Left ventricular diastolic function and its relationship with the renin-angiotensin-aldosterone system and amino-terminal prohormone B-type natriuretic peptide: The African-PREDICT study

B Viana

orcid.org/ 0000-0002-7477-9227

Dissertation submitted in fulfilment of the requirements for the degree Master of Health Sciences in Cardiovascular Physiology at the

North-West University

Supervisor:	Prof R Kruger
Co-supervisor:	Prof JM van Rooyen
Co-supervisor:	Dr LF Gafane-Matemane

Graduation: May 2018

Student number: 23414707

PREFACE

This dissertation forms part of the Master of Health Sciences in the Cardiovascular Physiology program and consists of four chapters. Chapter 1 contains the background and literature overview about left ventricular diastolic function, the renin-angiotensin-aldosterone system and amino-terminal prohormone B-type natriuretic peptide. A comprehensive methodology of this study is provided in Chapter 2. The research article in Chapter 3 is written according to the instructions of the *American Journal of Cardiology*. Chapter 4 summarizes the main findings of this study and includes the final conclusions with recommendations. All references at the end of each chapter are indicated according to the style of the designated journal.

ACKNOWLEDGEMENTS

I would like to extend my appreciation and express thanks to the following people who have contributed to making this study possible:

- My supervisor, **Prof. R Kruger**, for freely sharing your knowledge and wisdom. Thank you for your endless support and patience, valuable scientific input and professional guidance concerning this dissertation. Thank you for inspiring me as a young researcher. I am beyond grateful for your kindness, encouragement and for believing in me.
- My co-supervisors, **Prof. JM van Rooyen** and **Dr. LF Gafane-Matemane**, for your intellectual, technical and accommodative input. Thank you for your support and kindness, and for sharing your unique insight regarding the field of physiology.
- To all the **participants** and **researchers** that took part in the African-PREDICT study, thank you for your time and willingness to take part in this study.
- My wonderful **parents**, thank you for your support, guidance and prayers. Thank you for your unconditional love and the sacrifices you have made to help me achieve my goals.
- **Ockert Vermeulen**, you have been a great pillar to me. Thank you for believing in me, your unconditional love, encouragement and tremendous support. No words can express the love and appreciation I have for you.
- **Grammatically**, for language editing of the dissertation.
- **Smart Servier Medical Art**, thank you for making your illustrations available for use in my dissertation.

CONTRIBUTIONS OF AUTHORS

Miss B Viana: Responsible for data collection and capturing of data into final database, literature overview, design and planning of the dissertation, statistical analyses, interpretation of results and writing all sections of this dissertation.

Prof. R Kruger: Responsible for the unique design of the research article, intellectual and technical input, data collection, evaluation of statistical analyses. Supervised the writing of this dissertation and initial design and planning of this dissertation.

Prof. JM van Rooyen: Responsible for overseeing the writing of the dissertation and research article and providing scientific and professional input.

Dr LF Gafane-Matemane: Responsible for overseeing the writing of the dissertation and research article and providing scientific and professional input.

The following statement from the co-authors confirms their individual involvement in this study and gives their permission that the relevant research article may form part of this dissertation:

Hereby, I declare that I approved the abovementioned dissertation and that my role in this study (as stated above) is representative of my contribution towards the manuscript and supervised postgraduate study. I also give my consent that this manuscript may be published as part of the *Master of Health Science* dissertation of Bridget Viana.

Prof. R Kruger **Prof. JM van Rooyen**

Dr LF Gafane-Matemane **Miss Bridget Viana**

SUMMARY

Motivation

The association of left ventricular (LV) diastolic function markers with the renin-angiotensin-aldosterone system (RAAS) and amino-terminal prohormone B-type natriuretic peptide (Nt-proBNP) in older and diseased populations are known. Our study was motivated by the lack of evidence in young, healthy adults regarding the associations of LV diastolic function markers with the RAAS and Nt-proBNP to establish early manifestations of cardiovascular compromise.

Aim

To compare the cardiovascular characteristics along with the RAAS and Nt-proBNP levels, as well as to explore the associations of LV diastolic function with the RAAS and Nt-proBNP in young apparently healthy black and white South Africans.

Methodology

Cross-sectional data of the first 400 participants (age between 20–30 years) from the African prospective study on the early detection and identification of cardiovascular disease and hypertension (African-PREDICT) was used in this sub-study. Participants with missing data (n=55), as well as individuals with identified left or right bundle branch block (n=9) were excluded. This study obtained approval from the Health Research Ethics Committee of the North-West University (NWU-00032-17-A1) and complied with the Declaration of Helsinki (2008). Ambulatory blood pressure was measured along with a 12-lead electrocardiogram. A standard transthoracic echocardiography procedure was followed, to acquire variables of LV diastolic function including: E/A (peak early filling E-wave/late diastolic filling A-wave) ratio, E/é (mitral peak velocity of early filling/early diastolic mitral annular velocity) ratio, left atrium to aortic root ratio (LA/Ao) and LV end-diastolic volume. Anthropometric measurements included body height and weight, while body mass index and body surface area were additionally calculated. Among other biomarkers, renin, prorenin, aldosterone and Nt-proBNP were analyzed.

Results

Age and body composition were lower in the black group (all $p<0.005$) compared to the white group. Blood pressures were comparable between the groups. The LV end diastolic volume was lower in the black ($p<0.0001$) compared to the white group. The E/A and E/e' ratios were higher in the black (both $p<0.05$) compared to the white group, whereas heart rate and the LA/Ao ratio were similar in both groups. Total renin was higher in the black group ($p=0.010$), whereas aldosterone and Nt-proBNP were lower in the black group (all $p<0.005$) compared to the white group. In multiple regression analysis with covariates age, sex, body surface area (except for LV end-diastolic volume), systolic blood pressure, heart rate, gamma-glutamyl-transferase, C-reactive protein, cotinine, total cholesterol-to-high density lipoprotein cholesterol ratio,

estimated glomerular filtration rate and activity energy expenditure the following associations were found. The E/A ratio associated positively with prorenin in the black group (adj. R^2=0.201; β=0.15; p=0.049) and total renin in the white group (adj. R^2=0.131; β=0.16; p=0.042), whereas the LA/Ao ratio associated positively with prorenin (adj. R^2=0.050; β=0.18; p=0.032) in the white group only. No associations were evident between markers of LV diastolic function and Nt-proBNP in either group.

General conclusion

In conclusion, our study indicated that diastolic function markers associated adversely with components of the RAAS, in both groups. Our findings may indicate that higher E/A and LA/Ao ratios may be attributed to potential changes in the RAAS. This may suggest that both groups are prone to premature RAAS modifications, probably due to lifestyle risk factors, which may lead to future diastolic dysfunction.

Keywords: Left ventricular diastolic function, renin-angiotensin-aldosterone system, amino-terminal prohormone B-type natriuretic peptide, ethnicity

TABLE OF CONTENTS

APPENDICES

LIST OF ABBREVIATIONS

ABPM:	Ambulatory Blood Pressure Monitoring
ACE:	Angiotensin-converting enzyme
African-PREDICT:	African prospective study on the early detection and identification of cardiovascular disease and hypertension
Ang II:	Angiotensin II
BHS:	British Hypertension Society
BMI:	Body Mass Index
BNP:	B-type natriuretic peptide
BSA:	Body Surface Area
DBP:	Diastolic blood pressure
E/A ratio:	Peak early filling E-wave/late diastolic filling A-wave
ECG:	Electrocardiography
E/e' ratio:	Mitral peak velocity of early filling/early diastolic mitral annular velocity
eGFR:	Estimated glomerular filtration rate
GGT:	Gamma-glutamyl-transferase
HART:	Hypertension in Africa Research Team
HDL cholesterol:	High density lipoprotein cholesterol
HPCSA:	Health Professions Council of South Africa
LA/Ao ratio:	Left atrial diameter to aortic root ratio
LV:	Left ventricular
NHANES:	National Health and Nutritional Survey
Nt-proBNP:	Amino-terminal prohormone B-type natriuretic peptide
RAAS:	Renin-Angiotensin-Aldosterone System
RPR:	Rate in Pressure Rise
SBP:	Systolic blood pressure

LIST OF TABLES

Chapter 2

Chapter 3

LIST OF FIGURES

Chapter 1:

CHAPTER 1

Background, literature overview, aim, objectives and hypotheses

1.1 Introduction

High blood pressure is one of the main cardiovascular disease risk factors,[1,2] and a widespread problem in sub-Saharan African populations.[3] According to The National Health and Nutritional Survey (NHANES), black populations (32%) have an increased predisposition to develop hypertension, compared to the white population (23%).[3,4] Demographics, lifestyle and genetic factors are frequently associated with hypertension.[5,6] Furthermore, overweight and obesity, diabetes mellitus, increased sympathetic nervous system activity,[7,8] over-stimulation of the renin-angiotensin-aldosterone system (RAAS), and abnormal sodium balance, contribute to the development of hypertension.[9,10] Hypertension is also a significant risk factor identifying left ventricular (LV) diastolic dysfunction.[11] The Heart of Soweto study found that LV diastolic dysfunction occurs in approximately 25% of South Africans.[12] Other studies explored ethnic differences in LV structure and found that the black African population has a higher predisposition to develop increased relative wall thickness[13] and promotes LV hypertrophy, which is also linked to LV diastolic dysfunction (Figure 1).[14]

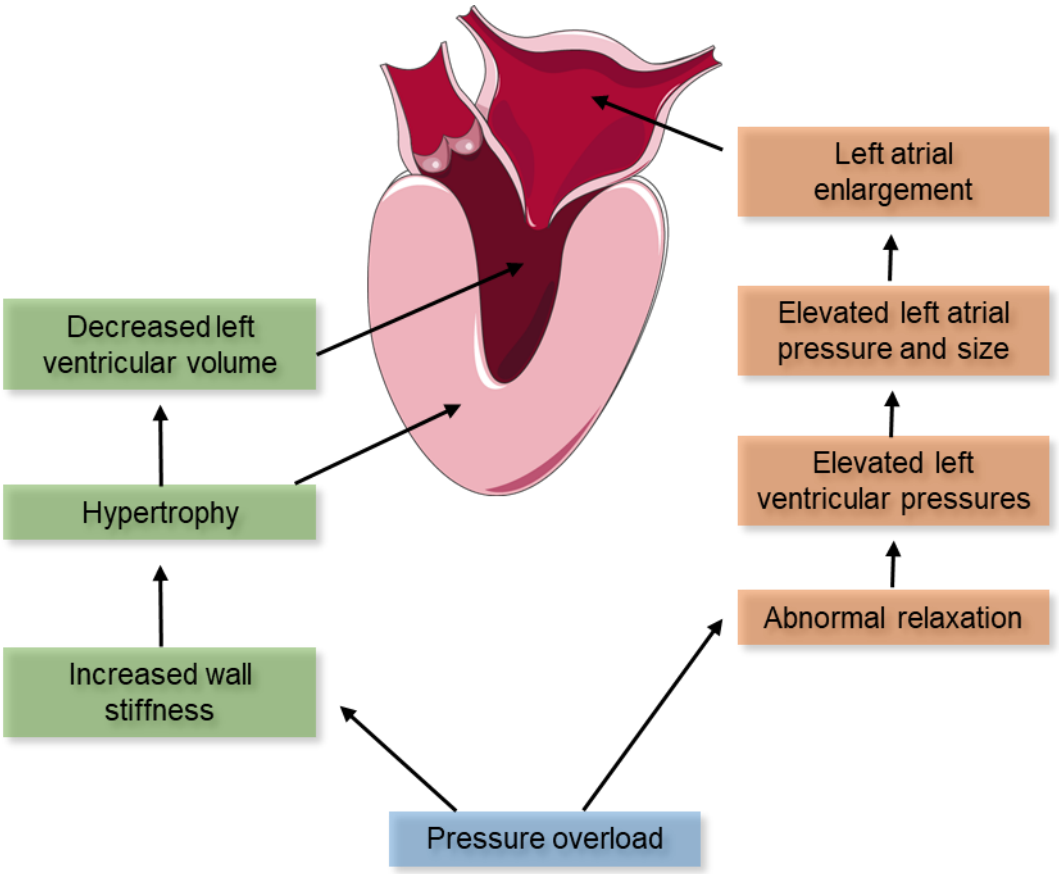


Figure 1: Illustration of left ventricular diastolic dysfunction.

LV diastolic dysfunction is characterized by thickening of the ventricular muscle and a decrease in LV volume since the ventricle fills with less blood then normal. Furthermore, the ventricle is not able to relax normally.

1.1.1 Left ventricular diastolic function

Diastole is the time period during which the myocardium loses its ability to contract but returns to an unstressed length and force,[15] thus the normal relaxation state where the cardiac muscle is perfused.[16] Myocardial relaxation is nearly complete at minimal LV pressure in normal hearts with a normal load.[17] However, elevated preload will cause a delay in myocardial relaxation, thereby contributing to elevated LV filling pressures.[17,18] Diastole can be divided into four phases, namely; isovolumetric relaxation, early rapid ventricular filling after the opening of the mitral valve, diastasis (a period of low blood flow during mid-diastole), and lastly, late rapid filling during atrial contraction.[16,19] Isovolumetric relaxation is caused by the closing of the aortic valve while the mitral valve is opening.[19] Impaired isovolumetric relaxation and decreased LV compliance, leads to LV diastolic dysfunction.[16,20] Doppler echocardiography is the primary clinical method for the evaluation of different variables of LV diastolic function, including LV filling pressure, relaxation and stiffness.[21-23]

The primary measurements of mitral inflow include the peak early filling (E-wave) and late diastolic filling (A-wave) velocities, E/A ratio, deceleration time of early filling velocity and isovolumetric relaxation time, and is generally used to assess LV filling.[17] However, markers of LV diastolic function include the E/A ratio, E/e' ratio (peak E velocity to e-prime), and mitral deceleration time.[17] The left atrial (LA)-LV pressure gradient during early diastole is reflected by the mitral E-wave velocity and thus affected by preload and alterations in LV relaxation.[22] However, the LA-LV pressure gradient during late diastole is reflected by the mitral A-wave, and thus affected by LV compliance and contractile function of the left atrium.[17] Mitral inflow patterns, including normal LV relaxation and filling, impaired LV relaxation, pseudonormal LV filling and restrictive filling, are identified by the mitral E/A ratio and mitral deceleration time.[17] The E/A ratio is the most common marker used in clinical practice to assess LV diastolic function,[24] whereas the E/é ratio is useful to estimate LV filling pressure.[17,25]

1.1.2 Left ventricular diastolic dysfunction

One of the consequences of prolonged and undetected hypertension is LV diastolic dysfunction which generally leads to heart failure with preserved LV ejection fraction.[26,27] The presence of abnormal filling and relaxation patterns of the left ventricle is described as LV diastolic dysfunction,[28,29] and is associated with cardiovascular morbidity and mortality.[30-32] Sustained overload on the myocardial wall contributes to the development of LV hypertrophy, which may lead to heart failure and symptomatic hypertensive heart disease,[26] as well as a broad spectrum of other LV functional abnormalities, such as severe left atrial dilation and/or significantly decreased systolic function.[33]

Myocyte hypertrophy and myocardial fibrosis is a compensatory and adaptive mechanism in response to LV pressure overload, caused by hypertension.[34,35] LV wall stress increases, as a result of hypertension, and initiates an increase in the contractile units of cardiac myocytes leading to myocyte hypertrophy and thickening of the LV wall.[34,36,37] However, excessive myocyte hypertrophy and myocardial fibrosis results from a persistent increase in cardiac workload, and is responsible for increased myocardial stiffness and impaired LV filling and relaxation in hypertensives.[34,37] Several other factors also contribute to the development of LV diastolic dysfunction in hypertensive individuals. These factors include older age, black/white ethnicity, increased dietary sodium, overweight and obesity, type 2 diabetes mellitus and chronic kidney disease.[11]

Diastolic dysfunction is one of the earliest detectable manifestations,[32] in individuals with hypertension, type 2 diabetes mellitus, and the elderly.[15,29,38] The most underlying and frequent cause of diastolic dysfunction are changes in LV myocardial structure or impaired elastic properties involved in diastolic filling.[27,28] Diastolic dysfunction is characterized by increased chamber stiffness, and a reduced capacity to fill at low diastolic pressures (Figure 1).[39] The basic underlying mechanism of diastolic dysfunction may be intrinsic to the cardiomyocytes due to abnormal calcium homeostasis, or it may be as a result of abnormalities in the extracellular matrix due to alterations in collagen.[36] The increase in myocardial mass and abnormalities of the extracellular matrix may also contribute to increased stiffness in the ventricle.[36] Consequently, there is a reduction in LV compliance, the dynamics of LV filling are altered and end-diastolic pressure is increased.[15,40] There are three distinctive patterns of diastolic dysfunction depending on the severity (Figure 2).[27,28,41] The first pattern is the delayed relaxation pattern, which represents alterations of the early LV active relaxation properties, whereas the pseudo-normal (second) and restrictive (third) patterns represent more severe diastolic dysfunction along with elevated LV filling pressures and LV stiffness.[28,41]

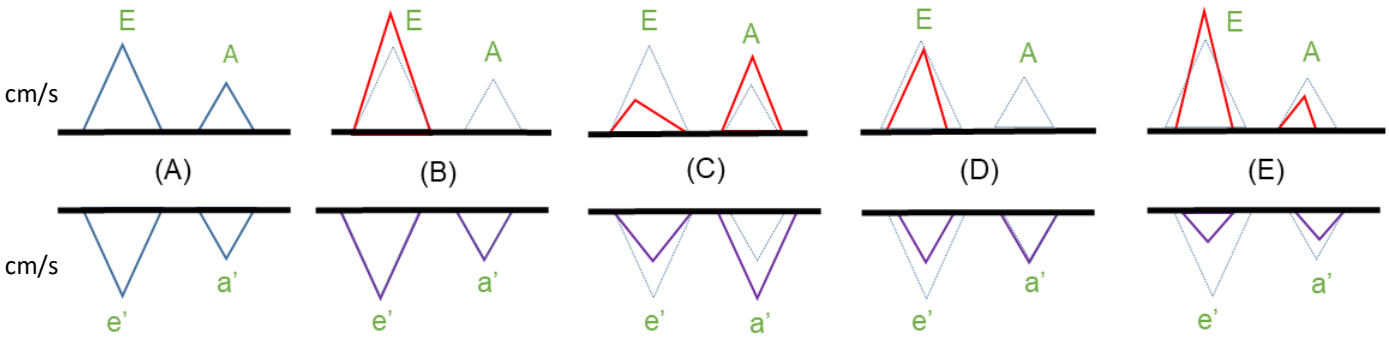


Figure 2: Different patterns of diastolic dysfunction depending on the degree of severity.

(A) Normal diastolic function, (B) Super normal filling, seen young and physical active individuals, (C) Impaired relaxation (Grade I diastolic dysfunction), (D) Pseudonormal filling pattern (Grade II diastolic function), (E) Reversible restrictive filling pattern (Grade III diastolic function).

A delay in myocardial relaxation may be due to age-related changes in diastolic function, which contributes to the development of diastolic heart failure in older individuals.[17] Therefore, age should always be taken into consideration, especially with regards to the E/A and E/é ratios.[17] With an increase in age, the mitral E-wave velocity and E/A ratio decreases, whereas the deceleration time and A velocity, late diastolic velocity (é) and the E/é ratio increases.[17,42] However, a higher E/A ratio, along with a short IVRT and short deceleration time (especially if it persists after preload reduction) relates to advanced diastolic dysfunction, as it indicates restrictive filling patterns (Figure 2).[17,43,44] However, in young, healthy adults a higher E/A ratio is indicative of normal diastolic filling. Young, healthy adults tend to have a decrease in LV minimal diastolic pressure due to rapid LV relaxation, thus causing a higher E/A ratio (Figure 2 (B)).[40] Furthermore, healthy adults with normal myocardial relaxation tend to have a proportional increase in the mitral E-wave and é velocities, whereas the E/é ratio will remain unchanged or even slightly reduced.[45]

Individuals with LV diastolic dysfunction are sensitive to certain precipitants, such as uncontrolled hypertension, atrial fibrillation, renal insufficiency, and high salt-intake, since there are significant alterations in LV diastolic pressure and a reduction in LV volume.[36,46] These factors can also contribute to fluid retention and promote heart failure. Individuals with diastolic dysfunction and diastolic heart failure exhibit exercise intolerance due to elevated LV diastolic and pulmonary pressures, and LV hypertrophy.[36] There is a reduction in lung compliance as a result of elevated LV diastolic and pulmonary pressures, which increases the rate of breathing and induces dyspnea.[36] Due to LV hypertrophy, these individuals exhibit increased relative wall thickness and reduces end-diastolic volume, and cannot produce a normal stroke volume because the chamber volume is decreased.[36,47] These mechanisms lead to a limited preload and cardiac output during exercise, which leads to lactate accumulation, and functional and structural abnormalities of skeletal muscles.[36] However, different factors can contribute to the progression and development of LV diastolic dysfunction. Among these are the RAAS and elevated natriuretic peptides, such as amino-terminal prohormone B-type natriuretic peptide (Nt-proBNP).[27]

1.1.3 The Renin-Angiotensin-Aldosterone System

The RAAS is typically viewed as an enzymatic cascade, which through the biosynthesis and release of prorenin and renin, finally leads to the production of angiotensin II (Ang II).[48,49] Under normal physiological conditions, the RAAS functions through anti-natriuretic and vasopressive effects to ensure the homeostatic balance of blood pressure regulation, water and salt balance, and tissue perfusion.[48-50] The biosynthesis and regulated secretion of renin, synthesized from the proenzyme or renin precursor known as prorenin, activates the RAAS.[48,49] Unprocessed prorenin is released from the kidney via a constitutive pathway, whereas active renin is released via an exocytic process due to a stimulus-secretion coupling into the renal and systematic circulation.[49]

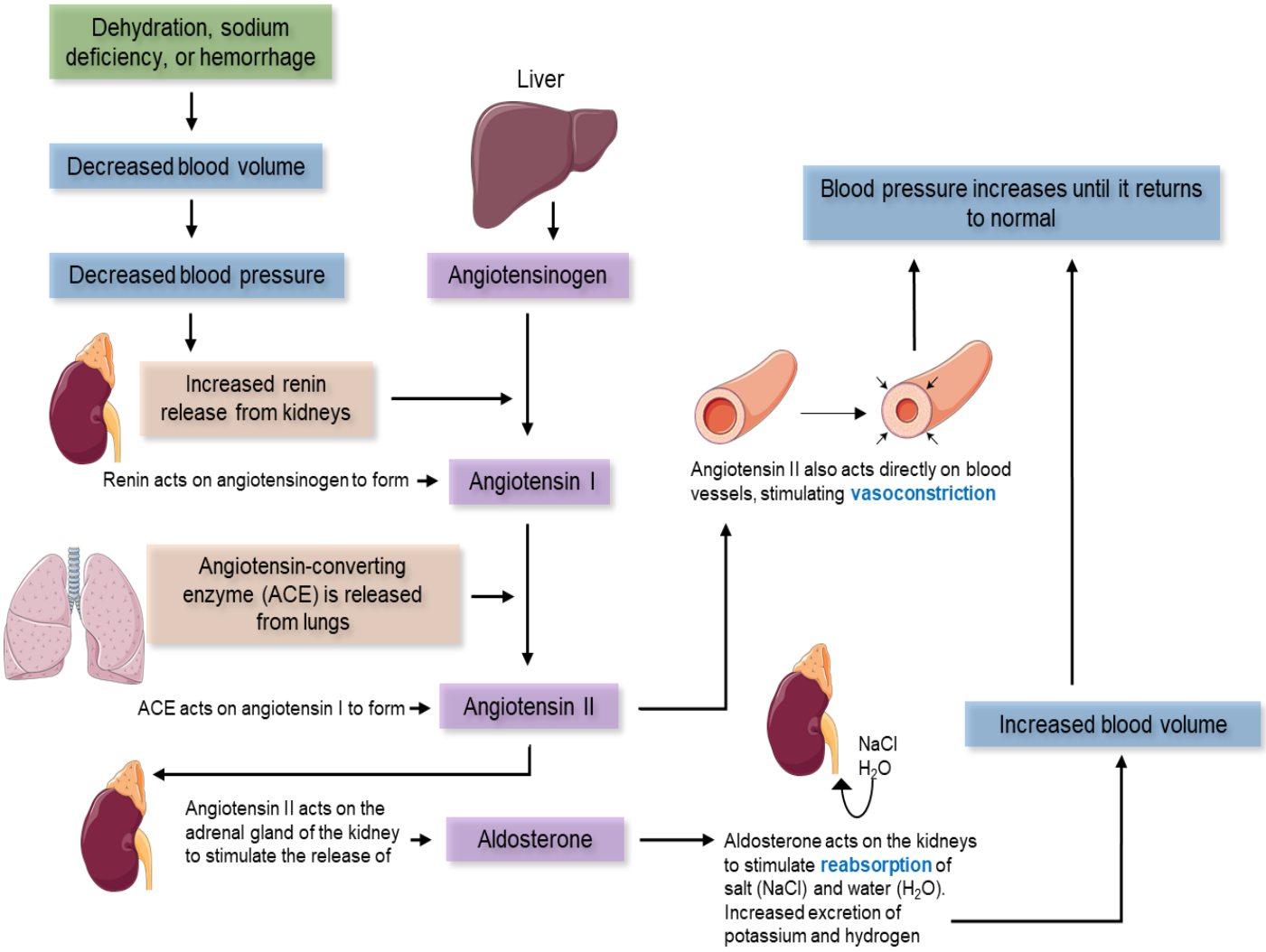


Figure 3: The Renin-Angiotensin-Aldosterone System.

Adapted from http://antranik.org/the-renin-angiotensin-aldosterone-reflex/
and https://www.britannica.com/science/renin-angiotensin-system (Date of access 15 November 2017).

The secretion of active renin is stimulated by a reduction in renal perfusion pressure and afferent arteriolar pressure, a reduction in tubular fluid sodium chloride concentration, or elevated sympathetic activity in the kidney (Figure 3).[48,49] Renin catalyzes cleavage of angiotensinogen, released from the liver, to form inactive angiotensin I (Ang I).[51] Ang I is converted by angiotensin-converting enzyme to the biologically active Ang II,[51] which can mediate various primary actions of the RAAS in the heart, brain, kidney and blood vessels.[48] The biological functions of Ang II include the homeostasis of the cardiovascular system, vasoconstriction, blood pressure regulation, and salt and water balance.[48,49,52] The binding of Ang II to the Ang II type I receptors in the smooth muscle cells consequently leads to an increase in blood pressure by producing acute vasoconstriction and increased peripheral resistance,[51,53] thereby stimulating the production of aldosterone (Figure 3).[49,54] Aldosterone plays a significant role in regulating extracellular fluid volume, through the regulation of the sodium and potassium balance.[49,51]

Aldosterone promotes reabsorption of sodium and water, thereby enhancing potassium excretion.[49,55] Therefore, Ang II extracellular fluid volume and potassium levels are regarded as the main regulators of aldosterone.[49] Acute stimulation of Ang II and aldosterone on their target organs causes an increase in blood pressure and extracellular fluid volume, thus restoring renal perfusion and blood pressure until normal and consequently inhibiting renin secretion (Figure 3).[48,49,51]

Furthermore, the RAAS has a pathophysiological contribution in the development of alterations in myocardial structure, functional abnormalities and impaired LV diastolic filling.[56-58] The RAAS can modulate cellular growth, proliferation and differentiation, which promotes cardiomyocyte hypertrophy, cardiac fibrosis and remodeling, mainly by acting through autocrine and paracrine signals.[27,56-58] Ang II is regarded as the primary effector molecule of the RAAS system, and is an essential hormone that affects the function of the heart, kidney, vasculature and brain.[59] Chronic stimulation of Ang II will, however, promote hyperplasia and hypertrophy of vascular smooth muscle cells,[59-61] and also plays a vital role in cardiac hypertrophy and remodeling.[59] Accumulation of the extracellular matrix is also promoted by chronic RAAS activation, thereby contributing to the increase in myocardial stiffness and diastolic dysfunction.[62,63] Moreover, chronic RAAS activation along with inflammatory processes, may contribute to the development of myocardial fibrosis and stiffness by impairing endothelial function in the vasculature of the heart, consequently leading to diastolic dysfunction.[27,64] Chronic RAAS activation is furthermore associated with the progression from diastolic dysfunction to heart failure.[65]

The pathogenesis of various hypertensive disorders, including essential hypertension, may be caused by the dysregulation of RAAS functioning.[49,53] Large variation in plasma renin levels was observed in essential hypertensive patients, in which approximately 15% of these individuals showed mild to moderately elevated renin activity along with increased sympathetic activity and a slight depletion in extracellular fluid volume.[49,66] In addition, the same study showed that younger American males have a higher prevalence of high-renin essential hypertension, whereas older individuals with hypertension, women,[49] the African population[67,68] and individuals with type 2 diabetes are more susceptible to low-renin hypertension.[49]

1.1.4 Amino-terminal prohormone B-type natriuretic peptide

Irregular diastolic filling pressure is the main functional abnormality in diastolic heart failure and may result in the release of cardiac neurohormones, which include natriuretic peptides.[69] Natriuretic peptides are considered important biomarkers in cardiovascular disease,[70,71] and can accurately detect diastolic dysfunction and heart failure.[69,72] B-type natriuretic peptide (BNP) is a peptide hormone, synthesized from ventricular myocytes, as an inactive prohormone, that is split into active BNP and the inactive split-product Nt-proBNP.[73,74] Both BNP and Nt-proBNP are secreted from cardiac myocytes as a result of myocardial wall stretch, commonly seen in cardiac failure, caused by elevated cardiac pressure and volume overload.[70,75-77]

The secretion of BNP induces vasodilation and diuresis as a protective mechanism against volume overload and hypertension.[75,78,79] Nt-proBNP, a cleavage product of BNP, is often used as a biomarker of cardiac volume and pressure load,[69,80] and can detect all degrees of diastolic dysfunction in symptomatic patients.[69]

The biological effects of Nt-proBNP include vasodilation, natriuresis, diuresis, inhibition of renin and aldosterone secretion, as well as inhibition of cardiac and vascular myocyte growth.[73,78,81] The upregulation of Nt-proBNP acts in a counteractive manner to reduce cardiac pressure and volume overload caused by the activation of the RAAS.[82,83] An abnormal or increased activity of circulating RAAS contributes to the development of volume overload, a decrease in cardiac output and vasoconstriction, consequently leading to elevated LV diastolic filling pressures and a condition of intravenous congestion.[62,84] LV diastolic dysfunction causes the natriuretic peptide system to maximally activate to protect the body against volume overload and hypertension, by opposing the RAAS.[75] However, while counteracting the effects of the RAAS, these natriuretic peptides also have an impact on the sodium balance,[78,85,86] and may promote volume overload.[78,80] These factors subsequently contribute to elevated blood pressure.[78] Some studies found an association between elevated plasma natriuretic peptide concentrations and a decrease in LV diastolic and systolic function.[87,88]

Hemodynamic stress load can cause severe damage to cardiac myocytes, and promote the secretion of natriuretic peptides.[73] LV diastolic dysfunction is believed to be the pathophysiological process underlying elevated BNP and Nt-proBNP levels,[73] especially in individuals with cardiovascular risk factors such as hypertension or type 2 diabetes mellitus or in the elderly.[89,90] Individuals with impaired LV relaxation, pseudo-normal or restrictive patterns, may have progressively higher Nt-proBNP levels, considering that the levels increase according to the severity of LV diastolic dysfunction. [69,89,91,92] The concentration of Nt-proBNP may increase with increasing age in healthy individuals and can be higher in men compared to women.[93,94]

In a South African study, higher Nt-proBNP levels were reported in black individuals compared to their white counterparts.[95] Other studies reported that black South Africans have lower plasma renin and aldosterone levels,[96] which may be a contributing factor to hypertension.[3] All these South African studies included older individuals in whom risk factors, such as high blood pressure, were already pronounced, which contributed to their risk for developing hypertension or related cardiovascular diseases.

1.1.5 The impact of the RAAS and Nt-proBNP on LV diastolic dysfunction

Both the RAAS and natriuretic peptides relate to cardiovascular disease and may influence renal function, endocrine function, and cardiovascular cell growth.[97] Many pathologies can be attributed to the over activation of the RAAS, such as hypertension, end-organ damage related to hypertension or type 2 diabetes mellitus, and atherosclerosis.[10] Hypertension can cause an increase in Ang II and aldosterone levels, and contributes to increased vascular permeability, activation of different myocyte pathways, an increased burden of oxidative stress and cytokine secretion, consequently leading to tissue fibrosis, myocyte hypertrophy and diastolic dysfunction.[34,41,98] The secretion of cytokines, due to increased oxidative stress, promotes and maintains inflammation and stimulates the production of collagen (type I),[99-102] which progressively accumulates, and thereby promotes myocardial stiffness, hypertrophy and diastolic dysfunction.[99-103] Thus, the RAAS is involved in the development of LV hypertrophy and myocardial fibrosis, which is also linked to the development of LV diastolic dysfunction.[34,90,104,105] Additionally, continued RAAS activation also contributes to the progression from diastolic dysfunction towards the development of cardiac heart failure.[65] Impaired LV filling, as seen in LV diastolic dysfunction, activates the RAAS even further, which contributes to LV remodeling and fluid retention, and consequently deteriorates diastolic function.[27] Furthermore, these pathological changes caused by the abnormal RAAS activity contributes to the development of volume overload and vasoconstriction, which leads to elevated LV diastolic filling pressure,[27,62,84] and stimulates the production and secretion of Nt-proBNP.[89,90]

Nt-proBNP demonstrates a similar pattern to BNP, therefore Nt-proBNP levels can also increase according to the severity of LV diastolic dysfunction.[69,106] The Nt-proBNP levels may be progressively higher in individuals who showed mitral valve flow velocity patterns of impaired LV relaxation, pseudo-normalization or restriction.[92,106,107] Elevated levels of Nt-proBNP increases the risk of developing congestive heart failure as well as LV diastolic dysfunction, and strongly predicts cardiovascular events in individuals with LV hypertrophy.[69,90,104,105] Hypertensive individuals, especially with LV hypertrophy or LV diastolic dysfunction, have higher Nt-proBNP levels compared to normotensive individuals.[108] The underlying pathophysiological process for elevated Nt-proBNP levels is LV diastolic dysfunction, as a result of increased wall stress and myocardial ischemia.[109] However, the association of LV diastolic function markers along with the RAAS and Nt-proBNP in young, healthy individuals is still unclear. Our study is motivated by the lack of evidence regarding the associations of LV diastolic function markers with the RAAS and Nt-proBNP to establish early manifestations of cardiovascular compromise.

1.2 Aim

The aim of this study is to compare the cardiovascular characteristics along with the RAAS and Nt-proBNP levels, as well as to explore the associations of LV diastolic function with RAAS and Nt-proBNP in a young black and white South African cohort (aged between 20-30 years old).

1.3 Objectives

In a study population of young black and white men and women, our objectives are:

i. To phenotype the study sample according to their LV diastolic function markers (E/A ratio, E/é ratio, LA/Ao ratio and end-diastolic volume), RAAS (prorenin, renin, and aldosterone), and Nt-proBNP levels

ii. To determine the associations of LV diastolic function markers with RAAS and Nt-proBNP.

1.4 Hypotheses

In a study population of young black and white men and women, our hypotheses are:

i. (a) Black individuals have higher E/A, E/e' and LA/Ao ratios, but a lower LV end-diastolic volume compared to their white counterparts.

(b) Black individuals have lower renin and aldosterone levels, but higher prorenin levels compared to their white counterparts.

(c) Black individuals have higher NT-proBNP levels compared to their white counterparts.

ii. (a) The E/A ratio will associate negatively with renin, prorenin, and aldosterone.

(b) LV end-diastolic volume will positively associate with Nt-proBNP which is a reliable marker of cardiac overload, whereas the E/A and LA/Ao ratios show no association with Nt-proBNP since these ratios reflects LV filling pressure and left atrium dilation.

1.5 References

1. Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense HW, Joffres M, Kastarinen M, Poulter N, Primatesta P, Rodríguez-Artalejo F. Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. *The Journal of the American Medical Association.* 2003;289(18):2363-2369.

2. Worls Health Organization. The world health report 2002: reducing risks, promoting healthy life. World Health Organization, Switzerland. 2002.

3. Opie LH, Seedat YK. Hypertension in sub-Saharan African populations. *Circulation.* 2005;112(23):3562-3568.

4. Burt VL, Cutler JA, Higgins M, Horgan MJ, Labarthe D, Whelton P, Brown C, Roccella EJ. Trends in the prevalence, awareness, treatment, and control of hypertension in the adult US population data from the health examination surveys, 1960 to 1991. *Hypertension.* 1995;26(1):60-69.

5. Steyn K, Gaziano TA, Bradshaw D, Laubscher R, Fourie J. Hypertension in South African adults: results from the Demographic and Health Survey, 1998. *Journal of Hypertension.* 2001;19(10):1717-1725.

6. Yusuf S, Reddy S, Ôunpuu S, Anand S. Global burden of cardiovascular diseases: Part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies. *Circulation.* 2001;104(23):2855-2864.

7. Murphy JK, Alpert BS, Moes DM, Somes GW. Race and cardiovascular reactivity. A neglected relationship. *Hypertension.* 1986;8(11):1075-1083.

8. Wang W, Lee ET, Fabsitz RR, Devereux R, Best L, Welty TK, Howard BV. A longitudinal study of hypertension risk factors and their relation to cardiovascular disease. *Hypertension.* 2006;47(3):403-409.

9. Redon J, Cifkova R, Laurent S, Nilsson P, Narkiewicz K, Erdine S, Mancia G. Mechanisms of hypertension in the cardiometabolic syndrome. *Journal of Hypertension.* 2009;27(3):441-451.

10. Steckelings UM, Rompe F, Kaschina E, Unger T. The evolving story of the RAAS in hypertension, diabetes and CV disease–moving from macrovascular to microvascular targets. *Fundamental & Clinical Pharmacology.* 2009;23(6):693-703.

11. Nadruz W, Shah AM, Solomon SD. Diastolic dysfunction and hypertension. *Medical Clinics.* 2017;101(1):7-17.

12. Stewart S, Libhaber E, Carrington M, Damasceno A, Abbasi H, Hansen C, Wilkinson D, Sliwa K. The clinical consequences and challenges of hypertension in urban-dwelling black Africans: insights from the Heart of Soweto Study. *International Journal of Cardiology.* 2011;146(1):22-27.

13. Hinderliter AL, Light KC, Willis PW. Racial differences in left ventricular structure in healthy young adults. *The American Journal of Cardiology.* 1992;69(14):1196-1199.

14. Sharp A, Tapp R, Francis D, McG TSA, Hughes AD, Stanton AV, Zambanini A, Chaturvedi N, Byrd S, Poulter, NR. Ethnicity and left ventricular diastolic function in hypertension an ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial) substudy. *Journal of the American College of Cardiology.* 2008;52(12):1015.

15. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I diagnosis, prognosis, and measurements of diastolic function. *Circulation.* 2002;105(11):1387-1393.

16. Satpathy C, Mishra TK, Satpathy R, Satpathy HK, Barone E. Diagnosis and management of diastolic dysfunction and heart failure. *American Family Physician.* 2006;73(5):841-846.

17. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA, Evangelista A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Journal of the American Society of Echocardiography.* 2009;22(2):107-133.

18. Leite-Moreira AF, Correia-Pinto J, Gillebert TC. Afterload induced changes in myocardial relaxation: a mechanism for diastolic dysfunction. *Cardiovascular Research.* 1999;43(2):344-353.

19. Ahmed A, Nanda NC, Weaver MT, Allman RM, DeLong JF. Clinical correlates of isolated left ventricular diastolic dysfunction among hospitalized older heart failure patients. *The American Journal of Geriatric Cardiology.* 2003;12(2):82-89.

20. Mandinov L, Eberli FR, Seiler C, Hess OM. Diastolic heart failure. *Cardiovascular Research.* 2000;45(4):813-825.

21. Garcia MJ, Thomas JD, Klein AL. New Doppler echocardiographic applications for the study of diastolic function. *Journal of the American College of Cardiology.* 1998;32(4):865-875.

22. Appleton CP, Hatle LK, Popp RL. Relation of transmitral flow velocity patterns to left ventricular diastolic function: new insights from a combined hemodynamic and Doppler echocardiographic study. *Journal of the American College of Cardiology.* 1988;12(2):426-440.

23. Hoit BD, Walsh RA. 1994. Diastolic function in hypertensive heart disease. Left Ventricular diastolic dysfunction and heart failure*.* Philadelphia: Lea and Febiger. p.354-372.

24. Mottram PM, Marwick TH. Assessment of diastolic function: what the general cardiologist needs to know. *Heart.* 2005;91(5):681-695.

25. Rivas-Gotz C, Manolios M, Thohan V, Nagueh SF. Impact of left ventricular ejection fraction on estimation of left ventricular filling pressures using tissue Doppler and flow propagation velocity. *American Journal of Cardiology.* 2003;91(6):780-784.

26. Bauersachs J, Widder JD. Role of the renin-angiotensin-aldosterone system in diastolic dysfunction and heart failure1. *Kardiovaskuläre Medizin.* 2009;12(3):74-77.

27. Sciarretta S, Paneni F, Palano F, Chin D, Tocci G, Rubattu S, Volpe M. Role of the renin–angiotensin–aldosterone system and inflammatory processes in the development and progression of diastolic dysfunction. *Clinical Science.* 2009;116(6):467-477.

28. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: part II. *Circulation.* 2002;105(12):1503-1508.

29. Gaasch WH, Little WC. Assessment of left ventricular diastolic function and recognition of diastolic heart failure. *Circulation.* 2007;116(6):591-593.

30. Redfield MM, Jacobsen SJ, Burnett Jr JC, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *The Journal of the American Medical Association.* 2003;289(2):194-202.

31. Schillaci G, Pasqualini L, Verdecchia P, et al. Prognostic significance of left ventricular diastolic dysfunction in essential hypertension. *Journal of the American College of Cardiology.* 2002;39(12):2005-2011.

32. Thomas JD, Weyman AE. Echocardiographic Doppler evaluation of left ventricular diastolic function. Physics and physiology. *Circulation.* 1991;84(3):977-990.

33. Eroglu S, Bozbas H, Muderrisoglu H. Diagnostic value of BNP in diastolic heart failure. *Biochemia Medica.* 2008;18(2).

34. Kai H, Kuwahara F, Tokuda K, Imaizumi T. Diastolic dysfunction in hypertensive hearts: roles of perivascular inflammation and reactive myocardial fibrosis. *Hypertension Research.* 2005;28(6):483-490.

35. Brilla CG, Weber KT. Reactive and reparative myocardial fibrosis in arterial hypertension in the rat. *Cardiovascular Research.* 1992;26(7):671-677.

36. Gaasch WH, Zile MR. Left ventricular diastolic dysfunction and diastolic heart failure. *Annual Review of Medicine.* 2004;55:373-394.

37. Weber KT, Brilla CG, Janicki JS. Myocardial fibrosis: functional significance and regulatory factors. *Cardiovascular Research.* 1993;27(3):341-348.

38. Zanchetti A, Cuspidi C, Comarella L, Rosei EA, Ambrosioni E, Chiariello M, Leonetti G, Mancia G, Pessina AC, Salvetti A. Left ventricular diastolic dysfunction in elderly hypertensives: results of the APROS-diadys study. *Journal of Hypertension.* 2007;25(10):2158-2167.

39. Gaasch WH. Diagnosis and treatment of heart failure based on left ventricular systolic or diastolic dysfunction. *The Journal of the American Medical Association.* 1994;271(16):1276-1280.

40. Nishimura RA, Tajik AJ. Evaluation of diastolic filling of left ventricle in health and disease: Doppler echocardiography is the clinician's Rosetta Stone. *Journal of the American College of Cardiology.* 1997;30(1):8-18.

41. Wilson EM, Spinale FG. Myocardial remodelling and matrix metallotxoteinases in heart failure: turmoil within the interstitium. *Annals of Medicine.* 2001;33(9):623-634.

42. Ha JW, Lulic F, Bailey KR, Pellikka PA, Seward JB, Tajik AJ, Oh JK. Effects of treadmill exercise on mitral inflow and annular velocities in healthy adults. *American Journal of Cardiology.* 2003;91(1):114-115.

43. Parthenakis FI, Kanoupakis EM, Kochiadakis GE, Skalidis EI, Mezilis NE, Simantirakis EN, Kanakaraki MK, Vardas PE. Left ventricular diastolic filling pattern predicts cardiopulmonary determinants of functional capacity in patients with congestive heart failure. *American Heart Journal.* 2000;140(2):338-344.

44. Bella JN, Palmieri V, Roman MJ, Liu JE, Welty TK, Lee ET, Fabsitz RR, Howard BV, Devereux RB . Mitral ratio of peak early to late diastolic filling velocity as a predictor of mortality in middle-aged and elderly adults. *Circulation.* 2002;105(16):1928-1933.

45. Ha JW, Oh JK, Pellikka PA, Ommen SR, Stussy VL, Bailey KR, Seward JB, Tajik AJ. Diastolic stress echocardiography: a novel noninvasive diagnostic test for diastolic dysfunction using supine bicycle exercise Doppler echocardiography. *Journal of the American Society of Echocardiography.* 2005;18(1):63-68.

46. Tsuyuki RT, McKelvie RS, Arnold JMO, Avezum Jr A, Barretto ACP, Carvalho ACC, Isaac DL, Kitching AD, Piegas LS, Teo KK. Acute precipitants of congestive heart failure exacerbations. *Archives of Internal Medicine.* 2001;161(19):2337-2342.

47. Aurigemma GP, Gaasch WH, McLaughlin M, McGinn R, Sweeney A, Meyer TE. Reduced left ventricular systolic pump performance and depressed myocardial contractile function in patients> 65 years of age with normal ejection fraction and a high relative wall thickness. *The American Journal of Cardiology.* 1995;76(10):702-705.

48. Volpe M, Savoia C, De Paolis P, Ostrowska B, Tarasi D, Rubattu S. The renin-angiotensin system as a risk factor and therapeutic target for cardiovascular and renal disease. *Journal of the American Society of Nephrology.* 2002;13(Suppl 3):S173-S178.

49. Atlas SA. The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. *Journal of Managed Care Pharmacy.* 2007;13(8 Suppl B):9-20.

50. Quiat D, Olson EN. MicroRNAs in cardiovascular disease: from pathogenesis to prevention and treatment. *The Journal of Clinical Investigation.* 2013;123(1):11.

51. Cagnoni F, Njwe CAN, Zaninelli A, Ricci AR, Daffra D, D'Ospina A, Preti P, Destro M. Blocking the RAAS at different levels: an update on the use of the direct renin inhibitors alone and in combination. *Vascular Health and Risk Management.* 2010;6:549.

52. Laragh JH, Sealey JE. 2011. Renin–angiotensin–aldosterone system and the renal regulation of sodium, potassium, and blood pressure homeostasis. Comprehensive Physiology. John Wiley & Sons, Inc. p.1409–1541.

53. Unger T. The role of the renin-angiotensin system in the development of cardiovascular disease. *The American Journal of Cardiology.* 2002;89(2, Supplement 1):3-9.

54. Weir MR, Dzau VJ. The renin-angiotensin-aldosterone system: a specific target for hypertension management. *American Journal of Hypertension.* 1999;12(S9):205S-213S.

55. Funder JW. New biology of aldosterone, and experimental studies on the selective aldosterone blocker eplerenone. *American Heart Journal.* 2002;144(5):S8-S11.

56. Brilla CG. The cardiac structure-function relationship and the renin-angiotensin-aldosterone system in hypertension and heart failure. *Current Opinion in Cardiology.* 1994;9(4):S12.

57. Sun Y, Weber KT. Cardiac remodeling by fibrous tissue: role of local factors and circulating hormones. *Annals of Medicine.* 1998;30:3-8.

58. Weber KT, Sun Y, Guarda E. Structural remodeling in hypertensive heart disease and the role of hormones. *Hypertension.* 1994;23:869-877.

59. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *American Journal of Physiology-Cell Physiology.* 2007;292(1):C82-C97.

60. Geisterfer AA, Peach MJ, Owens GK. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. *Circulation Research.* 1988;62(4):749-756.

61. Xi X-P, Graf K, Goetze S, Fleck E, Hsueh WA, Law RE. Central role of the MAPK pathway in Ang II–mediated DNA synthesis and migration in rat vascular smooth muscle cells. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 1999;19(1):73-82.

62. Dzau VJ. Tissue renin-angiotensin system in myocardial hypertrophy and failure. *Archives of Internal Medicine.* 1993;153(8):937-942.

63. Brilla CG, Zhou G, Rupp H, Maisch B, Weber KT. Role of angiotensin II and prostaglandin E2 in regulating cardiac fibroblast collagen turnover. *The American Journal of Cardiology.* 1995;76(13):8D-13D.

64. Izumiya Y, Kim S, Izumi Y, Yoshida K, Yoshiyama M, Matsuzawa A, Ichijo H, Iwao H. Apoptosis signal-regulating kinase 1 plays a pivotal role in angiotensin II–induced cardiac hypertrophy and remodeling. *Circulation Research.* 2003;93(9):874-883.

65. Wright JW, Mizutani S, Harding JW. Pathways involved in the transition from hypertension to hypertrophy to heart failure. Treatment strategies. *Heart Failure Reviews.* 2008;13(3):367-375.66. Laragh J. Laragh's lessons in pathophysiology and clinical pearls for treating hypertension. *American Journal of Hypertension.* 2001;14(9):837-854.

67. Sahay M, Sahay RK. Low renin hypertension. *Indian Journal of Endocrinology and Metabolism.* 2012;16(5):728.

68. Hamer M, Malan L, Schutte AE, Huisman HW, Van Rooyen JM, Schutte R, Fourie CMT, Malan,NT, Seedat YK. Plasma renin responses to mental stress and carotid intima-media thickness in black Africans: the SABPA study. *Journal of Human Hypertension.* 2011;25(7):437.

69. Tschöpe C, Kašner M, Westermann D, Gaub R, Poller WC, Schultheiss HP. The role of NT-proBNP in the diagnostics of isolated diastolic dysfunction: correlation with echocardiographic and invasive measurements. *European Heart Journal.* 2005;26(21):2277-2284.

70. de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. *The Lancet.* 2003;362(9380):316-322.

71. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *New England Journal of Medicine.* 1998;339(5):321-328.

72. Lainchbury JG, Campbell E, Frampton CM, Yandle TG, Nicholls MG, Richards AM. Brain natriuretic peptide and n-terminal brain natriuretic peptide in the diagnosis of heart failure in patients with acute shortness of breath. *Journal of the American College of Cardiology.* 2003;42(4):728-735.

73. Kragelund C, Grønning B, Køber L, Hildebrandt P, Steffensen R. N-terminal pro–B-type natriuretic peptide and long-term mortality in stable coronary heart disease. *New England Journal of Medicine.* 2005;352(7):666-675.

74. Schou M, Dalsgaard MK, Clemmesen O, Dawson EA, Yoshiga CC, Nielsen HB, Gustafsson F, Hildebrandt PR, Secher NH. Kidneys extract BNP and NT-proBNP in healthy young men. *Journal of Applied Physiology.* 2005;99(5):1676-1680.

75. Mair J, Friedl W, Thomas S, Puschendorf B. Natriuretic peptides in assessment of left-ventricular dysfunction. *Scandinavian Journal of Clinical and Laboratory Investigation.* 1999;59(Suppl 230):132-142.

76. Downie P, Talwar S, Squire I, Davies J, Barnett D, Ng L. Assessment of the stability of N-terminal pro-brain natriuretic peptide in vitro: implications for assessment of left ventricular dysfunction. *Clinical Science.* 1999;97(3):255-258.

77. Pemberton CJ, Johnson ML, Yandle TG, Espiner EA. Deconvolution analysis of cardiac natriuretic peptides during acute volume overload. *Hypertension.* 2000;36(3):355-359.

78. Bower JK, Lazo M, Matsushita K, Rubin J, Hoogeveen RC, Ballantyne CM, Selvin E. N-terminal pro-brain natriuretic peptide (NT-proBNP) and risk of hypertension in the Atherosclerosis Risk in Communities (ARIC) study. *American Journal of Hypertension.* 2015;28(10):1262-1266.

79. Omland T, Hagve TA. Natriuretic peptides: physiologic and analytic considerations. *Heart Failure Clinics.* 2009;5(4):471-487.

80. Yatabe J, Yatabe MS, Yoneda M, Felder RA, Jose PA, Sanada H. Hypertension-related gene polymorphisms of G-protein-coupled receptor kinase 4 are associated with NT-proBNP concentration in normotensive healthy adults. *International Journal of Hypertension.* 2012;2012.

81. Hall C. Essential biochemistry and physiology of (NT-pro) BNP. *European Journal of Heart Failure.* 2004;6(3):257-260.

82. Hystad M, Geiran O, Attramadal H, Spurkland A, Vege Å, Simonsen S, Hall C. Regional cardiac expression and concentration of natriuretic peptides in patients with severe chronic heart failure. *Acta Physiologica.* 2001;171(4):395-403.

83. Luchner A, Stevens TL, Borgeson DD, Redfield M, Wei CM, Porter JG, Burnett JC. Differential atrial and ventricular expression of myocardial BNP during evolution of heart failure. *American Journal of Physiology-Heart and Circulatory Physiology.* 1998;274(5):H1684-H1689.

84. Chinnaiyan KM, Alexander D, McCullough PA. Role of angiotensin II in the evolution of diastolic heart failure. *The Journal of Clinical Hypertension.* 2005;7(12):740-747.

85. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Wilson PWF, Vasan RS. Impact of obesity on plasma natriuretic peptide levels. *Circulation.* 2004;109(5):594-600.

86. Felder RA, White MJ, Williams SM, Jose PA. Diagnostic tools for hypertension and salt sensitivity testing. *Current Opinion in Nephrology and Hypertension.* 2013;22(1):65-76.

87. McDonagh T, Robb S, Murdoch D, Morton JJ, Ford I, Morrison CE, Tunstall-Pedoe H, McMurray JJV, Dargie HJ. Biochemical detection of left-ventricular systolic dysfunction. *The Lancet.* 1998;351(9095):9-13.

88. Groenning BA, Nilsson JC, Sondergaard L, Kjaer A, Larsson HB, Hildebrandt PR. Evaluation of impaired left ventricular ejection fraction and increased dimensions by multiple neurohumoral plasma concentrations. *European Journal of Heart Failure.* 2001;3(6):699-708.

89. Paulus WJ, Tschöpe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. *European Heart Journal.* 2007;28(20):2539-2550.

90. Ikonomidis I, Nikolaou M, Dimopoulou I, Paraskevaidis I, Lekakis J, Mavrou I, Tzanela M, Kopterides P, Tsangaris I, Armaganidis A. Association of left ventricular diastolic dysfunction with elevated NT-pro-BNP in general intensive care unit patients with preserved ejection fraction: a complementary role of tissue Doppler imaging parameters and NT-pro-BNP levels for adverse outcome. *Shock.* 2010;33(2):141-148.

91. Lubien E, DeMaria A, Krishnaswamy P, Clopton P, Koon J, Kazanegra R, Gardetto N, Wanner E, Maisel AS . Utility of B-natriuretic peptide in detecting diastolic dysfunction. *Circulation.* 2002;105(5):595-601.

92. Mottram PM, Leano R, Marwick TH. Usefulness of B-type natriuretic peptide in hypertensive patients with exertional dyspnea and normal left ventricular ejection fraction and correlation with new echocardiographic indexes of systolic and diastolic function. *The American Journal of Cardiology.* 2003;92(12):1434-1438.

93. Paulus W, Brutsaert D, Gillebert T, Rademakers FE, Stanislas U, Leite-Moreira AF, Hess OM, Jiang ZH, Kaufmann P, Mandinov L. How to diagnose diastolic heart failure. *European Heart Journal.* 1998;19(7):990-1003.

94. McDonagh T, Holmer S, Raymond I, Luchner A, Hildebrant P, Dargie H. NT-proBNP and the diagnosis of heart failure: a pooled analysis of three European epidemiological studies. *European Journal of Heart Failure.* 2004;6(3):269-273.

95. Kruger R, Schutte R, Huisman HW, Hindersson P, Olsen MH, Schutte AE. N-terminal prohormone B-type natriuretic peptide and cardiovascular function in Africans and Caucasians: the SAfrEIC study. *Heart, Lung and Circulation.* 2012;21(2):88-95.

96. Rayner BL, Myers JE, Opie LH, Trinder YA, Davidson JS. Screening for primary aldosteronism-normal ranges for aldosterone and renin in three South African population groups. *South African Medical Journal.* 2001;91(7):594-599.

97. Richards AM. The renin-angiotensin-aldosterone system and the cardiac natriuretic peptides. *Heart.* 1996;76(3 Suppl 3):36.

98. Brown NJ. Aldosterone and vascular inflammation. *Hypertension.* 2008;51(2):161-167.

99. Lim JY, Park SJ, Hwang HY, Park EJ, Nam JH, Kim J, Park SI. TGF-β1 induces cardiac hypertrophic responses via PKC-dependent ATF-2 activation. *Journal of Molecular and Cellular Cardiology.* 2005;39(4):627-636.

100. Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman M, Mann DL. Tumor necrosis factor-α provokes a hypertrophic growth response in adult cardiac myocytes. *Circulation.* 1997;95(5):1247-1252.

101. Hiraoka E, Kawashima S, Takahashi T, Rikitake Y, Kitamura T, Ogawa W, Yokoyama M. TNF-α induces protein synthesis through PI3-kinase-Akt/PKB pathway in cardiac myocytes. *American Journal of Physiology-Heart and Circulatory Physiology.* 2001;280(4):H1861-H1868.

102. Li Y, Feng Y, Kadokami T, McTiernan CF, Draviam R, Watkins SC, Feldman AM. Myocardial extracellular matrix remodeling in transgenic mice overexpressing tumor necrosis factor α can be modulated by anti-tumor necrosis factor α therapy. *Proceedings of the National Academy of Sciences.* 2000;97(23):12746-12751.

103. Nakagami H, Takemoto M, Liao JK. NADPH oxidase-derived superoxide anion mediates angiotensin II-induced cardiac hypertrophy. *Journal of Molecular and Cellular Cardiology.* 2003;35(7):851-859.

104. Kruger R, Schutte R, Huisman HW, Hindersson P, Olsen MH, Eugen-Olsen J, Schutte AE. NT-proBNP, C-reactive protein and soluble uPAR in a bi-ethnic male population: the SAfrEIC study. *PLoS One.* 2013;8(3):e58506.

105. Morrow DA, Braunwald E. Future of biomarkers in acute coronary syndromes. *Circulation.* 2003;108:250-252.

106. Paulus WJ, Tschöpe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. *European Heart Journal.* 2007;28(20):2539–2550.

107. Lubien E, DeMaria A, Krishnaswamy P, Clopton P, Koon J, Kazanegra R, Gardetto N, Wanner E, Maisel AS. Utility of B-Natriuretic Peptide in Detecting Diastolic Dysfunction. *Circulation*. 2002;105:595-601

108. Takeda T, Kohno M. Brain natriuretic peptide in hypertension. *Hypertension Research.* 1995;18(4):259-266.

109. Weber M, Hamm C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. *Heart.* 2006;92(6):843-849.

CHAPTER 2

Methodology

2.1 Introduction

Diastolic dysfunction is described as impaired left ventricular (LV) relaxation and filling,[1,2] and has been linked to increased cardiovascular morbidity and mortality in hypertensives and the elderly.[3] Different factors can contribute to the progression and development of LV diastolic dysfunction, among these are the renin-angiotensin-aldosterone system (RAAS) and elevated levels of natriuretic peptides, such as amino-terminal prohormone B-type natriuretic peptide (Nt-proBNP).[1] The RAAS play a major role in the development of LV diastolic dysfunction,[1] while Nt-proBNP is released to prevent volume overload and elevated blood pressure caused by abnormal RAAS activity.[4] However, the associations of LV diastolic function markers with the RAAS and Nt-proBNP in a young, healthy population are still unclear.

This chapter outlines the specific methodology with justifications for the biochemical analyses, cardiovascular, anthropometric and physical activity measurements used in the manuscript chapter to follow. This study may provide insight for better physiological and pathophysiological understanding of the relationships of LV diastolic function markers with RAAS and Nt-proBNP in a young normotensive population. This may be of public health importance as an initiative towards primary prevention strategies for the reduction of the cardiovascular disease burden.

2.2 Study design

This Master of Health Sciences (MHSc) study is a sub-study within the African prospective study on the early detection and identification of cardiovascular disease and hypertension (African-PREDICT). The African-PREDICT study is a longitudinal study that aims to identify and understand the early pathophysiological changes in cardiovascular function, as well as the specific predictors that contribute to the development of hypertension and target organ damage in apparently healthy young black and white South Africans.

The African-PREDICT study is currently screening participants to include a total of 1200 young, healthy individuals in the final database (aged between 20–30 years old). As the African-PREDICT study aims to track and evaluate the development and early stages of hypertension, individuals in the selected age group proved to be the ideal population as they are adults that are at the peak of health and at the stage prior to cardiovascular deterioration. The participants are from Potchefstroom and its surrounding areas in the North-West Province, South Africa. The inclusion criteria for participants in the larger African-PREDICT study are apparently healthy men and women; black and white ethnicity; a brachial systolic blood pressure (SBP) of <140 mmHg and a diastolic blood pressure (DBP) of <90 mmHg; HIV-uninfected; and no previous diagnosis or medication usage for chronic diseases.

For this sub-study we additionally excluded participants with identified left or right bundle branch block, since these individuals may have an increased risk of cardiac mortality.[5] Left and right bundle branch block were shown to associate with coronary artery disease and heart failure.[5,6]

In South Africa, several research teams including the Hypertension in Africa Research Team (HART) have shown that black individuals have an increased risk for the development of hypertension and are therefore included in this study. Men and women are equally distributed to determine whether sex differences exist. Normotensive or pre-hypertensive (SBP<140 and DBP<90mmHg) were based on the average of four blood pressure measures in one day. The guidelines of the American Society of Hypertension and the International Society of Hypertension were used to determine if a participant is hypertensive, pre-hypertensive or normotensive.[7]

Individuals with a self-reported previous diagnosis of a chronic disease (diabetes, liver disease, cancer, tuberculosis or renal disease) that may influence cardiovascular health, were excluded as the aim of this study is to track apparently healthy individuals over the course of 20 years. Pregnant or lactating women were excluded due to the known influence of pregnancy hormones on the cardiovascular system.[8] Women can develop hypertensive complications during pregnancy, for instance pre-eclampsia which causes significant maternal and fetal morbidity.[8] Pregnant women may also have an increased risk of cardiovascular disease due to elevated blood glucose and lipid levels.[9]

Cross-sectional data of the first 364 participants of the African-PREDICT study were used in this MHSc study, after excluding participants with missing data (n=27), as well as individuals with identified left or right bundle branch block (n=9). The purpose of this sub-study was to determine the independent relationship of LV diastolic function markers with the RAAS and NT-proBNP in a young, apparently healthy South African cohort.

2.3 Methodology

2.3.1 Organizational procedures

The African-PREDICT study obtained approval from the Health Research Ethics Committee of the North-West University in 2012 (NWU-00001-12-A1) and is endorsed by the National and Provincial Department of Health. This sub-study also obtained approval to conduct the study from the Health and Research Ethics Committee of the North-West University (NWU-00032-17-A1) and the study complied with the Declaration of Helsinki (2008) and conformed to the Medical Research Council guidelines of good clinical practice.[10]

All recruited individuals underwent a screening procedure (either at their place of work or at the Hypertension Research Clinic at the North-West University, Potchefstroom campus) to determine eligibility for the main research study, based on the inclusion and exclusion criteria as mentioned previously. Individuals that met the inclusion criteria were invited to join the research project and were given detailed information regarding the subsequent procedures. Individuals who were willing to participate, were contacted via telephone to confirm an appointment at the Hypertension Research Clinic at the North-West University, Potchefstroom campus. Participants were requested to fast for at least 8 hours, preferably overnight prior to the day of the study and were asked to arrive at the Research Clinic at 08h00. According to current guidelines, blood used for lipid profiles should be drawn after an 8- to 12-hour fast.[11] Plasma triglycerides can increase significantly postprandial, whereas a fasting period seemingly avoids the variability of triglycerides associated with meals. Fasting triglyceride levels provide a more stable estimate for risk assessment.[11]

The procedures of the measurements were explained to the participants and they had the opportunity to ask any questions. Only after written informed consent was obtained did the measurements begin. All procedures were performed in temperature controlled private rooms to ensure the participants' comfort and privacy. A maximum of four participants per day were accommodated to maintain the quality of an intense battery of measurements. After spot-urine and blood sampling, anthropometry, bio-impedance and a variety of cardiovascular measurements (Table 1), the participants received a light, balanced meal (excluding caffeine). After measurements were completed the participants received a gift voucher from a supermarket as a token of appreciation for their participation. Transport was provided to all participants after all the procedures were completed, at approximately 13h00.

Table 1: Summary of research measurements from the African-PREDICT study used in this MHSc sub-study

Measure:
Biological Sampling:
Fasted Blood Sample (87 ml)
Urine (24-hour sample + additional spot sample on a separate day)
Cardiovascular Measures:
Electrocardiography (ECG)
Echocardiography
24-hour Ambulatory blood pressure and ECG monitoring
Anthropometry:
Height and Weight
Waist, hip and neck circumferences
Body mass index (BMI)
Physical activity
7-day Accelerometry
Questionnaires:
General Health Questionnaire
24-hour dietary recall and salt frequency intake (on site and within 7 days)

2.3.2 24-Hour urine collection, blood sampling and biochemical analyses

Upon arrival of each participant at the Hypertension Research Clinic, each of the participants were informed about all procedures and consent was obtained, after which early morning fasting blood samples were taken by a registered nurse, and participants provided a spot urine sample. The venous blood samples were collected from the brachial vein branches, using a sterile winged butterfly infusion set and syringe. This is an invasive procedure and carries minimal risk for the participants. Once the registered nurse took the samples, a research assistant, trained in the handling of biological samples, collected the samples from the Hypertension Research Clinic and placed the tubes in a closed container according to pre-specified protocol. The biological samples (spot-urine sample, whole blood, serum and plasma) were taken immediately to the on-site temperature controlled laboratory. Samples were centrifuged according to standard procedures and then aliquoted into cryovials for short- and long-term storage in bio-freezers at –80°C.

All aliquoted samples were stored in a bio-freezer room on the North-West University Potchefstroom campus, which is protected by a secure alarm system. During all procedures, protective laboratory coats as well as latex gloves were worn, and students were particularly trained on how to handle biological samples from the moment the samples reached the laboratory until all samples are securely stored in the bio-freezers. All staff and students handling biological samples had to undergo extensive training, done by the laboratory manager to reduce any risk to the students working in the laboratory. They also received vaccination against Hepatitis B.

From these biological samples (serum, plasma, whole blood and urine) the following biomarkers were analyzed and used for this MHSc sub-study: total renin, prorenin, aldosterone, Nt-proBNP, total cholesterol, high-density lipoprotein cholesterol, glucose, C-reactive protein, gamma-glutamyl-transferase, cotinine and estimated glomerular filtration rate. All biochemical variables were tested for intra- and inter-assay variability to ensure the specificity, sensitivity and reliability. Intra-assay variability for all biochemical variables should be <10%, however the inter-assay variability for all biochemical variables should be <20%.

Renin is a circulating aspartic proteinase and a highly specific catalyst for the first step in the RAAS cascade. Renin cleaves to angiotensinogen (a peptide bond) to convert it to angiotensin I.[12] Total renin was measured using the Quantikine ELISA Kit (R&D system, Minneapolis, MN USA) and analyzed on Synergy H4 hybrid microplate reader (Biotek, Winooski, VT, USA). Intra- and inter-assay variability for total renin were 5.30% and 5.50%, respectively.

Prorenin is the precursor for renin, and increased levels may contribute to elevated volume loading and blood pressure that may consequently promote end-organ damage.[13,14] However, the prorenin to total renin ratio differs among individuals with various clinical conditions.[15] Prorenin was analyzed using the Human Prorenin ELISA Kit (Biovendor-Laboratorni medicina, Karasek, Czech Republic). Intra- and inter-assay variability for prorenin were 4.34% and 4.71%, respectively. The ratio of total renin to prorenin was additionally calculated.

Aldosterone, a steroid hormone, forms part of the RAAS and can have a direct effect on cardiovascular tissue.[16] Increased aldosterone levels may predict and contribute to hypertension and cardiovascular damage.[17] Aldosterone was analyzed using the RIA Aldosterone Kit (Beckman Coulter, Immunotech, Radiova, Czech Republic). Intra- and inter-assay variability for aldosterone were 2.13% and 2.42%, respectively.

Nt-proBNP is a cleavage product of the B-type natriuretic peptide, which can be used to detect all degrees of diastolic dysfunction.[4] Nt-proBNP was analyzed using the Electrochemiluminescence method on the E411 (Roche, Basel Switzerland). Intra- and inter-assay variability for Nt-proBNP were 2.58% and 4.60%, respectively.

To describe the general characteristics of our study population we included basic information including lipids, glucose, C-reactive protein, gamma-glutamyl-transferase and cotinine, which are known to promote the onset of cardiovascular disease such as atherosclerosis. [18,19]

High-density lipoprotein cholesterol (HDL cholesterol) plays an important role in maintaining cholesterol homeostasis. Atherosclerotic lesions may compromise this process, as there is an association between the development of atherosclerosis and low HDL cholesterol.[19] HDL-cholesterol is also a strong predictor for coronary heart disease in both men and women.[20] Total cholesterol and HDL cholesterol was analyzed using the Cobas Integra 400plus (Roche, Basel, Switzerland). Intra- and inter-assay variability for total cholesterol were 0.51% and 1.90%, respectively. Intra- and inter assay variability for HDL-cholesterol were 1.10% and 1.00%, respectively. The ratio of total cholesterol to HDL-cholesterol is a reliable indicator to predict cardiovascular diseases,[20] therefore we additionally calculated the total cholesterol to HDL-cholesterol ratio.

Individuals diagnosed with diabetes mellitus, impaired fasting glucose level or insulin resistance are at risk for developing cardiovascular disease.[21] Glucose was analyzed using the Cobas Integra 400plus (Roche, Basel Switzerland). Intra- and inter-assay variability for glucose were 1.80% and 2.10%, respectively.

C-reactive protein is a robust clinical marker for systemic inflammation, however it may also play a direct pathological role in the development of atherosclerosis.[18] C-reactive protein is an independent predictor for future cardiovascular events.[22] C-reactive protein was analyzed using the Cobas Integra 400plus (Roche, Basel, Switzerland). Intra- and inter-assay variability for C-reactive protein were 1.30% and 3.50%, respectively.

Gamma-glutamyl-transferase (GGT) is regarded as a biomarker of hepatobiliary disease, oxidative stress[23] and also increased alcohol consumption.[24,25] Several studies suggested an association between higher serum GGT with cardiovascular disease risk factors, such as C-reactive protein, diabetes, hypertension, dyslipidemia and the metabolic syndrome.[25-27] GGT was analyzed using the Cobas Integra 400plus (Roche, Basel, Switzerland). Intra- and inter-assay variability for GGT were 1.80% and 1.80%, respectively.

Cotinine is a nicotine metabolite, and considered as a biomarker of both passive exposure and active smoking.[28] Smoking is one of the major risk factors for both the development and progression of cardiovascular disease and vascular disease,[29] and is associated with atherosclerosis and increased inflammatory markers.[30] Cotinine was analyzed according to the Chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Intra- and inter assay variability for cotinine were 10.7% and 5.50%, respectively.

The process for 24-hour urine sample collection was explained and the participants received all the necessary equipment. Participants were instructed to collect a 24-hour urine sample on a day that was convenient for them which was noted by the research nurse. Participants were instructed to discard the first urine of the day and thereafter collect the urine throughout the day in the provided container, including the first urine of the following morning (day two). Participants had to record the start and end times as well. The protocol for 24-hour urine collection followed the Pan American Health Organization/World Health Organization protocol for population level sodium determination in 24-hour urine samples.[31] Incomplete urine collections were defined as a volume <300 mL per 24-hours and/or a 24-hour creatinine excretion of <4 mmol or >25 mmol in women, and <6 mmol or >30 mmol in men.[32] Urine albumin, creatinine, sodium, potassium and iodine were determined from 24-hour urine samples. Additionally, the blood serum levels were used to determine Cystatin-C and creatinine concentrations, which were then used to calculate the estimated creatinine clearance and estimated glomerular filtration rate (eGFR). Chronic Kidney Disease Epidemiology (CKD-EPI) Formula (eGFR – CKD-EPI creatinine + Cystatin-C) was additionally used to calculate the estimated glomerular filtration rate.[33]

2.3.3 Cardiovascular measurements

2.3.3.1 Electrocardiography

The Electrocardiography (ECG) was performed by a trained researcher and registered a standard 12 lead ECG (Norav Medical Ltd, PC 1200, v5.030, Israel), with the participant in supine position. The ECG recorded measurements at 5-minute intervals for 20 seconds for at least six cardiac cycles. The resting ECG was performed to identify participants with left and right bundle branch block (PC-ECG 1200, Norav Medical Ltd, Kiryat Bialik, Israel). Left bundle branch block was identified as a prolonged QRS of approximately 0.125 seconds in the presence of supraventricular rhythm, a QS or RS complex in lead V_1, and the absence of a Q-wave in lead I, V_5 or V_6 and an R-wave peak for approximately 0.06 seconds in the same lead.[34-37] Right bundle branch block was identified with an R-wave in lead V_1 and a small Q-wave in lead V_6, which would cause excitation to spread to the left ventricle, causing a S-wave in lead V_1 and a R-wave in lead V_6.[37]

2.3.3.2 Echocardiography

Echocardiography was performed by a clinical technologist registered by the Health Professions Council of South Africa (HPCSA). An experienced registered clinical technician obtained and analyzed the data. This procedure was performed in a semi-dark room and the participant was provided with a blanket, if they wished to cover their chest area.

A standard transthoracic echocardiography procedure was followed while each participant was in a partial left decubitus position with the head of the examining table moderately elevated. This position brings the heart chamber forward to the chest wall to ensure a clearer window for scanning. This is a non-invasive and important method used to assess and identify cardiac anatomy and function.[38,39] The General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Hearten, Norway) was used along with the 2.5 to 3.5 MHz transducer and a three-lead ECG for timing purposes. Standardized methods were employed to obtain high-quality recordings according to the current recommendations as outlined in the guidelines of the European Association of Echocardiography and the American Society of Echocardiography.[40,41]

Variables of LV diastolic function included: E/A (peak early filling E-wave/late diastolic filling A-wave) ratio, E/é (mitral peak velocity of early filling/early diastolic mitral annular velocity) ratio, left atrium to aortic root ratio (LA/Ao) and end-diastolic volume of the left ventricle.

Primary measurements of mitral inflow included the peak early filling (E-wave) and late diastolic filling (A-wave) velocities.[42] A 1-mm to 3-mm sample volume was placed between the tips of the mitral valve leaflets with parallel alignment of inflow. In addition, we performed tissue Doppler imaging to calculate the velocity of myocardial tissue movement in relation to mitral valve blood flow. Tissue Doppler imaging is a robust echocardiographic tool used for quantitative assessment of cardiac systolic and diastolic function, as well as the hemodynamics of LV filling.[39] The e' (e-prime) and a' (a-prime) were measured with continuous wave Doppler imaging, and we subsequently calculated the E/e' ratio (peak E velocity to e-prime) which reflects filling pressures and the rate of myocardial relaxation.[39,43] One of the earliest diastolic dysfunction markers is a decreased e', and is present in all the stages of diastolic dysfunction.[39] Continuous wave Doppler imaging is a non-invasive method used to calculate the left ventricular rate in pressure rise (RPR) from recordings of mitral regurgitation velocity curves.[44] This method also provides information regarding the instantaneous pressure gradients between the left ventricle and left atrium, as well as information relating to left ventricular function.[44] A pulsed-wave Doppler was performed in the apical 4-chamber view to obtain mitral inflow velocities for assessing LV filling.[45] Pulsed wave Doppler imaging provide velocity profiles of mitral annulus movements that reflect the rate of myocardial relaxation.[46]

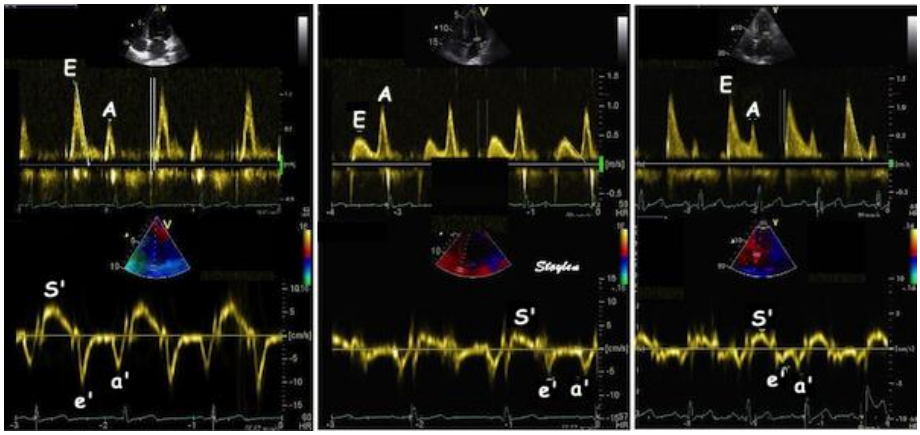


Figure 1: Tissue Doppler imaging of the E/A and E/e' ratio.

Left: Healthy individual with good diastolic function; high E and e', normal E/e'. Middle, patient with diastolic dysfunction without increased filling pressure; low E and e', normal E/e' ratio. Right, patient with diastolic dysfunction and increased filling pressure; high E, low e' and high E/e'.

M-mode imaging is a non-invasive method used to obtain quantitative dimensional measurements such as cardiac chamber size, wall thickness, wall motion velocities, vessel dimensions and valve motion.[47] We used M-mode imaging for obtaining the left atrium to aortic root (LA/Ao) ratio. The aortic root (Ao) diameter was measured at the end of diastole, between the anterior edges of the anterior and posterior aortic walls. The LA diameter was measured at the end of systole, from the anterior edge of the posterior aortic wall to the endocardial surface of the posterior aortic wall.[48] The left ventricular end-diastolic volume was measured using a two-dimensional echocardiography-guide M-mode approach and was measured from the apical four- and two chamber views.[49] Two-dimensional echocardiography-guide M-mode imaging enlarges the LV areas, while avoiding foreshortening of the left ventricle which can lead to miscalculating the volume of the left ventricle.[49] The LV end-diastolic and end-systolic volumes were calculated using the z-derived method as described by de Simone and colleagues.[50]

2.3.3.3 Ambulatory blood pressure measurements

Participants were fitted with a 24-hour ambulatory blood pressure monitoring (ABPM) (CardioXplore, MediTech, Hungary, British Hypertension Society (BHS) validated). The ABPM was fitted to each participant at approximately the same time every day (late morning). An appropriate sized cuff was fitted to the participant's non-dominant arm, and instructions were given to the participant on how to ensure successful inflations across the 24-hour period. The ABPM apparatus was programmed to take 24-hour pressure recordings every 30 minutes during the day (6 am to 10 pm) and every hour during the night (10 pm to 6 am). An ambulatory diary card was provided to complete by the participants during the 24-hour duration of the measurements. Only participants with >70% valid 24-hour blood pressure measurements, and >20-day time and >7-night time

measurements were included in the data analysis. For this MHSc sub-study we use 24-hour systolic and diastolic blood pressure profiles for each participant. Evidence suggests that home readings of blood pressure can more accurately predict cardiovascular events, than measurements taken in an unfamiliar setting.[51]

2.3.4 Anthropometric measurements

A trained research assistant used standard procedures to determine body height (m) by using the SECA 213 Portable Stadiometer (SECA, Hamburg, Germany), and body weight (kg) by using the SECA 813 Electronic Scales with a weighing capacity of up to 200kg (SECA, Hamburg, Germany). The privacy of the participants was taken into consideration by doing the measurements in a private, temperature-controlled room. All anthropometric measurements were performed according to the guidelines described in the International Society for the Advancement of Kinanthropometry.[52]

BMI is used as the current classification system for obesity[53] and was calculated by dividing weight (kg) with height (m^2). Obesity is known to associate with chronic conditions such as hypertension, high blood cholesterol levels and decreased levels of HDL-cholesterol in both men and women from different ethnic groups.[54,55] BSA was additionally calculated by using the Mosteller equation,[56] as BMI could overestimate obesity rates among a population including individuals performing intense training with a high muscle mass.

2.3.5 Physical activity measurements

After the participants were fitted with the ABPM and ECG apparatus, they were also fitted with an ActiHeart physical activity monitor (CamNtech Ltd., England, UK), in a temperature-controlled room to ensure the privacy of the participants. This is a compact, chest-worn monitoring device that records heart rate, inter-beat-interval and physical activity in one combined unit. It is designed for capturing heart rate variability data and for calculating and measuring activity energy expenditure. Activity energy expenditure was calculated using branched model equations and additionally divided by body weight and expressed as an index (kCal/kg/day). The ActiHeart device was worn for a maximum of 7 days.

Lifestyle modifications are effective for reducing the risk of cardiovascular disease. Much attention has been drawn to physical activity and its well-known effects on cardiovascular disease risk and all-cause mortality.[57] Regular physical activity has plenty of beneficial effects, such as prevention and treatment of atherosclerotic risk factors, which include hypertension, insulin resistance, glucose intolerance, increased triglyceride concentrations, low HDL-concentrations and obesity.[58]

2.3.6 General Health Questionnaire

Basic information regarding the participant's age, sex, ethnicity and current lifestyle factors; such as self-reported smoking, alcohol consumption, medication use and family history were collected by giving a standardized and validated General Health Questionnaire for the participants to complete. The participants completed the questionnaire on an Apple iPad (Hon Hai Precision Industry Co, Ltd) using a web-based program, and the questionnaire took approximately 15 minutes to complete.

2.3.7 Data management

Taking into consideration all ethical aspects related to the privacy of personal data, we used an automated, password protected, user friendly, secure web-based, electronic database, called REDCap, to capture all data.[59] When using this system all laboratory specimens, evaluation forms, reports, data and other records are identified only by the participant's number to maintain subject confidentiality. Apart from this system, data is also backed up on password protected hard drives – done by the Data Manager. The data is generated onto Excel spreadsheets from REDCap, and imported directly into IBM® SPSS® Statistics, Version 24 (IBM Corporation, Armonk, New York) to ensure the accuracy of the data analysis.

2.3.8. Statistical analyses

All statistical analyses were performed with the IBM® SPSS® Statistics, Version 24 (IBM Corporation, Armonk, New York). All variables were tested for normality by visual inspection of Q-Q plots and also reviewing the coefficients of skewness (–0.8; 0.8) and kurtosis (–3; 3). Appropriate interaction tests were performed for ethnicity and sex on the association of LV diastolic function markers with RAAS and Nt-proBNP. Significant interactions existed for ethnicity between the E/A ratio with aldosterone ($p=0.035$) and prorenin ($p=0.025$), therefore we divided the groups according to ethnicity. After standardizing all non-Gaussian variables by logarithmic transformation, we followed a Gaussian statistical approach which included independent samples T-test, Pearson and partial correlations, and standard multiple regression. Independent samples T-test is used to compare descriptive characteristics in a study population, as it accurately discriminates between higher and lower levels of specific variables. Therefore, we used independent samples T-test to determine differences between the black and white groups. The data is presented in table format and expressed as the arithmetic mean ± standard deviation for normally distributed variables and the geometric mean with 5^{th} and 95^{th} percentiles for non-Gaussian variables. Pearson and partial correlations were performed to explore the relationships of LV diastolic function markers with RAAS and Nt-proBNP. Multiple regression analyses were performed with LV diastolic function

as the dependent variable and RAAS and Nt-proBNP as the main independent variables. Confounding variables considered for entry in the multiple regression analysis included age, body surface area, blood pressure, C-reactive protein, total cholesterol, glucose, gamma-glutamyl-transferase, cotinine, estimated glomerular filtration rate and activity energy expenditure. Furthermore, we performed a sensitivity analysis to assess the contribution of the 24-hour sodium to potassium ratio on the associations of LV diastolic function markers with the RAAS and Nt-proBNP.

2.3.9. Student contribution

I have been involved in the initial screening phase of the African-PREDICT study, where I was responsible for urine dipstick analysis, glucose and cholesterol testing, blood pressure measurements as well as determining blood groups. These data contributed to the screening phase of the study, by which participants were included or excluded from the study. In addition, I contributed to laboratory work. I was involved in ensuring the blood samples were centrifuged, separated into individual cryovials and frozen afterwards. I am currently involved in the Exercise, Arterial Modulation and Nutrition in Youth South Africa (ExAMIN Youth SA) study, in which I am responsible for performing pulse wave analysis with the use of the validated oscillometric Mobli-o-Graph monitor (I.E.M GmbH, Germany) with integrated ARCSolver software. I also contributed to laboratory work, where I separated the urine and saliva samples into individual cryovials and froze them afterwards. Additionally, I underwent ethical and statistical training. I was responsible for data collection, capturing the data into the final database, all statistical analyses and interpretation of the results.

2.4 References

1. Sciarretta S, Paneni F, Palano F, Chin D, Tocci G, Rubattu S, Volpe M. Role of the renin–angiotensin–aldosterone system and inflammatory processes in the development and progression of diastolic dysfunction. *Clinical Science.* 2009;116(6):467-477.

2. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: part II. *Circulation.* 2002;105(12):1503-1508.

3. Aurigemma GP, Gottdiener JS, Shemanski L, Gardin J, Kitzman D. Predictive value of systolic and diastolic function for incident congestive heart failure in the elderly: the cardiovascular health study. *Journal of the American College of Cardiology.* 2001;37(4):1042-1048.

4. Tschöpe C, Kašner M, Westermann D, Gaub R, Poller WC, Schultheiss HP. The role of NT-proBNP in the diagnostics of isolated diastolic dysfunction: correlation with echocardiographic and invasive measurements. *European Heart Journal.* 2005;26(21):2277-2284.

5. Ghostine S, Caussin C, Daoud B, Habis M, Perrier E, Pesenti-Rossi D, Sigal-Cinqualbre A, Angel, CY, Lancelin B, Capderou A. Non-invasive detection of coronary artery disease in patients with left bundle branch block using 64-slice computed tomography. *Journal of the American College of Cardiology.* 2006;48(10):1929-1934.

6. Fantoni C, Kawabata M, Massaro R, Regoli F, Raffa S, Arora V, Salerno-Uriarte JA, Klein HU, Auricchio A. Right and left ventricular activation sequence in patients with heart failure and right bundle branch block: a detailed analysis using three-dimensional non-fluoroscopic electroanatomic mapping system. *Journal of Cardiovascular Electrophysiology.* 2005;16(2):112-119.

7. Weber MA, Schiffrin EL, White WB, Mann S, Lindholm LH, Kenerson JG, Flack JM, Carter BL, Materson BJ, Ram CVS . Clinical practice guidelines for the management of hypertension in the community. *The Journal of Clinical Hypertension.* 2014;16(1):14-26.

8. Robb AO, Mills NL, Din JN, Smith IBJ, Paterson F, Newby DE, Denison FC. Influence of the menstrual cycle, pregnancy, and preeclampsia on arterial stiffness. *Hypertension.* 2009;53(6):952-958.

9. Ness RB, Harris T, Cobb J, Flegal KM, Kelsey JL, Balanger A, Stunkard AJ, D'Agostino RB. Number of pregnancies and the subsequent risk of cardiovascular disease. *New England Journal of Medicine.* 1993;328(21):1528-1533.

10. Medical Research Council. MRC Guidelines for Good Clinical Practice in Clinical Trials. London: Medical Research Council. 1998.

11. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting Compared With Nonfasting Triglycerides and Risk of Cardiovascular Events in Women. *Journal of the American Medical Association.* 2007;298(3):309-316.

12. Unger T, Li J. The role of the renin-angiotensin-aldosterone system in heart failure. *Journal of Renin-Angiotensin-Aldosterone System.* 2004;5(1 suppl):S7-S10.

13. Ichihara A, Kaneshiro Y, Takemitsu T, et al. Contribution of nonproteolytically activated prorenin in glomeruli to hypertensive renal damage. *Journal of the American Society of Nephrology.* 2006;17(9):2495-2503.

14. Yoshimura M, Kawai M. Synergistic inhibitory effect of angiotensin II receptor blocker and thiazide diuretic on the tissue renin-angiotensin-aldosterone system. *Journal of the Renin-Angiotensin-Aldosterone System.* 2010;11(2):124.

15. Tu W, Eckert GJ, Pratt JH, Jan Danser A. Plasma levels of prorenin and renin in blacks and whites: their relative abundance and associations with plasma aldosterone concentration. *American Journal of Hypertension.* 2012;25(9):1030-1034.

16. Rocha R, Williams GH. Rationale for the use of aldosterone antagonists in congestive heart failure. *Drugs.* 2002;62(5):723-731.

17. Connell JM, Davies E. The new biology of aldosterone. *Journal of Endocrinology.* 2005;186(1):1-20.

18. Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes. *Circulation.* 2006;113(7):e152-e155.

19. Lewington S. The importance of cholesterol, blood pressure and smoking for coronary heart disease. *European Heart Journal*. 2003;24(19):1703–1704.

20. Castelli WP, Anderson K. A population at risk: Prevalence of high cholesterol levels in hypertensive patients in the framingham study. *The American Journal of Medicine.* 1986;80(2, Supplement 1):23-32.

21. Rubins HB, Robins SJ, Collins D, Nelson DB, Elam MB, Schaefer EJ, Faas FH, Anderson JW. Diabetes, plasma insulin, and cardiovascular disease: subgroup analysis from the Department of Veterans Affairs high-density lipoprotein intervention trial (VA-HIT). *Archives of Internal Medicine.* 2002;162(22):2597-2604.

22. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk. *Journal of the American College of Cardiology.* 2007;49(21):2129-2138.

23. Mason JE, Starke RD, Van Kirk JE. Gamma-Glutamyl transferase: a novel cardiovascular risk BioMarker. *Preventive Cardiology.* 2010;13(1):36-41.

24. Whitfield J. Gamma glutamyl transferase. *Critical reviews in Clinical Laboratory Sciences.* 2001;38(4):263-355.

25. Lee DS, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, Wang TJ, Benjamin EJ, D'Agostino RB, Vasan RS. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 2007;27(1):127-133.

26. Rantala A, Lilja M, Kauma H, Savolainen M, Reunanen A, Kesäniemi Y. Gamma-glutamyl transpeptidase and the metabolic syndrome. *Journal of Internal Medicine.* 2000;248(3):230-238.

27. Lee DH, Ha MH, Kim JH, hristiani DC, Gross MD, Steffes M, Blomhoff R, Jacobs DR. Gamma-glutamyltransferase and diabetes—a 4 year follow-up study. *Diabetologia.* 2003;46(3):359-364.

28. Whincup PH, Gilg JA, Emberson JR, Jarvis MJ, Feyerabend C, Bryant A, Walker M, Cook DG. Passive smoking and risk of coronary heart disease and stroke: prospective study with cotinine measurement. *British Medical Journal.* 2004;329(7459):200-205.

29. Conklin BS, Zhao W, Zhong DS, Chen C. Nicotine and Cotinine Up-Regulate Vascular Endothelial Growth Factor Expression in Endothelial Cells. *The American Journal of Pathology.* 2002;160(2):413-418.

30. Bakhru A, Erlinger TP. Smoking cessation and cardiovascular disease risk factors: results from the Third National Health and Nutrition Examination Survey. *PLoS Medicine.* 2005;2(6):e160.

31. World Health Organization. Protocol for Population Level Sodium Determination in 24-h Urine Samples. *Prepared by WHO/PAHO Regional Expert Group for Cardiovascular Disease Prevention through Population-Wide Dietary Salt Reduction: Sub-Group for Research and Surveillance.* 2010.

32. Stolarz-Skrzypek K, Kuznetsova T, Thijs L, Tikhonoff V, Seidlerová J, Richart T, Jin Y, Olszanecka A, Malyutina S, Casiglia E. Fatal and nonfatal outcomes, incidence of hypertension, and blood pressure changes in relation to urinary sodium excretion. *Journal of the American Medical Association.* 2011;305(17):1777-1785.

33. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T. A new equation to estimate glomerular filtration rate. *Annals of Internal Medicine.* 2009;150(9):604-612.

34. Sgarbossa EB, Pinski SL, Barbagelata A, Underwood DA, Gates KB, Topol EJ, Califf RM, Wagner GS. Electrocardiographic diagnosis of evolving acute myocardial infarction in the presence of left bundle-branch block. *New England Journal of Medicine.* 1996;334(8):481-487.

35. Wagner G. Marriott's Practical Electrocardiography 2008. Philadelphia, PA: Lippincott Williams & Wilkins.

36. Strauss DG, Selvester RH, Wagner GS. Defining Left Bundle Branch Block in the Era of Cardiac Resynchronization Therapy. *The American Journal of Cardiology.* 2011;107(6):927-934.

37. Hampton JR. 2013. The ECG Made Easy E-Book. 8th ed. Churchill, Livingstone: Elsevier.

38. Tanaka H, Shimada K, Yoshida K, Jissho S, Yoshikawa J, Yoshiyama M. The Simultaneous Assessment of Aortic Valve Area and Coronary Artery Stenosis Using 16-Slice Multidetector-Row Computed Tomography in Patients With Aortic Stenosis Comparison With Echocardiography. *Circulation Journal.* 2007;71(10):1593-1598.

39. Yu CM, Sanderson JE, Marwick TH, Oh JK. Tissue Doppler Imaging: A New Prognosticator for Cardiovascular Diseases. *Journal of the American College of Cardiology.* 2007;49(19):1903-1914.

40. Sahn D. The Committee on M-mode Standardization of the American Society of Echocardiography. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation.* 1978;58:1072-1083.

41. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise J. Recommendations for chamber quantification. *European Heart Journal Cardiovascular Imaging.* 2006;7(2):79-108.

42. Nagueh SF, Appleton CP, Gillebert TC, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Journal of the American Society of Echocardiography.* 2009;22(2):107-133.

43. Nagueh SF, Sun H, Kopelen HA, Middleton KJ, Khoury DS. Hemodynamic determinants of the mitral annulus diastolic velocities by tissue Doppler. *Journal of the American College of Cardiology.* 2001;37(1):278-285.

44. Bargiggia GS, Bertucci C, Recusani F, Raisaro A, De Servi S, Valdes-Cruz LM, Sahn DJ, Tronconi L. A new method for estimating left ventricular dP/dt by continuous wave Doppler-echocardiography. Validation studies at cardiac catheterization. *Circulation.* 1989;80(5):1287-1292.

45. Appleton CP, Jensen JL, Hatle LK, Oh JK. Doppler evaluation of left and right ventricular diastolic function: a technical guide for obtaining optimal flow velocity recordings. *Journal of the American Society of Echocardiography.* 1997;10(3):271-292.

46. Ommen SR, Nishimura R, Appleton CP, et al. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures. *Circulation.* 2000;102(15):1788-1794.

47. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation.* 1978;58(6):1072-1083.

48. Evans N. Diagnosis of patent ductus arteriosus in the preterm newborn. *Archives of Disease in Childhood.* 1993;68(1 Spec No):58-61.

49. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *European Heart Journal Cardiovascular Imaging.* 2015;16(3):233-271.

50. De Simone G, Devereux RB, Ganau A, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T. Estimation of left ventricular chamber and stroke volume by limited M-mode echocardiography and validation by two-dimensional and Doppler echocardiography. *American Journal of Cardiology.* 1996;78(7):801-807.

51. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz T, Sheps SG, Roccella EJ. Recommendations for blood pressure measurement in humans and experimental animals. *Circulation.* 2005;111(5):697-716.

52. Marfell-Jones MJ, Stewart A, de Ridder J. 2012. International Standards for Anthropometric Assessment. Wellington, New Zealand: International Society for the Advancement of Kinanthropometry.

53. Prentice AM, Jebb SA. Beyond body mass index. *Obesity Reviews.* 2001;2(3):141-147.

54. Brown CD, Higgins M, Donato KA, et al. Body Mass Index and the Prevalence of Hypertension and Dyslipidemia. 2000.

55. Kennedy L, Dickstein K, Anker S, Kristianson K, Willenheimer R, Group OS. The prognostic importance of body mass index after complicated myocardial infarction. *Journal of the American College of Cardiology.* 2005;45(1):156.

56. Mosteller R. Simplified calculation of body-surface area. *The New England Journal of Medicine.* 1987;317(17):1098.

57. Ahmed HM, Blaha MJ, Nasir K, Rivera JJ, Blumenthal RS. Effects of Physical Activity on Cardiovascular Disease. *The American Journal of Cardiology.* 2012;109(2):288-295.

58. Thompson PD, Buchner D, Piña IL, Balady GJ, Williams MA, Marcus BH, Berra K, Blair SN, Costa F, Franklin B. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. *Circulation.* 2003;107(24):3109-3116.

59. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics.* 2009;42(2):377-381.

CHAPTER 3

Research article

Summary of the instructions for authors: *American Journal of Cardiology*

A summary of the instructions for authors is provided below as adapted from http://www.ajconline.org/content/authorinfo (Date of access: 10 October 2017).

- The paper must be arranged as follows: (1) title; (2) abstract; (3) text; (4) acknowledgement; (5) references; (6) tables.
- The title page (page 1) should include the following: the title, the full names and academic degrees of the authors, and current affiliations of all authors.
- Add at the bottom of the page the phrase "Corresponding author" followed a telephone number, email address, and mailing address.
- Provide information about grant support if necessary, including the location (City/state/country). If work described is supported by a grant from a pharmaceutical company, that fact should be stated on the title page.
- An abstract (page 2) of no more than 250 words or a single page, with 2 to 4 key words must be provided.
- The 3 required major headings (Methods, Discussion and Results) must be used throughout the body of the text.
- Tables, figures, and references must be cited in numerical order throughout the manuscript.
- Each table must be numbered in Arabic numerals (Table 1, 2, etc.) and each table should consist of a title.

Exploring the links of left ventricular diastolic function with the renin-angiotensin-aldosterone system and amino-terminal prohormone B-type natriuretic peptide: The African-PREDICT study

Bridget Viana, BSc. Hons.[a]; Prof. Johannes Marthinus van Rooyen, DSc[a,b]; Dr. Lebo Francina Gafane-Matemane, PhD[a,b]; Prof. Ruan Kruger, PhD[a,b]*

a. *Hypertension in Africa Research Team (HART); North-West University, Potchefstroom, South Africa*
b. *MRC Research Unit for Hypertension and Cardiovascular Disease, North-West University, Potchefstroom, South Africa*

Short title: Cardiac function, RAAS and Nt-proBNP

***Corresponding author:**

Prof. Ruan Kruger, PhD

Hypertension in Africa Research Team (HART)

North-West University

Potchefstroom, 2531

South Africa

Phone: +27 18 299 2904

Fax: +27 18 285 2432

E-mail: ruan.kruger@nwu.ac.za

Statement of financial support

The research funded in this manuscript is part of an ongoing research project financially supported by the South African Medical Research Council (SAMRC) with funds from the National Treasury under its Economic Competitiveness and Support Package; the South African Research Chairs Initiative (SARChI) of the Department of Science and Technology and National Research Foundation (NRF) of South Africa; the Strategic Health Innovation Partnerships (SHIP) Unit of the SAMRC with funds received from the South African National Department of Health, GlaxoSmithKline R&D, the UK Medical Research Council and with funds from the

UK Government's Newton Fund; as well as corporate social investment grants from Pfizer (South Africa), Boehringer-Ingelheim (South Africa), Novartis (South Africa), the Medi Clinic Hospital Group (South Africa) and in kind contributions from Roche Diagnostics (South Africa). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF does not accept any liability in this regard.

Conflicts of interest

The authors report that they have no conflicts of interest.

Word count: 6126

Number of tables: 3

Number of figures: 0

Number of supplementary digital content files: 1

Abstract

Left ventricular (LV) diastolic dysfunction is linked to cardiovascular morbidity and mortality, with both the renin-angiotensin-aldosterone system (RAAS) and the amino-terminal prohormone B-type natriuretic peptide (Nt-proBNP) implicated in the pathophysiology. However, in young, healthy individuals the associations of LV diastolic function markers with the RAAS and Nt-proBNP has not been described yet. We aimed to compare LV diastolic function markers along with the RAAS and Nt-proBNP and to explore their associations in black and white participants. Cross-sectional data of 400 participants (aged between 20–30 years) were included from the African-PREDICT study after excluding individuals with missing data (n=64). Ambulatory blood pressure was measured and a standard transthoracic echocardiographic procedure followed. Among other biomarkers, we analyzed renin, prorenin, aldosterone, and Nt-proBNP. The E/A and E/e' ratios were higher in the black compared to the white group (both $p \leq 0.003$). Total renin was higher in the black group ($p=0.010$), whereas aldosterone ($p<0.001$) and Nt-proBNP ($p=0.006$) were lower compared to the white group. In multiple regression analyses, we found positive associations of the E/A ratio with total renin ($\beta=0.16$; $p=0.042$) and the LA/Ao ratio with prorenin ($\beta=0.18$; $p=0.032$) in the white group only, whereas the E/A ratio positively associated with prorenin ($\beta=0.15$; $p=0.049$) in the black group only. No associations were evident between markers of LV diastolic function and Nt-proBNP in either group. Our results may suggest that both groups are subjected to early changes in RAAS handling, which may promote cardiovascular alterations and lead to premature diastolic dysfunction in later life.

Keywords: Left ventricular diastolic function, renin-angiotensin-aldosterone system, amino-terminal prohormone B-type natriuretic peptide, ethnicity

Word count: 249

Introduction

Impaired left ventricular (LV) relaxation and filling, also described as left ventricular diastolic dysfunction,[1,2] has been linked to increased cardiovascular morbidity and mortality in hypertensives and the elderly.[3,4] Enhanced or dysregulated renin-angiotensin-aldosterone system (RAAS) activity, may contribute to volume overload and vasoconstriction, with subsequent increases in LV diastolic filling pressures.[5,6] Factors such as atherosclerosis, hypertension, ischemic injury, diabetes, metabolic disorders and obesity,[1,7,8] may alter the RAAS even further, and consequently promote cardiac remodeling through the development of LV hypertrophy and myocardial fibrosis.[1] The RAAS is also linked to vascular and myocardial inflammation that could lead to myocardial fibrosis and LV changes, therefore promoting diastolic dysfunction.[9] It is probable that there are other non-renal mediators contributing to volume retention, except the circulating RAAS. For instance, prorenin, the precursor of renin, is also associated with the activation of tissue RAAS, such as adrenal glands, brain, heart and blood vessels.[10] Tissue RAAS activation may contribute to increased volume loading and blood pressure that may lead to the suppression of circulating RAAS, and consequently promote end-organ damage.[11,12] Amino-terminal prohormone B-type natriuretic peptide (Nt-proBNP) is secreted in response to myocardial wall stretch, caused by elevated cardiac pressure and volume overload.[13,14] Nt-proBNP is secreted to protect the body from elevated cardiac pressure and volume overload caused by dysregulation of the RAAS.[15,16] The RAAS and elevated Nt-proBNP might play a major role in determining the development of LV diastolic dysfunction and its progression towards heart failure,[1] however less is known about the links of LV diastolic function markers with the RAAS and Nt-proBNP in healthy young individuals.[17] Therefore, we aimed to compare the cardiovascular characteristics along with the RAAS and Nt-proBNP levels, as well as to explore the associations of LV diastolic function with the RAAS and Nt-proBNP in young, apparently healthy black and white South Africans.

Methods

Cross-sectional data of the first 400 participants (aged between 20–30 years old) from the African prospective study on the early detection and identification of cardiovascular disease and hypertension (African-PREDICT) was used in this sub-study. The inclusion criteria for the African-PREDICT study was apparently healthy men and women; black and white ethnicity; a brachial systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg; HIV-uninfected; and no previous diagnosis or medication usage for chronic disease. Pregnant or lactating women were also excluded from participation Additionally, for the sub-study participants with missing data (n=55), as well as individuals with identified left or right bundle branch block (n=9) were excluded. We obtained approval to conduct the study from the Health and Research Ethics Committee of the North-West University (NWU-00032-17-A1) and the study complied with the Declaration of Helsinki (2008).

Participants were fitted with a 24-hour ambulatory blood pressure monitoring (ABPM) (Validated CardioXplore devices (CardioXplore, MediTech, Hungary). The ABPM was programmed to take 24-hour blood pressure recordings every 30 minutes during the day (6 am to 10 pm) and every hour during the night (10 pm to 6 am). The ABPM was fitted to each participant at approximately the same time every day (late morning), using an appropriately sized cuff. For each participant, 24-hour diastolic and systolic blood pressure profiles were used. A trained researcher performed and registered a standard 12 lead ECG (Norav Medical Ltd, PC 1200, v5.030, Israel), with the participant in the supine position. The ECG recorded measurements at 5 minute intervals for 20 seconds for at least six cardiac cycles. The resting ECG was performed to identify participants with left and right bundle branch blocks (PC-ECG 1200, Norav Medical Ltd, Kiryat Bialik, Israel).

A standard transthoracic echocardiography procedure was followed while each participant was in a partial left decubitus position with the head of the examining table moderately elevated. The General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Hearten, Norway) was used, along with the 2.5 to 3.5 MHz transducer and a three-lead ECG for timing purposes. Standardized methods were employed to obtain high-quality recordings according to the current recommendations as outlined in the guidelines of the European Association of Echocardiography and the American Society of Echocardiography.[18,19] An experienced registered clinical technician obtained and analyzed the data.

Variables of LV diastolic function include: E/A (peak early filling E-wave/late diastolic filling A-wave) ratio, E/é (mitral peak velocity of early filling/early diastolic mitral annular velocity) ratio, left atrium to aortic root ratio (LA/Ao) and end-diastolic volume of the left ventricle.[20] Primary measurements of mitral inflow included the peak early filling (E-wave) and late diastolic filling (A-wave) velocities.[20]

A 1-mm to 3-mm sample volume was placed between the tips of the mitral valve leaflets with parallel alignment of inflow. We additionally performed tissue Doppler imaging to calculate the velocity of myocardial tissue movement in relation to mitral valve blood flow. The e' (e-prime) and a' (a-prime) were measured with continuous wave Doppler imaging, and we subsequently calculated the E/e' ratio (peak E velocity to e-prime) which reflects filling pressures and the rate of myocardial relaxation.[21,22] A pulsed-wave Doppler was performed in the apical 4-chamber view to obtain mitral inflow velocities for assessing LV filling.[23] Pulsed wave Doppler imaging provide velocity profiles of mitral annulus movements that reflect the rate of myocardial relaxation.[24] We used M-mode imaging for obtaining the left atrium to aortic root (LA/Ao) ratio. The aortic root (Ao) diameter was measured at the end of diastole, between the anterior edges of the anterior and posterior aortic walls. The LA diameter was measured at the end of systole, from the anterior edge of the posterior aortic wall to the endocardial surface of the posterior aortic wall.[25] The left ventricular end-diastolic volume was measured using two-dimensional echocardiography-guide M-mode approach and was measured from the apical four- and two chamber views.[26] The LV end-diastolic and end-systolic volumes were calculated using the z-derived method as described by de Simone and colleagues.[27]

Anthropometric measurements included body height (m) and weight (kg) and were performed according to standard procedures as described by the International Society for the Advancement of Kinanthropometry.[28] Body mass index (BMI) was calculated and body surface area (BSA) was additionally calculated by using the Mosteller equation.[29] Participants were fitted with an ActiHeart physical activity monitor (CamNtech Ltd, England, UK), which measured and calculated activity energy expenditure. Activity energy expenditure was calculated using branched model equations and additionally divided by body weight and expressed as an index (kCal/kg/day).

Fasting blood samples were collected by a registered nurse, and participants provided a spot urine sample as well as 24-hour urine collection. The blood and urine samples were immediately taken to the on-site temperature-controlled laboratory and aliquoted into cryovials for short- and long-term storage in bio-freezers at –80°C. Total renin was measured using the Quantikine ELISA Kit (R&D system, Minneapolis, MN USA) and analyzed on Synergy H4 hybrid microplate reader (Biotek, Winooski, VT, USA). Prorenin was analyzed using the Human Prorenin ELISA Kit (Biovendor-Laboratorni medicina, Karasek, Czech Republic). The ratio of prorenin-to-renin was additionally calculated. Aldosterone was analyzed using the RIA Aldosterone Kit (Beckman Coulter, Immunotech, Radiova, Czech Republic). NT-proBNP was analyzed using the Electrochemiluminescence method on the E411 (Roche, Basel Switzerland). Total cholesterol and high-density lipoprotein (HDL) cholesterol were analyzed using the Cobas Integra 400Plus (Roche, Basel, Switzerland). The ratio of total cholesterol-to-HDL cholesterol was additionally calculated.

Glucose and C-reactive protein, were analyzed using the Cobas Integra 400plus (Roche, Basel Switzerland). Gamma-glutamyl-transferase was analyzed using the Cobas Integra 400plus (Roche, Basel Switzerland). Cotinine was analyzed according to the Chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Blood serum levels were used to determine Cystatin-C and creatinine concentrations, which were then used to calculate the estimated creatinine clearance and estimated glomerular filtration rate (eGFR). Chronic Kidney Disease Epidemiology (CKD-EPI) Formula (eGFR – CKD-EPI creatinine + Cystatin-C) was additionally used to calculate the estimated glomerular filtration rate. Intra- and inter-assay variability for all biochemical variables was <10.8%.

All statistical analyses were performed with the IBM® SPSS® Statistics, Version 24 (IBM Corporation, Armonk, New York). All variables were tested for normality. Non-Gaussian variables were logarithmically transformed. Appropriate interaction tests were performed for ethnicity and sex on the association of LV diastolic function markers with RAAS and Nt-proBNP. Significant interactions existed for ethnicity between the E/A ratio with aldosterone (p=0.035) and prorenin (p=0.025). Therefore, we divided the groups according to ethnicity. Descriptive statistics in table format were obtained by performing T-tests of independent samples and presented as the arithmetic mean ± standard deviation (for normally distributed variables) or the geometric mean with 5^{th} and 95^{th} percentiles (for logarithmically transformed variables). Pearson and partial correlations were performed to explore the relationships of LV diastolic function markers with RAAS and Nt-proBNP. Multiple regression analyses were performed with LV diastolic function as the dependent variable and RAAS and Nt-proBNP as the main independent variables. Confounding variables considered for entry in the multiple regression analysis included age, body mass index, blood pressure, C-reactive protein, total cholesterol, glucose, gamma-glutamyl-transferase, cotinine, 24-hour urinary sodium-potassium ratio, estimated glomerular filtration rate (eGFR) and activity energy expenditure. However, the following confounding variables used for entry in the multiple regression analysis included age, sex, BSA, systolic blood pressure (SBP), C-reactive protein, total cholesterol-to-HDL cholesterol ratio, gamma-glutamyl-transferase, cotinine, eGFR and activity energy expenditure.

Results

The general characteristics of the study population are described in Table 1. Age, height, weight, body mass index and body surface area were all lower in the black group (all p<0.05) compared to the white group. Blood pressures were comparable between the black and white groups. The end-diastolic volume was lower in the black group (p<0.001) compared to the white group. The E/A ratio and E/e' ratio were higher in the black group (both p<0.05) compared to the white group, whereas heart rate and the LA/Ao ratio were similar in both groups. Total renin and eGFR

were higher in the black group (both p<0.05), whereas aldosterone, Nt-proBNP, total cholesterol-to-HDL cholesterol ratio and glucose were lower in the black group (all p<0.05) compared to the white group. Gamma-glutamyl-transferase, cotinine and the activity energy expenditure were higher in the black group (all p<0.05) compared to the white group.

The single regression analysis is shown in Supplementary Table 1. Briefly, the E/A ratio inversely correlated with aldosterone (r=–0.15; p=0.046) and prorenin (r=–0.21; p=0.006), whereas a positive correlation existed with the prorenin to total renin ratio (r=0.19; p=0.012) in the black group only. A positive correlation existed between the E/A ratio with Nt-proBNP (r=0.15; p=0.046) in the white group only. The LA/Ao ratio positively correlated with prorenin (r=0.22; p=0.004) and the prorenin-to-total renin ratio (r=0.20; p=0.009) in the white group only. A positive correlation existed between the end-diastolic volume with renin in the black (r=0.15; p=0.043) and the white (r=0.29; p<0.001) groups, as well as with Nt-proBNP (r=0.27; p<0.001) in the white group only. However, end-diastolic volume inversely correlated with aldosterone (r=–0.18; p=0.020) in the white group only.

Partially adjusted correlations of LV diastolic function markers with RAAS and Nt-proBNP are shown in Table 2. After adjusting for age, sex, body surface area and eGFR the correlations of LA/Ao ratio with prorenin (r=0.21; p=0.005) and prorenin-to-total renin ratio (r=0.19; p=0.013) in the white group remained. No correlations were evident between markers of LV diastolic function and Nt-proBNP in either group.

Table 3 presents the standard multiple regression analyses of LV diastolic function markers with RAAS and Nt-proBNP. We found positive associations of the E/A ratio with renin (Adj. R^2=0.131; β=0.16; p=0.042) and the LA/Ao ratio with prorenin (Adj. R^2=0.050; β=0.18; p=0.032) in the white group only, whereas the E/A ratio associated positively with prorenin (Adj. R^2=0.201; β=0.15; p=0.049) in the black group only. No associations were evident between markers of LV diastolic function and Nt-proBNP in either group. In addition, certain covariates contributed to the associations observed in the black and white groups. The E/A ratio associated positively with cotinine (β=0.40; p<0.001) in the black group only. However, we found an inverse association of the E/A ratio with age (β=–0.17; p=0.027), body surface area (β=–0.30; p=0.019), total cholesterol-to-HDL cholesterol ratio (β–0.28; p=0.004), and activity energy expenditure (β–0.16; p=0.050) in the white group only.

Table 1: Descriptive characteristics of the study population

	Black (n=170)	White (n=166)	p-value
Age (years)	24.3 ± 3.36	25.4 ± 2.85	**0.002**
Height (cm)	163 ± 8.41	172 ± 8.76	**<0.001**
Weight (kg)	65.8 ± 13.1	77.9 ± 20.5	**<0.001**
Body mass index (kg/m^2)	24.6 ± 5.34	26.0 ± 5.89	**0.021**
Body surface area (m^2)	1.72 ± 0.18	1.91 ± 0.27	**<0.001**
Cardiovascular measurements			
Systolic blood pressure (mmHg)	116 ± 9.07	117 ± 9.62	0.20
Diastolic blood pressure (mmHg)	69 ± 5.81	69 ± 6.00	0.84
Pulse pressure (mmHg)	47 ± 6.73	48 ± 7.66	0.070
Mean arterial pressure (mmHg)	88 ± 6.50	88 ± 6.67	0.53
Heart rate (beats/min)	76 ± 11.0	74 ± 11.4	0.052
LV end-diastolic volume (ml/m)	60.5 ± 12.3	68.2 ± 14.3	**<0.001**
E/A ratio	2.30 ± 0.61	2.08 ± 0.51	**0.001**
E/e' ratio	6.70 ± 1.34	6.28 ± 1.21	**0.003**
LA/Ao ratio	1.13 ± 0.14	1.12 ± 0.15	0.30
Biochemical variables			
Total renin (pg/ml)	794 (326; 1667)	693 (308; 1339)	**0.010**
Prorenin (pg/ml)	676 (23.6; 3239)	765 (34.4; 4413)	0.39
Prorenin/renin ratio	9.52 (3.05; 16.0)	10.4 (3.39; 20.4)	0.074
Aldosterone (pg/ml)	64.4 (19.0; 190)	102 (31.4; 536)	**<0.001**
Nt-proBNP (pg/ml)	28.3 (10.2; 102)	34.9 (11.3; 126)	**0.006**
Total cholesterol/HDL ratio	3.01 (1.89; 5.42)	3.48 (2.18; 6.25)	**<0.001**
Glucose (mmol/L)	3.85 ± 0.85	4.73 ± 0.83	**<0.001**
C-reactive protein (mg/L)	1.29 (0.13; 9.39)	1.02 (0.12; 11.8)	0.12
Estimated GFR (ml/min/1.73m^2)	118 ± 14.7	101 ± 13.4	**<0.001**
Lifestyle factors			
Gamma-glutamyl-transferase (U/L)	25.5 (10.5; 86.6)	18.0 (7.43; 55.4)	**<0.001**
Cotinine (ng/ml)	4.83 (1; 319)	2.44 (1; 248)	**0.004**
Activity energy expenditure (kCal/kg/day)	6.88 ± 2.79	5.95 ± 2.89	**0.004**

Values are arithmetic mean ± standard deviation, geometric mean (5th & 95th percentiles) or number (n) of participants. LV end-diastolic volume was corrected for body height. Activity energy expenditure was corrected for body weight. *Abbreviations:* E/A ratio, peak atrial early filling/late diastolic filling ratio; E/e' ratio, mitral peak velocity during early filling/early diastolic mitral annular velocity ratio; LA/Ao ratio, left atrium diameter to aortic root ratio; Nt-proBNP, amino-terminal prohormone B-type natriuretic peptide; Total cholesterol/HDL ratio, total cholesterol-to-high density lipoprotein cholesterol ratio; Estimated GFR, estimated glomerular filtration rate.

Table 2: Adjusted correlations of left ventricular diastolic function markers with the renin-angiotensin-aldosterone system and Nt-proBNP

	E/A ratio		E/e' ratio		LA/Ao ratio		LV EDV (ml/m)	
	Black (n=170)	**White** (n=166)	**Black** (n=170)	**White** (n=166)	**Black** (n=170)	**White** (n=166)	**Black** (n=170)	**White** (n=166)
Renin (pg/ml)	r=0.033; p=0.67	r=0.13; p=0.084	r=–0.057; p=0.46	r=–0.15; p=0.053	r=–0.006; p=0.94	r=0.027; p=0.73	r=0.13; p=0.076	r=0.13; p=0.084
Aldosterone (pg/ml)	r=–0.13; p=0.094	r=–0.069; p=0.38	r=–0.011; p=0.88	r=0.12; p=0.12	r=0.039; p=0.62	r=–0.11; p=0.14	r=0.072; p=0.35	r=–0.12; p=0.10
Prorenin (pg/ml)	r=0.13; p=0.079	r=–0.044; p=0.57	r=–0.093; p=0.23	r=0.000; p=0.99	r=–0.098; p=0.21	**r=0.21; p=0.005**	r=0.062; p=0.42	r=–0.039; p=0.62
Prorenin/renin ratio	r=0.11; p=0.16	r=–0.090; p=0.25	r=–0.070; p=0.36	r=0.037; p=0.63	r=–0.086; p=0.27	**r=0.19; p=0.013**	r=0.009; p=0.91	r=–0.080; p=0.31
Nt-proBNP (pg/ml)	r=0.008; p=0.92	r=0.066; p=0.40	r=–0.004; p=0.96	r=0.043; p=0.58	r=0.010; p=0.17	r=0.018; p=0.82	r=0.081; p=0.30	r=0.043; p=0.58

All models were adjusted for age, sex, body surface area (except end-diastolic volume), estimated glomerular filtration rate. LV end-diastolic volume was corrected for body height. *Abbreviations:* E/A ratio, peak early atrial filling/late diastolic filling ratio; E/e' ratio, mitral peak velocity during early filling/early diastolic mitral annular velocity ratio; LA/Ao ratio, left atrium to aortic root ratio; LV EDV, LV end-diastolic volume; Nt-proBNP, amino-terminal prohormone B-type natriuretic peptide.

Table 3: Standard multiple regression analysis of left ventricular diastolic function markers with the renin-angiotensin-aldosterone system and Nt-proBNP

	Black (n=170)			**White** (n=166)		
	E/A ratio					
	Adj. R^2	**Std. β (±95% CI)**	**p-value**	**Adj. R^2**	**Std. β (±95% CI)**	**p-value**
Renin (pg/ml)	0.182	0.060 (–0.091; 0.21)	0.42	**0.131**	**0.16 (0.006; 0.32)**	**0.042**
Aldosterone (pg/ml)	0.188	–0.099 (–0.30; 0.065)	0.20	0.113	–0.084 (–0.20; 0.061)	0.28
Prorenin (pg/ml)	**0.201**	**0.15 (0.000; 0.32)**	**0.049**	0.108	–0.042 (–0.18; 0.10)	0.60
Prorenin/renin ratio	0.195	0.13 (–0.026; 0.30)	0.099	0.115	–0.093 (–0.22; 0.058)	0.24
Nt-proBNP (pg/ml)	0.181	–0.049 (–0.23; 0.12)	0.56	0.110	0.070 (–0.10; 0.23)	0.46
	E/e' ratio					
Renin (pg/ml)	–0.030	–0.060 (–0.22; 0.10)	0.48	0.024	–0.16 (–0.34; 0.006)	0.058
Aldosterone (pg/ml)	–0.034	–0.001 (–0.20; 0.20)	0.99	0.019	0.13 (–0.023; 0.27)	0.098
Prorenin (pg/ml)	–0.026	–0.093 (–0.26; 0.082)	0.29	0.001	–0.038 (–0.20; 0.12)	0.65
Prorenin/renin ratio	–0.030	–0.068 (–0.32; 0.14)	0.44	0.000	0.003 (–0.20; 0.21)	0.96
Nt-proBNP (pg/ml)	–0.033	0.024 (–0.17; 0.22)	0.80	0.007	0.10 (–0.090; 0.28)	0.30
	LA/Ao ratio					
Renin (pg/ml)	–0.020	0.015 (–0.014; 0.17)	0.85	0.021	0.036 (–0.015; 0.22)	0.68
Aldosterone (pg/ml)	–0.019	0.036 (–0.15; 0.23)	0.67	0.026	–0.082 (–0.23; 0.078)	0.32
Prorenin (pg/ml)	–0.012	–0.096 (–0.25; 0.075)	0.27	**0.050**	**0.18 (0.016; 0.36)**	**0.032**
Prorenin/renin ratio	–0.012	–0.093 (–0.26; 0.078)	0.29	0.042	0.15 (–0.008; 0.32)	0.062
Nt-proBNP (pg/ml)	–0.011	0.10 (–0.077; 0.29)	0.25	0.025	0.092 (–0.10; 0.29)	0.35
	LV end-diastolic volume (ml/m)					
Renin (pg/ml)	0.098	0.15 (–0.006; 0.25)	0.061	0.330	0.12 (–0.014; 0.30)	0.073
Aldosterone (pg/ml)	0.078	0.058 (–0.10; 0.22)	0.47	0.326	–0.10 (–0.23; 0.025)	0.11
Prorenin (pg/ml)	0.077	0.044 (–0.10; 0.18)	0.60	0.317	–0.047 (–0.20; 0.99)	0.50
Prorenin/renin ratio	0.075	–0.016 (–0.15; 0.13)	0.84	0.322	–0.086 (–0.23; 0.054)	0.21
Nt-proBNP (pg/ml)	0.092	0.14 (–0.028; 0.28)	0.10	0.316	0.042 (–0.12; 0.21)	0.61

All models were adjusted for age, sex, body surface area (except end-diastolic volume), systolic blood pressure, heart rate, gamma-glutamyl-transferase, C-reactive protein, cotinine, total cholesterol to high density lipoprotein cholesterol ratio, estimated glomerular filtration rate and activity energy expenditure. LV end-diastolic volume was corrected for body height. *Abbreviations:* E/A ratio; (peak early atrial filling / late diastolic filling) ratio; E/e' ratio; (mitral peak velocity during early filling/early diastolic mitral annular velocity) ratio; LA/Ao ratio, left atrium to aortic root ratio.

Supplementary Table 1: Unadjusted correlations of left ventricular diastolic function markers with the renin-angiotensin-aldosterone system and Nt-proBNP

	E/A ratio		E/e' ratio		LA/Ao ratio		LV EDV (ml/m)	
	Black (n=170)	**White** (n=166)	**Black** (n=170)	**White** (n=166)	**Black** (n=170)	**White** (n=166)	**Black** (n=170)	**White** (n=166)
Age (years)	**r=–0.21; p=0.005**	r=–0.14; p=0.057	r=0.077; p=0.31	r=0.080; p=0.30	r=0.060; p=0.43	r=0.12; p=0.10	r=0.14; p=0.058	r=0.039; p=0.61
Height (cm)	r=0.030; p=0.69	r=–0.14; p=0.062	r=–0.14; p=0.068	r=–0.094; p=0.23	r=–0.076; p=0.32	**r=–0.16; p=0.032**	**r=0.25; p=0.001**	**r=0.35; p<0.001**
Weight (kg)	**r=–0.16; p=0.036**	**r=–0.30; p<0.001**	r=0.12; p=0.12	r=0.15; p=0.052	r=0.078; p=0.31	r=–0.009; p=0.90	**r=0.24; p=0.001**	**r=0.58; p<0.001**
Body mass index (kg/m^2)	**r=–0.15; p=0.041**	**r=–0.27; p<0.001**	**r=0.18; p=0.017**	**r=0.22; p=0.004**	r=0.11; p=0.12	r=0.079; p=0.31	r=0.11; p=0.13	**r=0.51; p<0.001**
Body surface area (m^2)	**r=–0.15; p=0.050**	**r=–0.30; p<0.001**	r=0.078; p=0.31	r=0.10; p=0.17	r=0.059; p=0.44	r=–0.039; p=0.62	**r=0.29; p<0.001**	**r=0.59; p<0.001**
SBP (mmHg)	r=–0.004; p=0.96	**r=–0.17; p=0.022**	r=0.10; p=0.17	r=0.14; p=0.072	r=0.049; p=0.52	r=–0.011; p=0.88	**r=0.24; p=0.001**	**r=0.46; p<0.001**
DBP (mmHg)	r=–0.13; p=0.077	**r=–0.26; p<0.001**	r=0.064; p=0.41	r=0.095; p=0.22	r=0.071; p=0.35	r=–0.12; p=0.12	r=0.14; p=0.065	r=0.068; p=0.38
Heart rate (beats/min)	**r=–0.25; p=0.001**	**r=–0.21; p=0.006**	r=0.019; p=0.80	r=0.008; p=0.91	r=–0.009; p=0.91	**r=–0.19; p=0.011**	**r=–0.30; p<0.001**	**r=–0.22; p=0.003**
Renin (pg/ml)	r=0.046; p=0.55	r=0.023; p=0.77	r=–0.060; p=0.43	r=–0.12; p=0.098	r=–0.019; p=0.80	r=0.023; p=0.76	**r=0.15; p=0.043**	**r=0.29; p<0.001**
Aldosterone (pg/ml)	**r=–0.15; p=0.046**	r=0.000; p=0.99	r=0.025; p=0.75	r=0.091; p=0.24	r=0.059; p=0.44	r=–0.12; p=0.11	r=0.033; p=0.67	**r=–0.18; p=0.020**
Prorenin (pg/ml)	**r=–0.21; p=0.006**	r=–0.092; p=0.24	r=–0.11; p=0.12	r=0.006; p=0.93	r=–0.12; p=0.094	**r=0.22; p=0.004**	r=0.055; p=0.47	r=0.066; p=0.40
Prorenin/renin ratio	**r=0.19; p=0.012**	r=–0.10; p=0.18	r=–0.094; p=0.22	r=0.040; p=0.60	r=–0.11; p=0.14	**r=0.20; p=0.009**	r=–0.003; p=0.97	r=–0.037; p=0.63
Nt-proBNP (pg/ml)	r=–0.074; p=0.34	**r=0.15; p=0.046**	r=0.028; p=0.71	r=0.055; p=0.48	r=0.14; p=0.055	r=0.041; p=0.60	r=–0.022; p=0.77	**r=–0.27; p<0.001**
Total cholesterol/HDL ratio	r=–0.14 p=0.055	**r=–0.29; p<0.001**	r=0.093; p=0.22	r=0.015; p=0.85	r=0.048; p=0.53	r=–0.12; p=0.099	r=–0.062; p=0.42	**r=0.38; p<0.001**
Glucose (mmol/L)	r=–0.14; p=0.070	r=–0.15; p=0.055	r=0.12; p=0.10	r=–0.031; p=0.69	r=0.024; p=0.75	r=–0.059; p=0.45	r=0.058; p=0.45	**r=0.17; p=0.023**
C-reactive protein (mg/L)	r=–0.043; p=0.57	r=–0.094; p=0.22	r=0.009; p=0.90	r=0.052; p=0.50	r=0.059; p=0.44	r=–0.13; p=0.086	r=–0.078; p=0.31	r=0.065; p=0.40
eGFR (ml/min/1.73m^2)	r=0.036; p=0.64	r=–0.004; p=0.96	r=–0.008; p=0.91	r=0.041; p=0.60	r=–0.012; p=0.87	r=0.14; p=0.063	r=–0.070; p=0.36	**r=0.15; p=0.046**
GGT (U/L)	r=–0.039; p=0.61	r=–0.11; p=0.14	r=0.081; p=0.29	r=0.036; p=0.64	r=–0.064; p=0.40	r=–0.071; p=0.36	r=0.10; p=0.16	**r=0.32; p<0.001**
Cotinine(ng/ml)	**r=0.39; p<0.001**	r=–0.097; p=0.21	r=0.029; p=0.71	r=–0.048; p=0.53	r=0.040; p=0.60	r=–0.025; p=0.74	r=0.073; p=0.34	**r=0.18; p=0.020**
Self-reported smoking	**r=0.35; p<0.001**	r=–0.060; p=0.44	r=–0.066; p=0.93	r=0.062; p=0.43	r=0.029; p=0.70	r=–0.080; p=0.30	r=0.10; p=0.18	r=0.11; p=0.12
Self-reported alcohol use	**r=0.23; p=0.003**	r=–0.033; p=0.67	r=0.016; p=0.83	r=–0.032; p=0.67	r=0.007; p=0.93	r=–0.061; p=0.43	r=0.020; p=0.80	r=0.13; p=0.096

LV end-diastolic volume was corrected for body height. *Abbreviations:* E/A ratio, peak early atrial filling/late diastolic filling ratio; E/e' ratio, mitral peak velocity during early filling/early diastolic mitral annular velocity ratio; LA/Ao ratio, left atrium to aortic root ratio; LV EDV, LV end-diastolic volume; Nt-proBNP, amino-terminal prohormone B-type natriuretic peptide; Total cholesterol/HDL ratio, total cholesterol-to-high density lipoprotein cholesterol ratio; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyl-transferase.

Discussion

This study aimed to compare the cardiovascular characteristics along with the RAAS and Nt-proBNP levels, and explored the associations of LV diastolic function with the RAAS and Nt-proBNP in young, apparently healthy black and white South Africans. We found positive associations of the E/A ratio with prorenin and renin in the black and white groups. Additionally, there was a positive association between the LA/Ao ratio and prorenin in the white group only.

The positive association of the E/A ratio with prorenin and renin in the black and white groups, along with a positive association between the LA/Ao ratio with prorenin in the white group, may indicate that an increase in prorenin and renin levels may be involved in the early development of volume overload. An increase in prorenin and renin levels in the glomeruli is associated with renal tissue RAAS activation and potential vascular damage.[11] Prolonged RAAS activation, due to increased renin levels, may promote volume overload and vasoconstriction, followed by an increase in diastolic filling pressure.[1] Furthermore, it was suggested that the RAAS may contribute to myocardial and vascular inflammation, thus leading to myocardial fibrosis and the development of diastolic dysfunction.[1] Therefore, our association of LV diastolic function markers with components of the RAAS may indicate potential future and early onset diastolic dysfunction in these groups.

However, our study population is young and healthy, therefore our findings may suggest that an increase in prorenin in both groups and renin in the white group may lead to higher E/A and LA/Ao ratios, via the activation of the downstream components of the RAAS including angiotensin II and aldosterone. Angiotensin II is associated with endothelial dysfunction,[30] while aldosterone is associated with vascular inflammation.[31] Additionally, prorenin can also contribute to fibrosis and inflammation independent of angiotensin II, by stimulating the prorenin receptor.[32] Thus, an increase in prorenin and renin may contribute to a higher E/A ratio due to an increase in filling pressures, as well as a higher LA/Ao ratio which may indicate left atrial enlargement and an increase in left atrial pressure, thus suggesting potential diastolic dysfunction.[33,34] A higher E/A ratio relates to diastolic dysfunction in elderly and diseased individuals, as it indicates restrictive filling patterns.[35,36] However, in young, healthy adults, a higher E/A ratio is indicative of normal diastolic filling. Young, healthy adults tend to have a decrease in LV minimal diastolic pressure due to rapid LV relaxation, thus causing a higher E/A ratio.[37] Our findings may indicate that the young black and white participants have normal diastolic filling patterns, however potential changes in the RAAS may contribute to premature elevations of the E/A and LA/Ao ratios. The premature elevations in the E/A and LA/Ao ratio may suggest that both groups are prone to develop early cardiovascular alterations, such as hypertrophy, impaired myocardial relaxation and left atrial volume loading, which may lead to diastolic dysfunction in later life.

In addition, certain covariates contributed to the associations observed in the black and white groups. For instance, cotinine, a reliable metabolite of nicotine which defines smoking status,[38] may positively contribute to the link between the E/A ratio with prorenin in the black group. Although not excessively high, the cotinine values in the black group were higher compared to the white group. This may suggest that smoking, as a lifestyle risk factor, could be mediating the relationship observed between the E/A ratio and prorenin in the black group of this study.[39] However, age, body surface area, total cholesterol-to-HDL cholesterol ratio and activity energy expenditure may contribute to the link between the E/A ratio with renin in the white group. Body mass index is used as the current classification system for obesity,[40] which is known to associate with chronic conditions such as hypertension, high blood cholesterol levels and decreased HDL-cholesterol levels in both men and women from different ethnic groups.[41,42] HDL-cholesterol is also a strong predictor for coronary heart disease and atherosclerosis in both men and women,[43] thus the total cholesterol-to-HDL cholesterol ratio is commonly used as a reliable indicator to predict cardiovascular diseases.[43] Regular physical activity (measured by activity energy expenditure) has plenty of beneficial effects, such as prevention and treatment of atherosclerotic risk factors, which include hypertension, low HDL-concentrations and obesity.[44] This may suggest that ageing and lifestyle factors, such as obesity, could mediate the relationship between the E/A ratio and renin in the white population of this study.

We acknowledge several strengths and limitations in this study. To the best of our knowledge this is the first study conducted to show the association between LV diastolic function markers with the RAAS and Nt-proBNP in young black and white South Africans (aged between 20-30 years) without overt cardiovascular disease. All the measurements were performed in a controlled environment and we used a sizeable study group to ensure accurate results. In our study, the data for tri-plane three-dimensional left atrial volume was not obtained, which could better discriminate left atrial dilation or overload. Furthermore, we did not evaluate the influence of angiotensin II or other related RAAS enzymes, such as chymase enzyme or angiotensin-converting enzyme, on LV diastolic function markers. Therefore, we can only speculate on a potential mechanism for our results. Further studies are encouraged to incorporate these additional measures to confirm our results.

In conclusion, we found that diastolic function markers associated adversely with components of the RAAS, in both black and white groups. Our findings may indicate that a higher E/A ratio in both the black and white groups as well as a higher LA/Ao ratio in the white group, may be attributed to potential changes in the RAAS. This may suggest that both groups are prone to premature RAAS modifications, probably due to lifestyle risk factors, which may lead to future diastolic dysfunction.

Acknowledgements

The authors are grateful to the participants who took part in this study, the African-PREDICT research team, the field workers and supporting staff of the Hypertension in Africa Research Team (HART), and the North-West University campus. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF does not accept any liability in this regard.

References

1. Sciarretta S, Paneni F, Palano F, Chin D, Tocci G, Rubattu S, Volpe M. Role of the renin–angiotensin–aldosterone system and inflammatory processes in the development and progression of diastolic dysfunction. *Clinical Science.* 2009;116(6):467-477.

2. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: part II. *Circulation.* 2002;105(12):1503-1508.

3. Aurigemma GP, Gottdiener JS, Shemanski L, Gardin J, Kitzman D. Predictive value of systolic and diastolic function for incident congestive heart failure in the elderly: the cardiovascular health study. *Journal of the American College of Cardiology.* 2001;37(4):1042-1048.

4. Redfield MM, Jacobsen SJ, Burnett Jr JC, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *Journal of the American Medical Association.* 2003;289(2):194-202.

5. Chinnaiyan KM, Alexander D, McCullough PA. Role of angiotensin II in the evolution of diastolic heart failure. *The Journal of Clinical Hypertension.* 2005;7(12):740-747.

6. Dzau VJ. Tissue renin-angiotensin system in myocardial hypertrophy and failure. *Archives of Internal Medicine.* 1993;153(8):937-942.

7. Volpe M, Savoia C, De Paolis P, Ostrowska B, Tarasi D, Rubattu S. The renin-angiotensin system as a risk factor and therapeutic target for cardiovascular and renal disease. *Journal of the American Society of Nephrology.* 2002;13(suppl 3):S173-S178.

8. Ruggenenti P, Cravedi P, Remuzzi G. The RAAS in the pathogenesis and treatment of diabetic nephropathy. *Nature Reviews Nephrology.* 2010;6(6):319-330.

9. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *American Journal of Physiology-Cell Physiology.* 2007;292(1):C82-C97.

10. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension.* 2000;35(6):1270-1277.

11. Ichihara A, Kaneshiro Y, Takemitsu T, Sakoda M, Nakagawa T, Nishiyama A, Kawachi H, Shimizu F, Inagami T. Contribution of nonproteolytically activated prorenin in glomeruli to hypertensive renal damage. *Journal of the American Society of Nephrology.* 2006;17(9):2495-2503.

12. Yoshimura M, Kawai M. Synergistic inhibitory effect of angiotensin II receptor blocker and thiazide diuretic on the tissue renin-angiotensin-aldosterone system. *Journal of the Renin-Angiotensin-Aldosterone System.* 2010;11(2):124.

13. Pemberton CJ, Johnson ML, Yandle TG, Espiner EA. Deconvolution analysis of cardiac natriuretic peptides during acute volume overload. *Hypertension.* 2000;36(3):355-359.

14. de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. *The Lancet.* 2003;362(9380):316-322.

15. Tschöpe C, Kašner M, Westermann D, Gaub R, Poller WC, Schultheiss H-P. The role of NT-proBNP in the diagnostics of isolated diastolic dysfunction: correlation with echocardiographic and invasive measurements. *European Heart Journal.* 2005;26(21):2277-2284.

16. Brutsaert D. Diagnosing primary diastolic heart failure. *European Heart Journal* 2000;21(2): 94–96.

17. Lubien E, DeMaria A, Krishnaswamy P, Clopton P, Koon J, Kazanegra R, Gardetto N, Wanner E, Maisel AS. Utility of B-natriuretic peptide in detecting diastolic dysfunction. *Circulation.* 2002;105(5):595-601.

18. Sahn D. The Committee on M-mode Standardization of the American Society of Echocardiography. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation.* 1978;58:1072-1083.

19. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise J. Recommendations for chamber quantification. *European Heart Journal Cardiovascular Imaging.* 2006;7(2):79-108.

20. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA, Evangelista A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Journal of the American Society of Echocardiography.* 2009;22(2):107-133.

21. Yu CM, Sanderson JE, Marwick TH, Oh JK. Tissue Doppler Imaging: A New Prognosticator for Cardiovascular Diseases. *Journal of the American College of Cardiology.* 2007;49(19):1903-1914.

22. Nagueh SF, Sun H, Kopelen HA, Middleton KJ, Khoury DS. Hemodynamic determinants of the mitral annulus diastolic velocities by tissue Doppler. *Journal of the American College of Cardiology.* 2001;37(1):278-285.

23. Appleton CP, Jensen JL, Hatle LK, Oh JK. Doppler evaluation of left and right ventricular diastolic function: a technical guide for obtaining optimal flow velocity recordings. *Journal of the American Society of Echocardiography.* 1997;10(3):271-292.

24. Ommen SR, Nishimura R, Appleton CP, Miller FA, Oh KJ, Redfield MM, Tajik AJ. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures. *Circulation.* 2000;102(15):1788-1794.

25. Evans N. Diagnosis of patent ductus arteriosus in the preterm newborn. *Archives of Disease in Childhood.* 1993;68(1 Spec No):58-61.

26. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *European Heart Journal Cardiovascular Imaging.* 2015;16(3):233-271.

27. De Simone G, Devereux RB, Ganau A, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T. Estimation of left ventricular chamber and stroke volume by limited M-mode echocardiography and validation by two-dimensional and Doppler echocardiography. *American Journal of Cardiology.* 1996;78(7):801-807.

28. Marfell-Jones MJ, Stewart A, de Ridder J. 2012. International Standards for Anthropometric Assessment. Wellington, New Zealand: International Society for the Advancement of Kinanthropometry.

29. Mosteller R. Simplified calculation of body-surface area. *The New England Journal of medicine.* 1987;317(17):1098.

30. Weir MR, Dzau VJ. The renin-angiotensin-aldosterone system: a specific target for hypertension management. *American Journal of Hypertension.* 1999;12(S9):205S-213S.

31. Rocha R, Rudolph AE, Frierdich GE, Nachowiak DA, Kekec BK, Blomme EAG, McMahon EG, Delyani JA. Aldosterone induces a vascular inflammatory phenotype in the rat heart. *American Journal of Physiology-Heart and Circulatory Physiology.* 2002;283(5):H1802-H1810.

32. Satofuka S, Ichihara A, Nagai N, Yamashiro K, Koto T, Shinoda H, Noda K, Ozawa Y, Inoue M, Tsubota K. Suppression of ocular inflammation in endotoxin-induced uveitis by inhibiting nonproteolytic activation of prorenin. *Investigative Ophthalmology & Visual Science.* 2006;47(6):2686-2692.

33. Brown OR, Harrison DC, Popp RL. An improved method for echographic detection of left atrial enlargement. *Circulation.* 1974;50(1):58-64.

34. Pritchett A, Mahoney D, Jacobsen S, Rodeheffer R, Karon B, Redfield M. Diastolic dysfunction and left atrial volume: a population-based study. *Journal of the American College of Cardiology.* 2005;45(1):87.

35. Parthenakis FI, Kanoupakis EM, Kochiadakis GE, Skalidis EI, Mezilis NE, Simantirakis EN, Kanakaraki MK, Vardas PE. Left ventricular diastolic filling pattern predicts cardiopulmonary determinants of functional capacity in patients with congestive heart failure. *American Heart Journal.* 2000;140(2):338-344.

36. Bella JN, Palmieri V, Roman MJ, Liu JE, Welty TK, Lee ET, Fabsitz RR, Howard BV, Devereux RB. Mitral ratio of peak early to late diastolic filling velocity as a predictor of mortality in middle-aged and elderly adults. *Circulation.* 2002;105(16):1928-1933.

37. Nishimura RA, Tajik AJ. Evaluation of diastolic filling of left ventricle in health and disease: Doppler echocardiography is the clinician's Rosetta Stone. *Journal of the American College of Cardiology.* 1997;30(1):8-18.

38. Whincup PH, Gilg JA, Emberson JR, Jarvis MJ, Feyerabend C, Bryant A, Walker M, Cook DG. Passive smoking and risk of coronary heart disease and stroke: prospective study with cotinine measurement. *British Medical Journal.* 2004;329(7459):200-205.

39. Gu L, Pandey V, Geenen DL, Chowdhury SA, Piano MR. Cigarette smoke-induced left ventricular remodelling is associated with activation of mitogen-activated protein kinases. *European Journal of Heart failure.* 2008;10(11):1057-1064.

40. Prentice AM, Jebb SA. Beyond body mass index. *Obesity Reviews.* 2001;2(3):141-147.

41. Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E, Ernst ND, Horan M. Body Mass Index and the Prevalence of Hypertension and Dyslipidemia. 2000. *Obesity Research*. 2000;8:605–619.

42. Kennedy L, Dickstein K, Anker S, Kristianson K, Willenheimer R, Group OS. The prognostic importance of body mass index after complicated myocardial infarction. *Journal of the American College of Cardiology.* 2005;45(1):156.

43. Castelli WP, Anderson K. A population at risk: Prevalence of high cholesterol levels in hypertensive patients in the framingham study. *The American Journal of Medicine.* 1986;80(2, Supplement 1):23-32.

44. Thompson PD, Buchner D, Piña IL, Balady GJ, Williams MA, Marcus BH, Berra K, Blair SN, Costa F, Franklin B. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. *Circulation.* 2003;107(24):3109-3116.

CHAPTER 4

Summary of main findings and final conclusions

4.1 Introduction

In this conclusive chapter, a summary of the main findings from the research article as well as final conclusions are stated. The results from the research article are also explained and compared to relevant literature. This is followed by recommendations for future research regarding the links of left ventricular (LV) diastolic function with the renin-angiotensin-aldosterone system (RAAS) and amino-terminal prohormone B-type natriuretic peptide (Nt-proBNP).

4.2 Summary of main findings

In this sub-study, we aimed to compare the cardiovascular characteristics (especially related to LV diastolic function) along with the RAAS and Nt-proBNP levels, as well as explore the associations of LV diastolic function with the RAAS and Nt-proBNP in young apparently healthy black and white South Africans.

We hypothesized that, in a study population of young black and white men and women (aged between 20–30 years old):

i. (a) Black individuals have higher E/A, E/e' and LA/Ao ratios, but a lower LV end-diastolic volume compared to their white counterparts.

Our study showed that the E/A and E/e' ratios were higher in the black group, whereas the end-diastolic volume was lower in the black group compared to their white counterparts. However, the LA/Ao ratio were comparable between both the black and white groups. Therefore, the first hypothesis is partially accepted.

(b) Black individuals have lower total renin and aldosterone levels, but higher prorenin levels compared to their white counterparts.

The second hypothesis is partially accepted. Black individuals had lower aldosterone and higher prorenin levels compared to their white counterparts. However, black individuals had higher renin levels compared to the white group.

(c) Black individuals have higher NT-proBNP levels compared to their white counterparts.

Black individuals had lower Nt-proBNP levels in our comparative study than their white counterparts, therefore we reject the third hypothesis.

ii. (a) LV diastolic function makers (E/A ratio) negatively associates with renin, prorenin, and aldosterone.

We found positive associations of the E/A ratio in the black and white groups, respectively, along with a positive association between the LA/Ao ratio and prorenin in the white group only. Therefore, the first hypothesis is rejected.

(b) LV end-diastolic volume will positively associate with Nt-proBNP, a reliable marker of cardiac overload, whereas the E/A and LA/Ao ratios shows no association with Nt-proBNP, since these ratios reflect LV filling pressure and left atrial dilation.

We reject this part of our second hypothesis, as there were no associations evident between LV diastolic function markers with aldosterone in either group. Furthermore, no associations were evident between the LV end-diastolic volume, E/A ratio or LA/Ao ratio with Nt-proBNP in either group.

4.3 Comparison to relevant literature

When comparing the results of this study population with results from other study population groups, it is evident that certain findings are confirmed while others contradict previous observations. The Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) sub-study and two other studies that included black African Caribbeans and white Europeans, found that the African Caribbeans had a lower E/A ratio and a higher E/e' ratio compared to the white Europeans.[1-3] However, the ASCOT sub-study was performed on either untreated or treated hypertensive men (aged ≤55 years) with at least 3 cardiovascular risk factors. According to Opie and Seedat,[4] the black population have a higher prevalence of low renin levels compared to the white population. The difference in renin levels may be attributed to the environmental origin of the participants.[5] A study done by Rayner *et al.,* found that black individuals had low renin and aldosterone levels compared to their white counterparts. This may also suggest a salt-retaining tendency in black individuals.[6] The South African study regarding the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC) sub-study found that black South African men exhibit higher Nt-proBNP levels compared to the white group.[7] However, this sub-study involved hypertensive men (aged between 39-41 years). Our study population consisted of young, apparently healthy black and white South Africans, without overt cardiovascular disease, which may explain the different results we obtained, when compared to results of other studies.

Our study showed a positive association of the E/A ratio with prorenin in the black group, as well as with renin in the white group only. Additionally, the LA/Ao ratio associated positively with prorenin in the white group only. However, there is little or no information available about these specific LV diastolic function markers and RAAS, thus a comparison to relevant literature cannot be made. A study done by Yamamoto *et al.,* showed that the RAAS and endothelin system contributes to the development of excessive hypertrophy and ventricular fibrosis in rats with established hypertensive heart disease, thus promoting diastolic heart failure.[8] Furthermore, activation of the RAAS also contributes to LV structural alterations and impaired LV relaxation, which is linked to LV diastolic dysfunction, independently of the endothelin system.[8] In addition, findings from a study done on individuals (aged ≥65 years) with diastolic heart failure suggested that renin-angiotensin inhibition may be beneficial.[9] This study's findings showed that renin-angiotensin inhibition, with angiotensin converting enzyme inhibitors or angiotensin receptor blockers, associated with a significant reduction in all-cause mortality in older individuals with cardiac failure and chronic kidney disease.[9] Therefore, we can only speculate that a link exists between the RAAS and LV diastolic function markers.

4.4 Discussion of main findings

Our results indicated adverse associations of diastolic function markers with components of the RAAS (prorenin and renin), in both black and white young individuals. Our findings may suggest that the young black and white participants have normal diastolic filling patterns,[10] however potential changes in the RAAS may contribute to premature elevations of the E/A and LA/Ao ratios. The premature elevations in the E/A and LA/Ao ratio may suggest that both groups are prone to develop early cardiovascular alterations, such as hypertrophy, impaired myocardial relaxation and left atrial volume loading, which may lead to diastolic dysfunction in later life. The positive association of the E/A ratio with prorenin and renin in the black and white groups, respectively, along with a positive association between the LA/Ao ratio with prorenin in the white group, may indicate that an increase in prorenin and renin levels may be involved in the early development of volume overload. Although our study population is young and healthy, our findings may suggest that an increase in prorenin in both groups and renin in the white group may lead to higher E/A and LA/Ao ratios, via the activation of the downstream components of the RAAS including angiotensin II and aldosterone. However, we did not find any associations with aldosterone in either group. Thus, an increase in prorenin and renin may contribute to a higher E/A ratio as a result of increased LV filling pressures.[11,12] The higher LA/Ao ratio may also be attributed to the increase in prorenin, which may indicate left atrial enlargement and an increase in left atrial pressure, thus suggesting potential diastolic dysfunction.[11,12]

In addition, certain covariates contributed to the associations observed in the black and white groups. For instance, cotinine, a reliable metabolite of nicotine which defines smoking status,[13] may positively contribute to the link between the E/A ratio with prorenin in the black group only. Although not excessively high, the cotinine values in the black group were higher compared to the white group. Our findings may suggest that smoking, as a lifestyle risk factor, could be mediating the relationship observed between the E/A ratio and prorenin in the black participants.[14] However, age, body surface area, total cholesterol-to-high density lipoprotein (HDL) cholesterol ratio and activity energy expenditure may contribute to the link between the E/A ratio with renin in the white group only. Body mass index, total cholesterol-to-HDL cholesterol and activity energy expenditure are lifestyle factors that are frequently used as reliable indicators to identify obesity, which is known to associate with chronic conditions such as hypertension, high blood cholesterol levels and low HDL-cholesterol levels.[15-18] Thus, our findings may suggest that ageing and lifestyle factors, such as obesity, could mediate the relationship between the E/A ratio and renin in the white population of this study.

However, the lack of associations of LV diastolic function markers with Nt-proBNP may indicate that there may be other factors contributing to LV diastolic dysfunction and volume overload that we did not take into consideration. Although these findings may not be representative of the whole South African population, it contributes to the knowledge of the development of cardiovascular diseases in South Africa. Therefore, further studies are encouraged to investigate other potential and significant contributing factors.

4.5 Limitations, chance and confounders

It is essential to reflect on some of the factors that might have affected the results of this sub-study. With regards to methodology, there were various factors that could have influenced the outcome of this sub-study. All participants were recruited from Potchefstroom and the surrounding area in the North-West Province, which included both urban and rural individuals. Therefore, the results of the present sub-study cannot be representative of the general South African population, however these groups can reveal the general health of a specific geographical area in South Africa.

After performing appropriate interaction tests, it was found that ethnicity interacted significantly with the RAAS (aldosterone and prorenin). Therefore, our study population was divided into black and white groups. The number of participants in each group differed, with the black group being slightly larger than the white group. The presence of left atrial dilation, as a result of LV diastolic dysfunction, could not be assessed since the data for triplane three-dimensional left atrial volume was not available. The possibility of chance, regarding our results, should also be taken into consideration, especially since we tested multiple hypothesis.

Aside from performing a standard multiple regression analysis, there may be a possibility that certain associations reported may be due to chance. We adjusted for certain covariates such as age, sex, body surface area (except end-diastolic volume), systolic blood pressure, heart rate, gamma-glutamyl-transferase, C-reactive protein, cotinine, total cholesterol to high density lipoprotein cholesterol ratio, estimated glomerular filtration rate and activity energy expenditure. Consequently, these adjustments may have caused an over- or underestimation, of the associations observed by markers of LV diastolic function with the RAAS and Nt-proBNP. However, the associations were persistent and the results remained after rigorous multiple adjustments.

4.6 Recommendations

- Comparatively larger study populations are needed to explore the link of LV diastolic function markers with the RAAS and Nt-proBNP in a young study population.
- The data for tri-plane three-dimensional left atrial volume could possibly better discriminate between left atrial enlargement or volume overload. Tri-plane tissue Doppler imaging allows simultaneous acquisition of tissue Doppler imaging from all LV segments, and can additionally calculate three-dimensional volumes along with LV ejection fraction.[19] This method is also used to evaluate several cardiac valve complications.[20]
- Furthermore, the influence of angiotensin II (Ang II) and other related RAAS enzymes, such as chymase enzyme and angiotensin-converting enzyme (ACE), on LV diastolic function should be evaluated. Ang II, produced by chymase and ACE, plays an essential role in blood pressure regulation and salt and water regulation via the RAAS cascade. However, elevated levels of Ang II may lead to alterations in the structure and function of blood vessels,[21] and may therefore contribute to the development of hypertension.[21,22]
- Further studies on young apparently healthy individuals are encouraged to incorporate these additional measures to confirm our results.

4.7 Conclusion

In conclusion, our study indicates that in young apparently healthy individuals (aged 20 to 30 years), LV diastolic function markers (such as the E/A and LA/Ao ratios) associated with the RAAS (total renin and prorenin). The different results between the black and white groups may be attributed to the resultant effect of certain lifestyle behaviors, such as smoking, obesity and physical inactivity. These results are particularly relevant in South Africa as a developing country with a significant increase in non-communicable diseases, especially cardiovascular disease. The present sub-study provides relevant knowledge on potential premature adverse changes of the RAAS, due to certain lifestyle factors, and the resultant effects on LV diastolic function.

4.8 References

1. Sharp A, Tapp R, Francis DP, Thom SA, Hughes AD, Stanton AV, Zambanini A, Chaturvedi N, Byrd S. Poulter, NR. Ethnicity and left ventricular diastolic function in hypertension: an ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial) substudy. *Journal of the American College of Cardiology.* 2008;52(12):1015-1021.

2. Rittoo D, Monaghan M, Sadiq T, Nichols A, Richardson P. Echocardiographic and Doppler evaluation of left ventricular hypertrophy and diastolic function in black and white hypertensive patients. *Journal of Human Hypertension.* 1990;4(2):113-115.

3. Mayet J, Shahi M, Foale RA, Poulter NR, Sever PS, Thom SAM. Racial differences in cardiac structure and function in essential hypertension. *British Medical Journal.* 1994;308(6935):1011-1014.

4. Opie LH, Seedat YK. Hypertension in sub-Saharan African populations. *Circulation.* 2005;112(23):3562-3568.

5. Hoosen S, Seedat YK, Bhigjee AI, Neerahoo RM. A study of urinary sodium and potassium excretion rates among urban and rural Zulus and Indians. *Journal of Hypertension.* 1985;3(4):351-358.

6. Rayner BL, Myers JE, Opie LH, Trinder YA, Davidson JS. Screening for primary aldosteronism-normal ranges for aldosterone and renin in three South African population groups. *South African Medical Journal.* 2001;91(7):594-599.

7. van Rooyen J, Poglitsch M, Huisman H, Mels CMC, Kruger R, Malan L, Botha S, Lammertyn L, Gafane L, Schutte AE. Quantification of systemic renin-angiotensin system peptides of hypertensive black and white African men established from the RAS-Fingerprint®. *Journal of the Renin-Angiotensin-Aldosterone System.* 2016;17(4):1-7.

8. Yamamoto K, Masuyama T, Sakata Y, Mano T, Nishikawa N, Kondo H, Akehi N, Kuzuya T, Miwa T, Hori M. Roles of renin–angiotensin and endothelin systems in development of diastolic heart failure in hypertensive hearts. *Cardiovascular Research.* 2000;47(2):274-283.

9. Ahmed A, Rich MW, Zile M, et al. Renin-angiotensin inhibition in diastolic heart failure and chronic kidney disease. *The American Journal of Medicine.* 2013;126(2):150-161.

10. Nishimura RA, Tajik AJ. Evaluation of diastolic filling of left ventricle in health and disease: Doppler echocardiography is the clinician's Rosetta Stone. *Journal of the American College of Cardiology.* 1997;30(1):8-18.

11. Brown OR, Harrison DC, Popp RL. An improved method for echographic detection of left atrial enlargement. *Circulation.* 1974;50(1):58-64.

12. Pritchett A, Mahoney D, Jacobsen S, Rodeheffer R, Karon B, Redfield M. Diastolic dysfunction and left atrial volume: a population-based study. *Journal of the American College of Cardiology.* 2005;45(1):87-92.

13. Whincup PH, Gilg JA, Emberson JR, Jarvis MJ, Feyerabend C, Bryant A, Walker M, Cook DG. Passive smoking and risk of coronary heart disease and stroke: prospective study with cotinine measurement. *British Medical Journal.* 2004;329(7459):200-205.

14. Gu L, Pandey V, Geenen DL, Chowdhury SA, Piano MR. Cigarette smoke-induced left ventricular remodeling is associated with activation of mitogen-activated protein kinases. *European Journal of Heart Failure.* 2008;10(11):1057-1064.

15. Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E, Ernst ND, Horan M. Body Mass Index and the Prevalence of Hypertension and Dyslipidemia. *Obesity Research*. 2000;8:605–619.

16. Kennedy L, Dickstein K, Anker S, Kristianson K, Willenheimer R, Group OS. The prognostic importance of body mass index after complicated myocardial infarction. *Journal of the American College of Cardiology.* 2005;45(1):156.

17. Castelli WP, Anderson K. A population at risk: Prevalence of high cholesterol levels in hypertensive patients in the framingham study. *The American Journal of Medicine.* 1986;80(2, Supplement 1):23-32.

18. Thompson PD, Buchner D, Piña IL, Balady GJ, Williams MA, Marcus BH, Berra K, Blair SN, Costa F, Franklin B. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. *Circulation.* 2003;107(24):3109-3116.

19. Van de Veire NR, Yu C-M, Ajmone-Marsan N, Bleeker GB, Ypenburg C, De Sutter J, Zhang Q, Fung JWH, Chan JYS, Holman ER. Triplane tissue Doppler imaging: a novel three-dimensional imaging modality that predicts reverse left ventricular remodeling after cardiac resynchronisation therapy. *Heart.* 2008;94(3):e9

20. Leung KE, Bosch JG. Automated border detection in three-dimensional echocardiography: principles and promises. *European Journal of Echocardiography.* 2010;11(2):97-108.

21. Weir MR, Dzau VJ. The renin-angiotensin-aldosterone system: a specific target for hypertension management. *American Journal of Hypertension.* 1999;12(S9):205S-213S.

22. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *Journal of Clinical Investigation.* 1996;97(8):1916.

APPENDICES

Appendix A: Ethics approval for African-PREDICT study

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Faculty of Health Sciences
Health Sciences Ethics Office for Research, Training and Support
Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

6 September 2016

Prof AE Schutte
HART

Dear Prof Schutte

APPROVAL OF YOUR AMENDMENT REQUEST BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00001-12-A1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: African Prospective Study for the Early Detection and identification of Cardiovascular Disease and Hypertension (African-PREDICT Study)

Study leader/supervisor: Prof AE Schutte

You are kindly informed that your application to amend the single study was reviewed at the meeting held on 11/05/2016 of the HREC, Faculty of Health Sciences, and was approved on 06/09/2016.

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Dr Wayne Towers
HREC Chairperson

Prof Minrie Greeff
Ethics Office Head

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Letter templates\HREC Approval Letter - June 2016.docm
15 June 2016

File reference: 9.1.5.3

Appendix B: Ethics approval for the MHSc sub-study

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Faculty of Health Sciences
Health Sciences Ethics Office for Research, Training and Support
Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

31 May 2017

Prof R Kruger
Cardiovascular Physiology

Dear Prof Kruger

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00032-17-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: Left ventricular diastolic function and its relationship with the renin-angiotensin-aldosterone system and amino-terminal prohormone B-type natriuretic peptide: The African-PREDICT Study

Study leader/supervisor: Prof R Kruger

Student: B Viana

Application type: Single study

Risk level: Minimal

You are kindly informed that your application was reviewed at the meeting held on 19/04/2017 of the HREC, Faculty of Health Sciences, and was approved on 31/05/2017.

The commencement date for this study is 31/05/2017 dependent on fulfilling the conditions indicated below. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form at Ethics-HRECIncident-SAE@nwu.ac.za.

A monitoring report should be submitted within one year of approval of this study (or as otherwise stipulated) and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-Monitoring@nwu.ac.za. Annually a number of studies may be randomly selected for an external audit.

Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC, Faculty of Health Sciences. Ethics approval is required BEFORE approval can be obtained from these authorities.

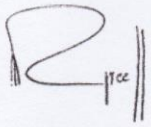
The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Prof Wayne Towers

HREC Chairperson



Prof Minrie Greeff

Ethics Office Head

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2017 Applications\Applications 03-19 April 2017\NWU-00032-17-S1(R Kruger-B Viana)\NWU-00032-17-S1(R Kruger-B Viana)-AL\NWU-00032-17-S1(R Kruger-B Viana)-AL.docm
7 June 2017

File reference: 9.1.5.3

Appendix C: Solemn declaration

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Higher Degrees Administration

SOLEMN DECLARATION AND PERMISSION TO SUBMIT

1. Solemn declaration by student

I, Bridget Viana

declare herewith that the thesis/dissertation/mini-dissertation/article entitled **(exactly as registered/approved title)**, Left ventricular diastolic function and its relationship with the renin-angiotensin-aldosterone system and amino-terminal prohormone B-type natriuretic peptide: The African-PREDICT study

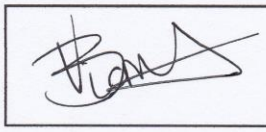
which I herewith submit to the North-West University, Potchefstroom Campus, is in compliance /partial compliance with the requirements set for the degree:

Master of Health Sciences in Cardiovascular Physiology

is my own work, has been language-edited in accordance with the requirements and has not already been submitted to any other university.

I understand and accept that the copies that are submitted for examination become the property of the University.

LATE SUBMISSION: If a thesis/dissertation/mini-dissertation/article of a student is submitted after the deadline for submission, the period available for examination is limited. No guarantee can therefore be given that (should the examiners' rports be positive) the degree will be conferred at the next applicable graduation ceremony. It may also imply that the student would have to re-register for the following academic year.

Signature of student  University number 23414707

Signed on this 20 day of November of 20 17

2. Permission to submit and solemn declaration by supervisor/promoter

- The undersigned declares that:

  the student is hereby granted permission to submit his/her mini-dissertation/ dissertation or thesis: Yes ☑ No ☐

- that the student's work has been tested by me for plagiarism (for example by TurnItIn) and a satisfactory report has been obtained: Yes ☑ No ☐

Signature/Supervisor/Promoter

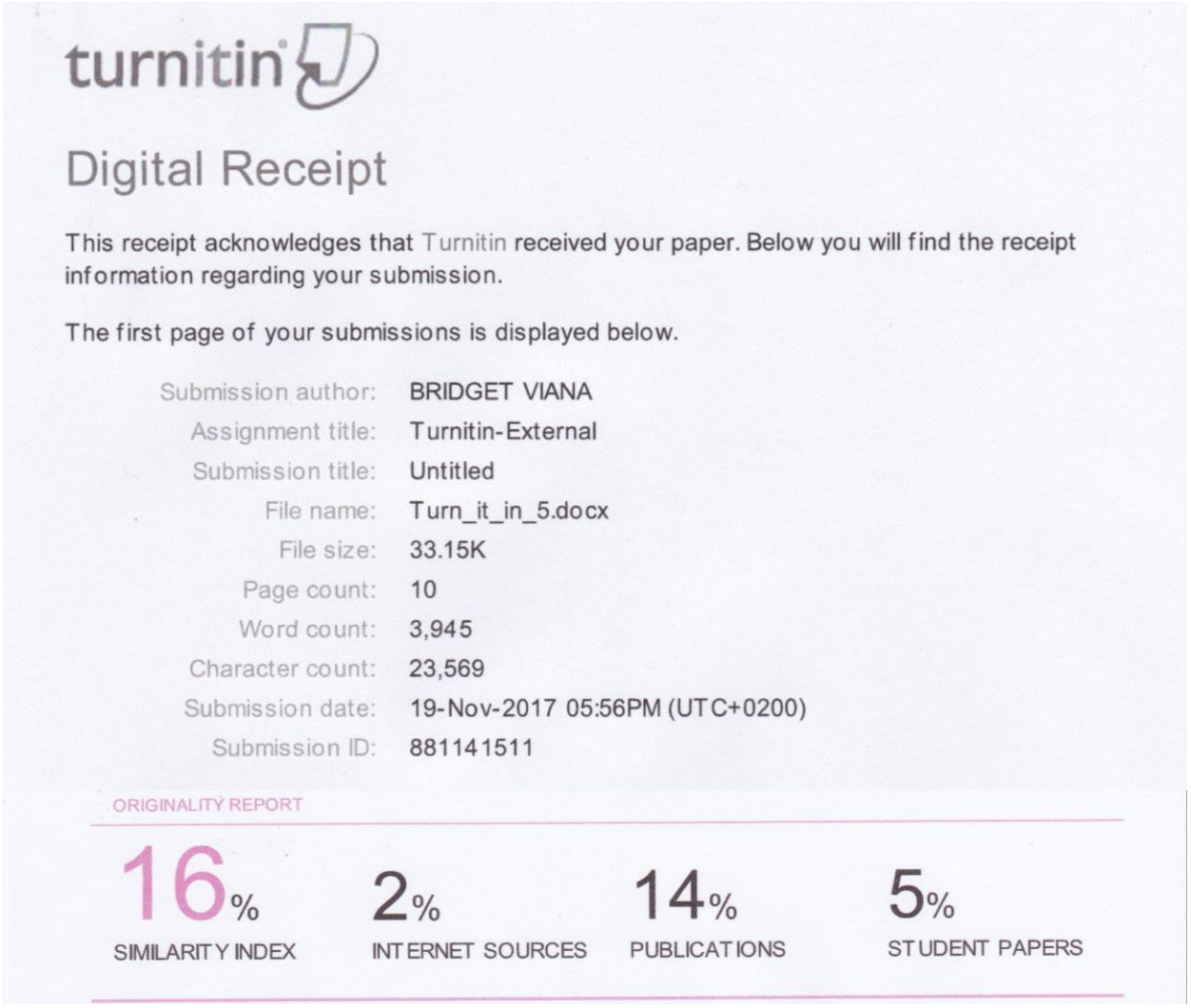
Prof Ruan Kruger Digitally signed by Prof Ruan Kruger Date: 2017.11.20 09:16:37 +02'00'

Date

20/11/2017

Original details: Marietjie Ackermann(10512187) R/SSupport doc's\SOLEMN DECLARATION AND PERMISSION TO SUBMIT.docm 28 july 2017
File reference: 7.1.11.3.2/3

Appendix D: Turn it in originality report



turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	BRIDGET VIANA
Assignment title:	Turnitin-External
Submission title:	Untitled
File name:	Turn_it_in_5.docx
File size:	33.15K
Page count:	10
Word count:	3,945
Character count:	23,569
Submission date:	19-Nov-2017 05:56PM (UTC+0200)
Submission ID:	881141511

ORIGINALITY REPORT

16%	2%	14%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

Appendix E: Confirmation of the editing of the dissertation

grammatically
just checked it!

Editing and Proofreading Service
If you can write it, we can work it!

Invoice: 110
Date: 16.11.2017

Invoice to:

Bridget Viana
North-West University
JAMA Reference Style
MHSc Health Dissertation
Due Date: 15.11.2017

Payment Details

EFT 10.11.2017 R750
EFT 16.11.2017 R750

PAID IN FULL

Word Count	Description	Unit Price	Total
1	Dissertation– SPECIAL	1500.00	1500.00
		Balance	1500.00

info@grammatically.co.za

www.grammatically.co.za

Cell: 076-199-0949

Banking Details
Nedbank
Acc: 1060489120
Branch Code: 194842
Reference: Your Name and Surname