DETERMINANT OF METABOLIC SYNDROME AND ITS CARDIOVASCULAR COMPLICATIONS AMONG PEOPLE OF AFRICAN ANCESTRY

Omotayo Alaba Eluwole

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DECLARATION

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

Omotayo Alaba Eluwole……..on this…….18th.....day of……May……2022

I certify that the studies contained in this thesis have the approval of the Committee for Research in Human Participants of the University of the Witwatersrand, Johannesburg. The ethics approval number is M190472.

Signed by Omotayo Alaba Eluwole on this……18th.....Day of……May……2022

Signature …… ……

We certify that the above statement is correct

Supervisor

Prof Joseph Muzà Maseko

Signature……

Co-supervisor

Prof. Rhian Touyz

Signature……

Co-Supervisor

Dr. Nqoba Tsabedze

Signature……

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DEDICATION

This thesis is dedicated to God; the general organizer of the whole universe, the source of my inspiration. I also dedicate this work to my late father, Deacon Samuel Kayode Eluwole.

PUBLICATIONS IN RELATION TO THE THESIS

The following publications on molecular cascades and mechanism of hypertension (book chapters) are review articles published by collaborative authors from University of Glasgow, myself and my supervisors.

i. **Touyz RM, Eluwole OA,** Camargo LL, Rios FJ, Alves-Lopes R., Neves KB, **Maseko, MJ,** Guzik JT, Petrie, J, and Montezano AC. Molecular mechanisms underlying vascular disease in diabetes. In Blood Pressure Disorders in Diabetes Mellitus. Eds. A. Berbari and G. Mancia. Springer Nature. 2023: 3 (2): 105-122.

ii. **Touyz RM**, Rios FJ, Montezano AC , Neves KB, **Eluwole OA**, **Maseko MJ**, Alves-Lopes R, Camargo LL. The vascular phenotype in hypertension. In The Vasculome: From Many to One. Ed. Z. Galis. 2022

iii. **Touyz RM** Camargo LL, Rios FJ, Alves-Lopes R, Neves KB, **Eluwole O**, **Maseko**, **MJ**, Lucas-Herald A, Blaikie Z, Montezano AC, Feldman RD. Arterial Hypertension. In Comprehensive Pharmacology. 2021; Chapter 00158

Abstract

Cardiovascular disease is now a leading cause of death globally. However, metabolic syndrome is an extremely critical healthcare issue worldwide due to progressive increase in obesity and its related factors. Obesity is strongly associated with insulin resistance and other components of metabolic syndrome. There is discrepancy in the use of parameters for the diagnostic criteria of metabolic syndrome due to genetic and environmental variability in different ethnicity. Body mass index and waist circumference (WC) are commonly used in the assessment of central obesity and abdominal obesity respectively. Fahed et al observed that waist circumference was employed because measurement was easy, however, waist circumference alone is inconclusive of abdominal adiposity and must be interpreted with body mass index. The two measurements (WC and BMI) have been documented to be strongly related to insulin resistance. (Fahed *et al*., 2022). However, there is controversial assessment of metabolic syndrome using either waist circumference (WC) or body mass index (BMI) or waist hip ratio (WHR) or combination of two measurements (Kassi *et al*., 2011; Fahed *et al*., 2022). Our study assessed the prevalence of metabolic syndrome among apparently healthy 1516 participants from African ancestry using seven established diagnostic criteria (NCEP-ATPIII- National Cholesterol Education Program Adult Treatment Panel III, WHO-World Health Organization, IDF-International Diabetes Federation, AHA/NHLBI- American Heart Association/National Heart, Lung and Blood Institute, EGIR - European Group for the study of Insulin Resistance, AACE- American Association of Clinical Endocrinology). The result revealed highest prevalence of metabolic syndrome when modified NCEP-ATPIII [National Cholesterol Education Program Adult Treatment Panel III (ATP III)] was considered. The predictive assessment of blood pressure and arterial stiffness may be useful in achieving early detection and prevention of target organ damage. This study further compared clinic blood pressure, ambulatory

blood pressure and central pressure using conventional blood pressure monitor, Spacelabs 90207 (Spacelabs Inc., Redmond, Washington, USA) and applanation tonometry Sphygmocor device respectively. The findings revealed that central blood pressure and ambulatory blood pressure are more predictive of cardiovascular events among people of African ancestry. Our findings are pointers to cardiovascular risk in the study population. Additionally, this study provides new insights to the role of obesity in the perturbation of left ventricular geometry of people of African ancestry with metabolic syndrome; using quantitative and comprehensive evaluation of biochemical and echocardiographic profile.

Aldosterone produced locally in adipose tissue, heart, kidney and vasculature increase the expression of cytokines and other fibrotic factors. Thus, role of the local renin angiotensin aldosterone system (RAAS) in the pathophysiology of atherosclerosis and cardio-renal fibrosis was evaluated in animal study. With high prevalence of metabolic syndrome and obesity in Africans, elevated aldosterone from diet may likely predispose African community to diastolic dysfunction; this may be a pointer to increased incidence of heart failure in groups of African ancestry. Hence, this study lends insights into the potential role of TRPM7; a novel non selective cation channel and chanzyme in aldosterone-induced cardiovascular fibrosis. This study concluded that modified NCEP-ATPIII has suitable components for the diagnosis of MS in people of African ancestry. Metabolic syndrome in people of African ancestry is strongly associated with factors such as sex, smoking and alcohol. Consequently, MS and other risk factors such as obesity, aldosterone and insulin resistance may lead to left diastolic dysfunction among individuals with MS. Experimentally, aldosterone-salt induced cardio-renal fibrosis, aggravated by TRPM7 might be the underlying pathogenesis of MS and its cardiovascular complications in Africans; thus suggests TRMP7 inhibitors has potential anti-fibrotic agents.

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My family: the Eluwoles 'e se modupe, eku aduroti.

STATEMENT OF MY CONTRIBUTION TO DATA COLLECTION AND ANALYSIS

The studies described in this thesis were self-designed with consultation with my supervisor. I performed all the data analysis for this thesis and interpreted these data.

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LIST OF ABBREVIATIONS

AA- African American

ABPM Ambulatory Blood Pressure Monitoring

AACE American Association of Clinical Endocrinology

AG/PP Augumentation index

Aldo Aldosterone

AHA/NHLBI American Heart Association/National Heart, Lung and Blood Institute

AT1 Ang II type 1 receptors

ARR Aldosterone renin ratio

BMI Body Mass Index

BP Blood Pressure

CBP Central Blood Pressure

CDP Central Diastolic Pressure

CHR Central Heart Rate

CI Confidence Intervals

CIMT Carotid artery intima-media thickness

CPP Central Pulse Pressure

CRP C-reactive protein

CRIBSA Cardiovascular Risk in Black South Africans

CSP Central Systolic Pressure

CV Cardiovascular

CVD Cardiovascular Disease

DBP Conventional Diastolic Blood Pressure

DBPD Daytime Diastolic Blood Pressure

DBPN Nighttime Diastolic Blood Pressure

DBP24 24-hr Diastolic Blood Pressure

DM Diabetes Mellitus

ECG Electrocardiogram

EDV End Diastolic Volume

EGIR European Group for the study of Insulin Resistance

ENaC Epithelial Sodium Channel

ESH European Society of Hypertension

ESV End Systolic Volume

EF Ejection Fraction

FS Fractional Shortening

Glu Glucose

IDF International Diabetes Federation

IL Interleukin

IR Insulin Resistance

HbA1c Glycated Haemoglobin

HDL High Density Lipoprotein

HTN Hypertension

HOMA-IR Homeostatic Model Assessment-Insulin Resistance

LDL Low Density Lipiprotein

LVEDD Left Ventricular End Diastolic Diameter

LVESD Left Ventricular End Systolic Diameter

LVH Left Ventricular Hypertrophy

LVM Left Ventricular Mass

LVMI Left Ventricular Mass Index

MR Mineralocorticoid Receptor

MS Metabolic Syndrome

NCC sodium-chloride co-transporter

NCEP-ATPIII National Cholesterol Education Program Adult Treatment Panel III

PVAT Perivascular adipose tissue

PP Pulse Pressure

PWV Pulse Wave Velocity

RAAS Renin-Angiotensin-Aldosterone System

SD Standard Deviation

SBP Conventional Systolic Blood Pressure

SBPD Daytime Systolic Blood Pressure

SBPN Nighttime Systolic Blood Pressure

SBP24 24-hr Systolic Blood Pressure

TG Triglyceride

TNF-α Tumor Necrosis Factor-a

TRPM7 Transient Receptor Potential Melastatin 7

WC Waist Circumference

WHO World Health Organization

VSMCs vascular smooth muscle cells

PREFACE

Hypertension is a complex, multifactorial and multisystem disorder and a leading cause of morbidity and premature death globally. Recent annual screening (USPSTF) recommendation defines hypertension as blood pressure equal or higher than 130/80 mmHg (Ferdinand and Brown, 2021). Hypertension is a very common disease with prevalence rates of about 30% in adults worldwide (Ferdinand and Brown, 2021). The incidence of hypertension is age-related. At younger ages, hypertension is more prevalent in males than females, but this trend is reversed by age 65 (Touyz *et al*., 2021). Heart Failure and related cardiovascular diseases are common in Africans despite advances in routine measurement of blood pressure, lifestyle modification and antihypertensive therapy. High salt intake is associated with hypertensive target organ damage, with oxidative stress and inflammatory process as the mediating factors (Touyz *et al*., 2021).

Evidence exists that obesity and hypertension have interwoven mechanism that may underlie cardiovascular and cerebrovascular damage (Mishra *et al*., 2018; Touyz *et al*., 2021). Human (invasive) studies involving time domain analysis of the intra-aortic BP waveform also confirmed that it is largely explicable by the transmission and reflection phenomena and closely associated with aortic wall stiffness (Omboni *et al*, 2019).

The Chapter 2 of this thesis evaluates the prevalence of metabolic syndrome using seven established criteria. Chapter 3, I evaluated the relationship between arterial stiffness and metabolic syndrome in South Africans using the WHO criteria to classify metabolic syndrome. In chapter 4, I assessed the relationship between metabolic syndrome, echocardiographic parameters and metabolic syndrom related biochemical parameters and metabolic syndrome. In Chapter 5, I evaluated effect of TRMP7 on aldosterone-salt induced cardio-renal fibrosis. Chapter 6 summarises the major findings and highlights the novelty of the study, it also outlines suggested recommendations and further studies.

CHAPTER ONE

Introduction and literature review

1.1 Introduction

Metabolic Syndrome (MS) is the aggregation of interconnected factors that collectively predict the risk for cardiovascular atherosclerotic diseases and type 2 diabetes mellitus (Biadgo *et al*., 2018). It was first named syndrome X by Reaver in 1988 due to its association with the metabolic and hemodynamic abnormalities (Reaver, 1988; Duque *et al*., 2020). It was later referred to as insulin resistance syndrome, mixed metabolic syndrome or cardiovascular dysmetabolic the syndrome, before the common terminology metabolic syndrome. First guideline for diagnosing MS was proposed by the World Health Organization (WHO) in 1998. The guideline emphasized important criteria for diagnosing MS in both men and women, the major components include; blood glucose level, lipid profile, increased blood pressure and anthropometric values for both sexes (Alberti, 2009; Simmons *et al*., 2010; Duque *et al*., 2020).

Metabolic syndrome is now a global pandemic (Adeboye *et al*., 2012). The increase in the prevalence of obesity in developed and developing countries has escalated the global burden of metabolic syndrome because obesity serves as a major component and simultaneously act as risk factor for other components; thus, aggravate diagnosis of MS and its cardiovascular complications (Mancia *et al*., 2010; Saklayen, 2018). Major components of MS are obesity, hypertension, hyperglycemia and dyslipidaemia. However, impaired insulin mediated glucose uptake is the most documented basic pathogenic mechanism of MS (Pucci *et al*., 2014). Obesity and dyslipidemia were documented to be the most common occurring components. Obesity is more common in females while hypertension tends to be more predominant in males (Elffers *et al*., 2017). Basically, the metabolic syndrome is defined in terms of body fat, blood pressure and the aggregation of lipids and lipoprotein aggregation. Notably, dyslipidaemia has been attributed to insulin resistance

and obesity. Myocardial triglyceride is associated with LV diastolic dysfunction in diabetes, it is not independently related to MS, thus, it seems to have a more complex role (Tan *et al*., 2013). Most of the underdiagnosed MS among Africans has been because of controversial lower prevalence of lipid abnormalities. However, previous research has suggested that by the time Africans are diagnosed, there is likely a more advanced condition of metabolic abnormalities, with extreme levels of inflammation, insulin resistance, oxidative stress and salt sensitivity than that seen in other racial/ethnic groups; these mechanisms culminate to the underlying cardio-renal pathology (Mancia *et al*., 2010; Lavie *et al*., 2013; Liu *et al*., 2020).

Fat deposition around the abdominal and central region of the body is a focus of pathogenesis of MS. It is mostly assessed by anthropometric measurements such as waist circumference (WC), body mass index (BMI) and waist-hip ratio (WHR). These measurements are frequently imprecise in Asians and Chinese population due to variation in techniques for the assessment of visceral fat (Van der Kooy *et al*., 1993; Wild and Byrne, 2005). However, waist circumference is still widely accepted as a good predictor of intra-abdominal fat mass (Feigenbaum and Longstaff, 2010; Kassi *et al*., 2011). Obesity and MS are interrelated. People from African origin have a body mass index (BMI) within the normal range meet the criteria for MS while obese patients are likely to be exempted by the European and other standard diagnostic criteria. It is important to understand the relation between different assessment of obesity and MS in obese populations (Okafor, 2012; Lee *et al*., 2019). Possibly, the risk of MS and /or cardiovascular complication may be more in Africans.

Alterations in insulin sensitivity play pathogenic role in metabolic syndrome. Over the years, hyperglycemia and/ insulin resistance have been reported to contribute significantly to

atherosclerotic changes (Burchfiel *et al*., 2005; Vander Merve and Pepper, 2006; Del Giorno *et al*., 2020). Theoretically, insulin plays a critical role in various biological events such as hepatic lipid synthesis, renal sodium reabsorption, arterial tissue growth, and vascular reactivity. The physiologic roles of insulin are evident in the development of obesity, hypertension and atherosclerosis through specific signalling pathways (Fig 1.2) (John, 2004; Gradidge *et al*., 2017). Evidences have shown that type 2 diabetes has an outcome of MS. Studies have shown that patients with type 2 diabetes (T2DM) have higher prevalence of MS than those without type 2 diabetes. Similarly, patients with metabolic syndrome are three to five times at risk of type 2 diabetes mellitus (Sattar *et al*., 2008; Maack *et al*., 2018).

Hyperaldosteronism is strongly associated with impaired insulin metabolic signalling. Increased aldosterone levels have been reported in individuals with obese hypertensive patients with high waist circumference (greater than 94cm in men and 102cm in women) and low HDL cholesterol levels less than 1.03 mmol/l in both sexes (Maack *et al*., 2018). In animal studies, deleterious effects of angiotensin II (Ang II) are mediated by the activation of Ang II type 1 receptors (AT1) and chronic infusion of Ang II causes hypertension and cardio-renal fibrosis. Excess aldosterone results in increased oxidative stress in cardiovascular tissue (Brown, 2013; Rockey *et al*., 2015; Touyz *et al*., 2022). Hypothetically, renin-aldosterone-angiotensin system (RAAS) involved in hypertension development and organ damage among Africans may be linked with cardiovascular complication of metabolic syndrome in Africans.

Clinically, hypertension is assessed using brachial cuff. It is defined according to 2007 ESH guideline (European Society of Hypertension–European Society of Cardiology) guidelines, that is, cut-off value of 140/90 mmHg (Mancia *et al*., 2013). Clinic assessment posed a challenge for the

clinicians on appropriate management of patients with or without hypertension because of white coat hypertension. Further studies on 24-h ABPM offered more information for making clinical decisions (Lurbe *et al*., 2016). Various studies on 24-h ambulatory BP monitoring (ABPM) and association between obesity and other parameters of cardiovascular risk such as conventional BP, central BP and pulse pressure (PP) amplification show to be more accurate assessment of interaction between body composition, metabolic syndrome and their clinical implications. Combination of metabolic and hemodynamic abnormalities characterizing the metabolic syndrome are associated with a prevalence of subclinical damage in a various body organ, such as left ventricular hypertrophy (LVH), thickening or atherosclerotic plaques of carotid arteries, microalbuminuria and deranged renal function (Han *et al*., 2017). Left ventricular hypertrophy (LVH) is a prevalent and potent independent predictor of cardiovascular morbidity and mortality in MS, due to increase demand of myocardial oxygen because of the increased impedance to left ventricular ejection (Tadic *et al*., 2012; DeBoer *et al*., 2017).

Abnormal daily salt intake (> 2g/day) remains a key predisposition to essential hypertension. South Africa implemented legislation in 2016 mandate sodium levels to 0.85g/day processed food in order to reduce the burden of hypertension. A study by Charlton also reported that annual cardiovascular mortality was reduced by 11% due to reduction in daily salt intake (Charlton *et al*., 2021). Impact of excessive salt intake on arterial stiffness and central blood pressure is an important determinant of cardiovascular (CV) disease. Amongst many other complications, arterial stiffness is the hallmark of metabolic syndrome, cardiovascular atherosclerotic diseases and diabetic vasculopathology in both old and young patients (Ebong *et al*., 2012). Arterial stiffness is a consequence of a complex interplay between a several independent as well as inter-dependent factors (Gkaliagkousi and Douma, 2009). Hardnening of the vessel wall is the

macroscopic manifestation of the glycemic state, LDL- induced endothelial dysfunction and anomaly of natriuretic peptides (Sonnenberg *et al*., 2004). Therefore, increase in biochemical parameters; such as lipid profile (TG > 1.7 mmol/l, LDL > 1 mmol/ l and HDL < 1.9 mmol/l), aldosterone-renin ratio, fasting blood sugar may signpost to atherosclerosis and cardio-renal complication (Teng-Yeow and Yao-Chung, 2012; Xanthakis *et al*., 2015). Likewise, an increase in serum gamma glutamyl transferase (GGT) > 85 ug/ml, is suggested to be a marker of metabolic and cardiovascular risk.

Carotid artery intima-media thickness (CIMT) has been described as a surrogate marker of atherosclerosis and heart failure (Adolphe *et al*., 2009; Larsen *et al*., 2018). It correlates with relative risk factors, anthropometric measurement, fasting plasma glucose, lipid profiles and cardiovascular disease (Teng-Yeow and Yao-Chung, 2012; Pasquele *et al*., 2015). However, aortic pulse wave velocity (PWV), the gold standard for assessment of arterial stiffness is strongly associated with all the major component of metabolic syndrome. It can independently cause wave reflections, arterial stiffness and endothelial dysfunction (Schillaci *et al*., 2015). The pathological effects reflect on central augmentation index and pulse wave velocity.

Race and ethnicity account for some of the differences in the definition and diagnosis of MS and its associated complications (Tan *et al*., 2013; Tu *et al*., 2017). Relationships between the diagnostic criteria of metabolic syndrome (obesity, hypertension and dyslipidemia) differ when comparing Africans and non-African populations (Sekokotla *et a*l., 2017). For example, a study by higher prevalence of the individual components (HDL cholesterol and waist circumference) was recorded in South Africans, the study suggested an increased risk for CVD, especially in patients over 55 years. (Saloojee *et a*l., 2016). In addition, only modified NCEP-ATP III considered use of antihypertensive as a component of metabolic syndrome. These may account for

misdiagnosing and inappropriate management in Africans. In this regard, larger percentages of cardiovascular complications in Africans may be attributed and can be traced to other non-traditional cardiovascular risk factors such as race, age, sex, alcohol and smoking (Osei, K., and Gaillard, 2017; Saklayen, 2018; Haverigen *et al*., 2021). Obesity epidemic in Africans may increase the risk of heart failure (HF) in this population, because, definitions and researches on MS are particular about absolute risk factors than relative risk factors (Osei, 2010); meanwhile, Africans are more prone to absolute risk than relative risk factors (race, age, sex, alcohol and smoking) of MS and heart failure (Pasquale *et al*., 2015; Csige *et al*., 2018). Although, there are discrepancies across different continents in the assessment of MS in individuals of black ancestry, research goal needs to delineate differential contributions to MS. Most importantly, the influence of aldosterone salt to the pathogenesis of its complications. This study proposed that most of these absolute risk factors contribute to metabolic syndrome in Africans; this may have direct myocardial effects due to their interrelationship with aldosterone/ salt (Taylor *et al*., 2008; Maggioni, 2017). Therefore, the aim of this study is to focus on the factors that contribute to cardiovascular complication of MS, that is, obesity, hypertension and aldosterone/salt. These will give cue to the management of heart failure in people of Africa ancestry.

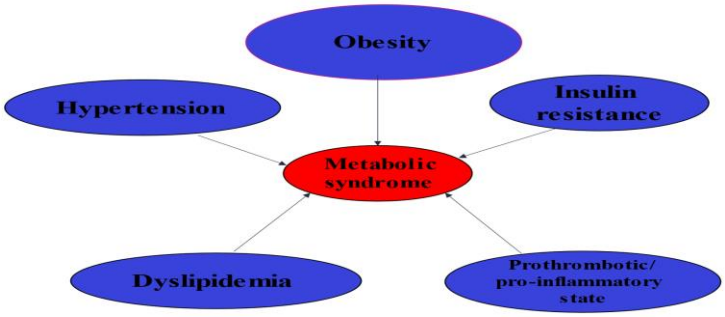


Figure 1.1a Major components of metabolic syndrome

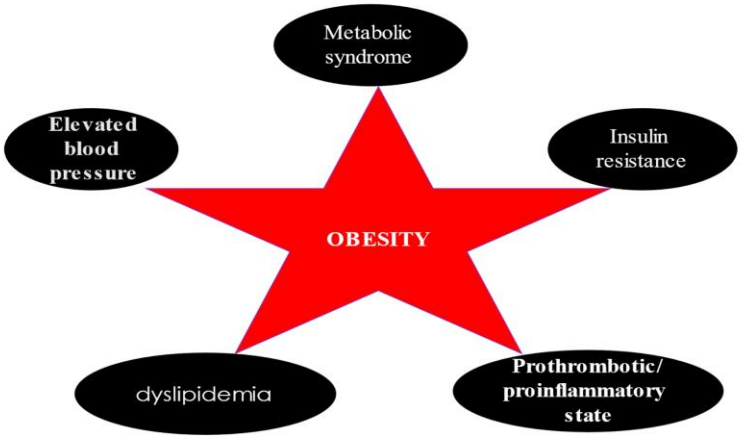


Figure 1.1b Obesity serves as a link to metabolic syndrome and other major component

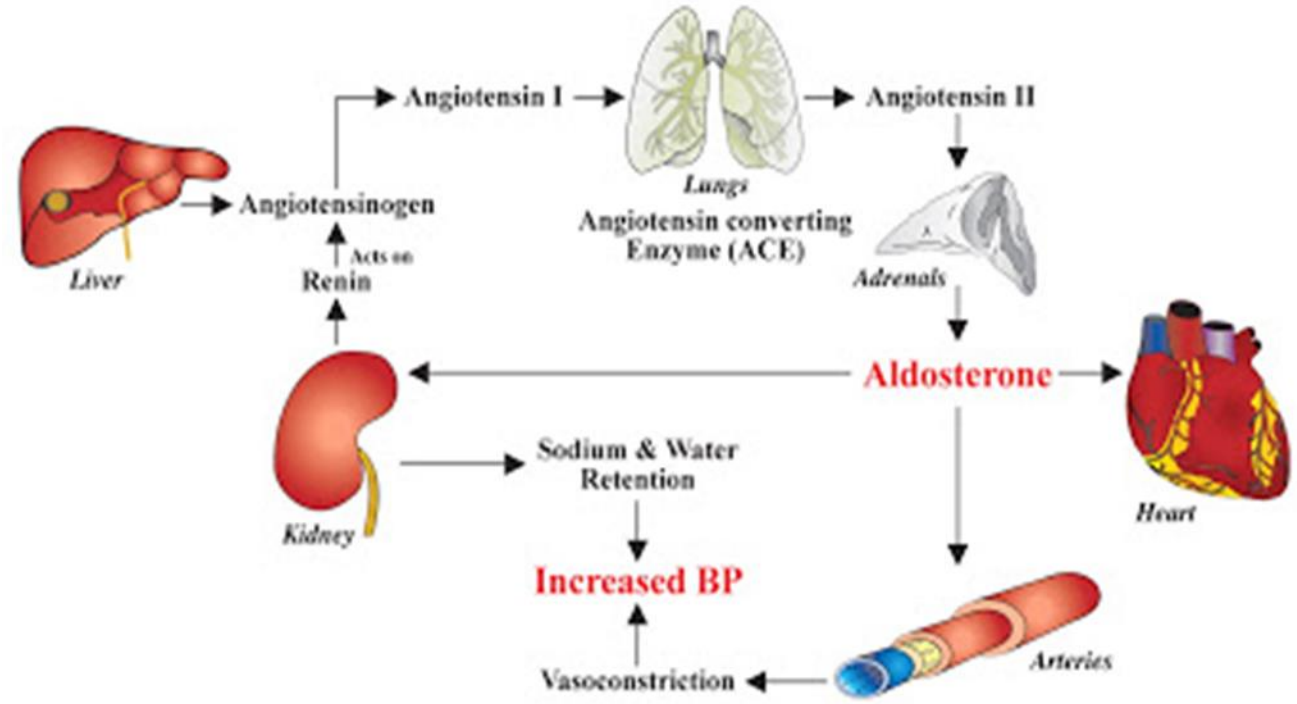


Fig 1.2 Renin angiotensin aldosterone system (RAAS) and its major role in the development of hypertension and atheroscleros

1.2 Literature review

1.2.1 Epidemiology

Metabolic syndrome is a complex multifactorial health disorder that increases the risk of cardiovascular atherosclerotic diseases and type 2 diabetes mellitus. For the past four decades, there has been a controversy on the significance of MS as a global pandemic and the correlation of individual components of MS to different communities (Kassi *et al*., 2011). The current definitions of the metabolic syndrome give an inconsistent picture of cardiovascular disease risk when applied to different ethnic groups (Kassi *et al*., 2011). In the general population, the prevalence of metabolic syndrome is estimated to be between 17% and 25%. The prevalence is increasing. It is more common in women than men and tends to increase with age. The prevalence of the metabolic syndrome varies between different populations (Shi *et al*., 2020). It was reported to be higher in developed countries than developing countries because of decreased physical activities and consumption of fatty foods; thus, leading to high rates of obesity. MS affects over 70–80 million Americans (Shi *et al*., 2020). Obesity has become epidemic globally especially in minority populations, particularly among Africans residing in urban and inner cities of the western world when compared with their African and non-African age group residing in rural areas in Africa. In fact, the prevalence of overweight and obesity approaches 70% in Africans when compared to 50% of non-Africans in United State of America (Shi *et al*., 2020). This also contradict the opinion documented several decades ago which considered MS rare among Africans, thus, reveals the burden of MS among African populations is becoming high (Saklayen, 2018). Prevalence of metabolic syndrome in African populations ranges from 0% to about 50% or even higher depending on the population setting. However, increase in the prevalence of metabolic syndrome in Africa settings is attributed to departure from traditional African to western lifestyles,

such that, it is no longer limited to adults but it is also becoming common among the young ones in Africa (Okafor, 2012), due to availability of facilities that reduce physical activities within communities, residential areas and workplaces combined with negative psyco-social perspective associated with obesity being regarded as 'fat is beautiful or sign of wealth and affluence'.

The prevalence of metabolic syndrome is increasing in African populations, and is particularly high in South African women (Gradidge and Crowther, 2017). This population group is also known to have the highest prevalence of obesity in the sub-Saharan African region (42%) when compared with women in the United Kingdom (23%) and the United States of America (36%). Abdominal subcutaneous fat, visceral fat, lean mass, adiponectin, leptin, vitamin D, smoking and menopausal status have been investigated for possible association with metabolic syndrome in African women (Gradidge and Crowther, 2017). In the CRIBSA study (Cardiovascular Risk in Black South Africans), high prevalence of metabolic syndrome among Africans in Cape Town was documented (Sweet *et al*., 2007; Csige *et al*., 2018). However, central obesity and low high-density lipoprotein cholesterol are metabolic syndrome components which were higher in women while raised blood pressure was the most frequent men (Peer *et al*., 2015). The prevalence of MS and its components also differ among racial and ethnic populations. Therefore, the International Diabetes Federation (IDF) released different guidelines and criteria based on gender, race and ethnicity. In this regard, National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP) criteria require three or more of the five components to constitute MS. Meanwhile, ATP III does not have race/ethnic based MS criteria and requires no prerequisite. According to Abiodun et al (2019) prevalence of metabolic syndrome among a group of hypertensive Nigerians was found to be 34.3% (ATP III), 35% (WHO), and 42.9% (IDF). These values generally were similar to that which emerged from non-diabetic Turkish adults where the prevalence rates were

as follows: 38% (NCEP-ATP III), 42% (ACE and IDF), 20% (EGIR), and 19% (WHO). There is a strong and convincing evidence to support ethnic and racial difference in MS and its components. However, these metabolic disparities in Africans make it difficult to point out the excess CVD and T2DM outcome in the group. In this regard, current classification of MS by the NCEP, WHO, and IDF in Africans was introduced. The new diagnostic criteria (Table 1) proposed by above mentioned international bodies predicted significant impact of MS on CVD morbidity and mortality in Africans. It has been observed that the predictive values are particularly relevant when using traditional metabolic risk factors rather than non-traditional biomarkers (Gradidge and Crowther, 2017). Therefore, our study assessed the prevalence of metabolic syndrome in study population and also evaluated the relative risk of metabolic syndrome among Africans.

Table 1.1: Diagnostic criteria of Metabolic Syndrome according to Modified NCEP-ATP III (Kassi *et al*., 2011).

Components of Metabolic Syndrome	Modified NCEP-ATP III
Abdominal obesity	Waist circumference ⩾102 cm for men and ⩾ 88 cm for women
Elevated Blood Pressure	⩾130/85 mmHg
High lipid density lipoprotein (HDL)	< 40 mg/dl (1.0 mmol/l) for men and < 50 mg/dl (1.3 mmol/l) for women
Fasting triglycerides (TG)	⩾150 mg/dl (1.7 mmol/l)
Fasting blood glucose	>100 mg/dl or 5.6 mmol/l
Others	Use of antihypertensive

Table 1.1 showing the major and minor criteria for diagnosing metabolic syndrome according to Modified NCEP-ATP III.

Moreover, definition of dyslipidaemia has markedly changed with time and recent ESH guidelines now considered thresholds of dyslipidaemia to be strictly applied to the definition of MS (Mancia

et al., 2013; Haverigen *et al*., 2021). Yet, the values do not offer a clear context-independent involvement of dyslipidaemia in diagnosing and management of metabolic syndrome and its cardiovascular complications.

Cardiovascular diseases (CVD) remain as the leading cause of mortality in the western world and have become a major health threat for developing countries (Saklayen, 2018). The World Heart Federation compiled extensive data in 1996 on the burden of CVD in major geopolitical regions of the world revealed that proportion of deaths attributable to circulatory conditions was estimated to be 29.0% worldwide, 45.6% in developed countries, and 24.5% in developing countries, the latter increasing from 16% or by one half as estimated by the World Health Organization (WHO) four decades ago (Saklayen, 2018).

Cardiac disease in the metabolic syndrome consists of both vascular and myocardial abnormalities (do Vale *et al*., 2020). The latter are characterised predominantly by diastolic dysfunction, which has been difficult to evaluate in spite of its prevalence. Due to the current obesity epidemic in sub-Saharan Africa, the prevalence of heart failure in people of African ancestry is likely to increase based on score of obesity and other modifiable risks such as smoking and alcohol (Gallagher *et al*., 2018). The high metabolic syndrome prevalence underscores the frequent clustering of cardiovascular risk factors which could culminate to atherosclerosis (Hoebel *et al*., 2010; Tan *et al*., 2013).

1.2.2. Pathophysiology of metabolic syndrome and its cardiovascular complication.

1.2.2.1 Role of oxidative stress and inflammation in the pathophysiology of metabolic syndrome.

Chronic hyperglycemia and insulin resistance play important role in the initiation of vascular complications of diabetes. The two factors are associated with obesity, particularly central obesity, but may be present in lean individuals with hypertension. However, visceral adipocytes are more susceptible to cellular death as they begin to enlarge, and their stromal vascular fraction becomes infiltrated with macrophages. Adipocyte inflammation may be essential for healthy adipose tissue (AT) because expansion and remodeling and lipid storage within AT macrophages may protect against obesity-linked adipocyte dysfunctions. However, in obesity, inflammatory or metabolically activated immune cells may contribute to AT dysfunctions, thus, accelerates fat spill over from AT to skeletal muscle and liver. This may result to ectopic fat deposition and systemic insulin resistance which play vital roles in the pathogenesis of type 2 diabetes mellitus (T2DM). Histologically, these macrophages around dead adipocytes form “crown- like structures that is associated with expression of cytokines (including tumor necrosis factor-a [TNF-a], interleukin-6 [IL-6]), and inducible nitric oxide synthase. These changes have been shown to coincide with the onset of insulin resistance and provide a pathophysiological link between metabolic and vascular disease. Perivascular adipose tissue (PVAT) is an active tissue that surrounds vessels. It is important to the control of vascular function and structure. It secretes a myriad of factors such as adipokines, inflammatory molecules and vasoactive substance involved in the obesity and diabetes mellitus pathogeny by several possible mechanisms, such as the release of free fatty acids, hyperglycemia, adipokines synthesis and metabolic inflammation. Mechanisms involved in fibrosis development are dependent of several intracellular pathways, including activation of

MAPK, transcription factors (NFkB, AP-1, Stat3, Smad2/3), Nox activation (Nox1, Nox4, Nox2) and ROS production, production of cytokines (IL-6, IL-11), activation of endothelin system (production of ET1 and expression of ETa and ETb), production of growth factors (TGFβ1) and downregulation of phosphatases (PPM1A, PTEN). Activation of these cascades change the cell phenotype in a process called epithelium-to-mesenchymal transition leading to fibrosis (Rockey *et al*., 2015). Fibrogenesis is a complex process involving production of reactive oxygen species (ROS), chronic inflammatory response, dysregulation of extracellular matrix and fibroblasts-to-myofibroblasts differentiation. Several and different intracellular mechanisms and soluble factors play important role in fibrosis development, which may be different according to the trigger fibrogenic mediator (Mokotedi *et al*., 2020; Touyz *et a*l., 2022). Hence, chronic/uncontrolled hypertension leads to cardiovascular injury and fibrosis.

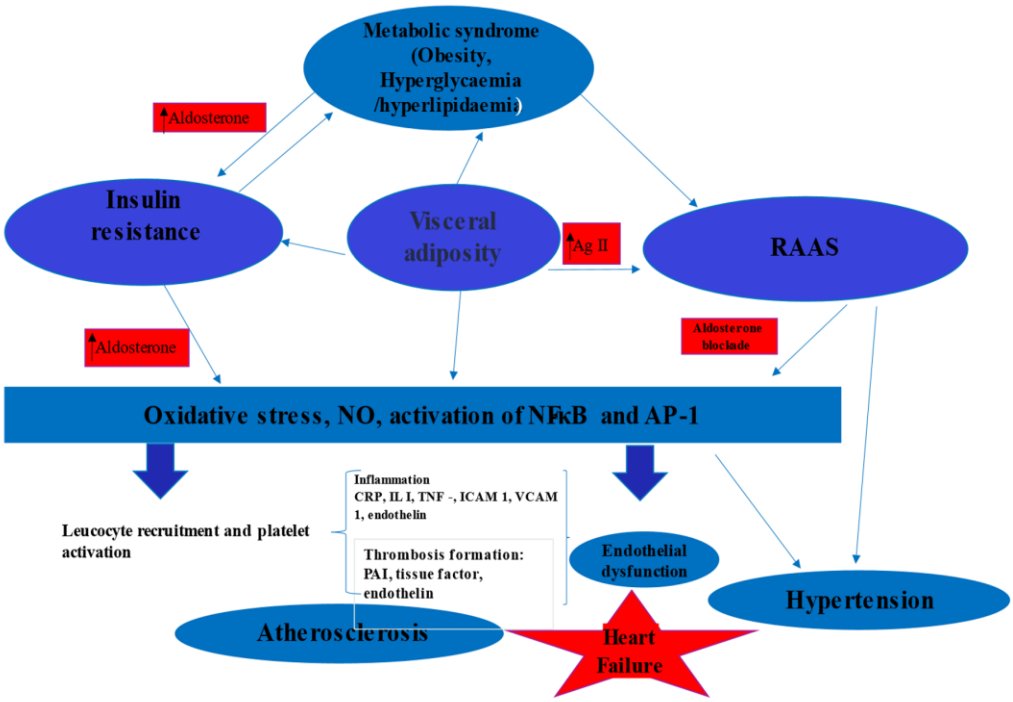


Figure 1.3 Pathophysiology of cardiovascular effect of metabolic syndrome

1.2.2.2 Role of renin-aldosterone-angiotensin system (RAAS) in hypertension and heart failure

The renin-aldosterone-angiotensin system (RAAS) is crucially involved in hypertension development and organ damage. Deleterious effects of Ang II are mediated by the activation of Ang II type 1 receptors (AT1) and chronic infusion of Ang II causes hypertension and fibrosis and kidney damage in animal models (Touyz *et al*., 2022). Aldosterone interacts with mineralocorticoid receptor (MR) and increase renal sodium reabsorption via activation of the sodium-chloride co-transporter (NCC) in the distal convoluted tubule and the epithelial sodium channel (ENaC) in the late distal convoluted tubule (Satoh *et al*., 2012; Rio *et al*., 2020). Systemic effects of aldosterone are also observed because of MR expression in cardiomyocytes, vasculature, kidney, adipocytes and immune cells. Aldosterone and Angiotensin II activate different receptors via different mechanisms to cause hypertension ad heart failure (Touyz *et al*., 2022). However, mechanisms involves in aldosterone or angiotensin II induced cardiac and renal fibrosis development is still elusive.

1.2.3 Specificity of the components of metabolic syndrome in people with African ancestry

1.2.3.1 Obesity and Dyslipidaemia

Obesity and dyslipidaemia are two interconnected attributes and components of metabolic syndrome. Obesity and dyslipidaemia seem to be the most common occurring components. Dyslipidaemia is defined as abnormalities in cholesterol, low density lipoprotein cholesterol (LDL), hypertriglyceridemia, and HDL. Alterations in lipids/lipoproteins have been associated with increased cardiovascular morbidity and mortality (myocardial infarction, stroke, and heart failure). Although, obesity appears more common in females, hypertension tends to be more predominant in males. However, both are independently linked to atherosclerotic disease. WHO

estimates global prevalence of hypercholesterolaemia (total cholesterol >5mmol/l) for men and women to be 37% and 40% respectively, with little change in the past 25 years. Historically, the lipid profiles of Africans were considered largely favourable, reflecting traditional lifestyles that include high levels of physical activity and diets low in fat thus, resulting in low rates of atherosclerotic disease. However, several studies from across Africa consistently report the phenomenon of low levels of HDL cholesterol in both men and women together with relatively high levels of LDL cholesterol and triglycerides. In Western African countries such as Nigeria and Sierra Leone, over 10% of individuals above 25 years had hypercholesterolaemia, while over 40% of those living in Northern African countries such as Tunisia and Egypt had hypercholesterolaemia. However, a South African survey reported that 28% of women and 19% of men older than 18 years had elevated total cholesterol, while 52% of men and 44% of women had low levels of HDL (Reiger *et al*., 2017). Generally, reports concerning prevalence of dyslipidaemia and awareness have been stagnant or slowly decreasing over the years globally especially in sub-Saharan Africa. Hence there is possibility of underestimation of dyslipidaemia among Africans which could have aggravated the incidence of cardiovascular complications.

Reduced HDL and hypertriglyceridemia are the two main types of dyslipidemia associated with metabolic syndrome. Dyslipidemia manifesting as reduced HDL was extremely common as demonstrated in Nigeria and Botswana. African-Americans (AA) with insulin resistance have lower triglycerides and higher HDL when compared to non-Africans. Clinically, high LDL level is harmful to human health while low HDL level is believed to be cardio-protective in non-Africans, however, the accuracy of the impact of high LDL in Africans remains controversial. Hypertriglyceridemia has also been demonstrated to contribute to dyslipidemia in Africans with metabolic syndrome but its contribution seems to be less frequent than reduced HDL as

demonstrated in Botswana and some part of Nigeria (Adeboye *et al*., 2012). According to Ogbera et al (2010), hypertriglyceridemia was also reported to be low or uncommon among people living in Cameroon and Cotonou (Ogbera *et al*., 2010). This pattern of dyslipidemia varied from the findings in north central Nigeria where a relatively high rate (62%) of raised TG was found. LDL was found to be the most common lipid abnormality followed by reduced HDL which occurred in about 60% of the individuals with MS (Ogbera, 2010), thus; there is a great need for dyslipidemia to be addressed in both lean and obese individuals because it is becoming common in metabolically healthy and metabolically unhealthy populations (Okafor, 2012).

Recent study by Liu et al (2020) suggested that increase in triglyceride is strongly associated with metabolic syndrome (MS); however, there are very limited studies integrating triglyceride (TG) and waist circumference (WC) into a continuous variable to investigate the predictive power of this phenotype (Liu *et al*., 2020).

However, in Africans with insulin resistance, serum triglyceride appears irrelevant to MS. A study reported that serum triglycerides is associated with only 17% prevalence of MS in non-diabetic African women in the United States. Studies have shown that the serum triglycerides > 1.7 mmol/l is not associated with changes in serum total cholesterol and LDL cholesterol levels in African American women (Osei, 2010; Peer *et al*., 2015). However, the study reported extremely low levels of serum triglycerides, despite the marked obesity as assessed by BMI (35 kg/m^2) and WC (> 102 cm) and percent body weight (40%) in non- diabetic African American women. Similarly, lower serum triglycerides levels have been reported in insulin-resistant, non-diabetic, obese black South African women when compared with white South African women and native Ghanaian/ Cameroonians women living in South Africa (Okafor, 2012; Peer *et al*., 2015). Thus, abnormal serum triglycerides seem to be a less powerful predictor of MS among people of African ancestry.

1.2.3.2. Insulin resistance among individuals from African ancestry

For decades Insulin resistance has remained the hallmark and key underlying pathophysiology of the metabolic syndrome (Amoah *et al*., 2003, Okafor, 2012). Insulin resistance is strongly associated with type 2 diabetes, obesity, hypertension and other cardio-metabolic diseases. Primarily, insulin maintains glucose homeostasis by integrated actions on carbohydrate, protein and lipid metabolism. These predominant actions take place in liver, skeletal muscle and adipose tissue. Glucose can alter insulin sensitivity in muscle and fat, as well as decrease insulin secretion from β cells of the pancreatic tissue. Pathogenesis of insulin resistance are numerous, however, one of the mechanisms involved elevated blood glucose which is induced by decreased glucose transport in skeletal muscle impairs adipose and hepatic insulin action. This is associated with inflammatory response and oxidative stress. Reports showed that Africans (youth and adults) manifest greater IR and insulin levels than their non-African counterparts. Factors that contribute to IR include genetics, obesity and lower physical activity, smoking which are higher in Africans. The relationships between IR and the components of MS still vary among different racial/ethnic populations. Despite the greater insulin resistance, Africans with insulin resistance manifest higher high-density lipoprotein cholesterol (HDLC) and lower serum triglycerides when compared with white counterparts (Osei and Gaillard, 2017). In the NHANES study, the prevalence of pre-diabetes/high fasting glucose meet the NCEP-ATP criteria, the changes in fasting glucose were insignificant over the three time-periods changes in fasting glucose over time among obese African American women (AAW), thus, obesity is considered a major contributor to glucose dysregulation and insulin resistance. Nevertheless, it may be possible that AAW are more sensitive to adverse changes in obesity given their higher prevalence of diabetes compared to non-Africans. Osei and Gaillard (2017) examined whether cardiovascular risk factors predicted future development of type

2 diabetes in 5 years. Their findings revealed that for each cardiovascular risk factor (hypertension, hypertriglyceridemia, low HDL, and impaired glucose tolerance) there was a doubling of risk for conversion to diabetes when compared to those without risk factors (Osei and Gaillard, 2017). Similarly, Africans lack significant or weak associations among insulin resistance and serum HDL-TG ratio, blood pressure and body composition and current data on MS are unable to explain the higher and excess CVD deaths among Africans (Peer *et al*., 2015). Notably, impaired glucose tolerance was the strongest risk factor associated with conversion to diabetes and cardiovascular disease in multi-ethnic population including African American.

1.2.3.3. Salt sensitivity and hypertension among people of African origin

Salt sensitivity is defined as an increase in blood pressure in response to sodium or salt intake, and commonly associated with a low circulating renin profile (Fujita, 2008; Bawa-Allah *et al*., 2019). Salt sensitivity is generally considered as hallmark of hypertension in people of African ancestry. (Svetkey *et al*., 1996). African descendants manifest a higher blood pressure response to change in salt intake than non-Africans independent of baseline blood pressure. Increased salt intake leads to a substantial rise in blood pressure that is consistent with enhanced salt sensitivity (Maseko *et al*., 2018). World Health Organisation recommended that salt intake should be limited < 5g/day while European Society of Cardiology guideline statement recommended that sodium intake should be limited to < 2g per day in general population as well as hypertensive patients.

It has been documented those individuals who exhibit salt sensitivity respond with substantial lowering of the blood pressure when dietary sodium is reduced (Svetkey *et al*., 1996). However, salt sensitivity has been associated with hypertension, the metabolic syndrome and target organ damage and it is beyond salt and water homeostasis (Rosengren, 2022). Therefore, research now

focus on possible mechanism and cross talk between salt sensitivity, insulin resistance, obesity and evidence of heritability.

Excess salt intake remains a key predisposition to essential hypertension in Africans (Maseko *et al*, 2006; Moinuddin *et al*., 2016). However, the blood pressure response to a change in salt intake might not be uniform due to variety of physiological, demographic, genetic and even environmental characteristics differentiate between salt sensitive and resistant population. Salt sensitivity appears to be determined by genetic factors, race/ethnicity, age, gender, body mass index (BMI) and salt diet (Fountain and Lappin, 2000). There was reduction in blood pressure due to intake of low-salt diet for two weeks, although, weight loss was also associated with a reduced rise in blood pressure in response to increased salt intake. However, salt sensitivity is specifically common in older adults, African Americans and hypertensive individuals with or without other comorbidities.

Furthermore, Weinberger et al (2006) observed that BP responses of both normotensive and hypertensive participants to salt depletion increased significantly as the age advanced with higher response in patients >30 years of age. Report has it that elderly people and patients with hypertension have a greater BP response to a change in salt than young adults and normotensive individuals (Weinberger *et al*., 2006; Mishra *et al*., 2018). Their study concluded that salt sensitivity was a predictor of subsequent, age-related BP increase. Numerous findings have established the fact that patients with hypertension were more salt sensitive than normotensives (Mishra *et al*., 2018). However, recent reflection of salt sensitive is discovered in females and obese individuals, although evidence for these associations is not clear, especially among Africans. Obesity increases sympathetic activity and salt sensitivity; the phenomenon appears to involve dysfunctional renal physiology. However, emerging novel evidence indicates that aldosterone production is sex-specifically heightened in salt-sensitive hypertensive women and female rodent

models, which may be regulated by intra-adrenal renin-angiotensin system activation and sex hormone receptors. In addition, new evidence that young females endogenously express higher levels of endothelial mineralocorticoid receptors (MRs) and that endothelial MR is a crucial mediator of endothelial dysfunction in females indicates that the aldosterone-endothelial MR activation pathway is a novel mediator of salt-sensitive hypertension (Faulkner *et al*., 2020). However, obese Asians were prone to hypertension than obese Caucasian; this is attributed to salt sensitivity. Similarly, Africans are also categorized as salt sensitive race, however, apart from racial influence; diet has also been attributed to salt sensitivity (Maseko *et al*, 2018). Using DASH (Dietary Approaches to Stop Hypertension) diet, BP response to dietary salt was found to be greater in the setting of a low-potassium intake and in the setting of poor-quality diet compared with the DASH (Dietary Approaches to Stop Hypertension) diet (Akita *et al*., 2003).

Plasma aldosterone and norepinephrine levels are closely related to the responsiveness of blood pressure to salt intake, suggesting an interaction between insulin sensitivity, the RAAS, and the sympathetic nervous system and their influence on the mechanism by which salt affects blood pressure. Both obesity and the metabolic syndrome are associated with insulin resistance. Earlier studies investigating the relationship between insulin resistance and salt sensitivity reported significantly higher levels of plasma insulin concentrations after an oral glucose challenge following a high-salt diet in normotensive and hypertensive individuals who exhibited salt sensitivity, compared with those who were salt resistant (Mishral *et al*., 2019). However, not all studies have shown a positive correlation between insulin resistance and salt sensitivity. An acute infusion of angiotensin II (Ang II) has been reported to improve insulin sensitivity in both rats and humans whereas chronic infusion of Ang II leads to insulin resistance mediated via oxidative stress. In animal studies, obesity with excessive production of angiotensinogen (AGT) in adipose tissue probably includes such a pathophysiological state, as mice overexpressing adipose AGT

have exhibited reduced insulin sensitivity, as estimated by the homeostasis model assessment index. Therefore, insulin resistance causes hypertension by the retention of sodium (Endo *et al*., 2009; Mishral *et al*., 2018). Summarily, the result supports the hypothesis that hyperinsulinemia associated with insulin resistance in obese patients causes hypertension due to sodium retention in the kidney. This explains why the prevalence of salt-sensitive type hypertension is significantly higher in patients with metabolic syndrome than those without metabolic syndrome. Additionally, obese hypertensive patients showed a greater depressor response to salt restriction and diuretics as compared to non-obese hypertensive patients (Mishral *et al*., 2018).

Central or abdominal obesity is first criterion attributed to metabolic syndrome. Crude assessment of anthropometric measures of visceral/abdominal obesity such as BMI, waist circumference (WC), and waist-hip ratio (WHR) are strong and consistent predictors for obesity related diseases such as type 2 diabetes mellitus, cerebrovascular and cardiovascular diseases. Body mass index (BMI) is the most commonly used parameter to determine abdominal obesity, this is assessed as weight in kilogram divided by square of height in meters, body mass index (BMI) greater than 30 kg/m^2 is classified as obesity. WHR optimal cut off values of 0.89 for men and 0.82 for men and women respectively was initially suggested by World Health Organization (WHO) as better anthropometric measure for estimating the risk of type 2 diabetes mellitus (Ahmad *et al*., 2016). Presently, World Health Organization (WHO) agreed on normal cut off value of WHR is > 0.9 and > 0.85 in men and women respectively, However, World Health Organization (WHO) guidelines further state that alternative measures that reflect abdominal obesity such as WC and waist-to-height ratio (WHtR) have been found to be superior to BMI. A study among Chinese population revealed that BMI and WC were found to be the important indices of obesity, but WC was found to be the best measurement of obesity whereas WHR could be used as an alternative

indicator for obesity (Yanga *et al*., 2006). Meanwhile, hypertensive patients had a significantly higher WHR (>0.9) as well as a significantly BMI >30 kg/m² when compared to the apparently normal individuals. Using the lowest cut off points recommended for Asians, a study among Indians found that the prevalence of abdominal obesity when WC was considered in men and women were 46% and 64% respectively, while the prevalence recorded when WHR was considered in each sex was about 12% in men and 68% in women (Kurpad *et al*., 2003). Moreover, according to study conducted by Ahmad and colleagues, prevalence of abdominal obesity for BMI among 669 adults living in three rural settings in Malaysia was almost similar for both gender across Caucasian and Asian BMI cut-off points. Based on Caucasian cut off points, the prevalence of abdominal obesity for WC was 23.8% (male) and 66.4% (female) while for WHR was 6.2% among males and 54.2% in females. Asian cut off points gave higher prevalence of abdominal obesity compared to that of WC among male respondents and WHR for both genders. Waist circumference showed strong and positive correlation with BMI compared to WHR in male and in female. The cut-off points of 92.5 cm in men and 85.5 cm in women was set as the optimal number for detection of abdominal obesity (Ahmad *et al*., 2016). Their study concluded that WC is the best indicator as compared with WHR for abdominal obesity for Malaysian adults.

However, two retro prospective studies that used the US National Health and Nutrition Examination Survey (NHANES III) database revealed that prevalence of metabolic syndrome was higher in obese than overweight and normal-weight men and women (Lee *et al*, 2016). Within each BMI category, there were differences in the proportion of total body fat between those with and without the metabolic syndrome. The study concluded that, obese women without metabolic syndrome had a significantly higher proportion of body fat than obese women with metabolic syndrome. Meanwhile, several groups of people who have normal body mass index (BMI) meet

the criteria for MS. Hence, waist circumference (WC) is reported to be greater predictor of MS in several populations, although the cut off points vary according to the IDF criteria. However, in Africans, WC is the most common component of MS. Meanwhile, the regional body fat distribution and composition tend to vary among ethnic and racial populations. For instance, when comparing two identical body mass index of Africans and non-Africans. A have lower intra-abdominal visceral adiposity (VAT) when compared to non-Africans. Even though, the corresponding subcutaneous fat depot (SAT) tends to be the same or greater in Africans than non-Africans in both genders. Thus, WC serves as a surrogate for intra-abdominal visceral adipose tissue (VAT) and an important determinant of insulin resistance and its concomitants for cardiovascular in non-African populations. This is more applicable and relevant in several non – Africans, but remains controversial in Africans. Research has shown that Africans with same BMI but living in different geographic locations have lower visceral adiposity despite increased insulin resistance when compared with non- Africans (Millen *et al*., 2013). Similar findings have been reported in black South Africans living in urban settlement. A study by Majane et al (2005) reported the relationship between waist circumference (WC) and conventional blood pressure (BP) is independent of other clinical indices of adiposity. Their findings showed that ambulatory BP may offer more prognostic information than conventional BP, they identified the association between the indices of central adiposity and ambulatory BP. The relationship between indices of adiposity (WC, waist-to-hip ratio, body mass index (BMI) or skin-fold thickness) and ambulatory or conventional BP was determined in 300 randomly selected individuals of African descent living in an urban developing community in South Africa. Relationships were determined with multiple indices of adiposity in the same regression model following adjusting for age, gender, alcohol and tobacco intake, the presence or absence of diabetes mellitus or inappropriate blood glucose control [haemoglobin A1c (HbA1c)], antihypertensive therapy and menopausal status. Sixty-five per cent

of participants were recorded as overweight or obese. In addition, increase in WC resulted in a 4.04 mmHg and 4.33 mmHg increase in 24-h systolic BP and in 24-h diastolic BP respectively. Interestingly, similar results were obtained in the subgroup of 237 participants not receiving antihypertensive therapy. The study concluded that WC is the only clinical index of adiposity that is associated with 24-h and conventional BP independent of other adiposity indices in a black community with a high prevalence of obesity (Majane *et al*., 2005). However, in most cases more women than men met the waist circumference criterion, but a higher proportion of non-African men than women were positive for the blood glucose criterion. Meanwhile, among Africans and non-African men, a higher proportion of non-Africans than Africans met waist circumference. The reasons for these discrepancies are yet to be investigated. However, increased waist circumference (WC) is now recognized as predictive factor for assessment of intra-abdominal fat and metabolic syndrome.

The measurement of waist circumference is widely advocated as simple anthropometric measurement, however, there is variability in the method according to different association and groups. In this study, the method of measurement of waist circumference is an adopted method that has been previously used by other principal investigators in our laboratory (Maseko *et al*., 2018; Bawa-Allah *et al*., 2019). Using a non-extensible tape, waist circumference was measured at the narrowest point between the lower costal (10th rib) border and the top of the iliac crest, perpendicular to the long axis of the trunk in our study (Hume, 2017; Bawa-Allah *et al*., 2019). It an acceptable method in South Africa. Diagnostic value for waist circumference was taken as $\geqslant$102 cm for men and $\geqslant$ 88 cm for women. Other crude assessment of subcutaneous fat such as hip circumference and skinfold measurement are obsolete and believed to be clinically irrelevant (Ayling, 2014; Hume, 2017). Modern analysis of body composition involves CT and MRI, that is,

use of dual X-ray densitometry (DXA) to show that the relation between adipose mass and lean body mass (Shuster *et al*., 2012; Mtintsilana *et al*., 2019).

Although many research works have been done worldwide on various aspects of anthropometric measurement to predict the risk of obesity-related cardiovascular diseases, the correlation of BMI with WC and WHR has seldom been addressed among in South Africans with metabolic syndrome. Currently, the biological significance of WC varies and remains debatable among racial and ethnic populations. This suggests that factors other than generalized adiposity are associated with metabolic syndrome and its complications in adults. However, one of the objectives of this study is to determine the correlation between BMI with WC and WHR in diagnosis of metabolic syndrome in people of African ancestry.

1.2.4. Sensitivity of the assessment of major diagnostic criteria

The metabolic syndrome (MS) is also defined by the constellation of lipids and lipoprotein and raise blood sugar. As mentioned earlier, a higher proportion of non-African men than women were positive for the blood glucose criterion while other components ascribed to metabolic syndrome were similar in non-African men and women. Among men, a higher proportion of non-African met serum triglyceride, and HDL cholesterol criteria, whereas African men had higher rates of elevated and abnormal blood glucose values. Meanwhile, among women, whites had higher rates of abnormal serum triglyceride levels and lower HDL cholesterol levels, whereas the African women had higher rates of hypertension, abnormal blood glucose levels, and large waist circumference. Thus, lipid abnormalities were nearly 2 times more common in non-Africans, while Africans had a higher prevalence of blood glucose abnormalities and hypertension than non-Africans (Goodpaster *et al*, 2005). Higher prevalence of cardiovascular disease in Africans compared to non-Africans have been attributed to other cardiovascular risk factors, such as

subclinical inflammation, increased low-density lipoprotein oxidation, lipoprotein a, adiponectin, and plasminogen activator inhibitor-1 (Han and Lean, 2016). Most importantly, it is believed that these adipocyte-derived cytokines and peptides could be the link between obesity, insulin resistance, MS, hypertension, type 2 diabetes and cardiovascular disease. Some studies in Africans have shown higher levels of these fat tissue–derived peptides including tumor necrosis factor-α, resistin, leptin, interleukin-6, C-reactive protein than non-Africans. Thus, to understand cardiovascular disease (CVD) and type 2 diabetes (T2DM) differences in African and non-African with MS, it is essential to explore the contributions of both cardiovascular and diabetic risk factors in people of African and Europoid ancestry. Reports by Mijkoviv-Gacic et al (2006) and Frazier-Wodds et al (2013) revealed that total, medium, and small VLDL particles in African-Americans and Afro-Caribbeans when compared to non-Africans, using NMR-derived lipoprotein, LDL particle sizes and numbers are consistent with the well accepted pathogenic role in atherosclerosis in non-African, but not so for Africans (Mijkovic-Gacic *et al*., 2006; Frazier-Wodds *et al*., 2013). Basically, alterations in lipids and lipoproteins play pathogenic role in atherosclerosis as well as major components of MS. Metabolic syndrome is associated with higher serum triglycerides and lower HDL and higher small dense low-density lipoprotein (LDL) (apo B) particles. This has been attributed in part to the underlying IR and obesity. Abnormal lipid profile are seen in patients with pre-diabetes and progresses with time. However, the relationships between IR and lipids and lipoproteins differ among racial and ethnic populations. In this regard, IR is associated with lower triglycerides and higher HDL in Africans and non-Africans. Thus, these relationships are inverse and paradoxical in Africans with MS than non-Africans residing in diverse geographic locations. Therefore, it has been postulated that Africans with and without MS have favourable anti-atherogenic lipid and lipoprotein profiles that could theoretically protect them against cardiovascular disease. Therefore, fasting triglycerides and TG/HDL ratios appear not to be

markers of IR in Africans. The reasons for this metabolic paradox remain unknown. Several reports have attributed the lower triglycerides in African to possible defective hepatic synthesis of very low-density lipoprotein (VLDL). Yet, it can be inferred that racial and ethnic differences in lipids and lipoprotein profile cannot explain the paradoxically higher and excess cardiovascular mortality and morbidity in Africans when compared to non-Africans. In support of the hypothesis, Dallas Heart Study and Multi-Ethnic Study of Atherosclerosis also agreed that the number of large HDL particles are greater in non-diabetic Africans (Lee *et al*., 2016).

Despite the greater insulin resistance, people of African ancestry individuals with insulin resistance manifest higher high-density lipoprotein cholesterol (HDL) and lower serum triglycerides when compared with white counterparts. Summarily, all the existing hypothesis and findings are yet to elucidate reason for higher and excess cardiovascular mortality and morbidity in Africans. Therefore, non-traditional risk factors should be integrated in defining MS as a predictor of MS and cardiovascular problems in Africans. Schutte and colleagues investigated the relationship between inflammation, obesity and cardiovascular disease in a South African population, they found significantly increased levels of leptin, high-sensitivity C-reactive protein (hsCRP) and fibrinogen ($p < 0.05$) in African women compared with their Caucasian counterparts (Schutte *et al*., 2006). Previous study by Schutte group investigated the differences in blood pressure (BP) for age- and BMI-matched African women and their Caucasian counterparts (Schutte *et al*., 2005). Their study revealed that obesity was strongly connected to cardiovascular risk in African women. A study conducted by Santo et al., (2008) reported increase prevalence of metabolic syndrome in females in lower social classes. However, the report is not specific about the race or ethnicity.

1.2.5. Variability in the assessment of hypertension and obesity

In both non-African and African, attention has been focused on hypertension and obesity, due to the high prevalence of these modifiable risk factors in the general population (Cuspidi *et al*., 2014). Hypertension is a major component of metabolic syndrome linked with an increase in visceral fat. There are racial and gender differences between MS-associated high blood pressure. Obesity is strongly associated with higher-than-optimal blood pressure. Hypertension is more frequent in obese subjects than in normal-weight subjects. Increase in blood pressure is greatest when the obesity is of abdominal distribution. Most patients with high blood pressure are overweight. Hypertension is about 6 times more frequent in obese subjects than in lean men and women (Omboni *et al*., 2016). Excess weight gain in young people is a potent risk factor for subsequent development of hypertension. A study revealed that increase in 10kg of body weight is associated with a 3.0 mmHg and 2.3 mmHg increase in systolic and diastolic blood pressure respectively (Poirier *et al*., 2006). Studies revealed that the risk of hypertension was up to five times higher among obese people than in their normal-weight counterparts (Sweet *et al*., 2007; Omboni *et al*., 2016). Obesity was also positively associated with type 2 diabetes and it was noted that nearly 90% of individuals who progressed to type 2 diabetes had BMIs greater than 30 kg/m². The compelling association between obesity, hypertension and diabetes among populations of African descent has also been documented. According to Coronary Artery Risk Development in Young Adults (CARDIA) study majority of African Americans develop high blood pressure in their mid-50s, that is, three quarter of Black Americans develop hypertension compared to little above half of white men and less than half of white women (Sweet *et al*., 2007). In the United States African Americans develop hypertension at an earlier age than whites, have much higher average blood pressure readings, a greater likelihood of refractory hypertension, and greater rates of premature hypertensive complications such as chronic kidney disease, stroke and heart disease. More

importantly, African Americans suffer from a three-fold higher death rate from hypertension with cardiovascular complications (William *et al*., 2014). This implies that Africans had a 1.5 to 2 folds higher risk for hypertension compared to non- Africans regardless of their blood pressure levels in young adulthood. This result to controversial revised American Heart Association/American College of Cardiology guidelines setting high blood pressure as 130 mmHg or higher diastolic and 80 mmHg or higher systolic. Based on these guidelines, the hypertension by the age of 55 in Africans and non-Africans varies such as 77.5% of African men,77.7% of African women, 54.5% of non-African men and 40.0% of non-African women were proposed to be hypertensive at age 55 (Thomas *et al*., 2018). Among South Africans, study by Norton team determined whether blood pressure (BP)-LVM relationships depend in-part on the influence of an excess adiposity and whether this translates into a greater effect of hypertension on LVM in obese as compared with lean people. The result revealed that adiposity-induced increases in LVM reflect an enhanced effect of BP on LV growth, an effect that may be mediated by leptin. This translates into an impact of untreated hypertension on LVMI in centrally obese, but not in lean people in groups of African descent in South Africa (Norton *et al*., 2009). Severe obesity is capable of producing hemodynamic alterations that predispose to changes in cardiac morphology and ventricular function. These include increased cardiac output, left ventricular hypertrophy and diastolic and systolic dysfunction of both ventricles. co- morbidities such as hypertension, and possibly certain neurohormonal and metabolic alterations/ abnormalities may predispose to left and right heart failure, a disorder known as obesity cardiomyopathy (Alpert *et al*., 2014). Obesity is independently associated with changes in cardiac output and peripheral vascular resistance. Increase in cardiac output may add to the increase in blood pressure in the obese individual because, an abnormal increase in blood pressure is primarily dependent on an increase in peripheral vascular resistance. Some of the mechanisms linking obesity and an increase in peripheral vascular resistance:

endothelial dysfunction, insulin resistance, sympathetic nervous system, substances released from adipocytes these include; IL-6, TNF-α (Touyz *et al*., 2018). Alterations in these mechanism and adipose tissue related pro-inflammatory effects culminate to pathognomonic atherosclerotic events.

A study by American Heart Association reports showed that prevalence of hypertension among African- Americans (AAs) is one of the highest is the world (O'Brien *et al*., 2003; Gillard *et al*., 2009; Stephane *et al*., 2016). Hypertension is more common in African-Americans than Americans. The group also report that African-Americans suffer disproportionally from higher rates hypertension and cardiovascular mortality when compared to non-African women. The rationale is yet to be documented. Although, insulin resistance has been associated with BP in several populations; however, there is a very weak relationship in Africans when compared to non-African (Gillard, 2018). However, it has been speculated that genetic and environmental factors, increased vascular reactivity and increase renal sodium reabsorption are contributing factors. Blood pressure responsiveness to high and low dietary salt intake is shown to exhibit significant heritability in African population. However, the trend of higher prevalence of high blood pressure with increasing BMI was similar for Africans and non-Africans of both genders, and the age-adjusted rates were highest among the latter at every level of BMI. It is well recognized that technical difficulties exist in the indirect measurement of blood pressure in the obese patient that may result in an overestimation of the level of blood pressure.

1.2.6. Conventional blood pressure and ambulatory blood pressure

Current values of brachial BP were used to define hypertension according to 2007 ESH (European Society of Hypertension–European Society of Cardiology guidelines. The VASOTENS Registry is an international telehealth-based repository of 24-hour ambulatory blood pressure monitoring (ABPM) obtained through an oscillometric upper-arm BP monitor allowing combined estimation

of some vascular biomarkers (Omboni *et al,* 2019). Common practice of using clinic/conventional measurements of brachial BP as pressure load imposed on the heart and on central circulation which include coronary and cerebrovascular arterial trees may be inaccurate. Central aortic pressure is a better predictor of cardiovascular outcome than peripheral pressure (Omboni *et al*., 2016). Evidence that antihypertensive treatment may have differential effects on brachial BP compared with central BP has been established. Various studies in hypertensive patients have provided preliminary evidence to suggest that changes in central BP by pharmacological therapy are more important than decreases in brachial BP in altering cardiac and vascular hypertrophy associated with hypertension (Cheng *et al*., 2019). Central blood pressure (BP) has been shown to predict cardiovascular disease clinical outcomes better than brachial BP in studies that demonstrate expected pulse pressure (PP) amplification. The likely underlying mechanism for this observation is a closer relation of central BP to preclinical cardiac and vascular disease because of its more accurate representation of loading conditions on the heart and coronary and cerebral vessels. Non-invasive central BP is a new parameter that involves using either carotid or radial artery applanation tonometry (Roman and Devereux, 2014; Cheng *et al*., 2019). Applanation tonometry measures the pressure at the aortic root. It accurately detects effects of elevated blood pressure on target organs. Elevated aortic pressure is acknowledged as accurate pressure causing target organs damaged. Therefore, preclinical hypertensive target organ damage, left ventricular (LV) mass and common carotid artery (CCA) geometry can be readily and accurately assessed using non-invasive techniques (Woodiwiss *et al*., 2009; Calderón *et al*, 2010; Roman and Devereux, 2014). Metabolic syndrome is a combination of structural and functional damage to the vasculature and interrelationship between these factors is critical in understanding the pathophysiology of cardiovascular diseases. In addition, the alteration in the central and peripheral pressures as a result

of arterial stiffness and endothelial dysfunction may also influence the interrelationship between these parameters in the central and peripheral vessels (Bradhwar *et al*, 2018).

1.2.7. Pulse Wave Analysis (PWA)

Previous study by Majane and colleagues explored the effect of age on the independent relationships between measurement of central adiposity; waist circumference (WC) and waist-to-hip ratio (WHR)) and carotid-femoral PWV, central augmentation index (AIc), or pressure wave amplification assessed from applanation tonometry measurements obtained at the radial, carotid, and femoral arteries in 508 randomly selected participants above the age of 16 years in a population sample of African ancestry with a high prevalence of excess adiposity. The report clarified that age influences the independent relationship between adiposity and pulse-wave velocity (PWV). In women of African ancestry, a greater independent effect of adiposity on PWV is noted in older as compared to younger women (Majane *et al*., 2008). One of the objectives of our study is to investigate vascular changes and arterial stiffness in Africans of varying age with MS.

Pulse wave analysis (PWV) is a direct measure of aortic stiffness and has been shown to be a risk factor in a number of cardiovascular-related diseases. The carotid-femoral PWV measurement can be determined by the mechanical properties of the aorta and a portion of the carotid artery, both of which are elastic arteries. It helps to identify the plaque characteristics using imaging modalities that help direct interventions in atherosclerosis with MS. Carotid intima media thickness (CIMT) assessed using high resolution B-mode US detects increased CIMT >1 mm, but cannot provide information about the biology of the plaque. However, CIMT methods are still being refined, it is important to know which part of the artery was measured (common carotid, internal carotid, or bulb) and whether near and far walls were both measured. Measurement can be reported as the average thickness or maximum thickness, although the average is more commonly reported. CIMT

measures the thickness of two layers (the intima and media) of the wall of the carotid arteries, the largest conduits of blood going to the brain. Carotid intima media thickness is thought to be an even earlier manifestation of atherosclerosis than coronary artery calcification because, thickening precedes the development of frank atherosclerotic plaque. However, aortic pulse pressure is shown to be a reliable indicator of the relationship between left ventricular stroke volume and aortic compliance/elasticity. Observed central (aortic) pulse pressure indicates heightened risk of adverse cardiovascular events such as ischemia/infarction. High aortic pulse pressure is also reported to be indicative of low aortic/arterial compliance or stiffness. Metabolic syndrome has also been associated with early vascular alterations, such as increased arterial stiffness and endothelial dysfunction (Omboni *et al*., 2019). The association with these vascular alterations might account for the cardiovascular risk of patients with MS, because both increased arterial stiffness and endothelial dysfunction. Therefore, this study evaluates the relationship between MS and central aortic pressure using aortic wave analysis to analyse central pulse wave among individuals with or without MS.

In patients with metabolic syndrome, PWV is aggravated, regardless of age and sex. A study by Omboni et al (2016) assessed pulse wave velocity (PWV) and augmentation index (AIx) in control and metabolic syndrome patients. Peripheral and central BP, PWV and AIx values were reported to increase in older participants (SBP only) and in those diagnosed with hypertension (SBP and DBP). BP was lower and PWV and AIx higher in females. Also, PWV was increased and BP unchanged in case of metabolic syndrome. The study results suggested that ambulatory pulse wave analysis in a daily life setting may help evaluate vascular health of individuals at risk for cardiovascular diseases (Omboni *et al*, 2019). Increased PWV reflects lower vessel distensibility and higher vascular stiffness. Vascular stiffness can be indexed as pulse wave velocity (PWV),

defined as the velocity of the systolic pressure waves propagating along the vessels. Pietri *et al*., (2009) also documented that metabolic syndrome is associated with a high risk of cardiovascular disease in hypertension. In their study, augmentation index and pulse wave velocity were determined with applanation tonometry in 391 untreated hypertensive patients and 166 controls. In their study, metabolic syndrome was defined according to the latest National Cholesterol Education Program Adult Treatment panel III. In untreated hypertensive patients, the presence of metabolic syndrome, which selectively impairs central pulse wave velocity, does not account for a further deterioration of peripheral wave reflection and conduit artery endothelial function. Their study revealed the association of metabolic syndrome with early vascular alterations, such as increased peripheral wave reflections and endothelial dysfunction, in untreated essential hypertensive patients (Pietri *et al*., 2009). Central pulse wave velocity was selectively impaired by metabolic syndrome in both genders. However, female gender was associated with increased augmentation index in the presence of the metabolic syndrome. The findings suggested that blood pressure, age and gender are important determinants of peripheral vascular structural and functional parameters in these subjects, irrespective of the metabolic syndrome. The finding further revealed that hypertensive patients showed significantly increased augmentation index and central pulse wave velocity, as well as reduced flow-mediated dilation, compared with controls (Pietri *et al*., 2009). However, no further impairment in augmentation index or flow-mediated dilation was observed between patients with or without the metabolic syndrome components and with increasing number of metabolic syndrome (Pietri *et al*., 2009). Hence, this study investigated the impact of metabolic syndrome and its major components on central waveform among people from African ancestry. Some epidemiological studies have suggested that MS per se might cause increased arterial stiffness and serve as independent predictor of cardiovascular morbidity and mortality in unselected populations. For instance, obese African-American (AA) women are at

high risk of hypertension and cardiovascular disease (CVD). Flow-mediated dilation and arterial augmentation index (AI) are measures of endothelial function and arterial stiffness (Bond *et al*., 2019). Whether endothelial function and arterial stiffness predict risk of HT or CVD in obese African-American women with, versus without, parental histories of HT and whether aerobic exercise is an effective countermeasure remain unclear. Greater AI may be found in normotensive-obese, young African- American women with hypertensive parents than in a matched control group with normotensive parents.

Commonly, applanation tonometry is a clinical method for estimating global PWV by measuring distance and the temporal shift between the carotid and femoral pressure waveforms (Herbert *et al*., 2014). Currently, local or regional PWV can be cardiac function, aortic geometry (diameter and arch length, widening, and curvature) and distensibility, compliance, elastic modulus and stiffness index. This direct measurement of the path length of pulse waves enables to evaluate local or regional PWV in specific vascular segments, as well as global PWV even in the presence of a tortuous vessel. The distance between measurement sites can be manually or semi-automatically assessed, and the transit time for wave propagation can be automatically determined using time-flow or time-velocity curves (Herbert *et al*., 2014). This study aims to gain further insight into possible atherosclerosis among African individual with MS using applanation tonometry device.

1.2.8. Cardio-renal fibrosis and mineralocorticoid pathway: Role of TRMP7 activation

In obesity, aldosterone production seems to be increased systemically by dietary salt intake and locally in adipose tissue by increased expression of cytokines. paracardial fat may exert a restrictive pressure on myocardial diastolic expansion. Para-renal, perirenal, and renal sinus fat accumulation may increase intrarenal pressures, sodium reabsorption, and hypertension via activation of the RAAS (Fang *et al*., 2016). In addition, the renal vasoconstriction attributable to

Ang II can induce ischemia, particularly at higher doses, long-term Ang II infusion more closely models the renal injury that accrues from chronic renal ischemia in human hypertension. For the past few years, the role of the local RAAS in specific tissues has been a focus of research, evidence suggests the importance of tissue RAAS in the heart, kidney and vasculature. Interplay of dietary sodium and renin-angiotensin-aldosterone system (RAAS) activity with the prevalence of left ventricular hypertrophy (LVH) in essential hypertension (Touyz *et al*., 2022). Hence, this study lends insights into the mechanisms that explain the variable relationship between aldosterone-salt and cardio-renal fibrosis; thus, suggests that aldosterone receptor blockade by TRPM7 may be an important modality of therapy in groups of African descent.

1.2.9. Target organ damage as a major consequence of obesity related metabolic syndrome

MS exacerbates arterial stiffness, and atherosclerosis (Nemcsik *et al*., 2017). Abdominal obesity appears to be an independent predictor of all-cause mortality in men and perhaps also in women. Heart failure, which is among the most common and disabling forms of cardiovascular disease, is closely associated with obesity. Current guidelines for the management of heart failure provide conflicting directions for the prognosis and management of BMI. American College of Cardiology (ACC)/AHA heart failure clinical practice guidelines for adults do not directly address the issue of BMI. The European Society of Cardiology recommends weight loss for overweight and obese patients with heart failure even though this recommendation is not supported by data from clinical trials (Han and Lean, 2016; Lippi and Sanchis-Gomar, 2020). Clinical and epidemiological studies have established that increase in BMI worsens medical conditions. However, body mass index-mortality analysis may be influenced by factors such as smoking, age, underlying pathologies and pharmacological management. For example, analysis from 7767 patients with stable heart failure enrolled in the Digitalis Investigation Group Trial (DIG) reported that higher BMI was associated

with lower mortality risk in patient on digitalis than those without digitalis (Lippi and Sanchis-Gomar, 2020). However, both obesity and metabolic syndrome are increasingly prevalent risk factors for cardiovascular disease and hypertension. The association of obesity with atherosclerotic heart disease has been widely known. MS is associated with presence and severity of coronary atherosclerosis. Arterial stiffness and microvascular dysfunction are markers of subclinical CVD and are associated with long-term cardiovascular morbidity and mortality. Studies have shown greater subclinical vascular dysfunction in African-Americans compared to non- Africans, even after adjustment for all cardiovascular risk factors. Although the impact of MS on vascular function has been reported, the strength of this association has been variable, and observations have been limited to largely non-Africans. Yet, it is unclear whether MS accurately identifies vascular dysfunction in both non-Africans and African-Americans. However, Africans without metabolic syndrome and hypertension have worse vascular function when compared with non-Africans. This shows that definition of metabolic syndrome may not capture the increased underlying risk that varies by race. This may aggravate or worsen incident of cardiovascular abnormalities in Africans. From the report of World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity, there is a revised set of criteria harmonizing the definition of the metabolic syndrome. However, recommendation of the use of ethnic or country-specific cut off points for central obesity is still paramount. Considering the fact that these definitions can be broadly grouped into those which require the measurement of insulin resistance and those which do not. Meanwhile, definitions such as NCEP, ACE, and IDF can easily be applied in a routine clinic setting and do not require measurement of insulin resistance have been reported by reported to be more useful than those requiring measurement of insulin levels (WHO and EGIR) as they identified twice more patients with insulin resistance and increased Framingham risk scores (Can and Bersot, 2007).

1.3 Justification of the study

Heart failure is a common complication associated with obesity, hypertension and metabolic syndrome. These components are related to aldosterone-salt induced fibrosis. Currently, there is rising prevalence of obesity, hypertension and heart failure in people of African ancestry. However, various definitions and the cut-off points for MS might not be favourably diagnose metabolic syndrome in people from African ancestry. Therefore, it is important to evaluate different diagnostic criteria and understand the suitable diagnostic criteria and possible factors underlying the development of metabolic syndrome and its cardiovascular complications in Africans; the outcome will enhance early diagnosis; thus, prevent metabolic-related cardiovascular complications.

1.4 Aims and objectives

The aim of this study was to determine the factors that contribute to the prevalence and cardiovascular complications in people of African ancestry.

Objectives

i. To evaluate the prevalence and determinants of metabolic syndrome among the population with high prevalence of obesity using different diagnostic criteria
ii. To evaluate the association between metabolic syndrome in WHO category and the assessment of blood pressure.
iii. To determine whether metabolic syndrome influences arterial stiffness when the WHO criteria is applied.
iv. To assess the impact of metabolic syndrome on left ventricular geometry and function.
v. To investigate the role of aldosterone-salt induced cardiac and renal fibrosis and also assessed role of TRMP7 on cardio-renal fibrosis and aortic vasculature.

CHAPTER TWO

Assessment of the prevalence of metabolic syndrome using different diagnostic criteria in people of African ancestry.

Abstract:

Introduction:

Metabolic syndrome is an accumulation of several disorders, which together raise the risk of developing neurological and cardiovascular complications. Over the years, metabolic syndrome was thought to be rare in Africa, but current trends are showing increase prevalence in both developed and developing countries. The prevalence of metabolic syndrome is believed to be on the increase in African populations due to westernization which results in the increase in the prevalence of obesity. Obesity plays major role in the pathogenesis of metabolic syndrome; it has interwoven mechanism with other components of metabolic syndrome. Based on the variation in the assessment of obesity combined with other relative factors, there may be discrepancy in assessment of metabolic syndrome in Africans. Hence, the aim of this study was to assess the prevalence of metabolic syndrome and to evaluate its determinants using different diagnostic criteria.

Methods:

One thousand, five hundred and sixteen (1518) participants were recruited, the cross-sectional study was conducted in 678 participants from African ancestry, with a minimum age of 18 years and no upper age limit. Obesity was assessed using body mass index (BMI), waist circumference (WC) and waist hip ratio (WHR), while conventional blood pressure was assessed using electronic blood pressure monitoring device (Omron, Kyoto, Japan), blood sample was taken for laboratory

parameters such as lipid profile [Triglyceride (TG), High lipid lipoprotein (HDL)], fasting blood sugar. Seven diagnostic groups (Modified NCEP-ATPIII, NCEP-ATPIII, IDF, WHO, EGIR, AHA/NHLBI and AACE diagnostic criteria) were used for assessment of prevalence and determinants of metabolic syndrome among the study group. Descriptive and inferential statistics were conducted using SPSS version 22.0.

Results:

Prevalence of metabolic syndrome in Modified NCEP-ATPIII, AACE, IDF, WHO, AHA/NHLBI, NCEP-ATPIII and EGIR diagnostic criteria were 24.2%, 22.4%, 21.4%, 20.4%, 18.7%, 16.6% and 14% respectively. Among the 668 selected participants, 36.8% were obese while 23.4% were overweight. All the indices for crude assessment of obesity increased prevalence of metabolic syndrome. Participants with increased waist circumference (WC) were 39.7%, those with BMI greater or equal to 30 kg/m^2 were 36.8%, while those with abnormal WHR were 36.2%. In this study, 24.7% had blood pressure of 130/85 mmHg while 15.2% had blood pressure greater than 140/90 mmHg. Prevalence of MS was higher in females compared to males in all the diagnostic groups except EGIR. The prevalence increases with age among all the groups.

Conclusion:

Prevalence of metabolic syndrome varies and depends on the criteria used in different diagnostic categories. WC (is an appropriate measure for crude assessment of central obesity among African with high prevalence of obesity. Hypertension and other relative factors also affected the prevalence of MS. This study concluded that WHO criteria is more suitable to determine the proportion of metabolic syndrome in Africans. This study also suggested that age and sex should be considered as criteria for diagnosing metabolic syndrome.

2.1 Introduction

Metabolic syndrome (MS) is the accumulation of several disorders, which together raise the risk of an individual developing atherosclerotic cardiovascular disease, insulin resistance, and diabetes mellitus and neurological complications (Elffers *et al*., 2017; Manaf *et al*., 2021). Irrespective of the discrepancies in various definitions, there is shocking figure of a vast proportion of the population being at high risk of development of cardiovascular complications of metabolic syndrome (Belete *et al*., 2021). The prevalence of metabolic syndrome is believed to be on the increase in African populations due to westernization and genetic factor (Okafor, 2012; Shi *et al*., 2020).

Globally, obesity is a major health problem in urban areas in diverse regions (Schutte *et al*., 2020). Overweight/obesity levels are rising in more developed and developing parts of Africa. Several studies have suggested that central obesity has a key role in the elevated cardiovascular risk associated with MS, however, the BMI is not a robust marker of central obesity (Tomiyama *et al*., 2003; Swarup *et al*., 2022). Hypertension and diabetes have been attributed to increased body fat (Schutte *et al*., 2020). Risk of comorbidities tends to rise with body mass index (BMI). Adiposity contributes significantly to cardiovascular disease (CVD) in both Africans and non-Africans. Obese people, especially African women have two to four times greater risk of CVD morbidity and mortality than non-Africans women. Clear evidences show that specific ethnic groups are particularly vulnerable to obesity (Peer *et al*., 2015; Liu *et al*., 2021). However, considerable variation exists across ethnic groups in the proportion of fat and lean tissue at equivalent BMIs and other environmental-genetic interactions, there is still unexplained variation in the association between comorbidities and higher BMIs differs among various ethnic groups.

However, a number of expert groups developed clinical criteria for the metabolic syndrome. The groups include, National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII), American Association of Clinical Endocrinology (AACE), International Diabetes Federation (IDF), American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) and European Group for the study of Insulin Resistance (EGIR). Three or more of the five components to constitute MS (Osei, 2010; Kassi *et al*., 2011). All the groups agreed on the major components of the metabolic syndrome these are, obesity, insulin resistance, dyslipidaemia and hypertension. Yet, there is still no universally accepted definition or criteria (Osei, 2010; Kassi *et al*., 2011). The prevalence of MS may be inappropriately assessed due to multiple definitions for the metabolic syndrome. The existing guidelines were either difficult to use or gave conflicting results when attempting to assess individuals from different ethnicity. Therefore, it is difficult to compare data from different studies where different definitions and/or population have been used to define or categorize metabolic syndrome.

In the past, most widely accepted definition were produced by World Health Organization (WHO), the European Group for the Study of Insulin Resistance (EGIR), and the National Cholesterol Education Program – Third Adult Treatment Panel (NCEP ATP III). Meanwhile, IDF group suggested that there must a universally acceptable diagnostic tool that should be accessible clinically. Therefore, the International Diabetes Federation (IDF) released different guidelines and criteria based on gender, race and ethnicity. However, till date, European cut off points are still recommended in most African populations where specific data on diagnostic criteria are not yet available (Fezeu *et al*., 2007; Jin-Kee *et al*., 2015).

Recently among some Africans, metabolic syndrome is being diagnosed according to the Modified National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria, i.e. at least

three of the following had to be present: blood pressure ≥ 130/85 mmHg; waist circumference ≥ 102 cm (men) or ≥ 88 cm (women); triglycerides ≥ 1.69 mmol/l or on drug treatment for elevated triglycerides; HDL cholesterol < 1.03 mmol/l (men) or < 1.29 mmol/l (women) or on drug treatment for reduced HDL cholesterol; fasting glucose ≥ 5.55 mmol/l or on drug treatment for elevated glucose. In other diagnostic groups, history or treatment of hypertension were not counted as criteria for metabolic syndrome as they applied to all other study populations. (Zidek *et al*., 2009).

Central or abdominal obesity is first major criterion attributed to metabolic syndrome (Alberti *et al.*, 2009). Crude assessment of anthropometric measures of central/abdominal obesity such as BMI, waist circumference (WC), and waist-hip ratio (WHR) are strong and consistent predictors for obesity related diseases such as type 2 diabetes mellitus, hypertension, metabolic syndrome, stroke e. t. c. Body mass index (BMI) is the most commonly used parameter to determine central obesity, this is assessed as weight in kilogram divided by square of height in meters, body mass index (BMI) greater than 30 kg/m^2 is classified as obesity. However, World Health Organization (WHO) guidelines state that alternative measures that reflect abdominal obesity such as WC, WHR and waist-to-height ratio (WHtR) have been found to be superior to BMI. Although, waist-hip ratio was initially suggested as better anthropometric measure for estimating the risk of type 2 diabetes mellitus, a study among Chinese population revealed that BMI and WC were found to be the important indices of obesity mellitus (Ahmad *et al*., 2016). Among Asians and Indians, the prevalence of abdominal obesity when WC was considered was found to be 46% and 68% respectively, while the prevalence recorded when WHR was considered were 12% and 64% respectively (Kurpad *et al*., 2003). However, majority believed WC is the best measurement of

obesity, whereas some agreed that WHR could also be used as an alternative indicator for obesity (Yanga *et al*., 2006).

Study has revealed that hypertensive patients had a significantly higher WHR (>0.9) as well as a significantly higher BMI (>25 kg/m²) when compared to the apparently normal individuals. Hypertension is also a core component of metabolic syndrome associated with obesity, sex and age. Coincidentally, prevalence of MS is increasing across the globe, and it tends to increase with age and varies with sex and race (Razzouk *et al*., 2009; Yang *et al*., 2019).

Generally, it has been observed that the predictive values of MS are particularly relevant when using traditional metabolic risk factors rather than non-traditional biomarkers (Gradidge and Crowther, 2017). Therefore, this study evaluated the prevalence and determinants of metabolic syndrome among the population with high prevalence of obesity using established diagnostic criteria.

Table 2.1: Assessment of metabolic syndrome using different diagnostic indices

Diagnostic criteria	Modified NCEP-ATP III	NCEP-ATP III	IDF	AHA	WHO	EGIR	AACE
WC (cm)	M≥102 F ≥88	M≥102 F ≥88	M≥102 F ≥88	M≥102 F ≥88	-	M≥102 F ≥88	-
BMI (kg/m^2)	-	-	-	-	>30	-	≥25
WHR	-	-	-	-	M >0.9 F>0.85	-	-
BP (mmHg)]	≥130/85	≥130/85	≥130/85	≥130/85	≥140/90	≥140/90	≥130/85
TG (mmol/L)	> 1.7	> 1.7	> 1.7	> 1.7	>1.7	> 1.7	> 1.7
HDL (mmol/L)	M<1.03 F<1.3	M<1.03 F<1.3	M<1.03 F<1.3	M<1.03 F<1.3	M<0.9 F<1.0	M<1.03 F<1.3	M< 1 F<1
Fasting Glucose, DM, IGT, or IFG (mmol/L))	≥ 5.6	> 6.l ± DM	≥ 5.6 +DM	≥ 5.6	DM	≥ 5.6 or IGT/IFG	IGT/IFG
Anti-hypertensive	Yes	No	No	No	No	No	No
Microalbuminuria	-	-	-	-	Yes	-	-

NCEP-ATPIII- National Cholesterol Education Program Adult Treatment Panel III, **WHO**- World Health Organization,

IDF- International Diabetes Federation, **AHA/NHLBI**- American Heart Association/National Heart, Lung and Blood Institute,

EGIR - European Group for the study of Insulin Resistance, **AACE**- American Association of Clinical Endocrinology, **BMI**- Body Mass Index, **TG**- Triglyceride, **HDL**- High Density Lipid Lipoprotein, **IGT**- Insulin Glucose Tolerance, IFG- **DM**- Diabetes Mellitus

2.2 Methods

2.2.1 Study design and population

This cross- sectional study was conducted among 753 male and 763 female participants (Total population was 1516) with a minimum age of 18 years and no upper age limit. The population size for the study is less than 10,000; therefore, the sample size was calculated using Leslie - Fischer's formula:

$$N = (Z_{1-\alpha})^2 * (P(1-P)/D^2$$

Where Z is the confidence level, P is the expected proportion of individuals with cardio-metabolic risk factors, and D is the margin of error. P was set at 0.40 and D at 0.05. The calculation was performed at the 95% confidence level. The required sample size for the study was calculated to be 450 participants. However, a total of 1516 participants were recruited in this study. Using matching random technique, one thousand, two hundred and sixteen (1216) apparently healthy participants were from Soweto, South Africa, and three hundred from the ongoing study at Human Nutrition Research Laboratory (HNRL), Johannesburg, South Africa.

2.2.2. Ethical clearance / approval

Ethical approval was obtained from the University of Witwatersrand, Human Research Ethics Committee [Medical (Reference number: M190472)]. Participants were provided with information sheets detailing the purpose and process of the study. Each participant gave written, informed consent for his/her voluntary participation in the study.

2.2.3 Clinical and demographic data

Six hundred and seventy-eight (678) participants with a minimum age of 18 years and no upper age limit were selected in the study. Acutely ill, psychotic, debilitated, pregnant or individuals with physical disability were excluded from the study. All participants were given informed consent form and comprehensive questionnaire. Detailed information about each participant's demographic data, family history, smoking habits, alcohol consumption, use of medication and other relevant history was obtained.

2.2.4. Measurements and analysis

2.2.4.1. Anthropometric measurement

Anthropometric measurement was used to assess the size, shape and body composition of adipose tissue. They were measured according to the international standards for anthropometric assessments (Stewart *et al*. 2011). Weight measurements were recorded using an electronic scale (Healthometer Professional) to the nearest 0.1 kg. During all the measurements, the participants were asked to take their shoes off, and minimal clothing was worn which allowed access to areas of measurements. Participants were asked to step on the scale and stand still without supporting themselves on any object and weight was recorded when the scale had stopped fluctuating. Height was measured using a stadiometer (Seca portable stadiometer) to the nearest 0.1 cm. When the measurement was taken the head of the participant was placed in the Frankfort plane with the body standing upright and a sliding headboard was lowered to the vertex of the head. Body mass index was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Participants were considered to be underweight if BMI is <18.5 kg/m^2, normal if BMI is between 18.5 and 24.9 kg/m^2, overweight if their BMI is greater than or equal to 25kg/m^2 and obese if their

BMI is greater than or equal to 30kg/m^2. Waist circumferences was determined using a tape measure with participants wearing minimal clothing on the abdominal region. Waist circumference was determined at the narrowest width midway between the iliac crest and the lowest rib to the nearest 0.1 cm at the end of a gentle expiration. Normal waist circumference was taken as ≤ 102 cm in men and ≤ 88 cm in women (Hume, 2017). Hip circumference was measured at the level of the greatest posterior protuberance, perpendicular to the long axis of the trunk (Ayling, 2014). Normal waist- hip ratio was taken as >0.9 in men and > 0.85 in women.

2.2.5 Conventional (clinic) blood pressure assessment:

Conventional blood pressure was measured using automated sphygmomanometer (Omron, Kyoto, Japan) after 10 min of rest in the seated position. Regular adult cuff of 9.5-12.5 inches (12 cm wide and 23cm long) was used for arm circumference less than 33 cm while large adult cuff of 13.5-16.5 inches was used for arm circumference that is greater than 33 cm, brachial blood pressure was recorded to the nearest 2 mmHg. Korotkov phases I and V were identified as systolic blood pressure (SBP) and diastolic blood pressure (DBP) respectively. Five consecutive BP readings were obtained. The average of the five readings were taken as the BP. Participants were classified as hypertensive if they were on antihypertensive therapy and/or if the mean value of blood pressure was higher than 130/85 mmHg or 140/90 mmHg, depending on the diagnostic group in Table 2.1.

2.2.6 Biochemical analysis

After an overnight fasting, 5ml of venous blood sample was collected from the cubital fossa using aseptic procedure, and the following parameters: lipid profile [Triglyceride (TG), high lipid lipoprotein (HDL)], fasting blood glucose were analysed at the Contact Laboratory Services (CLS).

2.2.7 Diagnosis of metabolic syndrome

Metabolic syndrome was defined according to seven different diagnostic groups: modified NCEP-ATPIII, AACE, IDF, WHO, AHA/NHLBI and EGIR diagnostic criteria (Table 2.1).

2.2.8 Statistical analysis

Statistical analyses were performed with IBM Corp. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp, (2013). Metabolic syndrome was determined as defined by different diagnostic groups using any three or more major criteria stated in Table 2.1. Frequencies were calculated for categorical variables and ranges were calculated for continuous variables. Chi-square tests were performed to compare categorical variables.

Results

Table 2.2 General characteristics of the study population according to BMI status

	Underweight	Normal BMI	Overweight	Obese
Number	63	517	341	543
Age (years)	25.7± 11.0	30.4 ±15.8	41.4±18.8	48.7±15.4
Female (%)	42.9	47.2	53.7	76.2
Alcohol intake (%)	35.5	30.8	24.9	17.8
Smokers (%)	27	23.2	20.7	9.5
Hypertensive (%)	4.8	16.4	33.7	39.8
Diabetic (%)	0	3	6.6	11

BMI, body mass index; Normal BMI ≥ 18.5 and < 25 kg/m^2; overweight BMI ≥25 and < 30 kg/m^2; Obese BMI ≥30 kg/m^2; values expressed as a percentage or mean ±standard deviation; P value < 0.05 considered s

Results

Figure 2.1 shows prevalence of metabolic syndrome (MS) in the study population using different diagnostic criteria. Variation in measure of criteria result to discrepancies in the prevalence of metabolic syndrome among same population (study population). In this study the prevalence of MS using modified NCEP-ATP III was the highest (24.2%). Prevalence when NCEP-ATPIII, IDF, AHA/NHLBI, WHO, EGIR and AACE were considered revealed 16.6%, 21.4%, 18.7%, 20.4%, 14% and 22.4% respectively.

When waist circumference (WC) was considered using modified NCEP-ATPIII, NCEP-ATPIII, IDF,WHO, AHA and EGIR, prevalence of metabolic syndrome in the study population were 24.2%, 16.6%, 21.4%, 18.7%, 18.7% and 14% respectively (Table 2.1 and figure 2.1). When BMI was considered in WHO and AACE prevalence of MS was 20.4% and 22.4% respectively, while prevalence of MS when WHR was considered was 20.4% (Table 2.2 and figure 2.1). WHO considered both BMI and WHR, but highest prevalence was recorded when modified NCEP-ATPIII was considered in people from African ancestry. However, EGIR recorded 14% prevalence despite consideration of WC while WHO recorded 20.4%. Notably, modified NCEP-ATPIII with highest prevalence considered those on anti-hypertensive therapy and fasting glucose of 5.6mmol (instead of fasting blood sugar of >6.1 mmol) which was considered in NCEP-ATPIII. This study revealed that the prevalence of MS using modified NCEP-ATP III was higher (24.2%) than NCEP-ATPIII (16.6%). Moreover, the study further revealed lowest MS prevalence in EGIR criteria (14%), despite considering WC and blood pressure of 140/90 mmHg compared to AHA (18.7%) criteria where the only adjustment was reduction of 10 mmHg in systolic pressure and 5mmHg in diastolic pressure.

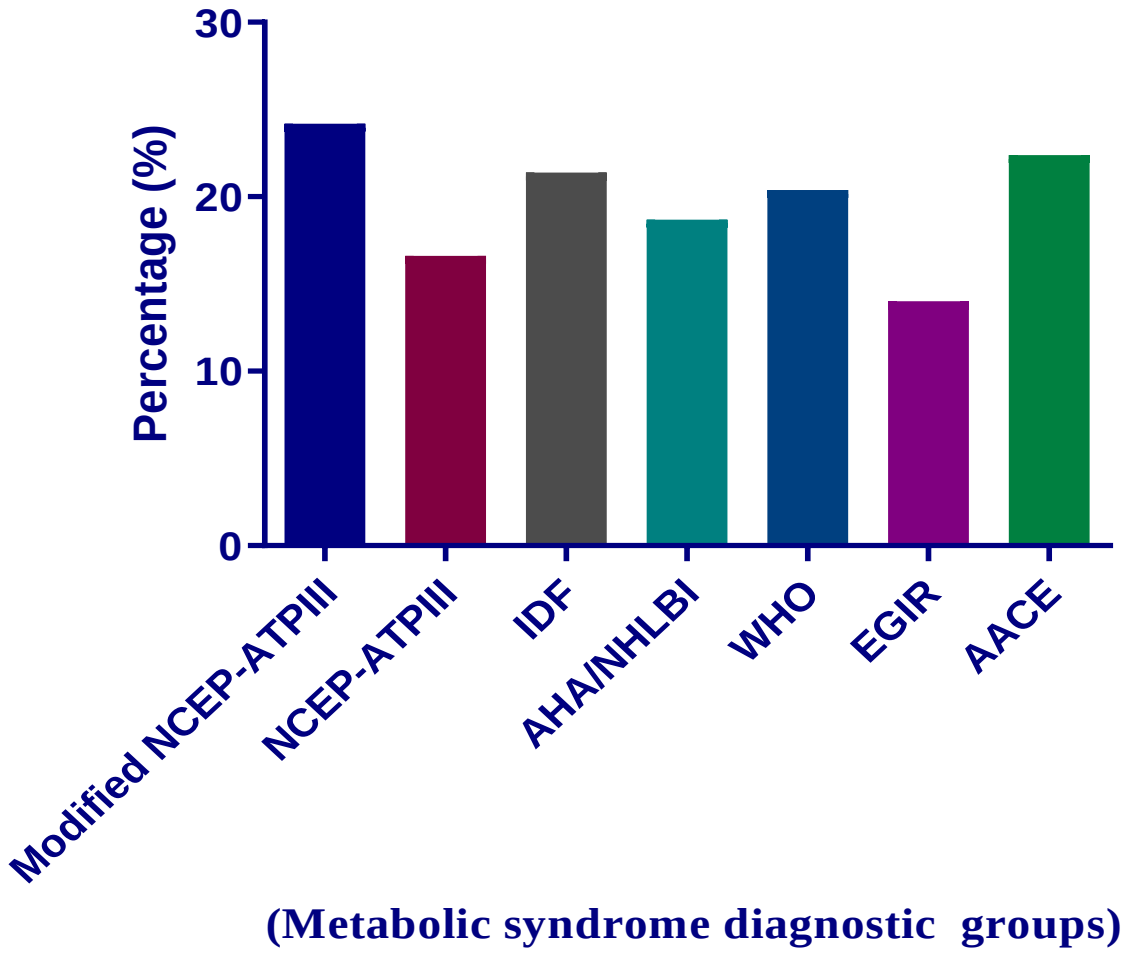


Figure 2.1 prevalence of metabolic syndrome in the study population using different diagnostic criteria.

NCEP-ATPIII- National Cholesterol Education Program Adult Treatment Panel III, **WHO**- World Health Organization, **IDF**- International Diabetes Federation, **AHA/NHLBI**- American Heart Association/National Heart, Lung, and Blood Institute, **EGIR** - European Group for the study of Insulin Resistance, **AACE**- American Association of Clinical Endocrinology

In figure 2.2, 4% were underweight, 35.8% had normal weight, and 23.4% and 36.8 % were overweight and obese respectively.

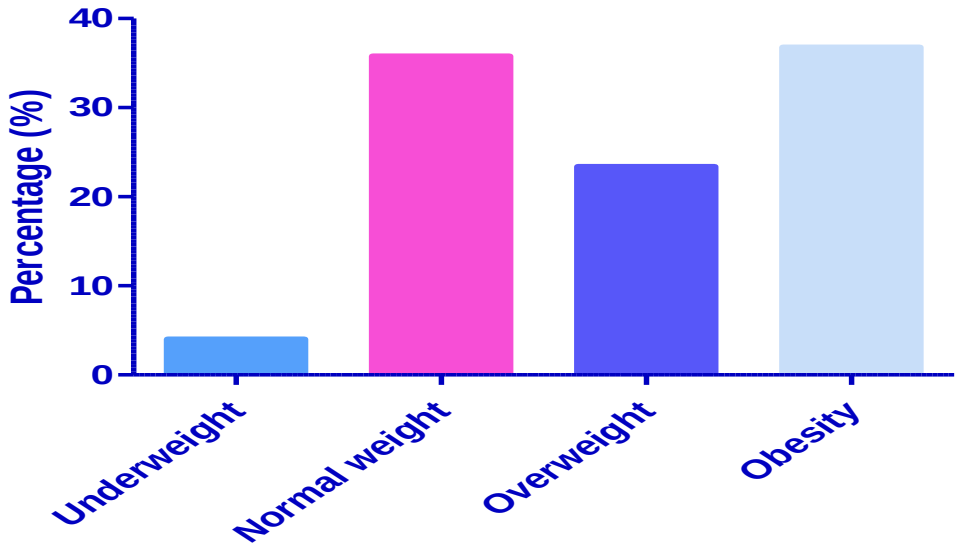


Figure 2.2: percentage of underweight, normal weight, overweight and obese individuals in the study population.

Fig 2.3: Gender variation in different diagnostic categories

The prevalence of metabolic syndrome is higher in females than males in all the diagnostic groups except EGIR group where males had higher prevalence than male.

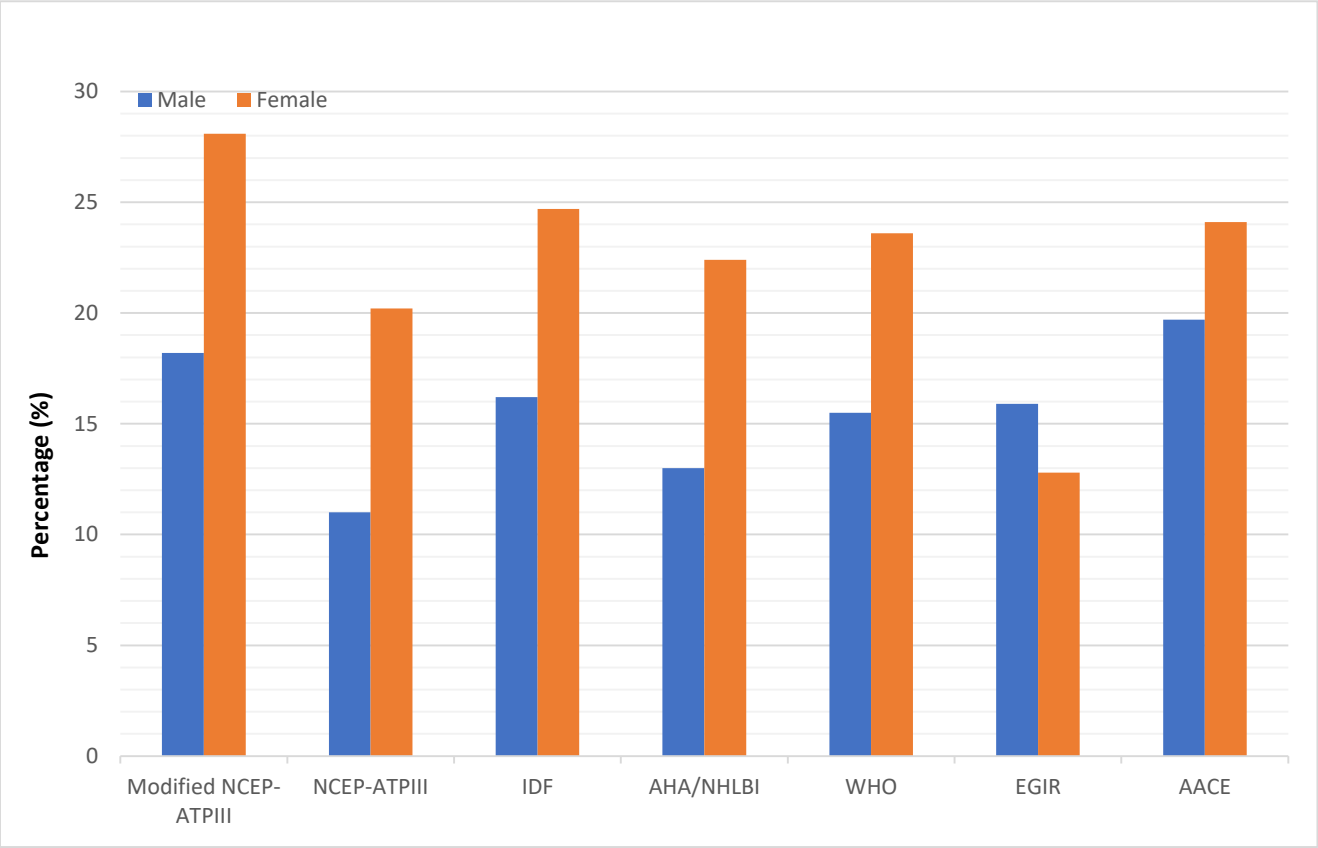


Fig 2.3 showing gender variation in different diagnostic categories

NCEP-ATPIII- National Cholesterol Education Program Adult Treatment Panel III, **WHO**- World Health Organization, **IDF**- International Diabetes Federation, **AHA/NHLBI**- American Heart Association/National Heart, Lung, and Blood Institute, **EGIR** - European Group for the study of Insulin Resistance, **AACE**- American Association of Clinical Endocrinology

Figure 2.4: Frequency and percentage of major diagnostic criteria among study population

Fig 2.4 Among the study population, 34.5% had HDL while 11.4 while 13.2% and 20.9% had fasting blood glucose >6.1mmol and >5.6mmol respectively. Notably, less than 10% of the study population had diabetes mellitus (MS). However, 33.4% and 20.6% had SBP >130 mmHg and >140 mmHg respectively while 24.7% and 34% had DBP of > 90 and >85 mmHg respectively. While 15.2% had BP >140/90 mmHg, 24.7% of the population had BP > 130/85 mmHg while 16.9% were on antihypertensive therapy.

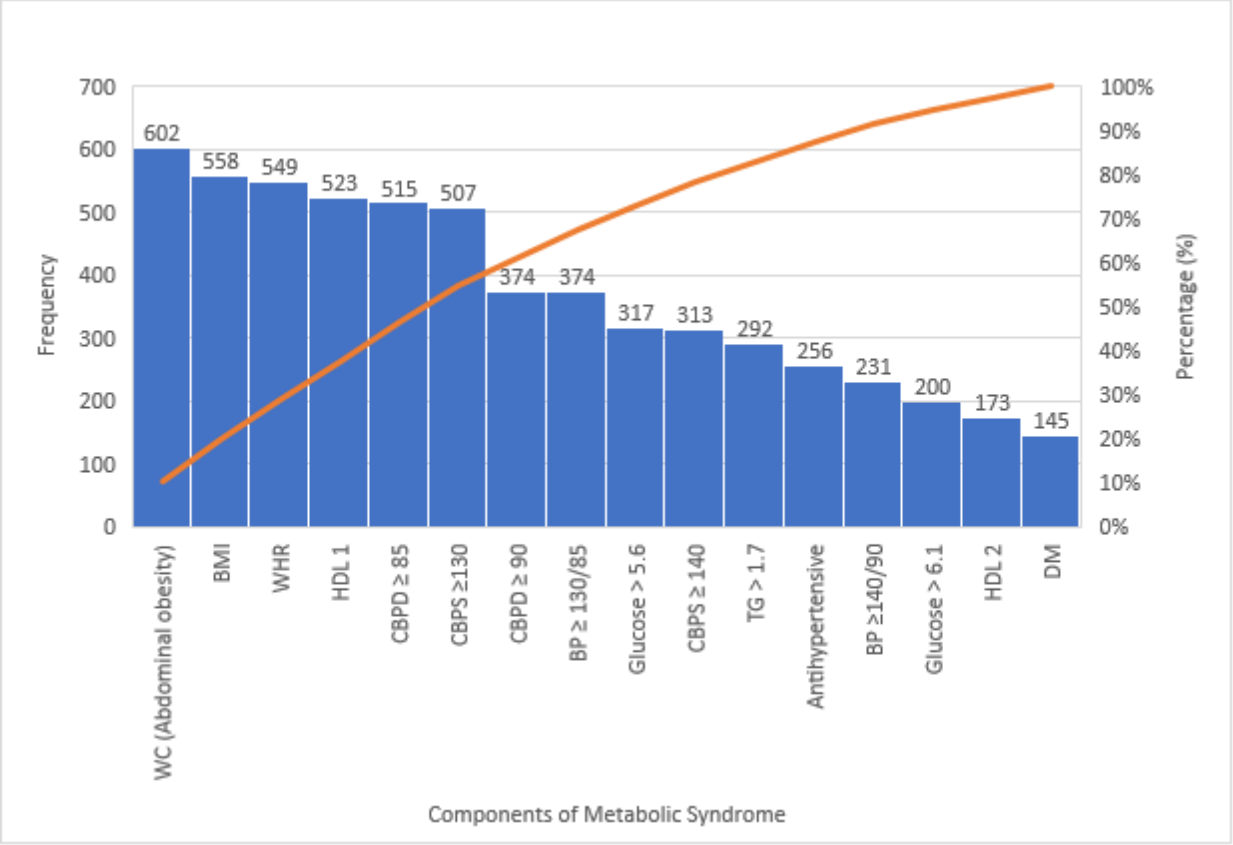


Fig 2.4: **Frequency and percentage of components of metabolic syndrome among the study population**

WC- waist circumference, **WHR**- waist Hip Ratio, **HDL1**- High Lipid Lipoprotein <1.03 mmol/l in male and female < 1.3 mmol/l, **TG**- Triglyceride, **BP**- blood pressure, **CBPS**- Conventional Blood pressure (Systolic**), CBPD**- Conventional Blood pressure (diastolic), **HDL2**- WHO High Lipid Lipoprotein value, < 0.9 mol/l in male, <1.0 mmol/l in female, **DM**- Type 2 diabetes mellitus.

Figure 2.5: Age distribution within the study population

Figure 2.5 shows age distribution of study participants and revealed that those between 20 and 29 years had highest percentage (30.4%) while age group below 20 years had lowest percentage (11.1%). However, age group of 40 years and above constituted about 50% of the study population, that is, 40-49, 50-59 and 60 and above respectively.

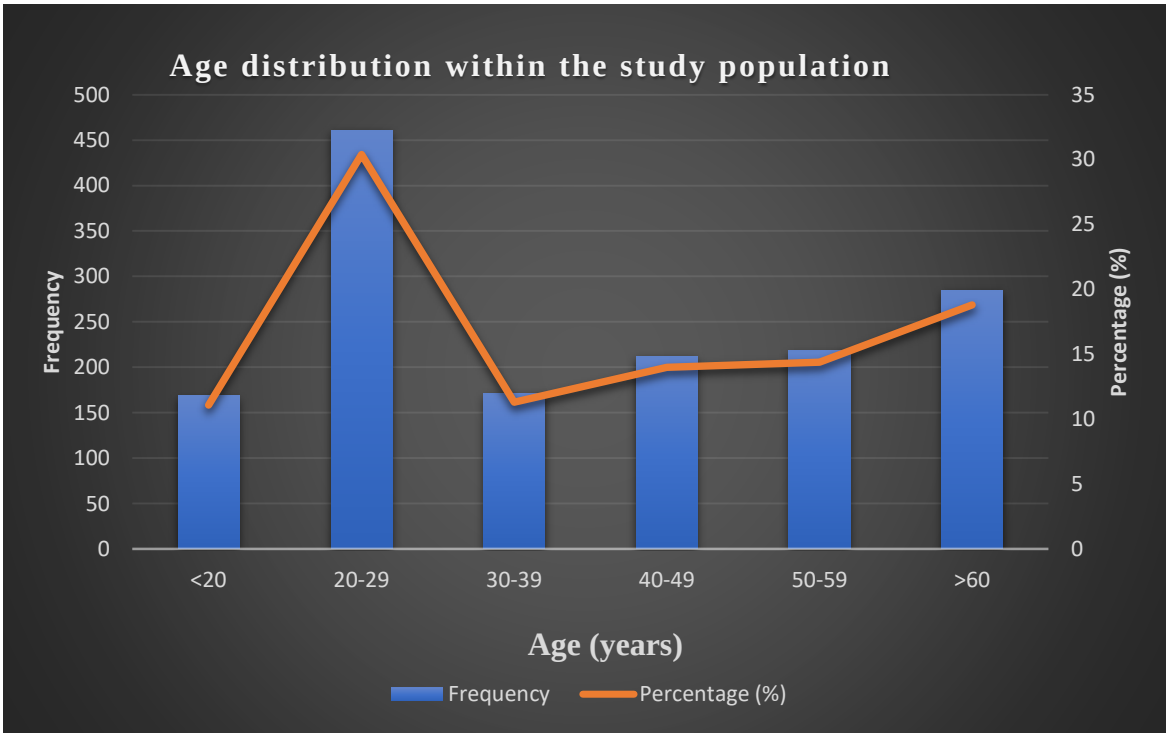


Figure 2.5: Age distribution within the study population

Table 2.3: **Age distribution and metabolic syndrome**

Table 2.3 shows variation between the age distributions among those with metabolic syndrome following different diagnostic criteria. Among all the groups there is increase in prevalence of metabolic syndrome with age. There is highest prevalence of 25.9%, 50.5 % and 53.7% among individuals between age 40 - 49 years, 50-59 years, 60 years and above respectively in modified NCEP-ATP III. However, there was lowest prevalence (0.6%) among those less than 20 years when WHO diagnostic criteria were considered.

Table 2.3: Percentage of Age Distribution among Different Diagnostic Criteria of Metabolic Syndrome

Age group	Modified NCEP-ATPIII	NCEP-ATPIII	IDF	AHA/NHLBI	WHO	EGIR	AACE
< 20 years	3%	1.2%	1.8%	1.8%	0.6%	1.2%	4.1%
20 - 29 years	4.6%	2.4%	4.8%	3.9%	3.3%	2.6%	6.1%
30 - 39 years	13.5%	9.4%	12.3%	11.7%	14%	5.8%	15.2%
40 - 49 years	25.9%	21.7%	24.5%	23.6%	24.5%	16%	25.9%
50 - 59 years'	50.5%	35.3%	44%	37.6%	43.1%	31.7%	43.6%
60 years and above	53.7%	34.7%	45.6%	38.9%	43.5%	29.8%	44.9%

NCEP-ATPIII- National Cholesterol Education Program Adult Treatment Panel III, **WHO**- World Health Organization,

IDF- International Diabetes Federation, **AHA/NHLBI**- American Heart Association/National Heart, Lung, and Blood Institute, **EGIR** - European Group for the study of Insulin Resistance, **AACE**- American Association of Clinical Endocrinology.

Figure 2.6: Influence of Smoking and Alcohol on Prevalence of Metabolic Syndrome

In figure 2.6 there was increase in the rate of smoking and alcohol intake among those with metabolic syndrome. This study revealed that rate of smoking was high in AACE (19%), and modified NCEP-ATP III (17.2%) while lowest rate was recorded in EGIR (12.2%) and NCEP-ATPIII (11.1%). Similarly, rate of alcohol intake was high in AACE (21.6%), modified NCEP-ATP III (20.5%) while lower rate was recorded in EGIR (14.1%) and NCEP-ATPIII (13.9%).

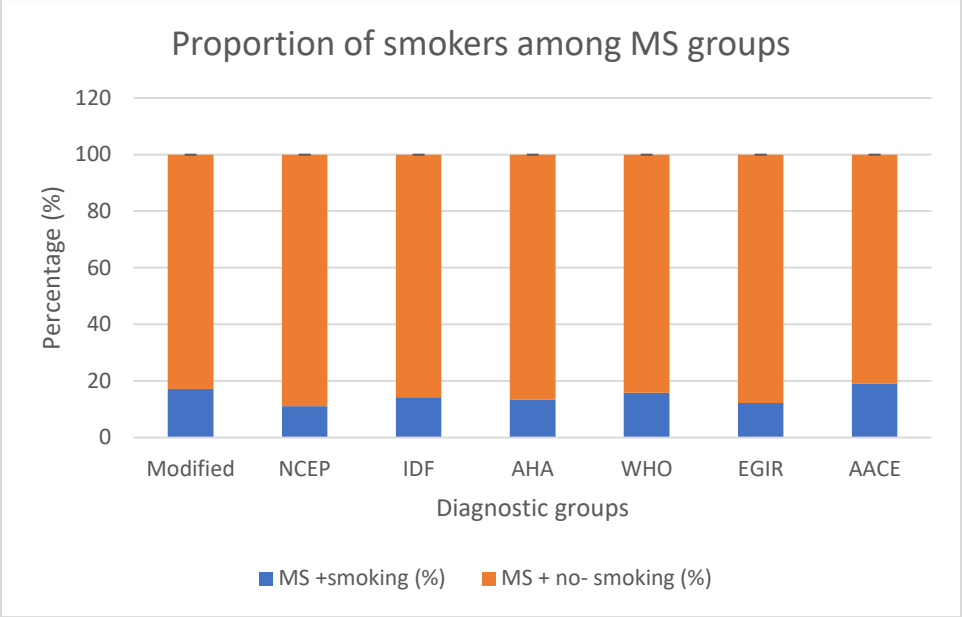


Fig 2.6a shows the percentage of smokers among each diagnostic group

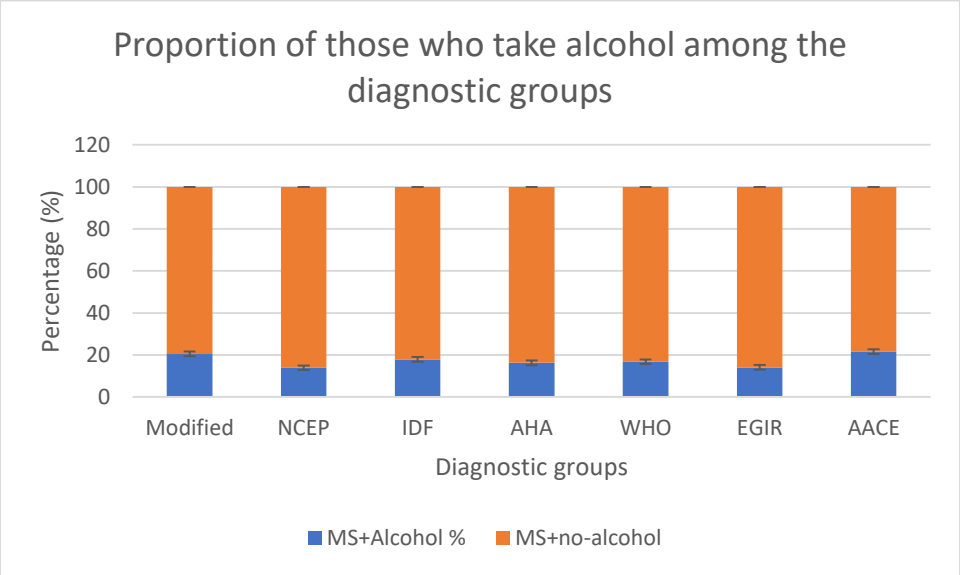


Fig 2.6b shows percentage of those who take alcohol among the diagnostic groups

NCEP-ATPIII- National Cholesterol Education Program Adult Treatment Panel III, **WHO**- World Health Organization, **IDF**- International Diabetes Federation, **AHA/NHLBI**- American Heart Association/National Heart, Lung, and Blood Institute, **EGIR** - European Group for the study of Insulin Resistance, **AACE**- American Association of Clinical Endocrinology

2.3. Discussion

This study is the first documentation where seven (7) diagnostic criteria were used to assess prevalence of metabolic syndrome in a community sample of African ancestry. In this population, there was high prevalence of obesity and overweight individuals (Table 2.1 and figure 2.2). Our study is in support of Maseko et al (2018) which reported that 71% of Africans in South Africa were either overweight or obese, with women having a higher prevalence of obesity (75%) compared to men (47%) [Maseko *et al*., 2018]. Similarly, this study also revealed (Table 2.1) higher percentage of obesity among female (76.2%). Our findings (table 1) also showed significant increase in number of hypertensive and diabetic individuals among those who are overweight and obese. Meanwhile, there was significant increase in alcohol intake and smoking among underweight individuals; which may be due to the effect of alcohol and smoking on nutritional status. Another explanation for this trend could that overweight/obese individuals have reduced their smoking and alcohol intake because they are aware of their poor health status.

Some studies have reported controversial assessment of obesity in the diagnosis of metabolic syndrome, this study revealed that all the measures of obesity influenced the prevalence of the syndrome, this implies that obesity is important in the development of metabolic syndrome. Irrespective of various criteria there was high prevalence of MS in the study group. Highest prevalence of MS (24.2%) was recorded when modified NCEP-ATP III diagnostic criteria was considered, in this group, antihypertensive therapy was recognized as major criteria (figure 2.1). This indicates that hypertension is another important determinant of metabolic syndrome in this population. Additionally, our results show that there was higher prevalence when blood pressure of 130/85 mmHg (e. g AACE, IDF) was considered than groups where 140/90 mmHg was

considered (e. g WHO, EGIR). The significant difference in prevalence is attributed to the change in systolic and diastolic blood pressure of 10 mmHg and 5 mmHg respectively. In previous study by Lee and colleagues, high BP was prevalent for both genders, though slightly more in males (Lee *et al*., 2016). Studies in Turkey and China reported that most frequently observed component of MS was high BP (Lee *et al*., 2016). Therefore, to achieve early diagnosis and management of cardiovascular diseases, this study is in agreement with recent annual screening (USPSTF) recommendation; defining hypertension as blood pressure equal or higher than 130/80 mmHg in people with African ancestry (Ferdinand and Brown, 2021). However, pathological role of insulin resistance in the diagnosis of metabolic syndrome among Africans cannot be underestimated.

Gender is an important factor in metabolic syndrome (Razzouk and Muntner, 2009; Lee *et al*., 2016). Currently, there is no specific gender prevalence in all documented data, but most studies reported a higher prevalence of the metabolic syndrome in women compared with men (Lee *et al*., 2016; Yang *et al*., 2019). In this study population, metabolic syndrome is common in women compared to men except in EGIR group (Figure 2.3). The higher prevalence of MS in women may be due to the higher indices of obesity in women in this population. This is confirmed by the findings of this study which show that there is a higher percentage of overweight and obese women in women compared to men (Table 2.1). Furthermore, in this population, indices of obesity play a major role in the in the pathogenesis of MS, followed by BP (Fig 2.4). This indicates that the indices of obesity and BP should be used as classical determinants MS in this population. Hence, diagnostic criteria that focus on indices of obesity and BP are more relevant for this population which has a high incidence of obesity and hypertension (Figure 2.4).

In studies by both Lee et al (2016) and Yang et al (2018) indicate that lipid changes contribute to increase in prevalence of metabolic syndrome in women (Lee *et al*., 2016; Yang *et al*., 2019). Interestingly, majority of the participants had increased waist circumference followed by indices of blood pressure assessment (figure 2.4); This implies that abdominal obesity and body mass index are important contributors to metabolic syndrome; they are likely responsible for cardiovascular disease among Africans.

Apart from the indices of obesity and hypertension, age contributes significantly to MS. Our results show that the prevalence of MS increases with increasing age until the age of 60 years. This further confirms that the indices of obesity increase with age until the age of 60 years. After the age of 60 years the prevalence of MS increases slightly or it decreases (Table 2.3). This trend has also been shown with BMI. A number of studies have shown that BMI increases with increasing age until the age of 60 years (Kim *et al*., 2018; Yang *et a*l 2021). After 60 years BMI either decreases or remains constant. The findings of this study further confirm that the indices of obesity are the main determinants of MS in this population. Other studies have also shown this positive association between age and MS. A study conducted in the United States, shows that the prevalence of metabolic syndrome in adults 18 years and older is significant (Swarup *et al*., 2020). When we compared the different diagnostic criteria, participants from age 18 to 39 had the lowest prevalence according to the WHO criterion.

Smoking and alcohol intake are modifiable risk factors associated with cardiovascular problem such as atherosclerosis, heart failure, myocardium infarction e. t. c. Highest proportion of smokers and those who take alcohol was recorded in AACE group with MS prevalence of while lowest proportion of those who take alcohol and those who smoke was recorded in EGIR group

and NCEP-ATPIII group respectively (Fig 2.6a and 2.6b); this implies that smoking and alcohol categorically influenced the prevalence of metabolic syndrome (Fig. 2.1).

2.4. Conclusion

Our results indicate that obesity is the main determinant of MS, better assessed by waist circumference. Although, WHO is the only criterion that incorporates two indices of obesity (BMI and WHR) and the stringent threshold of BP ≥ 130/85 mmHg has not been adopted in South Africa, our findings revealed that modified NCEP-ATPIII criterion is the more suitable for diagnosis of MS in people with African ancestry than the other six criteria because of the high prevalence of obesity and hypertension in this population. In addition, age, sex and influence of smoking and alcohol should be considered in the assessment of MS in Africans.

CHAPTER THREE

The impact of Metabolic Syndrome as defined by the WHO on Arterial Stiffness

Abstract:

Introduction:

Arterial stiffness is one of the leading contributors to the rising incidence of cardiovascular diseases in African communities. A number of studies have shown strong association between arterial stiffness and metabolic syndrome. However, the accuracy of this association is dependent on the criterion that is used to classify metabolic syndrome. As shown in the previous chapter, the WHO criteria is the most accurate measure of metabolic syndrome in populations with a high prevalence of obesity. Previous studies have shown that there is a high prevalence of obesity in people of African ancestry living in South Africa, hence, the relevance of the WHO in this population. Therefore, in this study we evaluated the relationship between arterial stiffness and metabolic syndrome in people of African ancestry using the WHO criteria to classify metabolic syndrome.

Methods

The cross-sectional study was conducted among 668 participants of African ancestry. The minimum age of the participants was 18 years and there was to upper age limit. Obesity was assessed using body mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR), while conventional blood pressure was assessed using electronic blood pressure monitoring device, ambulatory 24-hr day and night BP were determined using SpaceLabs monitors. Aortic pulse wave velocity was determined using applanation tonometry and SphygmoCor software. Blood sample was taken for laboratory for assessment lipid profiles [triglyceride (TG), high lipid lipoprotein (HDL)], fasting blood glucose and plasma hormone concentration. Metabolic syndrome was defined according WHO criteria. All analyses were

conducted using SPSS software for Windows, version 11.0J (SPSS, Chicago, USA) and STATA.

Results

Our results show the age of participants with metabolic syndrome was significantly higher ($p = 0.02$) than those without metabolic syndrome, confirming the positive association between age and metabolic syndrome. Metabolic syndrome was also significantly higher 72% in females compared to males (59.7%). As a confirmation of previous studies, the prevalence of both hypertension and diabetes was higher in the participants with metabolic syndrome compared to those without metabolic syndrome. After correcting for confounding variables, PWV was significantly associated with both BP (24- hour, night-time, daytime and conventional systolic and diastolic BP) and the indices of obesity (WC, BMI, WHR) in the population. Most importantly PWV was significantly higher in participants with metabolic syndrome compared to those without metabolic syndrome.

Conclusion

In this population of African ancestry, PWV is strongly associated both BP and the indices of obesity, both of which were shown to be the strongest predictors of metabolic syndrome in the previous chapter. Therefore, in this population with a high incidence of obesity, any effort to reduce cardiovascular diseases should employ both pharmaceutical (BP control through hypertension medication) and non-pharmaceutical (lifestyle changes through weight reduction). The combination of these two approaches will result in the reduction of the incidence of metabolic syndrome related large artery pathology in this community.

3.1 Introduction:

Aortic pulse wave velocity (PWV) is usually measured between the carotid and femoral artery. It is related to Moens -Korteweg equation. PWV is directly proportional to arterial wall width and Young modulus, a measurement of elastin and collagen concentration or arterial stiffness. It is also inversely proportional to vessel diameter and blood viscosity. Higher arterial wall width and stiffness will lead to higher PWV. Hence, higher PWV corresponds to lower vessel distensibility and compliance (Pai *et al*., 1999; Shahmirzadi *et al*., 2012; Pierra et al., 2015).

Moens -Korteweg equation: $\mathbf{c0 = \sqrt{Eh/2R\rho}}$

Where; c0 is the wave speed, E is Young modulus in the circumferential direction, h is wall thickness, R is vessel radius, and ρ is the density of fluid.

PWV is associated with endothelia dysfunction in physiological and pathological conditions such as aging, metabolic syndrome and obesity. Pulse wave velocity increases proportionally to the number of cardiovascular risk factors present. Atherosclerotic risk factors lead to vascular remodeling, creating arterial stiffness within the aorta and other large arteries. It is usually age dependent and also associated with cardiovascular events, diabetes, metabolic syndrome and cardiovascular events. So far, no study has evaluated the variability of PWV, blood pressure assessment and indices of obesity in a population with high prevalence of obesity and risk of hypertension. Therefore, this study investigated the impact of PWV and role of blood pressure assessment in a population with high prevalence of obesity and essential hypertension.

3.2 Pathophysiology of wave reflection

Wave reflection is an integral part of the central pulse waveform. The interface between the larger arteries and resistance vessels will instantaneously reflect the incoming cardiac pressure

pulse wave. A pressure wave returning during the systolic ejection period due to an increased PWV, a more proximal site of wave reflection will likely augment systole. The pressure wave contour in any artery is the result of the summation of the forward transmission of the cardiac pressure impulse and a backward reflection generated by the peripheral vascular system at the interface between large arteries and resistance vessels (arteries and arterioles). The reflected wave has high velocities and is reflected back to the central arteries during the same ejection cycle of the heart. Pressure recorded anywhere in the arterial system is thus the sum of the forward wave and the reflected wave and is dependent on these three3 factors: the amplitude and duration of ventricular ejection, the amplitude of the reflected wave, and the velocity of the reflected wave from the periphery. As shown in figure 3.1, the arterial pulse waveform is the sum of the forward pressure wave generated by left ventricular ejection and a backward propagating wave that is subsequently reflected from the peripheral site, and the time point at which these forward and backward propagating waves merge and the amplitude of the reflected (backward) wave affect the level of central BP (McEniery *et al*., 2005; Shahmirzadi *et al*., 2012). Presently, no study has evaluated the variability of PWV, blood pressure assessment and indices of obesity in a population with high prevalence of obesity and risk of hypertension. Hence, this study aimed to determine whether metabolic syndrome influences arterial stiffness when WHO criteria is applied.

In an effort to reduce the rising epidemic of cardiovascular diseases, focus has mainly been on BP control. However, a report from observational cross-sectional survey conducted in twelve European countries to assess the cardio-metabolic risk profile in patients with essential hypertension; GOOD survey (Global Cardiometabolic Risk Profile in Patients with Hypertension Disease) showed that blood pressure control was significantly worse in

hypertensive patients with metabolic syndrome and/or diabetes mellitus than in those with essential hypertension only. Zidek et al (2009) further revealed that visceral obesity and dyslipidemia are two major components of metabolic syndrome associated with resistance to antihypertensive treatment (Zidek *et al*., 2009). The high incident of obesity in the black South African population could be the reason for the poor BP control in this community. Therefore, for effective control of BP in this community, focus should be on both pharmaceutical (BP medication) and non-pharmaceutical (weight reduction). This was confirmed in the previous chapter where our results indicate that contrary to the belief that perturbation in glucose metabolism is the main pathophysiology of metabolic syndrome our results show that waist circumference, BMI and waist-to-hip ratio circumference are the main contributors to metabolic syndrome in our study population.

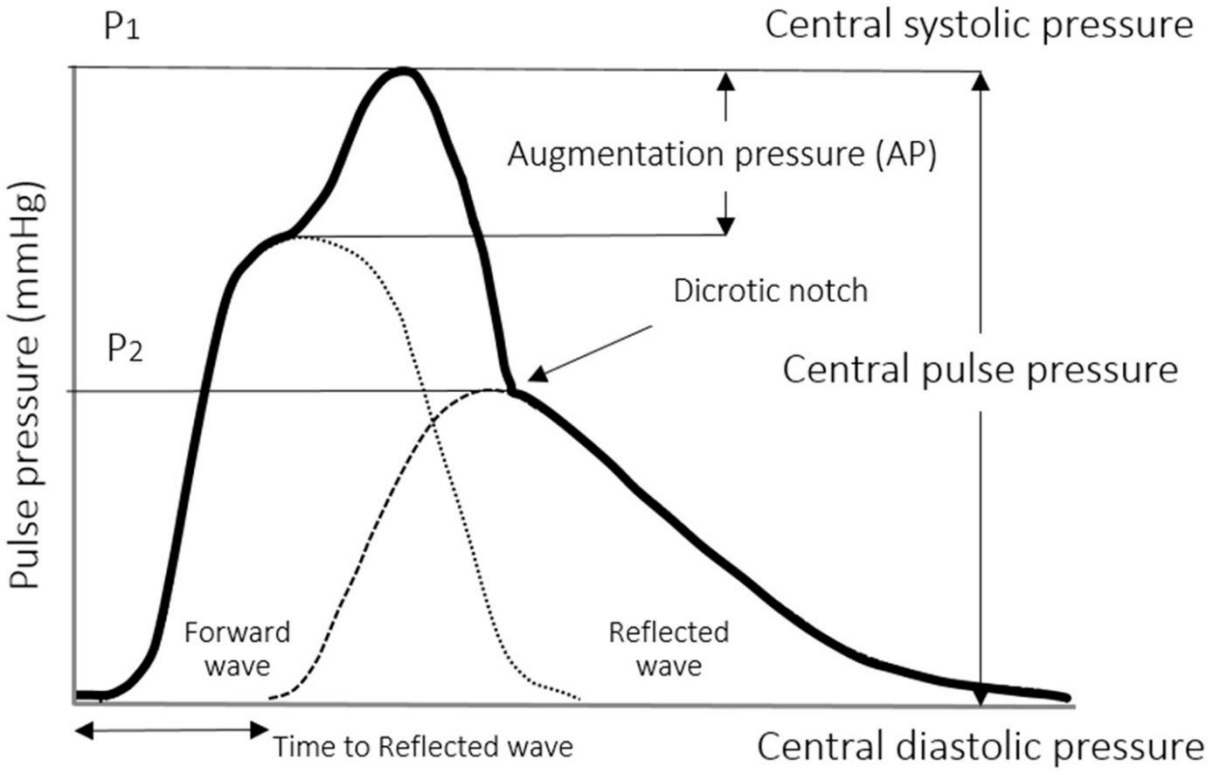


Figure 3.1: Central pressure waveform

Arterial stiffness is a marker of arterial damage and its increase has been shown to be associated with an increased risk of cardiovascular events. Recently, attention has been paid to the metabolic syndrome (MS) as a potent atherogenic state, and the presence of this syndrome has been reported to be associated with poor cardiovascular outcomes (Tomiyama *et al*., 2003). Raised blood pressure and raised fasting plasma glucose are components of MS, and previous prospective studies have demonstrated that presence of raised blood pressure and raised plasma glucose may synergistically lead to progression of arterial stiffness. Therefore, adequate knowledge of central pressure waveform will help in the prevention of arterial stiffness and related diseases. Central Blood Pressure (CBP) is defined as blood pressure within the ascending aorta. It expresses blood pressure in the thoracic or abdominal aorta; indicating systolic and pulse pressure exerted at the level of the heart, brain, and kidney. Difference in brachial blood pressure and central blood pressure is due to the phenomenon of pulse pressure (PP) amplification. This variability depends on factors such as age, exercise, and pathological conditions (Hashimoto, 2017).

Central (aortic and carotid) pressures are patho-physiologically more relevant than peripheral pressures for the pathogenesis of cardiovascular disease. It is aortic systolic pressure that the left ventricle encounters during systole (afterload), and the aortic pressure during diastole is a determinant of coronary perfusion. Furthermore, the distending pressure in the large elastic-type arteries (aorta and carotid) is a key determinant of the degenerative changes that characterize accelerated aging and hypertension. In contrast, the muscular peripheral arteries, such as the brachial and the radial ones, are less influenced by these changes. The pressure wave generated by the left ventricle travels down the arterial tree and then is reflected at multiple peripheral sites, mainly at resistance arteries (small muscular arteries and arterioles).

Consequently, the pressure waveform recorded at any site of the arterial tree is the sum of the forward traveling waveform generated by left ventricular ejection and the backward traveling wave, the pattern of the incident wave reflected at peripheral sites. When the large conduit arteries are healthy and compliant, the reflected wave merges with the incident in the proximal aorta during diastole, thereby augmenting the diastolic BP and aiding coronary perfusion. In contrast, when the arteries are stiff, pulse wave velocity increases, accelerating the incident and reflected waves; thus, the reflected wave merges with the incident wave in systole and augments aortic systolic rather than diastolic pressure (Zidek *et al*., 2009). As a result, left ventricular afterload increases and normal ventricular relaxation and coronary filling are compromised. Apart from changes in the timing of the waveforms merging, changes in the magnitude of the reflected wave and central pressures may result from changes in the proportion of the incident wave that is reflected.

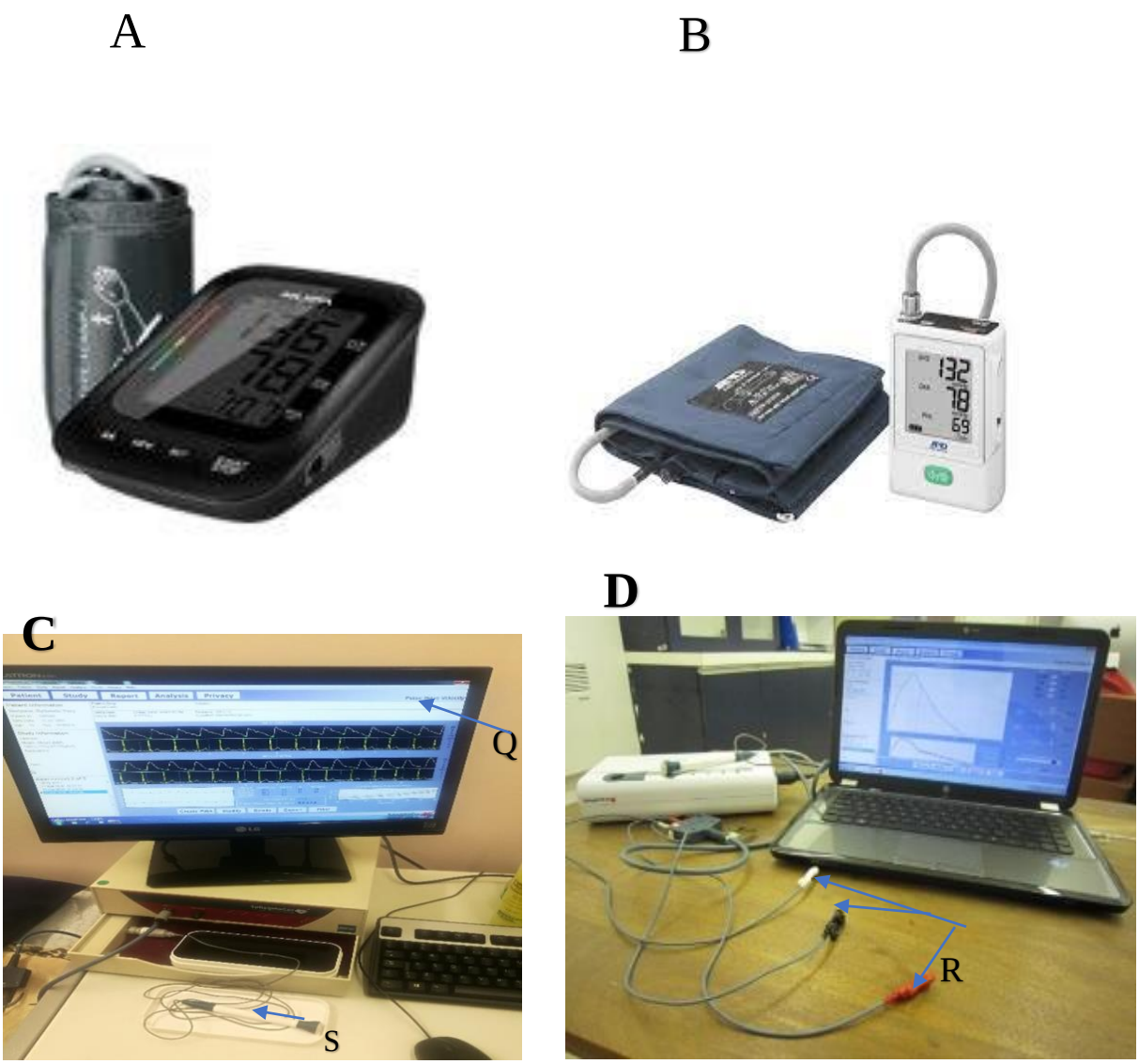


Figure 3.2 showing pictures of A. Conventional/clinic blood pressure monitor, B= Ambulatory blood pressure monitor, C and D = Applanation tonometry and Sphygmocor device setup (to access central pressure and to determine timing of pulse waves); Q = Computer showing PWV analysis, R= ECG electrodes to determine timing of pulse wave, S = Applanation tonometer to detect PWV.

3.3. Methods:

3.3.1. Study design and population

Details about the study population and design is as written in chapter 2 of this thesis. Participants were stratified according to WHO criteria for metabolic syndrome. Of the 1516 participants, 668 fulfilled the stipulated requirements.

Ethical approval was obtained from the University of Witwatersrand, Human Research Ethics Committee [Medical (Reference number: M190472)]. Participants were provided with information sheets detailing the purpose and process of the study. Each participant gave written, informed consent for his/her voluntary participation in the study.

3.3.2 Measurements and Biochemical analysis

3.3.2.1 Anthropometric measurement

Assessment of anthropometric measurement is as previously explained in chapter 2 (2.2.4.1).

3.3.2.2 Conventional (clinic) blood pressure assessment

Conventional blood pressure was measured using automated sphygmomanometer (Omron, Kyoto, Japan) after 10 min of rest in the seated position. Regular adult cuff of 12 cm wide and 30 cm long was used for arm circumference less than 33 cm while large adult cuff of 16 cm wide and 36 cm long was adult used for arm circumference that is greater than 33 cm (Bawa-Allah *et al*., 2019), brachial blood pressure was recorded to the nearest 2 mmHg. Korotkov phases I and V were identified as systolic blood pressure (SBP) and diastolic blood pressure (DBP) respectively. Five consecutive BP readings were obtained. The average of the five

readings were taken as the BP. Participants were classified as hypertensive if the mean value of blood pressure was higher than 140/90 mmHg (WHO standard for metabolic syndrome).

3.3.2.3 Ambulatory blood pressure monitoring

Ambulatory 24-hr day and night BP was determined using SpaceLabs monitors (model 90207; Spacelabs, Redmond, WA). The cuff size is the same as that used in conventional BP measurements. Monitors was programmed to measure 24-hr blood pressure at 15 minutes intervals during the day time (6:00 - 22:00) and at 30 minutes interval during the night time (22:00 - 06:00). Fixed clock time periods as opposed to actual in-bed and out of bed periods was statistically analysed to ensure that similar day and night time periods was selected for comparison among individuals (Pickering *et al,* 2005).

3.3.2.4 Pulse wave measurement and analysis

Aortic pulse wave velocity was determined using applanation tonometry and SphygmoCor software (Shiburi *et al.,* 2006). The participants rested for 15 minutes in a supine position. Arterial waveforms with an electrocardiogram recording were recorded at carotid-femoral pulse simultaneously by applanation tonometry during a 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Houston, TX) interfaced with a computer employing SphygmoCor, version6.21 software AtCor, Medical, West Ryde, New South Wales, Australia). Recordings were discarded when systolic or diastolic variability of consecutive waveforms exceeds 5% or when the amplitude of the pulse wave signal is < 80 mV. The time delay in the pulse wave between the carotid and femoral sites was determined using the R wave of electrocardiograph recordings as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. Distance from the suprasternal notch to the carotid sampling site (distance A) and from the suprasternal notch to the femoral artery (distance B) will be measured. Pulse

wave velocity was calculated as distance B minus distance A. Aortic PWV was calculated as distance (meters) divided by transit time (Redelinghuys *et al*., 2010; Maunganidze *et al*., 2013).

3.3.2.5 Biochemical analysis

After an overnight fasting, 5ml of venous blood sample was collected from the cubital fossa using aseptic procedure. Lipid profiles [Triglyceride (TG), low lipid density lipoprotein (LDL), High lipid lipoprotein (HDL)] and fasting blood sugar were analysed at the Contact Laboratory Services (CLS):

3.3.2.6 Diagnosis of metabolic syndrome

Metabolic syndrome was defined according to WHO criteria; that is; insulin resistance defined as type 2 diabetes mellitus (DM) or impaired fasting glucose (IFG) (> 100 mg/dl) or impaired glucose tolerance (IGT), plus two of the followings:

- Abdominal obesity (waist-to-hip ratio > 0.9 in men or > 0.85 in women, or body mass index (BMI) ≥ 30 kg/m^2.
- Triglycerides 150 mg/dl or greater, and/or high-density lipoprotein (HDL)-cholesterol < 40 mg/dl in men and < 50 mg/dl in women.
- Blood pressure (BP) 140/90 mmHg or greater.
- Microalbuminuria (urinary albumin secretion rate 20 μg/min or greater, or albumin-to-creatinine ratio 30 mg/g or greater) [WHO, 1998; Kassi *et al*., 2011]

3.3.2.7 Statistical analysis

All analyses were conducted using SPSS software for Windows, version 11.0J (SPSS, Chicago, USA) and STATA. P value of <0.05 was considered to denote statistical significance. Continuous data were reported as mean ± SEM. Results from the participants was compared between two groups using Student's *t*- test. The χ^2 statistic was used to compare means and proportions. Stata/MP 16.0 (StataCorp, College Station, TX, USA) was used to analyse the association between metabolic syndrome and changes different assessment of blood pressure. Multiple regression and pearson's correlation coefficient was used to determine the association between metabolic syndrome status and changes in peripheral blood pressure, central blood pressure and ambulatory blood pressure monitoring. P value < 0.05 considered significant. All models were also adjusted for the all the assessment of PWV.

Results

Table 3.1: Shows the general characteristics of the study population according to MS status using WHO criteria. Hypertension and diabetes were significantly higher in those with metabolic syndrome. Participants with MS were older than those without metabolic syndrome. Lifestyle factors, smoking and alcohol intake were not significantly different between the two groups.

Table 3.2: Shows haemodynamic characteristics of the study population according to MS status. The result revealed that SBP24, DBP24, SBPN, DBPN, SBPD, DBPD, SBPC, DBPC, DBPC were significantly higher in those with metabolic syndrome compared to those without metabolic syndrome.

Table 3.3: Shows the association between MS and BP in the WHO category of MS. The result revealed that SBP24, DBP24, SBPN, DBPN, SBPD, DBPD, SBPC and DBPC were significantly associated with PWV.

Table 3.4 shows multivariate association between PWV and the indices of obesity. The analysis shows that the three indices of obesity (WC, BMI and WHR) were significantly associated with PWV.

Figure 3.1: Shows the differences in PWV in the WHO category of MS. The PWV of participants with metabolic syndrome was significantly higher than those without metabolic syndrome.

Results

Table 3.1 General characteristics of the study population according to MS status (WHO criteria)

	Total population	Without MS	With MS	P value
Number	668	588	80	
Age (years)	48.5±18.1	42.4±17.9	56.2±14.4	0.0200*
Female (%)	61.7	59.7	76.2	0.0030*
Alcohol intake (%)	19.3	18.9	22.5	0.2611
Smokers (%)	16.6	17	13.8	0.2887
Hypertensive (%)	45.7	40.6	82.5	<0.0001*
Diabetic (%)	9.6	3.9	51.2	<0.0001*

MS-Metabolic syndrome, * P value <0.05 depicts significant difference between those with and without MS

Table 3.2 Haemodynamic characteristics of the study population (mm Hg) according to MS status

	Total population	Without MS	With MS	P value
SBP24	662	117.1 ± 14.1	125.4 ±17.8	<0.001*
DBP24	662	72.2 ± 9.6	76.0 ± 11.0	0.100
SBPN	662	110.4 ±16.2	119 ±21.0	0.011*
DBPN	662	64 ± 11.2	68.6 ± 12.7	0.020 *
SBPD	662	121.3 ± 13.7	128.5 ± 16.4	0.001*
DBPD	662	77.1 ± 9.5	80.5 ± 10.6	0.170
SBPC	688	127.2 ± 20.9	140.1 ± 24.1	0.005*
DBPC	688	82.6 ±11.5	88.4 ±14.9	0.001*
C_SBP	647	117.7 ±23.5	126.6 ±22.6	0.002*
C_DBP	647	83.32 ± 12.9	87.5 ± 11.4	0.006*

SBP24, 24-hour systolic BP; SBPD, 24-hour diastolic BP; SBPN, night-time systolic BP; DBPN night-time diastolic BP; SBPD, daytime systolic BP; DBPD, daytime diastolic BP; SBPC, conventional systolic BP; DBPC, conventional diastolic BP; C_SBP, central systolic BP; C_DBP, central diastolic BP. *P value < 0.05 was considered significant. values expressed as a mean ±standard deviation; p value < 0.05 considered significant.

Table 3.3The association between MS and BP (mm Hg) in the WHO

	R^2	CI	P value
PWV vs			
SBP24	0.171	0.061 to 0.091	< 0.001*
DBP24	0.097	0.062 to 0.110	< 0.001*
SBPN	0.163	0.051 to 0.077	< 0.001*
DBPN	0.109	0.058 to 0.099	< 0.001*
SBPD	0.142	0.056 to 0.088	< 0.001*
DBPD	0.064	0.046 to 0.095	< 0.001*
SBPC	0.303	0.059 to 0.074	<0.001*
DBPC	0.106	0.055 to 0.086	<0.001*

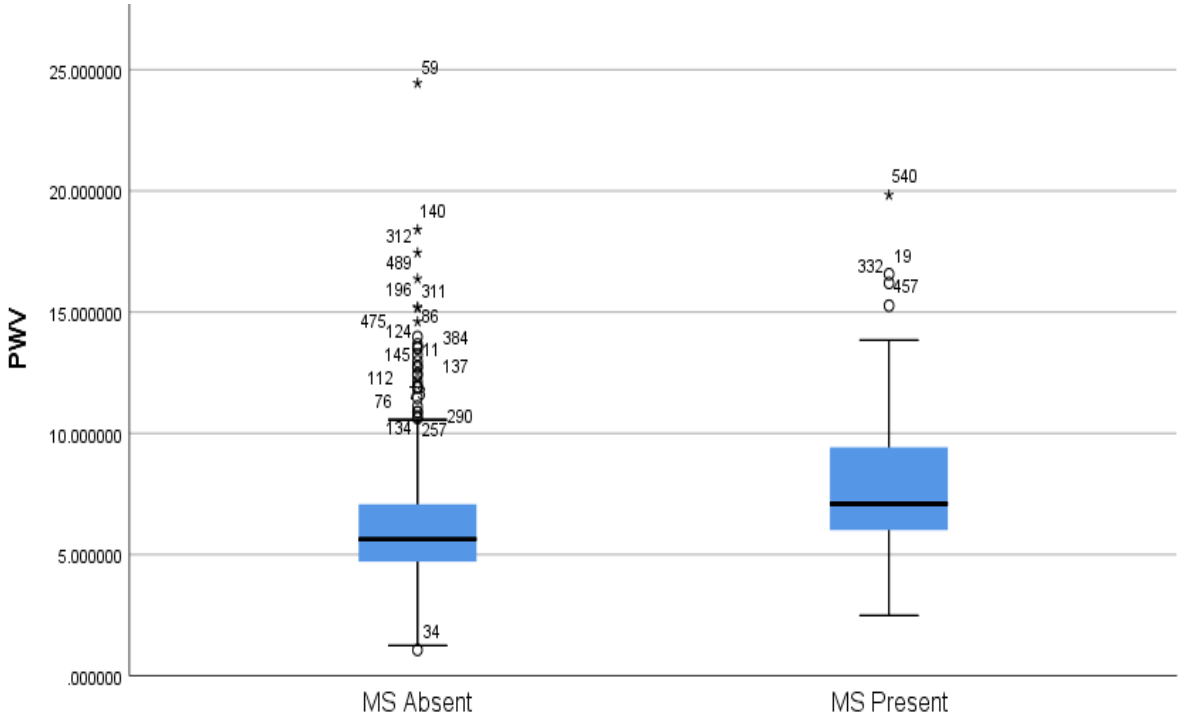
CI, confidence intervals, **SBPC**- Conventional Systolic Blood Pressure, **DBPC**- Conventional Diastolic Blood Pressure, **SBP 24** - 24-hour Systolic Blood pressure, **DBP24** - 24-hour-Diastolic Blood Pressure, **SBPD**-Daytime Systolic Blood Pressure, **DBPD**- Daytime Diastolic Blood Pressure, **SBPN** - Nighttime Systolic Blood Pressure, **DBPN** - Nighttime diastolic blood pressure, **PWV**- pulse wave velocity, *****means there was significant p value when compared with metabolic syndrome category, $p < 0.05$. Values expressed as a percentage or mean ±standard deviation; p value < 0.05 considered significant.

Table 3.4 multivariate association between PWV and the indices of obesity

	R^2	CI	P value
PWV vs			
WC (cm)	0.090	0.038 to 0.062	< 0.001*
BMI (kg/m^2)	0.043	0.038 to 0.062	<0.001*
WHR	0.054	2.754 to 5.338	<0.001*

PWV- Pulse waist velocity, WC- waist circumference, BMI-body mass index. Corrected for age, sex, smoking and alcohol intake; CI, confidence intervals, values expressed as a mean ±standard deviation; p value < 0.05 considered significant.

Fig 3. 3 showing the differences in PWV in the WHO category of MS



PWV- Pulse waist velocity, MS- metabolic syndrome, WHO- World Health Organization

3.4 Discussion

Our results revealed that people with metabolic syndrome are significantly older that those without metabolic syndrome. This is an indication that metabolic syndrome progresses with age. A number of studies (Lee *et al*., 2016; Kim *et al*., 2018; Yang *et al*., 2019) have shown that indices of obesity (WC, BMI and WHR) increase with increasing age. Hence the observed age- related increases in metabolic syndrome are mediated by the indices of obesity, confirming our findings in the previous chapter which show that indices of obesity are the most important determinants of metabolic syndrome in this population. Our results contradict previous findings which indicated that insulin resistance is the most important component of metabolic syndrome (Kassi *et al*., 2011). The difference in these results is that our study population has a high incidence of obesity. Hence, in our population, the main factors that drive the rising incidence of MS are the indices of obesity as indicated in our previous chapter. Our results further indicate that the role of hypertension in the pathophysiology of metabolic syndrome cannot be underestimated in this population (Table 3.1). Twenty-four ambulatory BP measurements results show that 24-hour and daytime systolic BP are significant higher in participants with metabolic syndrome. Interestingly, both systolic and diastolic nighttime BP was significantly higher in people with metabolic syndrome compared to those without metabolic syndrome (Table 3.2 and 3.3). This confirms previous study by Bawa-Allah et al (2019) which indicated that night-time BP is more closely related to cardiovascular target organ damage than daytime BP in this population (Bawa-Allah *et al*., 2019). The main reason for the normal diastolic 24-hour and daytime BP in individuals with MS despite high systolic BP is influenced by the age of this group. As stated above, participants with MS are significantly older than those without MS. Previous studies have shown that isolated systolic hypertension, where systolic BP increases despite normal diastolic BP, is more prevalent in older

individuals (Omboni *et al*., 2016). The two risk factors, smoking and alcohol intake, were not significantly different between the two groups, indicating that these factors contribute to MS in this study population. Similarly, Wang et al. (2022) reported that active smoking is associated with increased prevalence of MS, especially among those less than 70 years. (Wang *et al*., 2022). Yu et al. (2014) also documented that smoking and alcohol contribute to increase in prevalence of metabolic syndrome in Chinese men only. However, in a cohort study by a Norwegian group alcoholic beverages intake was observed to have protective effect in women with MS (Yu *et al*., 2014). This shows that smoking and alcohol should be included be considered as diagnostic criteria for classification of MS among Africans.

In this study, there was positive correlation between PWV and all the haemodynamic parameters (table 3.3) in this population. This confirms earlier studies by Pietri *et al*., and Omboni *et al*, 2019 which observed increased peripheral wave reflections and endothelial dysfunction in untreated essential hypertensive patients and suggested that ambulatory pulse wave analysis may help evaluate vascular health of individuals at risk for cardiovascular disease (Pietri *et al*., 2009: Omboni *et al*., 2019).

Persistent MS and its components were associated with accelerated progression of arterial stiffening (Fig 3.1). Our results show that PWV is significantly higher in individuals with MS compared to those without MS. This corroborates findings from different ethnicities which revealed that increased existence of metabolic syndrome results to increase in the progression of arterial stiffening, that is, persistent metabolic syndrome is strongly associated with acceleration of arterial stiffening (Tomiyama *et al*., 2003; Schutte *et al*., 2005; Taher *et al*., 2019). This implies that, resolution of metabolic syndrome may be associated with attenuation of the progression of arterial damage and associated complications.

Our study further revealed that there is a correlation between all the indices of obesity and arterial stiffness (Table 3.4). Metabolic syndrome is a multifactorial syndrome that majorly include insulin resistance, abdominal obesity and hypertension. Its pathophysiology involves several signaling pathways that includes NO and ROS signaling pathway which contribute to the subsequent endothelial dysfunction underlying arterial stiffness (Ruiz *et al.*, 2020). Moreover, these key factors affect the metabolism of advanced glycation end products which is an important pathway in the development of arterial stiffness, thus increase cardiovascular risk associated with the presence of MS (Versari *et al*., 2009; Wang *et al*., 2014; Ruiz *et al.*, 2020).

3.5 Conclusion:

Our study observed that obesity, hypertension and diabetes remain important risk factors for metabolic syndrome in Africans. Moreover, MS is associated with arterial stiffness. This means in this population, intervention strategies aimed at reducing endothelial dysfunction, should primarily target body weight reduction and hypertension control.

CHAPTER FOUR

The Association between Metabolic Syndrome and Left Ventricular Geometry

Abstract:

Introduction

Previous studies have shown that there is a high prevalence of obesity and hypertension in people of African ancestry living in South Africa, and arterial stiffness is one of the leading contributors to the rising incidence of cardiovascular diseases in African communities. Obesity, hypertension, insulin resistance coupled with other underlying biochemical derailment have the tendency to result to cardiac remodeling characterised by increased left ventricular mass index. Therefore, in this study we investigated the relationship between left ventricular geometry and metabolic syndrome in Black South Africans using the WHO criteria to classify metabolic syndrome.

Methods

The cross-sectional study was conducted among 668 participants of people of African ancestry. The minimum age of the participants was 18 years and there was to upper age limit. Obesity was assessed using, body mass index (BMI), waist circumference (WC) and waist to-to-hip ratio (WHR), while conventional blood pressure was assessed using electronic blood pressure monitoring device, ambulatory 24-hr day and night BP was determined using SpaceLabs monitors. Aortic pulse wave velocity was determined using applanation tonometry and SphygmoCor software. Blood sample was taken for biochemical parameters such as lipid profile; triglyceride (TG), high lipid lipoprotein (HDL)], fasting blood glucose. Metabolic syndrome was defined according WHO criteria. All analyses were conducted using SPSS software for Windows, version 11.0J (SPSS, Chicago, USA) and STATA.

Results

When participants were classified according to gender, there was no significant age difference between men and women. There was also no significant difference in the incidence of diabetes, hypertension, alcohol intake and smoking between the two genders. However, BMI and the incidence of MS were significantly higher in women compared to men. When the participants were classified according to MS status, LVMI was significantly higher in people with MS compared to those without MS and E/A ratio (marker of the function of the left ventricle of the heart) was significantly lower in participants with MS compared to those without MS.

Conclusion

The higher prevalence of obesity in women is not age related because there is no age difference between men and women. The incidence of MS in women was double that of men. Furthermore, our results show that MS is associated with both preclinical left ventricular hypertrophy and diastolic dysfunction in this population.

4.1. Introduction:

Obesity, diabetes mellitus and hypertension are three major components of metabolic syndrome that are on the increase in both developed and developing country (Gaillard *et al*., 2017).

Insulin resistance has been the center of mechanism involved in the pathophysiology of metabolic syndrome and its complications. Interrelationship between obesity and hypertension are also believed to result to insulin resistance (IR). Oxidative stress and inflammation are central to the relationship between obesity, IR and hypertension. Research is now focusing on other mechanisms involving mineralocorticoid mechanisms (RAAS), oxidative and inflammatory processes as the main pathophysiology of cardiovascular complication of metabolic syndrome especially atherosclerosis and left diastolic dysfunction (Touyz *et al*., 2021).

Obesity and its related cardiovascular risks factor have been documented to predict metabolic syndrome and insulin resistance (Alkholy *et al*., 2016). Leptin is involved in numerous physiological and pathological processes some of which inflammation and fibrosis, are pivotal contributing to pathophysiological mechanisms in the development and progression cardiac abnormalities due to arterial stiffness and diastolic dysfunction, notably (Alkholy *et al*., 2016; Touyz *et al*., 2021). Adipose cells are capable of yielding a local RAAS that contributes to inflammation. This is characteristically observed in experimental and human obesity. This has pathophysiological significance in the cardiovascular system because perivascular adipose tissue is an important player in vascular dysfunction in obesity-associated cardiovascular disease. Insulin-resistance also determines the hyperactivation of other systems such as renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system, with consequent hemodynamic and no hemodynamic effects (Touyz *et al*., 2021). A study by Ceravolo and colleagues demonstrated that there was a strong association between increased RAAS activation, circulating insulin levels and diastolic dysfunction (Ceravolo *et al*., 2003).

Furthermore, pathological activities within epicardium fat and myocardium may lead to signalling mechanism, insulin resistance and other processes that underlie cardiac dysfunction. In obesity, diabetes, metabolic syndrome, or conditions where there is activation of the RAAS, the cardioprotective arm of the RAAS may be decompensated, leading to a proinflammatory phenotype of the epicardial adipose tissue. Study has shown possible mechanisms whereby ACE2 negatively regulates epicardial adipose tissue and cardiac function (Fig 4.1) [(Touyz *et al*., 2016)].

The constellation of various components of MS is associated with higher left ventricular mass index (LVMI) and prevalence of left ventricular hypertrophy (LVH) which may result to heart failure (Gupta *et al*., 2022). Homeostasis model assessment of insulin resistance (HOMA-IR) is an index used to evaluate insulin resistance (fasting serum insulin (μU/ml) × fasting plasma glucose (mmol l-1)/22.5). A study reported by Gayoso-Diz, et al (2013) suggested the possible effects of two absolute factors of metabolic syndrome (age and gender) on HOMA-IR and their association with cardio- metabolic risk (Gayoso-Diz, *et al*., 2013). However, the following echocardiographic parameters; end systolic volume (ESV), end diastolic volume (EDV), stroke volume (SV), left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), ejection fraction (EF), E/A ratio (the marker of the function of the left ventricle of the heart) defined as the ratio of peak velocity blood flow from left ventricular relaxation in early diastole (the E wave) to peak velocity flow in the late diastole caused by atrial contraction (Christopher, 2008) and fractional shortening (FS) have been featured as the reliable prognostic indicator left ventricular dysfunction (Gaudon *et al*., 1993; Gupta *et al*., 2022). Therefore, this study aimed to assess the impact of metabolic syndrome on left ventricular geometry and function in people of African ancestry.

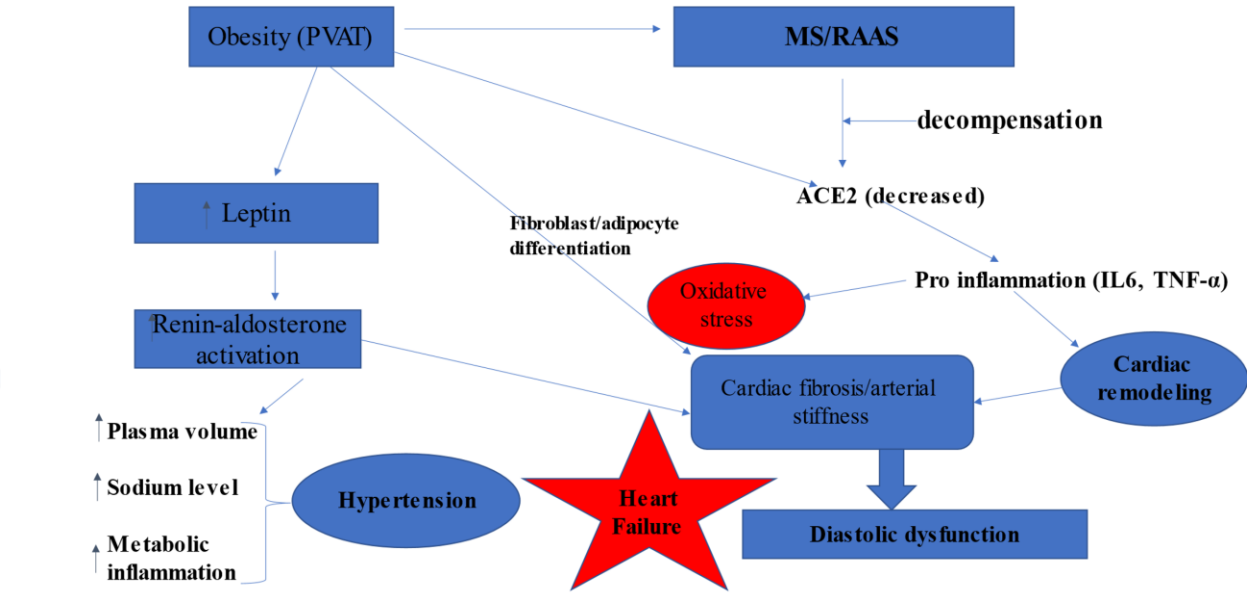


Fig 4.1

Figure 4.1 integrative relationship between obesity, metabolic syndrome and renin-angiotensin aldosterone system (RAAS).

4.2 Methods

4.2.1 Study design and population

This cross- sectional study and sample size are as explained in Chapter 2, section 2.2 and 2.2.1 respectively.

4.2.2. Ethical clearance / approval:

Ethical approval was obtained from the University of Witwatersrand, Human Research Ethics Committee [Medical (Reference number: M190472)]. Participants were provided with information

sheets detailing the purpose and process of the study. Each participant gave written, informed consent for his/her voluntary participation in the study.

4.2.3 Measurements and biochemical analysis

4.2.3.1 Anthropometric measurement

Assessment of anthropometric measurement is as previously explained in chapter 2, section 2.2.4.1.

4.2.3.2 Blood pressure assessment

Blood pressure assessment is as previously explained in chapter 2, section 2.2.4.

4.2.3.3 Biochemical analysis

Biochemical analysis is as previously explained in chapter 2, section 2.2.4.

4.2.4 Echocardiograph

Participants were placed in left lateral decubitus position. Echocardiographic measurements were assessed to determine cardiac structure (including heart chamber dimensions and wall thickness), as well as left ventricular mass. Two-dimensional targeted M-mode echocardiography (SonoSite R Inc., Bothell, WA, USA) in parasternal long axis view was used to determine left ventricular dimensions, following the method described by Nunez et al. (2005). The M-mode variables was analysed according to the American Society of Echocardiography convention (Lang *et al*., 2015). All readings were recorded on videotape and analysed by an experienced echocardiographer who was part of the study. The echocardiographer was blinded to the details and clinical data of the subjects (Skudicky *et al*., 2002).

4.2.5 Diagnosis of metabolic syndrome

As explained in chapter 3, metabolic syndrome was defined according to WHO criteria.as explained in chapter 3, section 3.3.4.

4.2.6 Statistical analysis

Windows version 11.0J (SPSS, Chicago, USA) software was used for grouping and bivariate analysis. A p value of < 0.05 was considered to denote statistical significance. Continuous data will be reported as mean ± SEM. Results from the participants was compared between two groups using Student's *t*- test. The χ^2 statistic was used to compare means and proportions. Multivariate analyses using Stata/MP 16.0 (StataCorp, College Station, TX, USA) was used to analyse the association between metabolic syndrome and changes in echocardiograph parameters, biochemical parameters, relative factors and major component of metabolic syndrome among the selected groups. All models were adjusted for age, sex, alcohol and smoking. Pearson's correlation coefficient was used to determine the association between metabolic syndrome status and measurement of obesity; lipid profile and echocardiograph. P value < 0.05 considered significant.

RESULTS

Table 4.1: Shows general characteristics of the study population stratified by gender. The prevalence of metabolic syndrome was higher among women (76.2%) in the study population compared to men (23.8%). The result also showed that BMI and WC were significantly increased in women compared to men, 31.2±7.5 cm and 92.4±16.6 respectively.

Table 4.2: shows cardiac parameters stratified according to MS status. Those with metabolic syndrome had significant decrease in E/A ratio and ESV ($p= 0.022$ and $p < 0.001$ respectively) compared to those without metabolic syndrome. Moreover, those with metabolic syndrome had significant increase in LVMI ($p<0.001$). There was no significance in EDV, SV, LVEDD, LVESD, FS and EF.

Table 4.3 shows association between relative components among those with and without metabolic syndrome. Participants with metabolic syndrome (MS) had significantly increase HOMA-IR concentration (0.5 ±0.4) compared to participants without MS (0.2 ±0.5). Furthermore, participants with metabolic syndrome had significantly high renin concentration (69.0±10.2) compared to participants without MS (37.5 ±10.2).

Table 4.4 Shows ROC curve analysis of the relationship between MS and cardiac parameters. There was significant difference in HOMA-IR, Aldo, leptin and renin, aldosterone, concentrations ($p= 0.001$, $p = 0.017$, 0.025 and $p = 0.043$ respectively) in those with metabolic syndrome. The predictive value (AUC) of HOMA-IR, Aldo, leptin and renin, aldosterone in metabolic syndrome were 0.705, 0.645, 0.636, and 0.623 respectively.

Note: **ESV**= End Systolic Volume, **EDV**= End Diastolic Volume, **SV**= Stroke Volume, **LVESD**= Left Ventricular End Systolic Diameter, **LVEDD** = Left Ventricular End Diastolic Diameter, **E**

Table 4.1 General characteristics of the study population stratified by gender

	Men	Women	P value
	Mean ± SD	Mean ± SD	
Number	256	412	---------
Age	43.3 ±18.9	44.5 ±17.6	0.385
BMI	25.1 ±4.8	31.2 ±7.5	<0.001*
WC	86 ±13.3	92.4 ±16.6	<0.001*
WHR	0.8 ±0.1	0.8 ±0.2	0.777
Hypertensive (%)	46.1	45.6	0.936
Diabetic (%)	8.2	10.4	0.207
% alcohol intake	20.3	18.7	0.615
% smoking	16.4	16.7	1.000
MS (%)	7.4	14.8	0.005*

MS defined according to the WHO criterion; BMI, body mass index etc. *means there was significant p value when compared with metabolic syndrome category, $p < 0.05$

Table 4.2 Cardiac parameters stratified by MS status

	Total population	Without MS	with MS	P value
Mean ± SD	Mean ± SD			
ESV (ml)	661	31.6 ±13.4	36.1 ± 16.5	0.022*
EDV (ml)	661	95.3 ±28.1	100.9 ±28.8	0.162
SV (ml)	661	63.7 ± 19.5	64.8 ± 18.6	0.691
LVEDD (mm)	661	4.7 ±0.6	4.8 ±0.7	0.071
LVESD (mm)	661	2.8 ±0.5	2.9 ±0.6	0.117
FS (%)	661	37.7 ±6.8	35.9 ±7.0	0.660
EF (%)	661	67.2 ±8.7	65.9 ±9.6	0.326
E/A ratio	661	1.3 ±0.5	1.0 ±0.3	<0.001*
LVMI (g/m^2)	661	37.9±5.7	53.9±7.0	<0.001*

MS defined according to the WHO criterion; **ESV,** end systolic volume**; EDV,** end diastolic volume. **LVESD**; Left Ventricular End Systolic Diameter, **LVEDD;** Left Ventricular End Diastolic Diameter, **EF**; Ejection Fraction, **E/A ratio**- marker of the function of the left ventricle of the heart (defined as the ratio of peak velocity blood flow from left ventricular relaxation in early diastole (the E wave) to peak velocity flow in the late diastole caused by atrial contraction (Christopher, 2008), **FS**; Fractional Shortening. Keans corrected for age, gender, hypertension status, diabetes, smoking and alcohol intake. *Means there was significant p value when compared with metabolic syndrome category, $p < 0.05$.

Table 4.3 Biochemical parameters stratified by MS status

Mean ± SD	Total population Mean ± SD	Without MS	with MS	P value
Aldo (pg/mL)	631	205.62 ± 29.14	219.8 ± 36.9	0.126
ARR	631	23.15 ±36.4	25.8 ±15.4	0.630
Galectin	631	8.9 ±4.1	9.6 ±3.5	0.201
HOMA IR	631	0.2 ±0.5	0.5 ± 0.4	<0.001*
Renin (ng/mL)	631	37.5 ±10.2	69.1 ±10.2	0.003*
Insulin (mmol/l)	631	12.7 ±15.6	15.3 ±19.4	0.186
Leptin (ng/mL)	631	22.0 ±24.4	29.3 ±19.8	0.131
Aldo-renin (ng/mL)	631	26.15 ±36.7	31.0 ±10.6	0.514

MS defined according to the WHO criterion**; Aldo**: Aldosterone, **ARR**: Aldosterone renin ratio, **HOMA-IR**= Homeostatic Model Assessment-Insulin Resistance, *means there was significant p value when compared with metabolic syndrome category, $p < 0.05$. Keans corrected for age, gender, hypertension status, diabetes, smoking and alcohol intake.

Table 4.4.

Table 4.4: ROC curve analysis of the relationship between MS and biochemical parameters

	AUC	CI	P value
Aldo (pg/mL)	0.645	0.512 to 0.777	0.017*
ARR	0.446	0.322 to 0.570	0.374
Galectin	0.522	0.405 to 0.638	0.722
HOMA IR	0.705	0.617 to 0.793	0.001*
Renin (ng/mL)	0.623	0.510 to 0.735	0.043*
Insulin (mmol/l)	0.608	0.518 to 0.699	0.075
Leptin (ng/mL)	0.636	0.536 to 0.735	0.025*
Aldo-renin	0.447	0.323 to 0.571	0.381

MS defined according to the WHO criterion**; Aldo**: Aldosterone, **ARR**: Aldosterone renin ratio, **HOMA-IR**= Homeostatic Model Assessment-Insulin Resistance, *means there was significant p value when compared with metabolic syndrome category, $p < 0.05$. Keans corrected for age, gender, hypertension status, diabetes, smoking and alcohol intake. AUC, area under the curve; CI, confidence intervals etc. Corrected for age, gender, hypertension status, diabetes, smoking and alcohol intake, *means there was significant p value when compared with metabolic syndrome category, $p < 0.05$.

Note: AUC of 0.5 suggests no discrimination, 0.7 to 0.8 is considered acceptable while 0.8 to 0.9 is excellent and > 0.9 is outstanding (Jayawant, 2010). AUC greater than 0.75 shows that there is higher prediction/ probability among those with MS than those without MS.

Table 4.5 ROC curve analysis of the relationship between MS and cardiac parameters

	AUC	CI	P value
ESV (ml)	0.676	0.488 to 0.664	0.066
EDV (ml)	0.566	0.483 to 0.648	0.111
SV (ml)	0.519	0.410 to 0.596	0.653
LVEDD (mm)	0.517	0.483 to 0.658	0.086
LVESD (mm)	0.554	0.466 to 0.642	0.0190
FS (%)	0.417	0.333 to 0.501	0.044*
EF (%)	0.461	0.375 to 0.548	0.350
E/A ratio	0.272	0.204 to 0.340	<0.001*
LVMI	0.683	0.512 to 0.777	<0.001*

AUC, area under the curve; CI, confidence intervals etc. Corrected for age, gender, hypertension status, diabetes, smoking and alcohol intake. Note: AUC of 0.5 suggests no discrimination, 0.7 to 0.8 is considered acceptable while 0.8 to 0.9 is excellent and > 0.9 is outstanding (Jayawant, 2010). AUC greater than 0.75 shows that there is higher prediction/ probability in those with MS than those without MS.

4.3 Discussion

In this study population, 76.2% of those with metabolic syndrome were women while men with metabolic syndrome were 3.9%. Likewise, there was significant increase in BMI and WC of women compared to men (Table 4.1). Body mass index and waist circumference are major risk factor for the diagnosis of metabolic syndrome. This is a pointer to adiposity as a contributory mechanism underlying the development of metabolic syndrome and its complications. Waist circumference and BMI are measure of obesity positively associated with LVMI (Rashid *et al*. 2014). There is increasing evidence that adiposity is strongly linked with cardio-metabolic risk and cardiac changes. This may be due to hypertrophied adipocytes as a result of dysregulated adipokine secretion, increased recruitment of inflammatory cells and impaired metabolic homeostasis that eventually results in the development of systemic insulin resistance (Atawia *et al*., 2019). There is also production of Nitric oxide synthase (NOS) which functions to maintain vascular and adipocyte homeostasis. Other reactive oxygen species (ROS) are hydrogen peroxide

($H2_2O_2$), superoxide (O_2-), hydroxyl radical (OH), high levels of nitric oxide (NO) and peroxynitrite (ONOO−). These ROS are products of numerous enzymatic reactions that lead to subcellular damage in the heart, kidney and vasculature (Atawia *et al*., 2019). Moreover, leptin has ability to induce oxidative stress, SNS activation, release of endothelin 1 and reduced NO-bioavailability (Tsai *et al*., 2016). This infers that, elevated serum leptin levels evident in this study population are likely associated with impaired vasodilation. Also, leptin increases proliferation and migration of vascular smooth muscle cells through its ability to stimulate phosphorylation and activate mitogen-activated protein kinases. These changes result to vascular, peripheral resistance and vasoconstriction (Muñoz-Durango *et al*. 2016).

This study also revealed an association between HOMA-IR and metabolic syndrome. There was highest predictive value of HOMA-IR in those with metabolic syndrome (table 4.3). According to Matthew et al. (1985), insulin resistance can be ascertained by a homeostatic model assessment (HOMA) of insulin resistance. Index value of HOMA-IR above 1.9 (normal range is 0.5-1.4) indicates insulin resistance (Matthews *et al*., 1985). Our study revealed high prediction of metabolic syndrome with HOMA-IR (Table 4.4).

The release of renin is under the control of numerous cellular, hormonal and hemodynamic factors, there is increasing interest in exploring inappropriate activation of the renin-angiotensin-aldosterone system (RAAS) as a unifying mechanism between insulin resistance and other components of the metabolic syndrome (Rochlani *et al*., 2017). Some studies have suggested that elevations in plasma aldosterone levels are associated with the insulin resistance independent of other components of angiotensin II (Ang II) [(Norman *et al*., 2020; Swarup *et al*., 2022)]. This relationship has been observed in studies exploring the association between primary aldosteronism, in which there are low levels of renin activity and Ang II, and insulin resistance

(Norman *et al*., 2020). Our study revealed high predictive value between renin and metabolic syndrome. Similarly, aldosterone had high predictive value for metabolic syndrome (Table 4.4). There is evidence to indicate that renin-aldosterone ratio could be used for a more targeted pharmacological treatment of Africans with essential hypertension. This shows that low renin profile in Africans provides a basis for the greater reduction in blood pressure in response to dietary sodium restriction. (Sagnella, 2001, Maseko *et al*., 2018).

In this study, we observed that leptin had high predictive value of metabolic syndrome. Insulin resistance is strongly related to obesity, a major component of metabolic syndrome (Gaillard *et al*., 2017). According to Fang et al (2020), they documented that peri-renal fat thickness is an independent predictor of kidney dysfunction in people with type 2 diabetes, this may be due to hyperstimulation of paracrine gland; thus, affect renal circulation via pro-inflammatory cytokines. Similar effect applies to cardiovascular diseases (Fang *et al*., 2020). Therefore, metabolic consequences of obesity/overweight have been attributed to biological activity of the adipocytes and adipose tissues unique to the fat distribution such as visceral and subcutaneous adiposity other than hemodynamic of salt and water retention alone.

Unique to this study is the prospective analysis of the interrelationship between the development of metabolic syndrome and preclinical diastolic dysfunctions. The increasing number of MS criteria is associated with cardiac diastolic dysfunction. Our finding showed decrease in E/A ratio and reduced ESD in those with metabolic syndrome (Table 4.3). Obesity is an independent risk factor for LVH, but it is also associated with other risk factors, such as hypertension and diabetes; these can amplify the effect of obesity on cardiac remodeling related to combination of RAAS mechanism and inflammatory process as earlier discussed. The consequences of the processes on coronary endothelium may have negative influence on left ventricular diastolic performance

(Cuspidi *et al*., 2014). There was increased LVMI among those with metabolic syndrome. Notably, studies have reported increase in LVMI in obese and hypertensive individuals (Karakan and Inan, 2015; Alkholy *et al*., 2016). This study is in agreement with the study conducted in Hispanics/Latinos with metabolic syndrome, the study revealed increase risk of decreased left ventricular diastolic and systolic function among participants with metabolic syndrome (Burroughs-Peña *et al*., 2017). Obesity, hypertension and hyperinsulinemia are independent cardiovascular risk factor that determines LVMI associated with MS. These are associated with activation of the SNS that may affect LVM directly (De-Simeon *et al*., 2009; Shereef and Kandeel, 2019). Our findings are in support of Libhaber et al (2009) who revealed that arterial stiffness predicts LVMI among people of African ancestry, particularly women. This is also similar to study by Bidulescu et al (2011), their findings also highlights the importance of assessing factors that influence LVMI on an ethnic level (Bidulescu *et al*. 2011).

4.4 Conclusion

Adipose-related cardiovascular events unique to this study population was investigated. In this study, BMI, WC and WHR were used as indices of obesity to assess the impact of adiposity on left ventricular geometry. This study concluded that cardiac damage in Africans is influenced by obesity, diabetes and hypertension; we further corroborated the importance of leptin, renin, aldosterone and insulin in the assessment of preclinical diastolic dysfunctions in Africans.

CHAPTER FIVE

Aldosterone and cardiovascular fibrosis – role of TRPM7

ABSTRACT

Cardiac and renal fibrosis are strongly associated with obesity and metabolic dysfunction which may contribute to increase incidence of atrial arrhythmias, progressive nephropathy, heart failure and sudden death. There are experimental and observational evidences linking obesity to myocardial and renal fibrosis in animal models and human patients, all focusing on the fundamental pathophysiological alterations that may trigger several cellular and molecular fibrogenic signalling pathways that may culminate to fibrotic response. Aldosterone and Angiotensin II (Ang II) activate different receptors and different mechanisms regarding the fibroblast activation. The renin-aldosterone-angiotensin system (RAAS) is crucially involved in hypertension development and organ damage. The Touyz lab has demonstrated an important role for the non-selective cation channel, TRPM7 in the pathophysiology of hypertension and associated target organ damage. In particular, it seems that decreased activity of TRPM7 and perturbed Mg2+ regulation may be important. Deleterious effects of Ang II are mediated by the activation of Ang II type 1 receptors (AT1) and chronic infusion of Ang II causes hypertension, cardio-renal fibrosis in animal models. However, whether chronic infusion of aldosterone or Ang II activate similar or distinct mechanisms regarding to myocardial and renal fibrosis development is still elusive, therefore, this experiment investigated the role of aldosterone- salt on cardiac and renal tissue, particularly focusing on the potential role of TRPM7 in aldosterone-induced cardiovascular fibrosis.

Methods

Studies were performed in aldosterone-salt-treated wild-type mice to induce hypertension and cardiovascular fibrosis and in TRPM7-deficient mice ($TRPM7^{+/\Delta kinase}$) to explore the role of TRPM7.

Results

In this chapter, results related to cardiovascular and renal fibrosis and remodeling are presented. Results demonstrated that aldosterone-salt treatment causes significant vascular, cardiac and renal collagen deposition and fibrosis with associated vascular remodeling (media thickening). These effects were amplified in mice induced with TRPM7/aldo-salt mice.

Conclusion

This study shows that aldosterone-salt impact aortic vasculature, cardiac and renal tissues, leading to cardio-renal and aortic fibrosis which is aggravated by TRMP7. Possibly, TRMP7 may serve as therapeutic target for the management of aldosterone-salt induced atherosclerosis and heart failure.

5.1 Introduction

Obesity is associated with a wide range of pathophysiological alterations (such as pressure and volume overload, metabolic dysregulation, neurohumoral activation, and systemic inflammation); their relative role in mediating cardiac fibrosis is poorly defined (Cavalera *et al*., 2014). Activation of fibroblasts likely plays a major role in obesity-associated fibrosis; however, inflammatory cells, cardiomyocytes, and vascular cells may also contribute to fibrogenic signalling. Several molecular processes have been implicated in regulation of the fibrotic response in obesity. Activation of the renin-angiotensin-aldosterone system, induction of transforming growth factor β, oxidative stress, advanced glycation end-products, endothelin 1, Rho-kinase signaling, leptin-mediated actions, and upregulation of matricellular proteins may play a role in the development of fibrosis in models of obesity and metabolic dysfunction (Prescott *et al*., 2000; Cavalera *et al*., 2014; Touyz *et al*., 2018). Moreover, experimental evidence suggests that obesity and insulin resistance profoundly affect the fibrotic and remodelling response after cardiac injury (Elagizi *et al*., 2020). Understanding the pathways implicated in obesity-associated fibrosis may lead to the development of novel therapies to prevent heart failure and attenuate post-infarction cardiac remodelling in patients with obesity. Basically, thickening of the arteries has been implicated in uncontrolled/ longstanding hypertension. Arterial stiffness and endothelial dysfunction are phenotypic diffuse vascular damage, which is associated with the progression of atherosclerosis (focal vascular damage). Although, angiotensin II (Ang II) has pleiotropic effects on vascular smooth muscle cells (VSMCs) [Touyz *et al*., 2006; Touyz *et al*., 2022)]. It has been demonstrated to promote the proliferative phenotype of VSMCs in mouse ascending aorta, but the underlying mechanisms is not well understood.

Cardiac fibrosis is a progressive pathological process that often result to delayed atrio-ventricular conduction, electrical heterogeneity, defaulted systolic ejection, hypoxia, myocardial and arterial stiffness. These are associated with the development of arrhythmia, heart failure and sudden cardiac death (Vassort and Alvarez, 2009). Pathologically, cardiac fibroblasts (CFs) are the principal responsible cells in the fibrogenesis cascade of events. Experimental balloon injury model revealed that adventitial fibroblasts (AFs) can be transformed into myofibroblasts (MFs) and migrate into the neointima of arteries after endoluminal injury. Over the years, the contributory roles of adventitia in artery remodeling are becoming area of interest. Similarly, kidney injury responses to hypertension are characterized by renal fibrosis, tubular hypertrophy, and glomerular alterations (Touyz *et al*., 2023). Hypertensive renal fibrogenesis is a complex process involving various pathological scarring processes such as dysregulated extracellular matrix (ECM) assembly, anchoring or degradation, inflammatory cytokines formation, failed regeneration of tubular epithelium, microvascular rarefaction, and fibroblasts activation (Touyz *et al*., 2023).

Aldosterone plays an important role in regulating NaCl reabsorption and blood pressure (Satoh *et al*., 2012). It is a steroid hormone that regulates blood pressure by enhancing Na^+ reabsorption in the distal convoluted tubule, connecting tubule, and collecting duct of the nephron as well as the homeostatic maintenance of $Mg^{2+}$and K^+ (Covelli, 2012). However, studies have shown that mineralocorticoid plus salt treatment increases endothelin expression in the heart and vasculature (Rockey, 2009; Touyz *et al*., 2018). Clinical data suggests that aldosterone enhances the deleterious effects of Ang II in animal model by amplifying other pathways such as post-receptor signal-transduction pathways.

TRPM7 was identified over two decades ago and has been known to play a critical role in tissue fibrosis, tumor progression and anoxic neuronal death (Yogi *et al*., 2011; Hu *et al*, 2021).

Transient receptor potential melastatin 7 (TRPM7), a novel channel kinase, has been recently identified in the vasculature. TRPM7 has been shown to be expressed in the human vascular system and to play a role in vascular functions (Liu *et al*., 2017; Falcőn *et al*., 2019). The interaction of transient receptor potential melastatin 7 with macrophages promotes vascular adventitial remodeling in transverse aortic constriction rats (Valinsky *et al*., 2016). Various studies are pointer to the fact that TRPM7 might be involved in cardiovascular diseases endothelial related cardiovascular diseases (Yu *et a*l., 2014, Liu *et al*., 2017: Rios *et al*., 2020). In vascular endothelial cells, TRPM7 modulates cell growth and proliferation as well as calcium and magnesium homeostasis. Notably, expression of TRPM7 expression in endothelial cells can be upregulated by high glucose, oxidative stress, and laminar shear stress (Negri et al., 2020); these can be influenced by obesity and hypertension.

TRPM7, a member of the TRPM (Melastatin) subfamily, is a non-selective cation channel which permeates both calcium and magnesium (Duan *et al*., 2018). TRPM7 was identified over two decades ago and has been known to play a critical role in balance of some micro elements such as magnesium, calcium, zinc e. t. c. Basically, TRPM6 and TRPM7 are important regulators of magnesium homeostasis. However, these channels also transport other cations such as cobalt and in the absence of both calcium and magnesium, they can mediate sodium flux (Yogi *et al*., 2011). TRPM7 channels play a key role in the functional change of cardiac fibroblasts (CFs) induced by angiotensin II (Ang II). Study by Yu et al (2014) demonstrated TRPM7 expression in CFs, and 2-APB (TRPM7 inhibitor), inhibited Ang II-induced CTGF, α-SMA expression and CFs proliferation (Yu *et al*., 2014). Moreover, knocking down TRPM7 by shRNA, the research team proved that TRPM7 mediated both calcium and magnesium changes in cardiac fibroblasts which

contribute to fibrosis progress. Their study suggested that TRPM7 should play a pivotal role in cardiac fibroblast functions associated to cardiac fibrosis development (Yu *et al*., 2014).

On the contrary, Rio et al (2020) documented that chanzyme TRPM7 protects against cardiovascular inflammation and fibrosis, their findings suggested that anti-inflammatory and anti-fibrotic role for TRPM7 are mediated via Mg2+-sensitive mechanism (Rio *et al*., 2020). Upregulation of TRPM7 during inflammatory renal damage has been proposed as pharmacological intervention, targeting TRPM7 may also be protective in progressive kidney fibrosis (Suzuki *et al*., 2020). The interaction of transient receptor potential melastatin 7 with macrophages also promotes vascular adventitial remodeling in transverse aortic constriction rats (Vanilinski *et al*., 2016). Additionally, TRPM7 may also play a major role in vesicular trafficking, membrane reorganization, and neurotransmitter release e. g acetylcholine, thus play vital role in neurodegenerative diseases (Sun *et al*., 2015; Zou *et al*., 2020).

Emerging evidence suggests that vascular TRPM7 function might be altered in hypertension (Yogi *et al*., 2011). Aldosterone increases TRPM7 function. According to Vanillnski et al (2016) aldosterone was shown to increase intracellular magnesium levels and alter inflammatory signaling in TRPM7-expressing HEK293 cells. Their study assessed effects related to aldosterone-mediated increase of TRPM7 current and/or plasma membrane localization and Aldosterone significantly increase TRPM7 current by 48% while and TRPM7 plasma membrane protein expression by 34% aldosterone induces TRPM7 macroscopic current via the mineralocorticoid (MR) and serum and glucocorticoid-regulated kinase 1 (Vanllnski *et al*., 2016). This study investigated the role of aldosterone-salt induced cardiac and renal fibrosis. We also assessed the role of TRMP7 on aortic vasculature. Possibly, TRMP7 may serve as therapeutic target for salt sensitive individuals.

5.2 Methods:

The experiments were conducted by Dr. Francisco Rios in the laboratory of Prof. Touyz, University of Glasgow. I contributed to the studies by examining vascular fibrosis and collagen status in the different experimental groups.

5.2.1 Animals

Animal experiments were approved by the University of Glasgow Animal Welfare and Ethics Review Board in accordance with the United Kingdom Animals Scientific Procedures Act 1986 (Licence No. 70/9021) and with ARRIVE Guidelines. Wild type (WT) mice (C57BL/6J and SV129 mixed background) and mice heterozygous for the deletion of the TRPM7-kinase ($TRPM7^{+/\Delta kinase}$), generated by the gene-targeting vector technique. Reverse transcription PCR analysis was used to identify wild-type (WT) (TRPM7+/+) and heterozygous ($TRPM7^{+/\Delta kinase}$ (TRPM7+/Δ)) animals.

We used male, 18–22-week-old mice. Wild type (WT) mice (C57BL/6J and SV129 mixed background) and mice heterozygous for the deletion of the TRPM7-kinase ($TRPM7^{+/\Delta kinase}$), generated by the gene-targeting vector technique. Mice were maintained in controlled room temperature (25°C) with a 12-h light/dark cycle and food and water *ad libitum.* Genotyping was performed using the REDExtract-N-AmpTM Tissue PCR Kit (Sigma-Aldrich, Dorset, UK) according to manufacturer's instructions. The PCR protocol was as following: 1 cycle of 94 ^{o}C (heat start), and 35 cycles of: 10 sec at 94 ^{o}C (denaturation), 30 sec at 62 ^{o}C (annealing), 2 min at 68 ^{o}C (extension). One final cycle of 5 min at 68 ^{o}C was performed, followed by temperature of 4 ^{o}C. PCR products were visualized using agarose gel electrophoresis (Yogi *et al*., 2011; Zou *et al*., 2019; Rios *et al*., 2020).

5.2.2 Animal treatment

WT and TRPM7$^{+/\Delta kinase}$ male mice, 12-16 weeks of age were studied. WT and TRPM7$^{+/\Delta kinase}$ mice were divided into 4 groups and treated for 4 weeks as follow: Group 1 - vehicle controls (veh, WT n=14; TRPM7$^{+/\Delta kinase}$ n= 17); Group 2 - aldosterone-infused (aldosterone group, WT n=10; TRPM7$^{+/\Delta kinase}$ n= 9) (600 µg/kg/day) (Sigma-Aldrich, Dorset, UK) by Alzet osmotic mini-pumps (model 2004, Alzet, CA, USA); Group 3 - 1% NaCl drinking water (salt group, WT n=7; TRPM7$^{+/\Delta kinase}$ n= 9); Group 4 - aldosterone-infused + 1% NaCl drinking water group (aldosterone-salt, WT n=13; TRPM7$^{+/\Delta kinase}$ n= 15). Mice were euthanized after 4 weeks of treatment by overdose of anesthesia, followed by cardiac puncture. aorta, heart and kidneys were dissected for further studies.

5.2.3 Histology

Hearts, aortas, and kidneys were fixed in 10% buffered-formalin solution and processed for histological inclusion in paraffin. Five-µm thick tissue sections were stained with PicroSirius red for light microscopy. Fibrosis was further analysed in PicroSirius red stained samples using polarized light microscopy. Fifteen randomly selected non-overlapping fields (x200) were analysed using the package, Image J 1.44p (Wayne Rasband, NIH, USA).

5.2.3.1 Histopathological staining

Mice were sacrificed postoperatively at 14th day after perfusion with 4% paraformaldehyde under physiological pressure. The tissues (heart, kidney and aorta) were carefully removed and embedded in optimum cutting temperature tissue freezing medium (Sakura, Torrance, CA, USA) or in paraffin after formalin fixation for 24h. For morphometric analysis, paraffin-embedded tissue was sectioned at 5mm thickness and stained with picrosirius stain for 60 mins (Kiernan, 1999). Tissues were mount in a resinous medium (DPX), then followed by examination under light

microscopy (Carl Zeiss, Jena, Germany). In bright-field microscopy, collagen is red on a pale-yellow background. When examined through circularly polarised light the larger collagen fibres (Type 1) are bright yellow or orange, and the thinner ones, including reticular fibres, are green (Type 3) Junqueira *et al*., 1979, the birefringence is highly specific for collagen (Puchtler, 1973, Whittaker, 1995). In normal kidney the glomerular basement membranes were red. However, tissue fibrosis (collagen deposition) and thickness of the aorta were measured using Image J software (32-bit).

5.2.4 Statistical analysis

Data was analysed using Graph pad 5.0 software version 5.0 (USA). The results obtained were presented as mean ± SEM (Standard error of the mean). Comparisons were made between treated and control groups using Student's t-test. One-way ANOVA was used to analyse significant difference of means with Student Newman Keuls post hoc test for multiple comparisons. P value < 0.05 was considered statistically significant.

Results

Percentage of collagen deposition in heart, kidney and aorta

There was significant increase in collagen deposition on the heart of WT, aldo-salt, TRMP7, and TRMP/aldo-salt when compared with WT. In figure 5.1b, there was significant increase in renal collagen deposition on the heart of the groups; aldo-salt, and TRMP/aldo-salt when compared with WT. There was also a significant increase in collagen deposition of heart, kidney and the aorta when TRPM-7-aldo-salt when compared with TRPM7 (Fig 5.1 a-c).

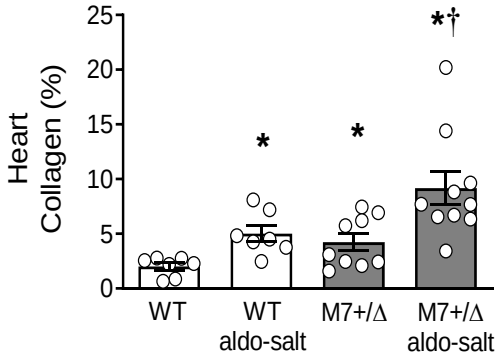


Fig 5.1a

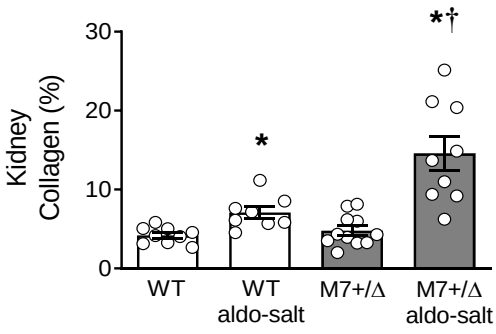


Fig 5.1b

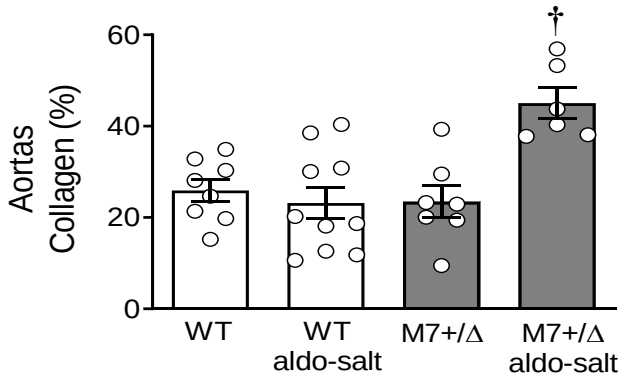


Fig 5.1 (a-c): Percentage of collagen deposition in heart, kidney and aorta

Figure 5.1 (a-c), showing the percentage of collagen deposition in heart, kidney and aorta where WT = Wild type, M7+/Δ- TRPM, aldo-salt = aldosterone-salt. †means significant when compared to M7+/Δ, p value is < 0.05.

Effect of aldosterone- salt on aorta thickness

There was significance increase in the thickness of the aorta in TRPM7-aldo-salt when compared with aldo- salt and TRPM7 (Fig 5.2). Similarly, the finding shows increase in the thickness of the aorta.

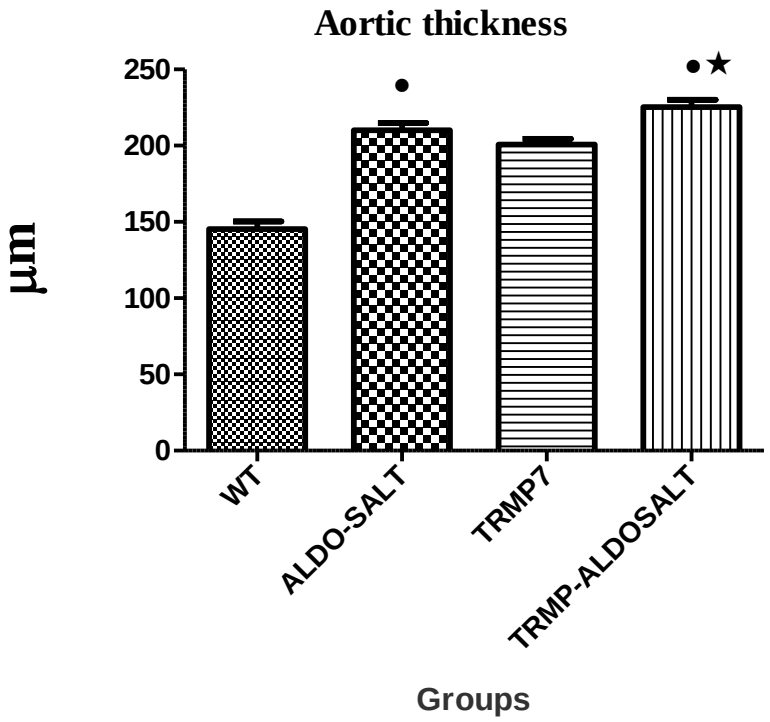


Figure 5.2: **Effect of aldosterone- salt on aorta thickness**

The thickness of the aorta where WT = Wild type, Aldo-salt = aldosterone-salt, where WT = Wild type, Aldo-salt = aldosterone-salt. ⁎means significant when compared to TRMP7, ● means significant when compared to WT, p value is < 0.05.

Micrograph of the heart x200

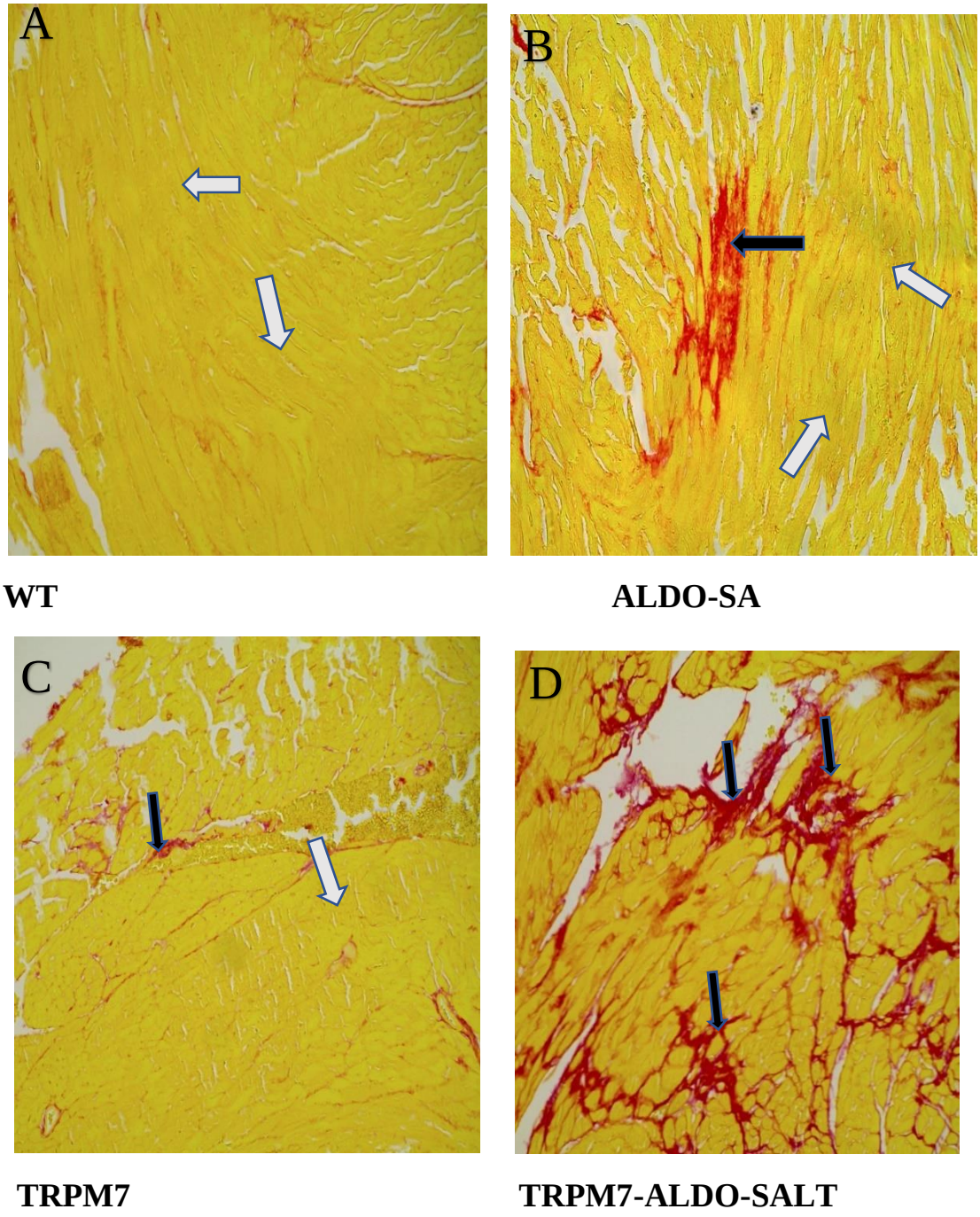


Figure 5.3 (A-D): Micrograph of the heart showing collagen deposition

Cardiac sections were stained with picrosirius red and collagen content was assessed using a standard grading scheme. Cardiac fibrillar collagen deposition was increased in aldosterone-treated animals (B and D) Collagen content was increased in the aldosterone-treated group and attenuated by TRMP7. Black arrows showing collagen deposition. A. WT- control; B, Aldo; C TRPM 7 D. TRPM7 Aldo-salt Magnification: x200**.**

Micrograph of the kidney x200

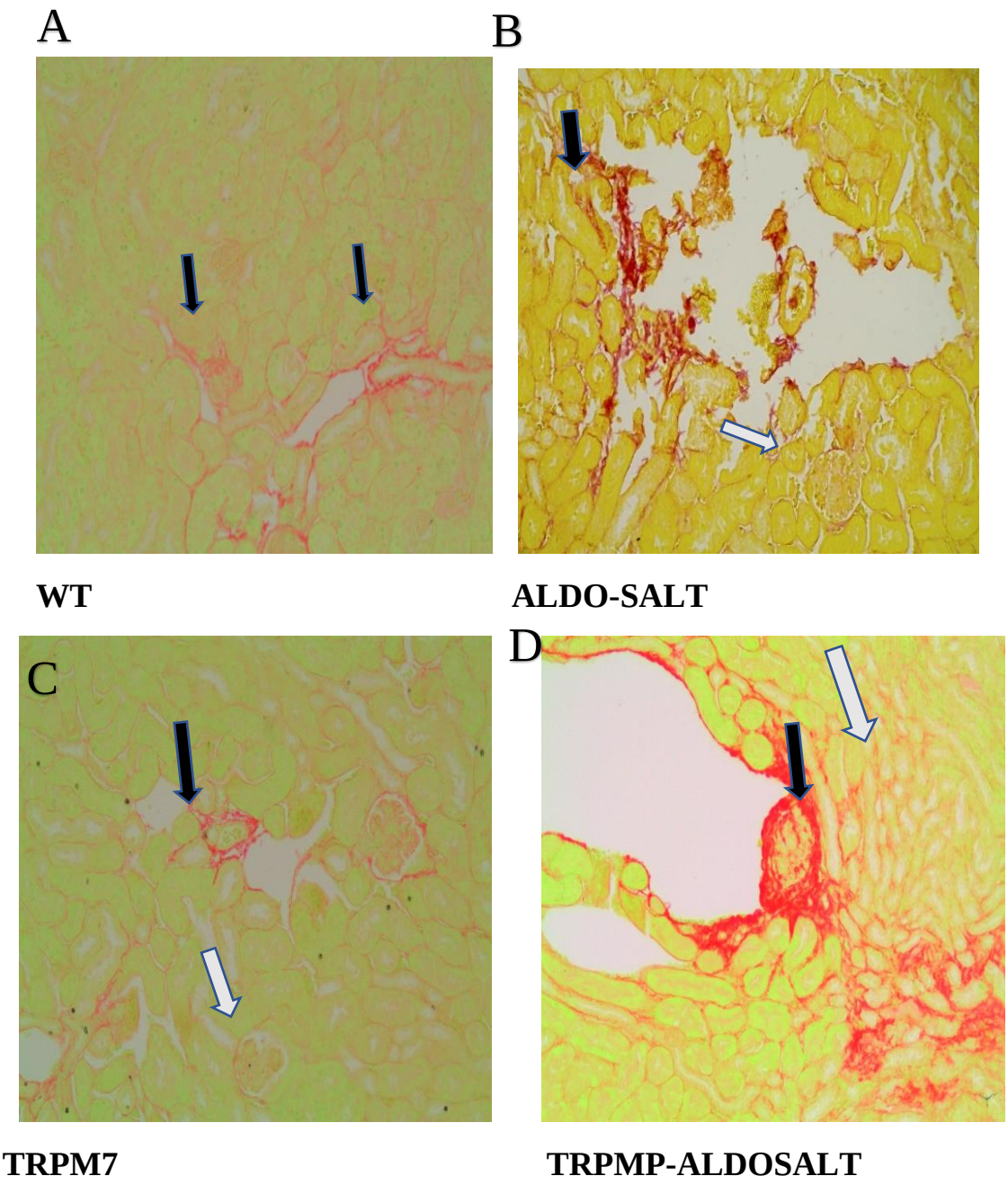


Figure 5.4 (A-D): Micrograph of the kidney showing collagen deposition

Renal sections were stained with picrosirius red, and collagen content was assessed using a standard grading scheme. Collagen content was increased in the aldosterone-treated groups (B and D) were aggravated by TRMP7. Black arrows show collagen deposition. Images are representative of 15 mice in each group. Magnification: x200

Micrograph of the aorta (x200)

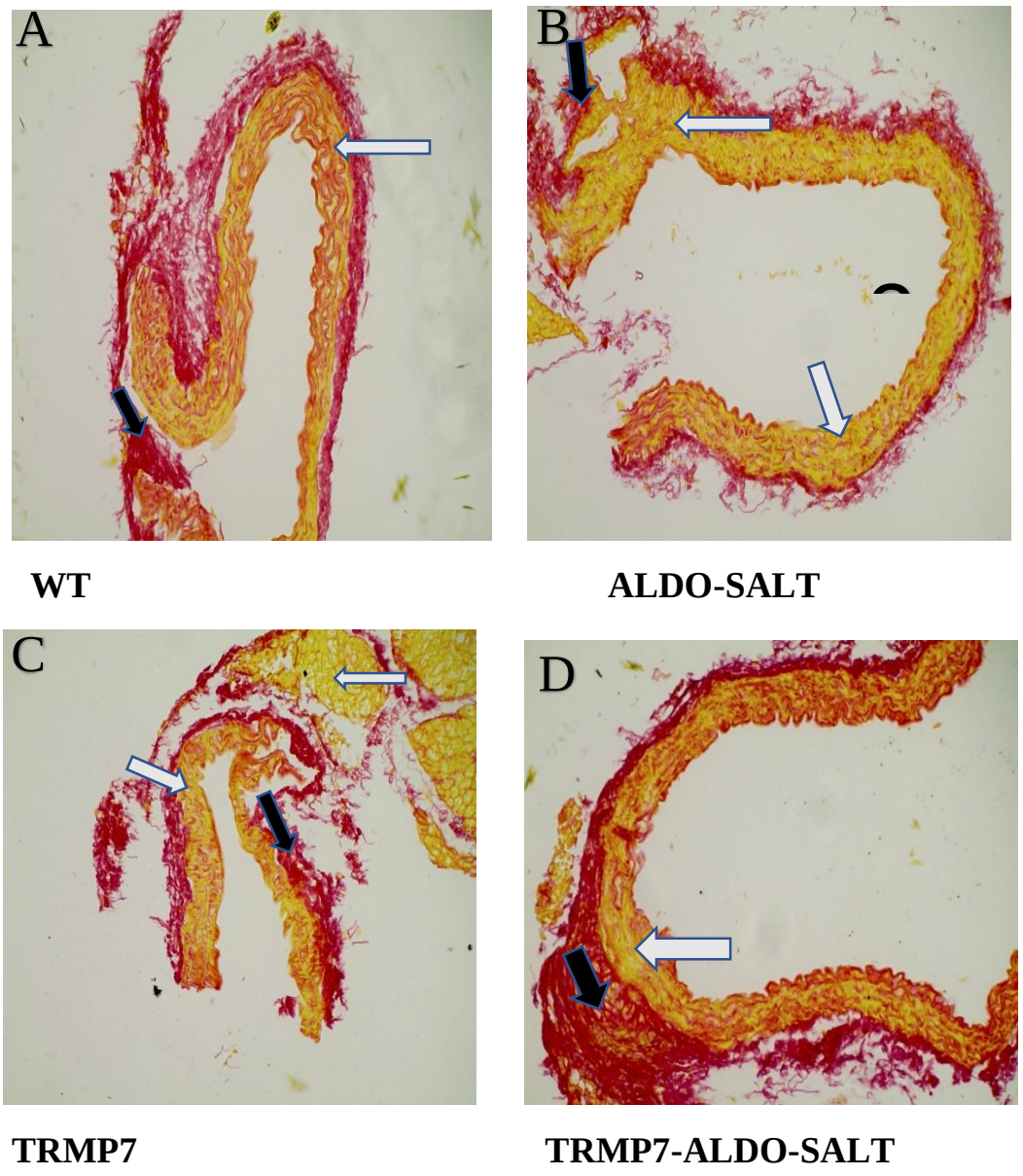


Figure 5.5 (A-D): Micrograph of the aorta showing collagen deposition

Aortic collagen content and in four groups. Vessel sections were stained with picrosirius red. Collagen content was increased in the aldosterone-treated groups (B and D) were aggravated by TRMP7. Black arrows show collagen deposition. Images are representative of 15 mice in each group. Magnification: x200.

5.3 Discussion

Histologically, this study revealed significant increase in collagen accumulation in the heart of the groups infused with only aldo-salt, TRPM7 and combination of aldo-salt and TRPM7 when compared with wild type only (control). The study also showed significant increase in collagen deposition in group treated with combination of TRPM7 and aldo-salt when compared with groups with TRPM7 treatment only (control). Similarly, there was significant increase in collagen deposition in the kidney of the group treated with combination of TRPM7 and aldo-salt when compared with group treated with either TRPM7 or aldo-salt alone. On the aorta, there was also significant increase in collagen deposition in group treated with combination of TRPM7 and aldo-salt when compared with groups with TRPM7 treatment only. However, there was no significant aortic changes in wild type treated with aldosterone-salt when compared with wild type without aldosterone-salt infusion.

Concerning fibrosis, evidence from animal experiments as well as Randomized Aldactone Evaluation (RALES) and Eplerenone Post-acute Myocardial Infarction Heart Failure Efficacy and Survival (EPHESUS) studies in patients with heart failure (HF) (with or without previous myocardial infarction) suggest that chronic mineralocorticoid receptor (MR) blockade markedly reduces blood collagen peptides. These studies suggesting that Aldo is an important determinant of cardiovascular collagen turnover, simply indicating aldosterone-salt as a biological marker of cardiovascular fibrosis. In rats, Aldo infusion concomitantly with a high-sodium intake increases arterial stiffness with development of extracellular matrix (ECM) protein synthesis and inflammation. These findings are reinforced at the cellular level in vascular smooth muscle cells (VSMCs) where aldosterone increases collagen synthesis via MR; thus, leading to increase in the thickness of the aorta (Figure 5.4). These studies suggest that aldosterone and salt are important

determinants of cardiovascular collagen turnover, simply indicating aldosterone and salt as a joint biological marker of cardiovascular fibrosis (Rios *et al*., 2020). Taken together, the most published data indicate the potential contribution of aldosterone in vascular remodeling as a collagen regulator (Touyz *et al*., 2022). This present study corroborates the fact that aldosterone-salt plays vital role in fibrosis. Although, the precise mechanisms responsible for Aldo-salt induced collagen synthesis in VSMCs is yet to be fully elucidated, interplay between calcium/magnesium and TRMP7 cannot be unrecognized. Therefore, this study suggests that TRMP7 has potential anti-fibrotic effects and that when it is dysregulated or downregulated cardiovascular and renal injury are exaggerated. Mechanisms causing TRPM7 dysregulation in the context of hyperaldosteronism are unclear and await further clarification.

5.4. Conclusion

Aldosterone-salt causes cardio-renal and aortic fibrosis, this is aggravated by chaenzyme; TRMP7. Our study suggest TRMP7 inhibitors as anti-fibrotic agent. Possibly, this may help in the therapeutic management of atherosclerosis and cardio-renal syndrome.

CHAPTER SIX

6.0. Conclusion and Recommendation

Obesity, hypertension and heart failure are rampant cardiovascular problem among Africans. However, prevalence of metabolic syndrome among people of African ancestry is better diagnosed using modified NCEP-ATP III. In addition, it is expedient to consider traditional factors such as age, gender, smoking and alcohol in Africans. More importantly, ambulatory blood pressure and PWV assessment are modern assessments of cardiovascular risks that are yet to be utilized clinically. These should be introduced to enhance early detection of hypertension and cardiovascular complications. Altogether, this study established the fact that intercalated mechanism involving inflammatory process and oxidative stress might be responsible for cardio-renal and aortic fibrosis leading to atherosclerosis and heart failure, aside salt -water retention associated with renin angiotensin aldosterone system. Our study suggests that further studies are needed to evaluate the mechanism between metabolic syndrome and TRMP7. The outcome might serve as a therapeutic regimen for cardiovascular complications in Africans.

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APPENDICES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Omotayo Alaba Eluwole

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190472

NAME: (Principal Investigator)	Miss Omotayo Alaba Eluwole
DEPARTMENT:	School of Physiology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Determinant of heart failure among African patient with metabolic syndrome
DATE CONSIDERED:	26/04/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Muzi Maseko
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	08/07/2019

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature

Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Home Office

No. PPL 7009021

ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986

PROJECT LICENCE.

to carry out a programme of scientific procedures
on living animals

In pursuance of the powers vested in him/her by the above Act, the Secretary of State hereby licences

Dr Delyth Graham
BHF Glasgow Cardiovascular Research Ctre
University of Glasgow
126 University Place
Glasgow
G12 8TA

to carry out the programme of work specified in the Schedule subject to the restrictions and provisions contained in the Act and subject also to the limitations and conditions contained in this licence and to such other conditions as the Secretary of State may from time to time prescribe.

Under this project licence number PPL 7009021 the Secretary of State grants authority only for the work specified on the Schedule. The procedures and animal types which may be used and the place or places at which the work may be carried out are specified in the Schedule. Authority is granted only for the severity limits attached to individual procedures specified in Part E of the schedule.

The Secretary of State has determined that a retrospective assessment of this licence is not required.

This licence, unless earlier revoked, shall be in force until 27 June 2021.

Home Office
2 Marsham Street
London SW1P 4DF

For the Secretary
of State

27 June 2016

NB. This licence does not authorise the project licence holder or any other person to carry out procedures on any animals unless s/he holds a personal licence issued under the Act which authorises him/her to carry out those procedures on the animals of those types.

Handling Instructions: Contains personal sensitive information, subject to confidentiality requirements under the Data Protection Act. This should only be circulated in accordance with ASPA Guidance and stored in a locked secure location. All government information may be subject to an FOI request and subsequent assessment.