner M.B., American Heart Association Statistics Committee and Stroke Statistics Subcommittee. (2015). Heart disease and stroke statistics--2015 update: a report from the American Heart Association, Circulation, 131, e29-322, doi: 10.1161/CIR.0000000000000152. Epub 2014 Dec 17.

MRC Working Party. (1992). Medical Research Council trial of treatment of hypertension in older adults: principal results, BMJ, 304, 405-412.

Mureddu G.F., Greco R., Rosato G.F., Cella A., Vaccaro O., Contaldo F., de Simone G. (1998). Relation of insulin resistance to left ventricular hypertrophy and diastolic dysfunction in obesity, Int J Obes Relat Metab Disord, 22, 363-368.

Nagano N., Nagano M., Yo Y., Liyama K., Higaki J., Mikami H., Ogihara T. (1994). Role of glucose intolerance in cardiac diastolic function in essential hypertension, Hypertension, 23, 1002-1005.

Nagueh S.F., Appleton C.P., Gillebert T.C., et al., (2009). Recommendations for the evaluation of left ventricular diastolic function by echocardiography, Eur J Echocardiogr, 10, 165-193.

Nagueh S.F., Bachinski L.L., Meyer D., Hill R., Zoghbi W.A., Tam J.W., Quiñones M.A., Roberts R., Marian A.J. (2001). Tissue Doppler imaging consistently detects myocardial abnormalities in patients with hypertrophic cardiomyopathy and provides a novel means for an early diagnosis before and independently of hypertrophy, Circulation, 104, 128-130.

Nagueh S.F., Middleton K.J., Kopelen H.A., Zoghbi W.A., Quiñones M.A. (1997). Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures, J Am Coll Cardiol, 30, 1527-1533.

Nagueh S.F., Smiseth O.A., Appleton C.P., Byrd B.F., Dokainish H., Edvardsen T., Flachskampf F.A., Gillebert T.C., Klein A.L., Lancellotti P., Marino P., Oh J.K., Popescu B.A., and Waggoner A.D. (2016). Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging, J Am Soc Echocardiogr 29, 277-314.

Nanayakkara S., and Kaye D.M. (2015). Management of heart failure with preserved ejection fraction: a review. Clin Ther, 37, 2186-2198.

Nichols W., O'Rourke M., and Vlachopoulos C. (Eds.). (2011). McDonald's blood flow in arteries: theoretical, experimental and clinical principles. CRC Press.

Nichols G.A., Gullion C.M., Koro C.E., Ephross S.A., and Brown J.B. (2004). The incidence of congestive heart failure in type 2 diabetes: an update, Diabetes Care, 27, 1879-1884.

Nicklas B.J., Cesari M., Penninx B.W., Kritchevsky S.B., Ding J., Newman A., Kitzman D.W., Kanaya A.M., Pahor M., and Harris T.B. (2006). Abdominal obesity is an independent risk factor for chronic heart failure in older people, J Am Geriatr Soc, 54, 413-430.

Nikolaidis L.A., Sturzu A., Stolarski C., Elahi D., Shen Y.T., Shannon R.P. (2004).The development of myocardial insulin resistance in conscious dogs with advanced dilated cardiomyopathy, Cardiovasc Res, 61, 297-306.

Noh H-M., Lee S.C., Park S.W., Sung J., and Song Y-M. (2015). Genetic influence on left ventricular structure and function: A Korean twin and family study. Twin Res and Hum Genet, 18, 281-289.

Norton G.R., Candy G., and Woodiwiss A.J. (1996). Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats, Circulation, 93, 1905-1912.

Norton G.R., Maseko M., Libhaber E., Libhaber C.D., Majane O.H., Dessein P., Sareli P., and Woodiwiss A.J. (2008). Is pre-hypertension an independent predictor of target organ changes in young-to-middle aged persons of African descent? J Hypertens, 26, 2279-2287.

Nunez E., Arnett D.K., Benjamin E.J., Liebson P.R., Skelton T.N., Taylor H., and Andrew M. (2005). Optimal threshold value for left ventricular hypertrophy in blacks: the Atherosclerosis Risk in Communities study, Hypertension, 45, 58-63.

O'Brien E., Asmar R., Beilin L., Imai Y., Mallion J.M., Mancia G., Mengden T., Myers M., Padfield P., Palatini P., Parati G., Pickering T., Redon J., Staessen J., Stergiou G., Verdecchia P., on behalf of the European Society of hypertension Working group on Blood Pressure Monitoring. (2003). European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement, J Hypertens, 21, 821-848.

Oh J.K., Park S.J., Nagueh S.F. (2011). Established and novel clinical applications of diastolic function assessment by echocardiography, Circ Cardiovasc Imaging, 4, 444-455.

Oki T., Tabata T., Yamada H., Wakatsuki T., Shinohara H., Nishikado A., Luchi A., Fukuda N., Ito S. (1997). Clinical Application of Pulsed Doppler Tissue Imaging for Assessing Abnormal Left Ventricular Relaxation, Am J Cardiol, 79, 921-928.

Olsen M.H., Hjerkinn E., Wachtell K., Hoieggen A., Bella J.N., Nesbitt S.D., Fossum E., Kjeldsen S.E., Julius S., Ibsen H. (2003). Are left ventricular mass, geometry and function related to vascular changes and/or insulin resistance in long-standing hypertension? ICARUS: a LIFE substudy, J Hum Hypertens, 17, 305-311.

Ommen S.R., Nishimura R.A., Appleton C.P., Miller F.A., Oh J.K., Redfield M.M., and Tajik A.J. (2000). Clinical Utility of Doppler Echocardiography and Tissue Doppler Imaging in the Estimation of Left Ventricular Filling Pressures : A Comparative Simultaneous Doppler-Catheterization Study, Circulation, 102, 1788-1794.

Ouwens D.M., Boer C., Fodor M., De Galan P., Heine R.J., Maassen J.A., Diamant M. (2005). Cardiac dysfunction induced by high fat diet is associated with altered myocardial insulin signalling in rats, Diabetologia, 48, 1229-1237

Owan T.E., Hodge D.O., Herges R.M., Jacobsen S.J., Roger V.L., and Redfield M.M. (2006). Trends in prevalence and outcome of heart failure with preserved ejection fraction, N Engl J Med, 355, 251-259.

Palmieri V., Bella J.N., Roman M.J., Gerdts E., Papademetriou V., Wachtell K., Nieninen M.S., Dahlöf B., and Devereux R.B. (2003). Pulse pressure/stroke index and left ventricular geometry and function: the LIFE study, J Hypertens, 21, 781-787.

Paulus W.J., and van Ballegoij. (2010). Treatment of heart failure wit normal ejection fraction. an inconvenient truth, J Am Coll Cardiol, 55, 526-537.

Pavlopoulos H., Grapsa J., Stefanadi E., Kamperidis V., Phillippou E., Dawson D., Nihoyannoppoulos P. (2008). The evolution of diastolic dysfunc- tion in the hypertensive disease, Eur J Echocardiogr, 9, 772-778.

Peterson[a] V.R., Norton G.R., Libhaber C.D., Maseko M.J., Sareli P and Woodiwiss A.J. (2016). Intra-Familial Aggregation and Heritability of Tissue Doppler Indexes of Left Ventricular Diastolic Function in a Group of African Descent, J Am Soc Hypertens, 10, 517-526.

Peterson[b] V.R., Norton G.R., Raymond A., Libhaber C.D., Millen A.M.E., Majane O.H.I., Maseko M.J. and Woodiwiss, A.J. (2016). Insulin Resistance-Associated Decreases in Left Ventricular Diastolic Function are Strongly

Modified by the Extent of Concentric Remodelling in a Community Sample, Int J Cardiol, 220, 349-355.

Peterson V.R, Norton G.R, Redelinghuys M., Libhaber C.D., Maseko M.J., Majane O.H.I., Brooksbank R., and Woodiwiss A.J. (2015). The intra-familial aggregation and heritability of left ventricular geometric remodeling is independent of cardiac mass in families of African ancestry, Am J Hypertens, 28, 657-663.

Peterson[c] V.R., Woodiwiss A.J., Libhaber C.D., Raymond A., Sareli P. and Norton G.R. (2016). Cardiac Diastolic Dysfunction is Associated with Aortic Wave Reflection, but not Stiffness in a Predominantly Young-to-Middle-Aged Community Sample, Am J Hypertens, 29, 1148-1157.

Pickering T.G., Hall J.E., Appel L.J., Falkner B.E., Graves J., Hill M.N., Jones D.W., Kurtz T., Sheps S.G., Roccella E.J. (2005). Recommendations for blood pressure measurement in humans and experimental animals. Part 1: Blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Council on High Blood Pressure Research, Hypertension, 45, 142-161.

Pieske B., Butler J., Filippatos G., Lam C., Maggioni A.P., Ponikowski P., Shah S., Solomon S., Kraigher-Krainer E., Samano E.T., Scalise A.V., Muller K., Roessig L., Gheorghiade M., SOCRATES Investigators and Coordinators. (2014). Rational and design of the Soluble Guanylate Cyclase Stimulator in Heart Failure Studies (SOCRATES), Eur J Heart Fail, 16, 1026-1038.

Pitt B, Pfeffer M.A., Assmann S.F., Boineau R., Anand I.S., Claggett B., Clausell N., Desai A.S., Diaz R., Fleg J.L., Gordeev I., Harty B., Heitner J.F., Kenwood C.T., Lewis E.F., O'Meara E., Probstfield J.L., Shaburishvili T., Shah S.J., Solomon S.D., Sweitzer N.K., Yang S., and McKinlay S.M., for TOPCAT Investigators. (2014). Spironolactone for heart failure with preserved ejection fraction, N Engl J Med, 370, 1383-1392.

Prichett A.M., Jacobsen S.J., Mahoney D.W., Rodeheffer R.J., Bailey K.R., Redfield M.M. (2003). Left atrial volume as an index of left atrial size: a population-based study, J Am Coll Cardiol, 41, 1036-1043.

Quiñones M.A., Otto C.M., Stoddard M., Waggoner A., and Zoghbi W.A. (2002). Recommendations for quantification of Doppler echocardiography: a report from the Doppler Quantification Task Force of the Nomenclature and Standards Committee of the American Society of Echocardiography, J Am Soc Echocardiogr, 15, 167-184.

Rayner B. (2010). Hypertension: detection and management in South Africa, Nephron Clinical Practice, 116, c269-c273.

Reaven G.M. (2003). Importance of identifying the overweight patient who will benefit the most by losing weight, Ann Intern Med, 138, 420-423.

Redelinghuys M., Norton G.R., Scott L., Maseko M.J., Brooksbank R., Majane O.H.I., Sareli P., and Woodiwiss A.J. (2010) Relationship between urinary salt excretion and pulse pressure and central aortic hemodynamics independent of steady state pressure in the general population, Hypertension, 56, 584-590.

Redelinghuys M., Norton G.R., Maseko M.J., Majane O.H.I., and Woodiwiss A.J. (2012). Intra-familial aggregation and heritability of aortic versus brachial pulse pressure after imputing pretreatment values in a community of African ancestry, J Hypertens, 30, 1144-1150.

Redfield M.M., Chen H.H., Borlaug B.A., Semigran M.J., Lee K.L., Lewis G., LeWinter M.M., Rouleau J.L., Bull D.A., Mann D.L., Deswal A., Stevenson L.W., Givertz M.M., Ofilli E.O., O'Connor C.M., Felker G.M., Goldsmith S.R., Bart B.A., McNulty S.E., Ibarra J.C., Lin G., Oh J.K., Patel M.R., Kim R.J., Tracy R.P., Velazquez E.J., Anstrom K.J., Hernandez A.F., Mascette A.M., Braunwald E., RELAX Trial. (2013). Effect of phophodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: a randomized clinical trial, JAMA, 309, 1268-1277.

Redfield M.M., Jacobson S.J., Burnett J.C., Mahoney D.W., Bailey K.R., Rodeheffer R.J. (2003). Burden of systolic and diastolic ventricular dysfunction in the community, J Am Med Assoc, 28, 194-202

Rodriguez C.J., Diez-Roux A.V., Moran A., Jin Z., Kronmal R.A., Lima J., Homma S., Bluemke D.A., and Barr R.G. (2010). Left ventricular mass and ventricular remodeling among Hispanic subgroups compared to non-Hispanic blacks and whites: The Multi-Ethnic Study of Atherosclerosis, J Am Coll Cardiol, 55, 234-242.

Roger V.L., Weston S.A., Redfield M.M., Hellermann-Homan J.P., Killian J., Yawn B.P., Jacobsen S.J. (2004). Trends in heart failure incidence and survival in a community-based population, JAMA, 292, 344-350.

Roman M.J., Okin P.M., Kizer J.R., Lee E.T., Howard B.V., and Devereux R.B. (2010). Relations of central and brachial blood pressure to ventricular hypertrophy and geometry: the Strong Heart Study, J Hypertens, 288, 384-388.

Russo C., Jin Z., Homma S., Rundek T., Elkind M.S., Sacco R.L., Di Tullio M.R. (2011). Effect of obesity and overweight on left ventricular diastolic function. A community-based study in an elderly cohort, J Am Coll, Cardiol, 57, 1368-1374.

Russo C., Jin Z., Homma S., Rundek T., Elkind M.S., Sacco R.L., Sacco R.L., Di Tullio M.R. (2010). Effect of diabetes and hypertension on left ventricular diastolic function in a high risk population without evidence of heart disease, Eur J Heart Fail, 12, 454-461.

Sahn D.J., De Maria A., Kisslo J., and Weyman A. (1978). Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurement, Circulation, 58, 1072-1083.

Scantlebury D.C., and Borlaug B.A. (2011). Why are women more likely than men to develop heart failure with preserved ejection fraction, Curr Opin Cardiol, 26, 562-568.

Schillaci G., Pasqualini M., Verdecchia P., Vaudo G., Marchesi S., Percellati C., de Simone G., Mannarino E. (2002). Prognostic significance of left ventricular diastolic dysfunction in essential hypertension, J Am Coll Cardiol, 39, 2005-2011.

Schunkert H., Brockel U., Hengstenberg C., Luchner A., Muscholl M.W., Kurzidim K., Kuch B., Doring A., Riegger G.A.J., and Hense H. (1999). Familial predisposition of left ventricular hypertrophy, J Am Coll Cardiol, 33, 1685-1691.

Senni M., Tribouilloy C.M., Rodeheffer R.J., Jacobsen S.J., Evans J.M., Bailey K.R., Redfield M.M. (1998). Congestive heart failure in the community: a study of all incident cases in Olmsted County, Minnesota, in 1991, Circulation, 98, 2282-2289.

Shah A.M., Claggett B., Sweitzer N.K., Shah S.J., Anand I.S., O'Meara C.E., Desai A.S., Heitner J.F., Li G., Fang J., Rouleau J., Zile M.R., Markov V., Ryabov V., Reis G., Assman S.F., McKinlay S.M., Pitt B., Pfeffer M.A., and Solomon S.D. (2014). Cardiac structure and function and prognosis in heart failure with preserved ejection fraction. Findings from the echocardiographic study of the treatment of preserved cardiac function heart failure with an aldosterone antagonist (TOPCAT) trial, Circ Heart Fail, 7, 740-751.

Sharp A.S., Tapp R.J., Thom S.A.M., Francis D.P., Hughes A.D., Stanton A.V., Zambanini A., O'Brien E., Chaturvedi N., Lyons S., Byrd S., Poulter N.R., Sever P.S., Mayet J., ASCOT Investigators. (2010). Tissue Doppler E/E ratio

is a powerful predictor of primary cardiac events in a hypertensive population: an ASCOT substudy, Eur Heart J, 31, 747-752.

Shim C.Y., Park S., Choi D., Yang W-I., Cho I-J., Choi E-Y., Chung N., and Ha J.W. (2011). Sex differences in central hemodynamics and their relationship to left ventricular diastolic function, J Am Coll Cardiol, 57, 1226-1233.

Sibiya M.J., Woodiwiss A.J., Booysen H.L., Raymond A., Millen A.M., Maseko M.J., Majane O.H., Sareli P., Libhaber E., and Norton G.R. (2015). Reflected rather than forward wave pressures account for brachial pressure-independent relations between aortic pressure and end-organ changes in an African community, J Hypertens, 33, 2083-2090.

Simek C.L., Feldman M.D., Haber H.L., Wu C.C., Jayaweera A.R., Kaul S. (1995). Relationship between left ventricular wall thickness and left atrial size: comparison with other measures of diastolic function, J Am Soc Echocardiogr, 8, 37-47.

Sliem H., and Nasr G. (2011). Left ventricular structure and function in prediabetic adults: Relationship with insulin resistance, J Cardiovasc Dis Res 2, 23-28.

Sohn D-W., Chai I-H., Lee D-J., Kim H-C., Kim H-S., Oh B-H., Lee M-M., Park Y-B., Choi Y-S., Seo J-D., and Lee Y-W. (1997). Assessment of mitral annulus velocity by Doppler tissue imaging in the evaluation of left ventricular diastolic function, J Am Coll Cardiol, 30, 474-480.

Spies C., Farzaneh-Far R., Na B., Kanaya A., Schiller N., Whooley M.A. (2009). Relations of obesity to heart failure hospitalization and cardiovascular events in persons with stable coronary heart disease (from the Heart and Soul Study), Am J Cardiol, 104, 883- 889.

Steyn K., and Damasceno A. (2006). Lifestyle and related risk factors for chronic diseases, Disease and Mortality in Sub-Saharan Africa. 2nd edition. Washington (DC): World Bank, 2006. Chapter 18.

Swan L., Birnic D.H., Padmanabhan S., Inglis G., Connell J.M., and Hillis W.S. (2003). The genetic determination of left ventricular mass in healthy adults, Eur Heart J, 24, 577-582.

Syed M., Rosati C., Torosoff M.T., El-Hajjar M., Feustel P., Alger S., Singh P., and Fein S. (2009). The impact of weight loss on cardiac structure and function in obese patients, Obes Surg, 19, 36-40.

Tang W., Arnett D.K., Devereux R.B., Province M.A., Atwood L.D., Oberman A., Hopkins P.N., Kitzman D.W. (2002). Sibling resemblance for left ventricular structure, contractility and diastolic filling, Hypertension, 40, 233-238.

Taylor H.A., Penman A.D., Han H., Dele-Michael A., Skelton T.N., Fox E.R., Benjamin E.J., Arnett D.K., Mosley T.H. (2007). Left ventricular architecture and survival in African-Americans free of coronary heart disease (from the Atherosclerosis Risk in Communities [ARIC] study), Am J Cardiol, 99, 1413-1420.

Thomas L., Boyd A., Thomas S.P., Schiller N.B., Ross D.B. (2003). Atrial structural remodelling and restoration of atrial contraction after linear ablation for atrial fibrillation, Eur Heart J, 24, 1942-1951.

Tsioufis C.P., Tsiachris D.L., Selima M.N., Dimitriadis K.S., Thomopoulous C.G., Tsiliqqiris D.C., Gennadi A.S., Syrseloudis D.C., Stefanadi E.S., Toutouzas K.P., Kallikazaros I.E., Stefanadis C.I. (2008). Impact of waist circumference on cardiac phenotype in hypertensives according to gender, Obesity, 17, 177-182.

Utz W., Engeli S., Haufe S., Kast P., Hermsdorf M., Wiesner S., Pofahl M., Traber J., Luft F.C., Boschmann M., Schulz-Menger J., Jordan J. (2011). Myocardial stetosis, cardiac remodelling and fitness in insulin-sensitive and insulin-resistant obese women, Heart, 97, 1585-1589.

van Heerebeek L., Borbely A., Niessen H.W., Bronzwaer J.G., van der Velden J., Stienen G.J., Linke W.A., Laarman G.J., Paulus W.J. (2006). Myocardial structure and function differ in systolic and diastolic heart failure, Circulation, 113, 1966-1973.

van Heerebeek L., Hamdani N., Handoko M.L., Falcao-Pires I., Musters R.J., Kupreishvili K., Ijsselmuiden A.J., Schalkwijk C.G., Bronzwaer J.G., Diamant M., Borbely A., van der Velden J., Stienen G.J., Laarman G.J., Niessen H.W., Paulus W.J. (2008). Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension, Circulation, 117, 43-51.

Vasan R.S., and Levy D. (2000). Defining diastolic heart failure: a call for standardized diagnostic criteria, Circulation, 101, 2118-2121.

Verdecchia P., Schillaci G., Borgioni C., Ciucci A., Battistelli M., Bartoccini C., Santucci A., Santucci C., Reboldi G., Porcellati C. (1995). Adverse prognostic significance of concentric remodeling of the left ventricle in hypertensive patients with normal left ventricular mass, J Am Coll Cardiol, 25, 871-878.

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

pubertal twins (the Medical College of Virginia twin study), Am J Cardiol, 68, 661-668.

Wada H., Shinjo D., Kameda S., Ono K., Satoh N., Morimoto T., Osakada G., Nakano T., Fujita M., Shimatsu A., Hasegawa K. (2010). Transmitral E/A ratio decreases in association with abdominal fat accumulation in patients with impaired glucose tolerance or mild diabetes without left ventricular hypertrophy, Heart Vessels, 25, 45-50.

Waggoner A.D., and Bierig S.M. (2001). Tissue Doppler imaging: a useful echocardiographic method for the cardiac sonographer to assess systolic and diastolic ventricular function, J Am Soc Echocardiogr, 14, 1143-1152.

Wan S-H., Vogel M.W., Chen H-H. (2014). Pre-clinical diastolic dysfunction, J Am Coll Cardiol, 63, 407-416.

Wang M., Yip G.W., Wang A.Y., Zhang Y., Ho P-Y., Tse M-K., Lam P.K., Sanderson J.E. (2003). Peak early diastolic mitral annulus velocity by tissue Doppler imaging adds independent and incremental prognostic value, J Am Coll Cardiol, 41, 820-826.

Watanabe K., Sekiya M., Tsuruoka T., Funada J., Kameoka H. (1999). Effect of insulin resistance on left ventricular hypertrophy and dysfunction in essential hypertension, J Hypertens, 17, 1153-1160.

Weber T, Auer J, O'Rourke MF, Punzengruber C, Kvas E, Eber B. (2006). Prolonged mechanical systole and increased arterial wave reflections in diastolic dysfunction, Heart, 92, 1616-1623.

Weber T., O'Rourke M.F., Ammer M., Kvas E., Punzengruber C., Eber B. (2008). Arterial stiffness and arterial wave reflections are associated with diastolic function in patients with normal ejection fraction, Am J Hypertens, 21, 1194-1202.

Westermann D, Kasner M, Steendijk P, Spillmann F, Riad A, Weitmann K, Hoffmann W., Poller W., Pauschinger M., Schultheiss H-P., and Tschöpe C. (2008). Role of left ventricular stiffness in heart failure with normal ejection fraction, Circulation, 117, 2051-2060.

Wilhelmsen L., Rosengren A., Eriksson H., and Lappas G. (2001). Heart failure in the general population of men: morbidity, risk factors and prognosis, J Intern Med, 249, 253-261.

Wong C-Y., O'Moore-Sullivan T., Leano R., Byrne N., Beller E., Marwick T.H. (2004). Alterations of left ventricular myocardial characteristics associated with obesity, Circulation, 110, 3081-3087.

Woodiwiss A.J., Libhaber C.D., Majane O.H.I., Libhaber E., Maseko M., and Norton G.R. (2008). Obesity promotes left ventricular concentric rather than eccentric geometric remodeling and hypertrophy independent of blood pressure, Am J Hypertens, 21, 1144-1151.

Woodiwiss A.J., Molebatsi N., Maseko M.J., Libhaber E., Libhaber C., Majane O.H.I., Paiker J., Dessein P., Brooksbank., Sareli S., and Norton G.R. (2009). Nurse-recorded auscultatory blood pressure at a single visit predicts target organ changes as well as ambulatory blood pressure, J Hypertens, 27, 287-297.

Woodiwiss A.J., and Norton G.R. (2015). Obesity and left ventricular hypertrophy: The hypertension connection, Curr Hypertens, Reports, 17, 539-547.

Wu C-K., Yang C-Y., Lin J-W., Hsieh H-J., Chiu F-C., Chen J-J., Lee J-K., Huang S-W., Li H-Y., Chiang F-T., Chen J-J., and Tsai C-T. (2012). The Relationship Among Central Obesity, Systemic Inflammation, and Left Ventricular Diastolic Dysfunction as Determined by Structural Equation Modeling, Obesity, 20, 730-737.

Yamamoto T., Oki T., Yamada H., Tanaka H., Ishimoto T., Wakatsuki T., Tabata T., and Ito Susumu. (2003). Prognostic value of the atrial systolic mitral annular motion velocity in pa- tients with left ventricular systolic dysfunction, J Am Soc Echocardiogr, 16, 333-339.

Yambe M., Tomiyama H., Hirayama Y., Gulniza Z., Takata Y., Koji Y., Motobe K., and Yamashina A. (2004). Arterial stiffening as a possible risk factor for both atherosclerosis and diastolic heart failure, Hypertens Res, 27, 625-631.

Yancy C.W., Lopatin M., Stevenson L.W., De Marco T., Fonarow G.C. (2006). Clinical presentation, management, and in-hospital outcomes of pa- tients admitted with acute decompensated heart failure with preserved systolic function: a report from the Acute Decompensated Heart Failure National Registry (ADHERE) Database, J Am Coll Cardiol, 47, 76-84.

Yancy C.W., Jessup M., Bozkurt B., Butler J., Casey D.E., Drazner M.H., Fonarow G.C., Geraci S.A., Horwich T., Januzzi J.L., Johnson M.R., Kasper E.K., Levy W.C., Masoudi F.A., McBride P.E., McMurray J.J., Mitchell J.E., Peterson P.N., Riegel B., Sam F., Stevenson L.W., Tang W.H., Tsai E.J., Wilkoff B.L., American College of Cardiology Foundation, American Heart Association Task Force on Practice Guidelines. (2013). 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, J Am Coll Cardiol, 62, e147-239

Yu C-M., Fung J.W-H., Zhang Q., Kum L.C., Lin H., Yip G.W-K., Wang M., and Sanderson J.E. (2005). Tissue Doppler echocardiographic evidence of atrial mechanical dysfunction in coronary artery disease, Int J Cardiol, 105, 178-185.

Yusuf S., Pfeffer M.A., Swedberg K., Granger C.B., Held P., McMurray J.J., Michelson E.L., Olofsson B., Ostergren J. (2003). Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial, Lancet, 362, 777-781.

Xu L., Jiang C.Q., Lam T.H., Yue X.J., Lin J.M., Cheng K.K., Liu B., Jin Y-L., Zhang W.S., and Thomas G.N. (2011). Arterial stiffness and left ventricular diastolic dysfunction. Guangzhou Biobank Cohort Study-CVD, J Hum Hypertens, 25, 152-158.

Zamani P., Bluemke D.A., Jacobs D.R., Duprez D.A., Kronmal R., Lilly S.M., Ferrari V.A., Townsend R.R., Lima J.A., Budoff M., Segers P., Hannan P., Chirinos J.A. (2015). Resistive and pulsatile arterial load as predictors of left ventricular mass and geometry: the multiethnic study of atherosclerosis, Hypertension, 65, 85-92.

Zhou Y-T., Grayburn P., Karim A., Shimabukuro M., Higa M., Baetens D., Orci L., Unger R.H. (2000). Lipotoxic heart disease in obese rats: Implications for human obesity, Proc Nat Acd Sci, 97, 1784-1789.

Zile M.R., Desantis S.M., Baicu C.F., Stroud R.E., Thompson S.B., McClure C.D., Mehurg S.M., and Spinale F.G. (2011). Plasma biomarkers that reflect determinants of matrix composition identify the presence of left ventricular hypertrophy and diastolic heart failure, Circ Heart Fail, 4, 246-256.

Zile M.R., Baicu C.F., and Gaasch W.H. (2004). Diastolic heart failure: abnormalities in active relaxation and passive stiffness of the left ventricle, N Engl J Med, 350, 1953-1959.

Zile M.R., and Brutsaert D.L. (2002). New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis and measurements of diastolic function, Circulation, 105, 1387-1393.