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THE IMPACT OF SALT ON BLOOD PRESSURE AND CARDIOVASCULAR TARGET ORGAN DAMAGE

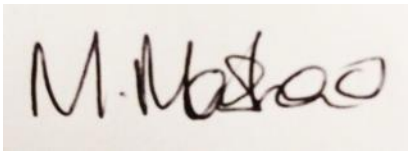
Mapula Mercy Mashao

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

Johannesburg, South Africa
2020

DECLARATION

I, Mapula Mercy Mashao, declare that "The impact of salt on blood pressure and cardiovascular target organ damage" hereby submitted to the University of the Witwatersrand, for the degree of doctor of philosophy, has not previously been submitted by me for a degree at this or any other University; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

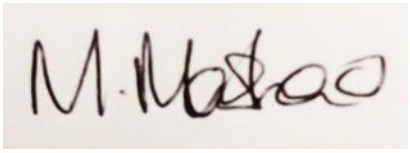


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Mapula Mercy Mashao

Signed on ___6th___ day of __October_____, 2020 in PARKTOWN

I certify that the studies included in this thesis have the approval of the Human Research Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics clearance number is M17-02-15.



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Mapula Mercy Mashao

Signed on __6th____ day of _____October_____, 2020 in PARKTOWN

DEDICATION

I would like to thank God of Israel for taking me through this long-awaited journey and giving me the strength and courage to make this thesis a living document. This thesis is dedicated to the Mashao and Majeke families. My grandmother Claribell Nompakamo Majeke for encouraging and supporting me throughout my journey, my parents (Lolly and Petrus Mashao) for their invaluable support. Next, my aunt Nelisiwe Majeke for consistently encouraging and motivating me. Nomonde and cousin Nokuthula, and uncle Thamsanqa. I also dedicate the thesis to my grandmothers Nokuzola Theresa Mkosana, and Diagracia Nolwandle Majeke, grandfather Victor Majeke, Mane Qaqambile and Luthando, Rakgadi Nteleki, Rangwane Mokapi, my siblings Lebo and Dineo. Finally, a special dedication to my daughter Kgaogelo Mahlogonolo Reneilwe.

ABSTRACT

Cardiovascular events such as hypertension, renal failure and arterial stiffness are attributed to dietary sodium intake as measured using urinary sodium excretion. In this thesis, I investigated the relationship between urinary sodium excretion and blood pressure and the relationship between urinary sodium excetion and arterial stiffness in a unique salt sensitive cohort of black South Africans.

Previous studies conducted to investigate the relationship between sodium and blood pressure in our cohort of African participants have yielded contradictory results. With the high prevalence of obesity in this population, it is possible that these contradictory findings are due to the masking effects of obesity on this relationship. Ambulatory blood pressure was determined using a Spacelabs 90207 (Spacelabs Inc., Redmond, Washington, USA) monitor. In a sample of 547 individuals, I demonstrated that there is no independent relationship between 24-hour Na+ excretion and blood pressure in the total population sample, however when participants were stratified according to body mass index (BMI) status, there was a significant association between 24-hour Na+ excretion and ambulatory blood pressure in the normal-BMI participants but not in the overweight/obese participants. I concluded that 24-hour urinary Na^+ excretion, was associated with increased ambulatory blood pressure but this relationship was masked because of a high proportion of overweight/obese individuals in this population.

Because I demonstrated a masking effect of obesity on the relationship between urinary sodium excetion and blood pressure in the previous chapter, I decided to investigate a possible masking effect of obesity on the relationship between urinary sodium excretion and arterial stiffness as measured using carotid-femoral pulse wave velocity. Increased arterial stiffness as measured by carotid femoral pulse wave velocity is an independent predictor of cardiovascular morbidity and mortality. In the present study, I measured arterial stiffness using carotid femoral pulse wave velocity. This was done using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a SphygmoCor software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia); version 9.0. In the present study, I demonstrate in 547 participants of African ancestry that obese individuals have higher pulse wave velocity when compared with normal weight individuals (7.0±3.0 vs 5.9±2.3). Urinary sodium excretion was significantly lower in overweight/Obese female participants compared to normal weight

individuals (99.5±68.6 vs 121.5±99.4). Leptin levels were also higher in the overweight/obese group when compared with the normal weight group irrespective of gender. More importantly, I show that 24-hour urinary sodium excretion is an independent predictor of arterial stiffness as measured by carotid femoral pulse wave velocity in normal weight females ($p = 0.0002$) but not in overweight/obese females. This relationship was however absent in males irrespective of BMI status. Therefore, the study suggests that obesity masks the relationship between urinary sodium excretion and pulse wave velocity in females in a cohort of black South Africans.

Due to a high prevalence of obesity in the Black South African population and because I have reported the masking effects of obesity on the relationship between urinary sodium excretion and blood pressure and the relationship between urinary sodium excretion and arterial stifness in the previous chapters, I decided to investigate the relationship between urinary sodium excretion and blood pressure in a cohort of normal weight black South Africans. I measured conventional blood pressure and collected 24-hour urinary Na^+ excretion in all participants. Anthropometric measurements were taken, and standard questionnaire was used to collect data on family medical history, use of medication and dietary information. Of the 750 participants with complete measurements, 236 participants had normal BMI and were included in the statistical analyses. The mean 24-hour urinary Na+ excretion was 114 mmol/day. Multivariate regression analyses showed a significant inverse relationship between systolic BP and 24-hour urinary Na+ excretion ($r = -0.16$; $p=0.0082$) and between diastolic BP and 24-hour urinary Na+ excretion ($r = -0.14$; $p=0.02$). The findings from this study reveal a negative relationship between 24-hour urinary sodium excretion and blood pressure, which is in complete contrast to previously established relationships. This might point to a unique renal sodium handling in this cohort. The results have far reaching implications, as they suggest that urinary sodium excretion cannot be used to estimate dietary sodium intake in this population as there might be some sodium retention going on despite increases in blood pressure. I believe that future research is needed to determine the mechanisms that govern the relationship between urinary sodium excretion and blood pressure in this population and that other methods should be used to estimate dietary sodium intake in this population.

In conclusion, the data presented in this thesis have identified a possible role of obesity in interfering with the relationship between urinary sodium excretion and blood pressure. I have also identified a possible role of obesity in interfering with the relationship between urinary sodium excretion and a cardiovascular target organ damage like arterial stiffness. The data in

this thesis also identified an inverse relationship between urinary sodium excretion and blood pressure in normal weight individuals of African ancestry. All these have added new and unique knowledge concerning obesity and cardiovascular disease.

PRESENTATIONS

The following presentations have arisen from this work.

Oral presentation:

Presented a Seminar at the School of Physiology, 26 March 2019, University of the Witwatersrand, Parktown, Johannesburg. **Seminar title**: "*The salty truth*".

Poster presentation:

Presented a research poster at the Faculty research day. 6 September 2018. University of the Witwatersrand, Parktown, Johannesburg. Poster title: The impact of obesity on the relationship between dietary salt intake and arterial stiffness in a South African community.

Presented a research poster at the Physiological Society of Southern Africa (PSSA) conference. 7-10 October 2018. Stellenbosch, South Africa. Poster title: The Association of Na+ Excretion with Arterial Stiffness is mediated by Leptin in African Females with a High Prevalence of Obesity.

PUBLICATION

The following publication has arisen from this work:

Maseko M, Mashao M, Bawa-Allah A, Phukubje E, Mlambo B, Nyundu T. Obesity masks the relationship between dietary salt intake and blood pressure in people of African ancestry: the impact of obesity on the relationship between Na+ and blood pressure. Cardiovasc J Africa, 2018, 29: doi:10.5830/CVJA-2018-011

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Prof M.J Maseko. Supervised the design and planning of the study (chapter 2) and intellectual input on the published research paper (chapter 3).
Prof. R Brooksbank. Co-supervised the design and planning of the protocol and intellectual input on the final writing of the thesis.

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Ms D. Nciweni, Data collection (drawing blood from participants).

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LIST OF ABBREVIATIONS

ABP	ambulatory blood pressure
ABPM	ambulatory blood pressure monitoring
ACE	angiotensin converting enzyme
AGEs	advanced glycation end products
ANOVA	analysis of variance
ARR	aldosterone to renin ratio
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CF-PWV	carotid femoral pulse wave velocity
CLS	clinical laboratory services
cm	centimetre
DBP	diastolic blood pressure
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
g	gram
g/day	gram per day
HDLc	high density lipoprotein cholesterol
HIV	human immunodeficiency virus
HR	heart rate
HREC	human research ethics committee
hs-CRP	high-sensitivity c-reactive protein
IL-6	interleukin-6
ISH	isolated systolic hypertension
kg	kilogram
kg/m^2	kilogram per $metre^2$
LDLc	low density lipoprotein c
LVH	left ventricular hypertrophy
m	metre
m/s	metre per second
MAP	mean arterial pressure

ml	millilitre
ml/day	millilitre per day
mmHg	millimetre of mercury
mmol	millimole
mmol/day	millimole per day
Na^+/K^+	urinary sodium to potassium excretion ratio
NaCl	sodium chloride
NDP	nocturnal non-dipping blood pressure pattern
NHLS	national health laboratory service
NOS	nitric oxide synthase
PWV	pulse wave velocity
RAAS	renin angiotensin aldosterone system
SBP	systolic blood pressure
SD	standard deviation
SNS	sympathetic nervous system
TGF-β	transforming growth factor beta
TNF	tumor necrosis factor
UK^+	twenty-four-hour urinary potassium excretion
UNa^+	twenty-four-hour urinary sodium excretion
VLDL	very low-density lipoprotein
vs	versus
WHR	waist to hip ratio

PREFACE

Cardiovascular diseases are presently a leading cause of death in South Africa and sub-Saharan Africa (SSA) (STATSSA 2013; Mensah *et al*., 2015; STATSSA 2018). The emerging prevalence of cardiovascular events occurring in young African populations is associated with the high prevalence of hypertension (Ezela-Adikaibel *et al*., 2012). Despite advances in routine measurement of BP at clinical settings, the prevalence of hypertension and stroke in black populations is on the rise (Maritz *et al*., 2016). However, evidence exists that lifestyle modification strategies such as weight management and reduced salt intake may reduce hypertension and its complications in urban communities of African ancestry (Norton and Woodiwiss, 2011).

In chapter 3 of this thesis, I assessed whether obesity interferes with the relationship between urinary salt excretion and blood pressure. I established that the high prevalence of obese females, is contributing to the masking effects of obesity on the relationship between sodium and blood pressure. In chapter 4, I assessed whether obesity affects the relationship between urinary sodium excretion and pulse wave velocity. Finally, I determined the relationship between 24-hour urinary Na^+ excretion and conventional systolic and diastolic BP in normal weight black South Africans.

In the present thesis, chapter 1 deals with the background and literature review. Chapter 2 outlines the methods for all three studies presented in chapters 3-6. This chapter includes sampling method(s), the study design, scope, and data collection. Chapters 3-6 are results and their discussions. Chapter 3 deals specifically with the impact of obesity on the relationship between urinary salt excretion and blood pressure which addresses aim (1). Chapter 4 addresses aim (2) which focuses on the relationship between 24-hour urinary excretion and pulse wave velocity. Chapter 5 focuses on the relationship between 24-hour urinary Na^+ excretion, conventional systolic and diastolic BP. Chapter 6 summarises the major findings and highlights the novelty of the study, and outlines suggested recommendations.

CHAPTER 1

INTRODUCTION

Current knowledge about urinary sodium excretion, blood pressure and obesity.

1.1 Introduction

Cardiovascular diseases (CVDs) are presently a leading cause of morbidity and mortality in South Africa and sub-Saharan Africa (SSA). Indeed, hypertension (HT) remains the most profound risk factor for CVD (STATSSA 2018; Mensah *et al*., 2015; Yan *et al*., 2015; Millen *et al*., 2013). In addition, population estimates indicate that in developed countries the prevalence of CVD is greater in populations of African descent compared to their European descent counterparts (Chaturvedi, 2003; Scott *et al*., 2011). Indeed, these disparities are reflected in the burden of strokes, left ventricular hypertrophy (LVH) and urine albumin to creatinine ratios (Scott et al., 2011; Steyn, 2006). In developing countries such as South Africa, mortality rates for the elderly indicate that HT is ranked number two in causing elderly death rates and other forms of heart related diseases are ranked number four in African elderly population (MACoD, 2016). However, ischemic heart diseases and other forms of heart diseases are ranked one and two respectively in Caucasians but, HT related deaths are ranked nine (MACoD, 2016). This suggests that HT is indeed a significant risk factor for CVD in Africans and that scrutiny of the lifestyle modification strategies may be a better tool for reaching treatment targets and effective management of HT in this population.

Hypertension is defined as a consistent elevation of arterial BP with a SBP of > 140 mmHg and/or DBP of > 90 mmHg (ESH, 2018; Seedat *et al*., 2014). Recently, the new ESH guidelines in management of BP concomitant with co-morbidity specific treatment cutoffs, recommend higher BP goals compared to the previous guidelines 2003, 2007, and 2013. In patients aged 18 to 65 years without major co-morbidities, and 65 years or older who have diabetes, chronic kidney disease (CKD), or both conditions, the treatment target BP level is ≥140/90 mmHg. In addition, in patients 65-79 years who do not have diabetes mellitus (DM) or CKD, the treatment target BP level is ≥140 mmHg and in patients 80 years or older (<150 mmHg) (ESH, 2018). Pre-hypertension is also a significant contributor of HT prevalence. Therefore, individuals with pre-hypertension have a BP falling between 120-139 mmHg SBP and 80-80 mmHg DBP (Singh *et al*., 2017). Treatment goals for this group recommend initiation of lifestyle intervention and drug therapy in individuals at risk of CVD (ESH, 2018).

Drug therapy for patients with HT is either mono- or combination therapy with a low-dose diuretic, calcium channel blocker (CCB) and an ACE inhibitor (ACEI) or angiotensin receptor blocker (ARB)s (Seedat *et al*., 2014). Approximately less than 50% of treated patients reach target BP levels in developed countries however, in developing countries such as South Africa, controlled target BP levels are still poor (Maseko *et al*., 2011; Scott *et al*., 2011). This may be attributed to the higher prevalence of HT in the African population. Moreover, there is evidence that indicates a pathophysiologic mechanism of low renin hypertension to aldosterone in this population and a resultant of poor response to ACEI and β-blockers as monotherapy (Scott *et al*., 2011, Maseko *et al*., 2011).

Presently, an incidence of infectious disease has transitioned to an era of non-communicable diseases (NCD)s. This is reflected in a South African context, where a typical prudent diet consisting of mostly traditional staples has been replaced by a western diet that consist of processed food that is high in salt (Charlton *et al*., 2013). Consequently, the prevalence of NCDs has resulted in a quadruple burden of diseases (Steyn, 2006). This burden of disease is also due to multi-morbidity in individuals wherein, HT is a significant contributor (Weimann *et al*., 2016, Jinabhai *et al*., 2003; Charlton *et al*., 2013).

Despite advances in monitoring and treatment of HT, its diagnosis and effective management is destitute, especially in developing countries (Steyn, 2011). South Africa as a developing country is also profoundly affected by the escalating prevalence of HT and its complications. This is evident in the increased incidences of stroke, coronary artery disease (CAD), heart failure (HF) and CKD (Swanepoel *et al*., 2016; Rayner *et al*., 2007; Charlton *et al*., 2005). In addition, the emerging prevalence of cardiovascular events occurring in young African populations is also associated with the high prevalence of HT (Ezela-Adikaibel *et al*., 2012). As a consequence, global estimates indicate that one billion adults are hypertensive and that the prevalence of HT is projected to be 1.5 billion by 2025 (Oparil, 2014).

1.2 Lifestyle risk factors for hypertension

Traditional risk factors of hypertension include aging, family history, ethnicity, gender, coexistence of other metabolic diseases, stress, obesity, high dietary salt intake and saturated fats,

tobacco use, sedentary lifestyle and hormonal contraceptives (Forman *et al*., 2009; Steyn, 2006). Ethnic and inter-ethnic disparities are among the most important contributors to the development of hypertension. These inter-ethnic disparities remain consistent even after adjusting for confounding factors (Kaufman *et al* 2007; Lê Cook *et al*., 2012; Hicken *et al*., 2014). For decades, studies have continued to investigate gene alleles that predominate in African descent population that might predispose to hypertension (Poch, 2001; Weinberger, 1996). Variants in the GRK4 and SLC4A5 genes have the strongest and most consistent genetic links to salt related hypertension (Dong, 2018). Currently, there are limited methods to assess these phenotypes in the clinical setting, moreover the practicality of these methods would require more time to effectively monitor diet using a crossover study design (Dong, 2018). Therefore, most studies have confirmed these disparities using less time constraining methods (Schutte *et al*., 2013; Bochud *et al*., 2009; Bankir *et al*., 2008).

1.3 Lifestyle modification strategies in the prevention and management of hypertension

The ESH, 2018 lifestyle recommendations on lowering BP include weight loss, reduction in Na^+ intake to less than 2.0 grams per day (<5.0g of salt), and at least 30 minutes of aerobic activity most days of the week. Consequently, obesity and dietary salt intake are two common modifiable risk factors targeted for prevention and management of hypertension (Woodiwiss and Norton, 2011); Moreover, the combined co-existence of these lifestyle risk factors impedes independent causal associations with hypertension and new drug targets for treatment. Because of this, the prevalence of new onset hypertension is on the rise (Forman *et al*., 2009). As will be discussed in subsequent sections, the two risk factors in question have been continuously contested for over 100 years.

1.3.1 Obesity as a risk factor for hypertension

Obesity is defined by the WHO as the excessive accumulation of fat in the body, resulting in adverse health effects such as the development of metabolic diseases (Osuji *et al*., 2010). Diseases associated with obesity are a major public health concern in both developed and developing

countries (Crowther and Norris, 2012). The prevalence of obesity is evident in countries undergoing nutritional and economic transition (Smit, 2012). Manifestations of obesity include abdominal obesity, which is a form of obesity associated with the metabolic syndrome and it presents as increased waist circumference (Ntyintyane *et al*., 2006). BMI and waist circumference (WC) have been identified as indices of adiposity that have a direct association with BP (Wai *et al*., 2012). Although WC has been shown to have a stronger association with BP compared to BMI, none the less BMI is widely used in epidemiological studies to define obesity at population level (Mahadevan and Ali, 2016). Furthermore, obesity can directly manifest on major regulatory internal organs including the heart, liver, lungs, blood vessels, brain and kidneys. The latter is imperative for further scrutiny, in that direct effects imposed on the kidneys are implicated in Na^+ handling (Jiang *et al*., 2016).

In addition, mechanisms in obesity related hypertension may be attributed to interactions of adipokine levels in the vascular wall especially those locally produced such as leptin (Martinez-Martinez *et al*., 2014). Obese individuals have been found to exhibit high levels of leptin; however, these high levels are unable to reduce adiposity indicating leptin resistance. In contrast, leptin deficiency in obese rats and individuals has been associated with insulin resistance, DM and other components of metabolic syndrome (Park and Ahima, 2015). Leptin deficient ob/ob mice develop obesity due to a lack of leptin receptors; however, BP is normal despite obesity (Bell and Rahmouni, 2017). Thuessen et al., 2015 postulated that the relationship between dietary salt intake and its link to hypertension may be mediated by obesity and thus in this instance, BMI should not be adjusted for as a co-variate. This is evident in that studies that failed to observe a relationship between salt intake and BP did not consider analyzing data with individuals classified according to BMI indices (Redelinghuys *et al*., 2010, Mitchel *et al*., 2014).

1.3.2 Blood pressure monitoring

Effectively monitoring blood pressure (BP) involves measuring both conventional and ambulatory BP. A key diagnostic feature of monitoring BP involves conventional measurements. Nevertheless, this method has limitations in estimating BP over time (Staessen *et al*., 2000). This

method of assessing BP is an example of misrepresentation of a selected subgroup of individuals with hypertension presenting with white coat hypertension. These patients exhibit raised in office BP, however, out of office BP is normal (Stergiou and Bliziotis, 2011). Likewise, another important distinction is found in patients with masked hypertension. This group of individuals often have normal office BP; nevertheless, their ambulatory BP is elevated (Katholi and Couri, 2011). Despite the fact that the prevalence of white coat and masked hypertension is considered insignificant in the general population (Márquez-Contreras *et al*., 2006), assessment of BP using both conventional and 24-hour ambulatory BP makes an imperative contribution to BP monitoring (Katholi and Couri, 2011). Importantly, with 24-hour ambulatory assessment, it permits other hemodynamic measurements beyond BP. Therefore, is deemed as a tool that predicts cardiovascular organ damage above conventional BP (Chen *et al*., 2018).

Importantly, various patterns of diurnal BP can be deduced from ambulatory BP measurements. Nighttime BP values should fall by at least 10% of the daytime BP, this phenomenon is known as "dipping" (Figure 1.1) (Okamoto *et al*., 2009). Conversely if BP doesn't fall, "non-dipping" ensues (Sachdeva and Weder, 2006). Non-dippers have been found to experience morphological and functional changes to a greater degree of severity. It is also common in chronic renal disease with the prevalence ranging between 74-82% (Sinha and Argawal, 2015). Hence, in these individuals, much of the Na^+ is excreted during the night and in order to maintain balance, the compensatory mechanism is Na^+ retention (Sachdeva and Weder, 2006). In a study conducted among individuals of African descent, poor day time Na^+ excretion was associated with increased nighttime BP and a blunted nocturnal BP dipping (Bankir *et al*., 2008). Moreover, Antic *et al*., 2001 observed a loss of nocturnal dipping of BP in obesity-induced hypertensive rabbits. Furthermore, the rabbits were treated with adrenergic beta blockers during the day which inhibited the sympathetic pathways and controlled BP; however, at night this feature was inactive leading to a decline in nocturnal BP fall (Antic *et al*., 2001). The decline in nocturnal dipping is evident in obese hypertensive individuals much of the Na^+ is excreted during the night (Sachdeva and Weder, 2006). In healthy individuals much of the Na^+ is excreted during the day and only less during the night (Bankir et al., 2008; Scott *et al*., 2011) resulting in augmentation of nocturnal BP fall.

Studies have reported that the inconsistency in variations between nocturnal fall and Na^+ excretion in overweight and obese individuals is associated with an increased risk of CVD (Kwagyan *et al*.,

2015). Shin *et al*., 2014 reported that there was an independent relationship between Na^+ excretion and nocturnal BP fall in a similar population (middle age general population) with a mean normal BMI. They further indicated that nighttime BP was an independent prognostic factor in predicting CVD in this population. In a group of eastern African descent population, Bankir *et al*., 2008 reported that a relationship between nighttime BP and nocturnal dipping associated with day time Na^+ excretion in slightly overweight individuals. Their participants were classified according to tertiles of day:night ratio of urinary Na^+ excretion. They reported that participants who were in tertile one, consisted of the heaviest BMI and a lower 24-hour-urinary electrolyte excretions (99, 106, 105 mmol/l) respectively across the three tertiles. The study highlighted that individuals in tertile one had a decreased capacity to excrete Na^+ during the day and subsequently an increased nighttime BP resulted in a blunted nocturnal BP dipping (Bankir *et al*., 2008).

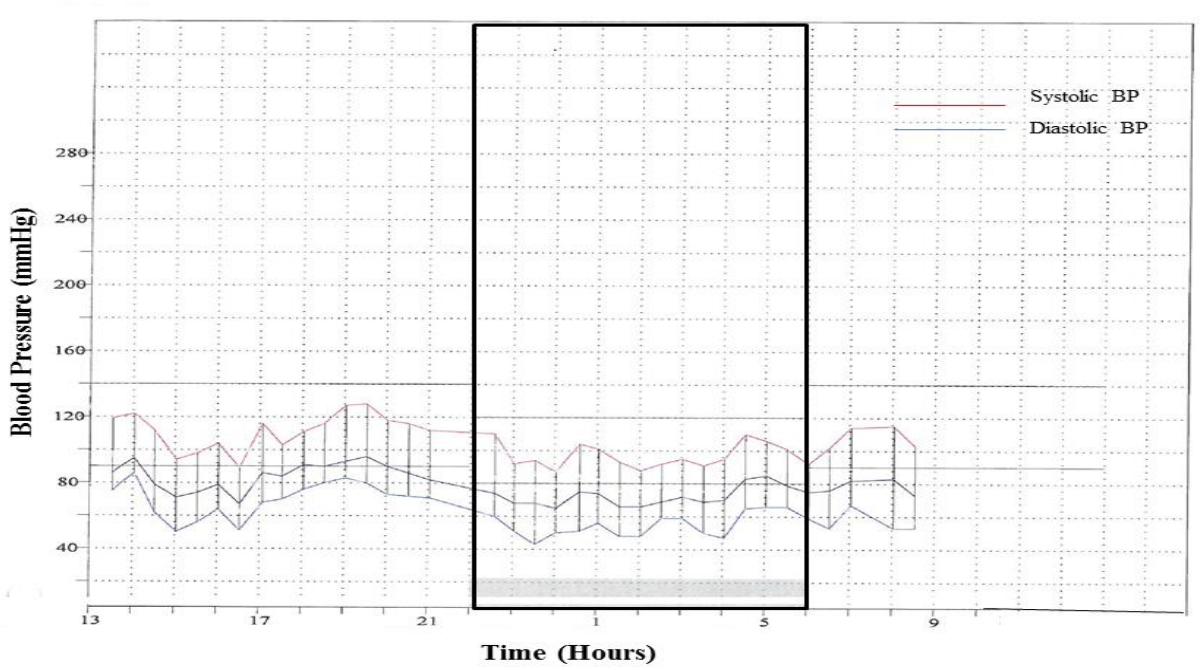


Figure 1.1 illustration showing the typical diurnal rhythm of blood pressure. It depicts changes in systolic and diastolic blood pressures over a 24-hour period. The highlighted area represents nighttime 'asleep' period during which blood pressure 'dips' relative to daytime values. BP, Blood pressure.

Importantly, non-dipping is crucial due to its association with target organ damage (Mezue *et al*., 2016). In hypertensive patients, 25% are non-dippers (Routledge *et al*., 2007). Studies have confirmed that hypertensive non-dippers are at even greater risk of cardiovascular morbidity (Selcoki *et al*., 2008; Fargart *et al*., 1995). These include myocardial infarction (MI), unstable angina, stroke, transient cerebral ischemia, symptomatic aortoiliac occlusive disease, congestive heart failure (CHF) and cardiac death (Routledge *et al*., 2007). Selcoki *et al*., 2007 found that even target organ markers such as left ventricular internal diameter in systole, intraventricular septum thickness in diastole, posterior wall thickness and left ventricular mass were significantly increased in hypertensive non-dippers compared to dippers. Because vascular structure and function is dependent on the use of central aortic BP and PWV as cardiovascular prognostic predictors, non-invasive measurements of central aortic BP are considerably important in assessing cardiovascular risk (Drawz *et al*., 2012).

1.3.3 Pulse wave velocity measurement

Central aortic BP is measured as pressure arising from the aorta. The measurement is relatively lower than brachial BP. Moreover, central BP (BPc) may be more predictive of complications associated with HT including target organ damage of major organs such as the heart, kidney and major arteries supplying the brain (Aziz, 2015). These organs are exposed to aortic pressures rather than brachial pressure (McEniery *et al*., 2014). The common method of estimating BPc is applanation tonometry measure of the common carotid or radial artery. When blood is ejected from the left ventricle to the aorta, the pressure wave that is generated is pushed away from the heart by the arteries. The pressure wave approaches the junction of the large conduit arteries and the high resistance arterioles; it is subsequently reflected to the heart by impedance. The resultant pressure wave then becomes the sum of the forward wave propelled from the heart towards the small vessels and the reflected wave moving backwards towards the heart (McEniery *et al*., 2014). Under normal circumstances, the reflected wave reaches the ascending aorta during diastole leading to an increased diastolic BP (DBP) (Safar *et al*., 2011).

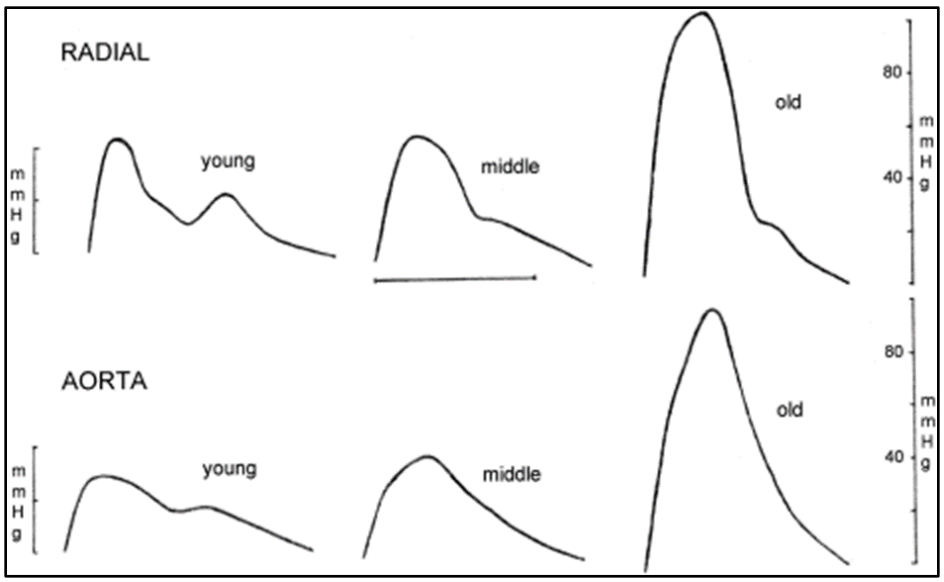


Figure 1.2: Pressure wave forms of young, middle-aged and old individuals in the aorta. Age effects on aortic and radial artery pressure waves. The figure depicts the combined effect of the aortic waveforms with age (Hashimoto and O'Rourke, 2007).

The changes in pressure waves result in complications associated with ventricular load and coronary perfusion. In such instances, increased pulse wave velocity (PWV) occurs due to the summing of the reflected wave and the forward travelling wave subsequently leading to the augmentation of central SBP and ventricular afterload (Figure 1.2). In the elderly, with systolic HT this maladaptive mechanism may result in reduced central DBP (DBPc) and coronary blood flow which is a prognosis of coronary ischemia (Safar *et al*., 2011). These pressure wave reflections between central and peripheral PP are distinguished in aging populations and suggest the development of arterial stiffness (Safar *et al*., 2011). Stiffening of the arteries occurs as an age-related outcome (Quinn *et al*., 2012). With increasing age, the elasticity of the arteries loses compliance (Figure 1.2) (Franklin, 2005). A decline in elasticity of the arteries results in increased PP, LVH (LVH), sub-endothelial ischemia, vessel endothelial dysfunction and cardiac fibrosis (Quinn *et al*., 2012).

1.3.4 Association of dietary salt intake with arterial stiffness

1.3.4.1 Arterial stiffness

Arterial stiffness is implicated in cardiovascular outcomes and it results in stroke, CAD and HF (Liao and Farmer, 2014). Arterial stiffness is described as an impaired ability of the artery to expand and recoil whenever there are pressure changes (Cecelja and Chowienczyk, 2012). Arterial stiffness is quantified by measuring PWV and augmentation index (AI) both of which are dependent on age (Figure 1.2) and BP. Moreover, AI is also dependent on heart rate and body habitus (Mahmud, 2007). Pulse wave velocity is one of the profound measurements identified as a marker for HT (Youssef *et al*., 2016). In addition, pulse wave analysis varies by age (Rogers *et al*., 2001). In young adults, it moves slowly hence it increases DBP due to the delayed reflective wave in the cardiac cycle. However, in older adults, PWV is faster and the reflected wave is earlier resulting in increased SBP (Wagenseil and Mecham, 2012). Moreover, diseases such as HT and diabetes mellitus (DM) cause stiffening of the vasculature.

High salt intake exerts direct structural changes. The physiologic levels of angiotensin II combined with concentration of Na^+ in the ECF, renders the stiffening of the endothelial cells and a decreased production of nitric oxide (NO) (Edwards and Farquhar, 2015). This results in endothelial dysfunction characterised by a decreased size of endothelial cells, surface area, volume, cytoskeleton, deformability and pliability (Imaizumi *et al*., 2016). As a consequence, endothelial cells respond to an increase in dietary salt intake by adapting a stiff morphology due to their physical nature of contact with plasma Na^+ (Hucke *et al*., 2016). Furthermore, the endothelium responds by producing more transforming growth factor beta (TGF-β) which is compensated by release of NO. However, this adaptor mechanism becomes impaired over time due to increased production of reactive oxygen species (ROS). The resultant effect; decreased vascular compliance, vasoconstriction and HT. In salt-sensitive populations this effect is exacerbated (Kanbay *et al*., 2011).

Furthermore, high salt intake may enhance scavenging of NO, moreover, NO may interact with high levels of superoxide leading to oxidation of tetrahydrobiopterin (BH_4) which is an important cofactor for eNOS by ROS (Figure 1.3) (Edwards and Farquhar, 2015). Increased production of ROS after activation by ischemia-reperfusion to repair tissue damage, counteractively results in

deleterious effects on the ventricular wall. In this case, increased salt intake stimulates the production of ROS through upregulation of NADPH and NADPH oxidase activity (Costa *et al*., 2012).

In addition, increased salt intake leads to an increase in vascular collagen deposition and TGF-β1 production. When the vasculature is challenged with shear stress (Figure 1.3), the endothelial cells respond to the situation due to their structural adaptability. They have sensors that can detect changes in blood flow and volume when there is increased salt loading (Sanders, 2009). With aging the elastic fibres become fragmented therefore; the cyclic load that creates pressure is then transferred to the collagen fibres. Due to this imbalance in elastin and collagen fibres, the tissues start to produce more collagen as elastin fibres can no longer regenerate during adulthood. The downside of collagen fibres is that they are stiffer than elastin fibres which in turn increase arterial stiffness significantly (Wagenseil and Mecham, 2012).

In a study investigating whether high salt intake exacerbates BP during the manifestation of HT through the sympathetic nervous system, Koga *et al*., 2008 reported that high salt intake resulted in increased BP and that the production of ROS in the rostral ventrolateral medulla (RVLM) was high. Due to the activation of the sympathetic nervous system by high salt intake, there was an increase in 24-hour urinary norepinephrine excretion rates in the rats fed high salt diet compared to rats that were fed regular salt (Koga *et al*., 2008). Consequently, in this study, Imaizumi *et al.*, 2016 hypothesized that high salt intake is associated with hypertensive target organ damage independent of BP and that oxidative stress is the mediating factor in this association. They studied, 369 Japanese adults and they found that urinary salt excretion was associated with the measure of renal target organ damage (TOD) irrespective of BP.

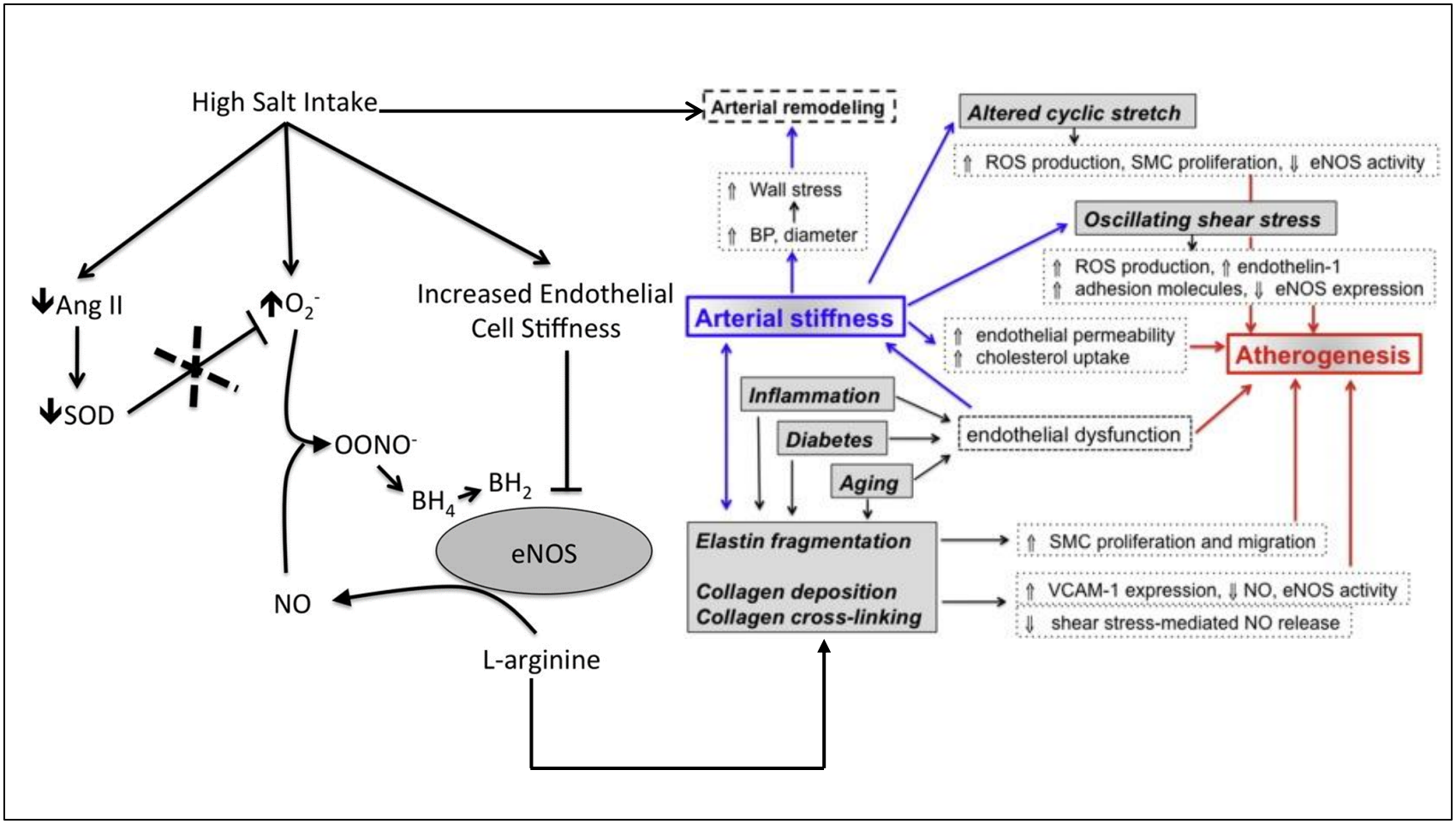


Figure 1. 3 Pathophysiological mechanisms relating to arterial stiffness induced by high salt intake. The figure depicts NO interacting with high levels of superoxide which eventually reduces the availability of NO. Furthermore oxidation of tetrahydrobiopterin (BH_4) which is an important cofactor for eNOS by ROS may further lead to NO scavenging decreased nitric oxide bioavailability leading to arterial stiffness through elastin fragmentation, collagen deposition and collagen cross-linking. Superoxide is the key radical in the production of ROS. It is regulated by scavenging through an enzyme superoxide dismutase (SOD). The expression and function of SOD is suppressed when salt intake is in excess (Edwards and Farquhar, 2017; Palombo and Kozakova, 2016).

Inflammatory markers may promote HT by causing endothelial dysfunction. In this study Schutte *et al*., 2013, investigated a group of African hypertensive men to determine whether alkaline phosphatase (ALP) is a preclinical marker associated with structure and function of large conduit arteries. Their findings indicated an independent association between ALP and 24-hour SBP, 24-hour PP and carotid intima media thickness (CIMT). Furthermore, three inflammatory markers c-reactive protein (CRP), tumour necrosis factor (TNF) and interleukin (IL)-7 have been implicated in the attenuation of NO production through inhibition of endothelial NO synthase (eNOs) resulting in endothelial dysfunction (Dinh *et al*., 2014). Nitric oxide is also important in the pathophysiology of the vasculature in that it regulates BP by facilitating relaxation of resistant vessels and by reducing the production of TGF-β. Increased salt intake disrupts the production of NO, resulting in salt retention and salt sensitive HT. However, studies confirm that restrictions in dietary salt intake enhance NO production which in turn promotes vasodilation of the afferent arteriole; halts glomerular filtration rate and augment Na^+ natriuresis curve subsequently retaining salt (Sanders, 2009).

The role of epithelial Na^+ channels (ENaC) is implicated in the association between Na^+ and endothelial dysfunction (Korte *et al*., 2014). As a consequence, activation of ENaC by aldosterone exacerbates the stiffening of endothelial cells by suppressing NO production. The glycocalyx situated in the inner surface of blood vessels contains ENaC and Na+-K+ adenosine triphosphatase (Na^+/K^+-ATPase) which binds to Na^+ as a protective barrier. If this feature is impaired, blood vessels have increased permeability to Na^+ thus resulting in increased vascular resistance (Imaizumi *et al*., 2016).

1.4 Dietary sodium and potassium intake in South Africa

Reducing salt intake has become a mandatory approach in preventing and managing HT (Karppanen and Mervaala, 2006; Charlton *et al*., 2013; Horn, 2015). Indeed, recommendations of reducing salt intake to <2000–2400 mg/day of which is still in the upper limit, indicates that majority of people are consuming salt in excess on a daily basis (Graudal, 2016). Reductions of salt < 2400mg/day were estimated to decrease SBP and DBP by 7 and 4mmHg; 4 and 2mmHg in hypertensive patients and normotensive individuals respectively. Moreover, the study concluded that the prevalence of stroke and CAD was further reduced by 18-24% at population level (Strazzullo *et al*., 2009).

In a meta-analysis including salt intakes in SSA, studies reviewed in South Africa with data collected dating back to 1978, found that SA is one of the countries with the highest salt intakes (Oyebode *et al*., 2016). Sever *et al* 1980, reported that there was no relationship between dietary Na^+ or urine Na^+/creatinine ratio and BP. Nevertheless, the rise in BP linearly with age was significant in urban residents of African descent. In addition, low plasma renin was common in this group and it was suggested to be heritable to the African descent population. In another group of Africans residing in SA, the serum and urine Na^+ excretion rates were similar across the normotensive, hypertensive and malignant hypertensive groups. Nonetheless, potassium excretion was significantly lower in the African population compared to the Caucasian population. Likewise, low plasma renin was significant in blacks with mild to moderate as well as severe HT compared to normotensives. However, plasma renin and aldosterone were significantly high in malignant hypertensive patients with renal failure (Cohen *et al*., 1982). Similarly, Barlow *et al*., 1982; studied 24-hour urine Na^+ and K+ excretions in 71 black and 34 white males. They reported a mean Na^+ of 126.8 mmol and 166.8 mmol of Na^+ respectively in blacks and whites. Moreover, K+ excretion was significantly lower in blacks (30.9 mmol), compared to whites (59.3 mmol). They concluded that the high Na^+ to K+ ratio in blacks compared to whites may be contributing to the development of HT. To further validate their findings, Barlow *et al*., 1986 conducted a follow-up study; they measured 48-hour faecal and urinary electrolyte excretion in normotensive black and white males. Indeed, 48-hour excretion rates were similar to the previous findings and in other studies that measured 24-hour urinary electrolyte excretions. Twenty-four-hour urinary K in blacks (38.3±12.4 mmol) was significantly lower compared to whites (78.3±7.3 mmol). However, the mean faecal K excretion in blacks (15± 7.3 mmol) was not significantly lower than that of their counterpart (20.8±10.8 mmol). They concluded their findings by highlighting that urinary K^+ excretion is a reflection of low K^+ intakes in blacks compared with whites. A consistent finding was reported in the rural and urban Zulu tribes compared to the Indians. Hoose *et al*, 1985; reported that there was no relationship between salt intake and BP within groups, however urinary K+ correlated negatively with BP in rural Zulus and Indians. The Na^+/K^+ ratio was not significantly different between Indians and Zulus. Nonetheless, the ratio was significantly lower in rural Zulus than in urban Zulus. Plasma renin levels were significantly lower in urban Zulus compared to rural Zulus and the difference was speculated to be an environmental factor.

To date, there are two large scale population studies that are consistent with the previously mentioned studies. These studies have been conducted from 2002 with data sets inclusive of all African tribes

especially the Nguni tribes (Mitchel *et al*., 2014, Millen *et al*., 2013; Charlton *et al*., 2013; Redelinghuys *et al*, 2010; Charlton *et al*., 2008; Charlton *et al*., 2005; Charlton *et al*., 2005). Maseko *et al*., 2006, investigated salt intake in Africans residing in urban communities in Johannesburg. They suggested that to a great extent these groups were consuming salt above the threshold limit, but they could not establish the relationship between dietary salt intake and BP. In one other study, the investigators showed an association between BP and the Na^+/K^+, which is also an index of dietary Na^+ intake, but they could not show any direct relationship between BP and dietary Na^+ (Redelinghuys *et al*., 2010). It is evident from these studies that interethnic disparities; lifestyle choices and urbanisation are contributing to the differences in BP within these communities. Previous studies conducted in this population have revealed a high prevalence of HT (Ntuli *et al*., 2015; Peer *et al*., 2013). Moreover, a number of studies have shown that the incidence of HT is increasing (Akpan *et al*., 2015; Lindhorst *et al*., 2007; Mbokazi, 2006). Nevertheless, there is still a gap in our knowledge on the role of dietary Na^+ on BP in black communities. Indeed, global strategies and population-based intervention studies including Dietary Approaches to Stop HT (DASH) have focused particularly on reduction of Na^+ intake as a means of lowering BP (BP) in a population (Davy *et al*., 2015; He and MacGregor, 2010; Charlton *et al*., 2005). This could be of benefit to people of African descent because a number of studies have shown that the incidence of HT is increasing (Akpan *et al*., 2015; Lindhorst *et al*., 2007; Mbokazi, 2006). Nevertheless, there is a gap in our knowledge on the role of dietary Na^+ on BP in African communities.

1.4.1 Regulation of sodium

Sodium is an essential nutrient of the body required for normal functioning of cells, tissues, organs and homeostasis of all systems. Na^+ can be introduced in the body as Na^+ chloride (NaCl) in three ways; through manufactured or processed foods, foods that naturally contain salt and table salt (De Kock *et al*., 2016). The average Na^+ content of an adult is nearly 92g, of which almost half of it is a constituent of the extracellular fluid (ECF) at a concentration of 135-145 mmol/L (Strazullo, 2014). Therefore, tight regulation of Na^+ and its interaction with other ions is crucial in maintaining homeostasis.

Sodium homeostasis is regulated by the balance between volume in the ECF and osmolarity. This entails movement of water from low to high solute concentrations (Patel, 2009). A slight change in ECF volume signals activation of Na^+-regulating mechanisms (Palmer, 2008). The kidneys serve as one of the pivotal organs in maintaining Na^+ balance together with other electrolytes (Karppanen and Mervaala, 2006). In the kidneys, Na^+ is filtered by the glomerulus and is reabsorbed along the segments of the nephron. The three segments regulating Na^+ reabsorption consist of the distal convoluted tubule (DCT), connecting tubule (CT) and collecting duct. This process is mediated by co-transporters NA^+-Cl^- (NCC) and eNaC. Subsequently Na^+ reabsorption is merged to K^+ secretion via the renal outer medullary K^+ channel (ROMK) (van der Wijst *et al*., 2018). Majority (50-75%) of Na^+ reabsorption is achieved via secondary active cotransporters in the proximal tubule. The thick ascending loop of henle accounts for 20-25% of Na^+ reabsorption and this is achieved through Na^+-K^+-$2Cl^-$ cotransporters. Only 2-5% of the filtered Na^+ is regulated under tight control of aldosterone in the aldosterone sensitive distal nephron (Bonny, 2007). This segment of the kidney also known as the late distal tubule (late distal convoluted tubule, the connecting tubule and the CCT), it determines how much of the filtered Na^+ will be excreted. Therefore, the regulation of Na^+ in the body enables maintenance of BP through different systems.

The body has vast physiologic systems that regulate BP. To mention a few, the Kallikrein-Kinin system, prostaglandins, baroreceptors, natriuretic peptides, the renin angiotensin aldosterone system (RAAS), the adrenergic nervous system, NO, endothelin etc., are involved in short term and long-term regulation of BP (Bonny, 2007). The Na^+ pressure-natriuresis is important in the long-term regulation of BP. This feature is impaired in individuals with essential HT (Hall et al., 1990). Pressure natriuresis is a process by which the kidneys decrease

raised BP by excreting more water and salt (Titze et al., 2005). This is achieved by increasing renal vascular resistance and filtration fractions. Whenever BP is elevated, this increase is transmitted into peritubular capillaries leading to increased hydrostatic pressure in the renal tubules and glomerulus thus reducing Na^+ and water reabsorption. This would consequently decrease intravascular volume which in turn returns capillary and arterial pressure back to normal. In this way, the negative feedback loop is closed. Resetting of the negative feedback loop is associated with persistent increased circulatory volume and elevated pressure (Hall et al., 1990).

In addition to the regulatory pathways involved in ECF/Na^+ homeostasis, the body employs other regulatory mechanisms to maintain BP. Whether these mechanisms regulate BP on a short- or long-term basis, is still a controversy. As will be discussed in section 1.6, the RAAS is a complementary hormonal system that regulates ECF/Na^+ homeostasis which in turn has an effect on BP.

1.5 The relationship between salt intake and hypertension

The relationship between salt intake and BP was first noted by a Chinese medical doctor in 2698-2598 BC, who described it in the Yellow Emperor's Classic of internal medicine stating, "therefore if large amounts of salt are taken, the pulse will stiffen or harden". In addition, Ambard and Beaujard, 1904 showed that whenever salt intake was reduced, a negative Na^+ balance was achieved. Nevertheless, the opposite occurred when salt intake was high. As a consequence, the BP increased (Ha, 2014). It was after the advent of these findings that the prospects of a low salt diet in lowering BP emerged. In 1948, Kempner developed a diet containing 20g of protein, low fat and ideally < 0.5 g salt. This diet contained rice and fruit with considerably reduced salt. In 500 hypertensive individuals treated only with the diet, there was an overall reduction in BP, heart size and reversal of the inverted T waves of the ECG. Nevertheless, the sustainability of this diet was not ideal in terms of palatability (Ha, 2014). However as will be highlighted, there is conflicting views with regards to dietary salt intake and BP in animal models and human studies. This has led to extensive research, investigating the underlying mechanisms associated with increased salt intake and hypertension (Olubodun *et al*., 1997). Moreover, this has been validated through a volume of epidemiologic and

experimental studies (Dahl and Love, 1954, 1960, Ball and Meneely, 1957, Isaacson *et al*., 1963, Charlton *et al*., 2013).

Of note, findings from animal models have confirmed a consistent relationship between Na^+ intake and BP. Epidemiologic and experimental studies had led to the debates over whether current salt reduction recommendations are too high from a health perspective. One of these studies suggested that certain levels of salt intake were necessary to induce HT (Dahl and Love, 1954; Dahl, 1960). They reported a significant positive correlation between salt and intake and the development of essential HT. Similarly, Ball and Meneely, 1957, showed that in groups of rats fed varying salt concentrations (0.15, 2.8, 5.6, 7.8 or 9.8%); BP increased proportionally with the amount of salt intake progressively over nine months.

Indeed, this linear relationship has also been confirmed in human studies. The study investigated 100 healthy black men, the mean 24-hour urinary Na^+ excretion corresponded to a minimum daily intake of 18.6 g salt. The study reported a corresponding linear relation between salt intake and the incidence of HT (Isaacson *et al*., 1963). Similarly, the INTERSALT study reported a significant positive correlation between 24-hour urinary Na^+ excretion and BP. The study investigated 10079 participants from 50 centres worldwide. They reported that Na^+ excretion was independently associated with BP (INTERSALT Study Cooperative research group., 1988). The study concluded that that the associated increases in BP with age are evident when salt intake is increased. Moreover, changes in BP due to age may favourably respond to a lower salt intake diet thus, reducing cardiovascular mortality (Ha, 2014).

1.6 Renal mechanisms for salt dependent hypertension

1.6.1 Increased salt intake activates RAAS

The RAAS contributes significantly to the development of HT (Aziz, 2015). Importantly, in groups of black African ancestry, the predominant phenotype causing HT is a low renin, salt sensitive state (Mitchel *et al*., 2014; Scott *et al*., 2011). Because of this, the exclusion of RAAS blockers in the treatment guidelines in this group, impedes effective control of BP in this group (Scott *et al*., 2011). It is without doubt, that RAAS plays an important role in BP control. The activity of the RAAS is controlled by the release of renin in response to salt intake. In this regard, acute and chronic salt loading suppress the activity of renin, however a decrease in salt

intake stimulates it (Shweda, 2015). Whenever salt intake increases, it reduces the activity of RAAS (salt-retaining hormones) and enhances the release of atrial natriuretic peptide (salt losing hormones). In an experimental study investigating the effects of aldosterone in rats, stroke-prone spontaneously hypertensive rats (SHRSP) were fed low, intermediate, and high salt diet with or without eplerenone. Summary of their findings showed, impaired pressure natriuresis where RAAS is implicated; twenty-four-hour Na^+ excretion was not altered by eplerenone; plasma aldosterone levels decreased with salt loading. Consequently, suggesting that when salt is low, aldosterone levels increases subsequently reducing end organ damage and aldosterone antagonism. The findings indicated that eplerenone inhibited vascular remodelling and cardiac fibrosis (Endemann *et al*., 2004).

In addition, increased or fixed low activity of the RAAS causes salt sensitivity (SS). Genetic studies in animal models have been effective in elucidating the mechanisms related to renin substrates and precursors for enzyme substrates required for activation if angiotensin II (Bernstein *et al*., 2018; Shroten *et al*., 2012; Scott *et al*., 2011), in attempts to explain the relationship between RAAS and BP. Moreover, circulating components of the RAAS such as angiotensin II (AngII) are implicated in causing HT through activation of AT1 kidney receptors (Hall, 2016). Therefore, mechanisms linking changes with increased renal tubular salt reabsorption and salt sensitive BP will now be reviewed.

1.6.2 Salt sensitive hypertension

Salt sensitivity is defined as the significant increase in BP in response to a change from low to high salt diet (Zanchetta *et al*., 1997). This feature is acquired in almost 50% of individuals with essential HT (Poch *et al*., 2001). Salt sensitive BP prevails when the levels of salt intake either increases or decreases BP (Farquhar *et al*., 2015). In this way individuals can even become salt resistant when there is no change in BP when salt intake is restricted (Farquhar *et al*., 2015). Dahl and Heine (1975), confirmed that in developed salt sensitive and salt resistant rat models, variable degrees of SS existed. On a low salt diet, the rats sustained a normal BP however on a high salt diet, the rats developed HT. In humans, salt-sensitivity arises from interaction of genes and the environment (Poch *et al*., 2001). In particular, SS is acquired from non-specific renal injury caused by uncontrolled HT, weight gain, low dietary K+ intake,

(Kaplan, 2010). In Africans the predisposition to SS is increased (Mitchel *et al*., 2014; Scott *et al*., 2011) therefore; an increased risk of future HT and CVD may ensue even in SS normotensives. In this section, I will review the mechanisms associated with salt induced HT.

Studies have suggested the notion that almost 30-50% hypertensive patients and 25% normotensive individuals are salt sensitive (Bloch, 2016; Poch 2001). In South Africa, several studies have suggested that essential HT in the African population is attributed SS (Mitchel *et al*., 2014; Scott *et al*., 2011; Redelinghuys *et al*., 2010). In this regard, the generalisability of much published research on salt induced HT is problematic. Despite these ongoing debates about salt induced HT, all these arguments are based on fundamental physiological concepts of BP regulation during salt loading. In particular, Hall, 2016 postulates that kidney dysfunction mediates salt induced HT. As a consequence, salt sensitive HT occurs due to increased plasma volume and cardiac output. In their summary, two key concepts including vasodysfunction and renal dysfunction are evident. Kidney dysfunction increases salt reabsorption due to a decreased glomerular capillary filtration coefficient or nephron injury. Consequently, systemic vasoconstriction may occur in response to pressure overload on the tissues and not necessarily the cause of increased BP, suggesting that the resetting of the normal pressure-natriuresis is essential for chronic increases in BP in salt sensitive individuals. Nevertheless, although this argument is constructive in defining that renal dysfunction rather than non-renal vascular dysfunction are the mechanisms involved in salt sensitive HT, Morris *et al*., 2016 seems to base their views on vasodysfunction that involves renal dysfunction rather than abnormally increased renal retention of Na^+. This hypothesis, postulates that salt sensitive HT occurs due to an unusual decrease in renal and peripheral vascular resistance in response to high salt intake. Likewise, salt sensitive and resistant individuals have increased plasma volume and CO however, SS individuals lack the capacity to decrease systemic vascular resistance to adjust BP to normal (Bloch, 2016). Traditionally it has been argued that the African gene theory which entails the slavery HT hypothesis does not robustly represent the theories relating salt induced HT, however from the two reviews it is clear that Hall *et al*., 2016, somewhat seems to agree with this view. In this regard, the slavery HT hypothesis argues that Na^+ retention is a heritable feature in populations of African descent. This view was first noted by researchers in 1960-70s. They hypothesized that a genetic mutation linked to natriuresis resulted in SS HT. Indeed, during those times where salt supplies were limited and the environment was arid and hot, a compensatory response was to retain salt. Therefore, the

aforementioned views deem imperative to suggest that this genetic predisposition has evolved particularly in individuals of African descent (Scott *et al*., 2011).

Consensus has been reached in most scientific platforms to reduce dietary salt intake to 5 g/day or less (Batuman, 2013). However, there is still scepticism and uncertainty on this move. Part of these controversies is implicated in contradictory findings on the relationship between dietary salt intake and BP. Previous studies revealed a modest association (Charlton *et al*., 2005), while others showed no associations (Oparil, 2014; O'Donnell *et al*., 2013). In other studies, the investigators showed an association between BP and the Na^+-to-K+ ratio, which is also an index of dietary Na^+ intake, but they could not show any direct relationship between BP and dietary Na^+ (Hoosen et al., 1985; Charlton *et al*., 2005). Moreover, recently in a Framingham study it was revealed that lower salt intakes are not associated with lower BP levels among Framinghan offspring adults. On the contrary, findings of the trials of HT prevention (TOHP) showed a dose response relationship associated with reduced Na^+ intake and the reduction of cardiovascular events. In addition, findings from an observational study indicate a 25–30% lower risk of cardiovascular events in a longitudinal data with baseline data collected 10–15 years earlier (Cook *et al*., 2007). Tuomilehto *et al*., 2001 reported on a study conducted in Finland in 1982 and 1987. The study showed that CAD, CVD and all-cause mortality frequency significantly rose with increased 24-hour Na^+ excretion independent of BP and cardiovascular risk (CV) risk. As highlighted, the conflicting data with regards to whether high or low dietary salt intake leads to HT and its cardiovascular complications is still a conundrum in this African population.

1.7 Overall conclusion

As previously discussed, the contradictory findings of these studies are indicative of the complex relationship between BP and Na^+. In this population the complexity of this relationship could be compounded by high prevalence of obesity as BMI has been shown to have a direct association with BP (Maseko *et al*., 2006). Almost 60% of the countries in the world do not have a strategy to reduce salt intake. Nevertheless, In South Africa, the Department of Health enforced salt reductions in different food categories such as bread, margarine and spreads; savoury snacks; pressed meats; soup powders and stock cubes. Importantly, it remains unclear whether the current salt reduction recommendations will favour this population that is known to be salt sensitive. This has raised uncertainties whether a) A possibility exists that obesity masks the relationship between BP and urinary Na^+ excretion in

this population; b) Whether there is a relationship with sodium and arterial stiffness (a marker of cardiovascular target organ damage). Therefore, in the present thesis I aimed:

1.8 Aims

The aims of this thesis are as follows:

1. To determine whether obesity is associated with the relationship between urinary sodium excretion and 24-Hour ambulatory blood pressure. The data are presented in chapter 3 of this thesis.

2. To determine whether obesity is associated with the relationship between urinary salt excretion and pulse wave velocity. The data are presented in chapter 4 of this thesis.

3. To determine the relationship between 24-hour urinary Na^+ excretion and conventional systolic and diastolic BP in nomal weight individuals. The data are presented in chapter 5 of this thesis

CHAPTER 2

Methods

2.1. Introduction

In this chapter, I provide the details of the materials and methodologies used throughout this thesis. Generalized information concerning statistical analysis performed throughout this thesis is also provided in this chapter. However, specific details concerning statistical analysis performed in each chapter are given in respective chapters.

The studies described in this chapter were carried out in a clinic, set up in the School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg except otherwise stated.

2.2. Study Population

The study design has been described previously by Maseko *et al*., 2018. Participants were Black-African ancestry, residing in South West Township (Soweto) of Johannesburg and Ekurhuleni metropolitan municipality. Participants were of Nguni, Tsonga, Venda and Sotho tribes. The minimum age of 18 years were implemented however, there was no upper age limit.

Seven hundred and fifty participants were randomly recruited for demographic, clinical, and anthropometric assessments. Of the 750 participants, 547 had complete 24-hour ambulatory BP (Chapter 3). Participants were stratified according to BMI status, according to the WHO standards. Of these participants, 547 participants had complete PWV measurements; 24-hour SBP and DBP, data presented in chapter 4. Finally, only 236 had normal BMI in relation to the conventional BP, the data is presented in chapter 5. The methodology was adapted from previous publication by Maseko *et al*., 2006.

2.3. Ethics

The study was approved by the University of the Witwatersrand for Research in Human Subjects (M17-02-15). The study was conducted at the University of the Witwatersrand. All participants gave informed written consent before participating in the study.

2.4. Demographic and clinical assessments

A standardized questionnaire was administered to all the participants (refer to the appendix). Demographic and clinical data were obtained from the questionnaire. This questionnaire included questions such as date of birth, sex, smoking status, alcohol consumption, previous medical history, family medical history of HT and CVD, existing medical conditions and previous and current drug therapy. The questionnaire was not translated to the languages spoken by the participants; however, study assistants who were familiar with the language ensured that the answers provided by the participants were related to the questions asked. This was done to avoid translational errors, as there are different dialects within the languages spoken in this specific community.

2.5 Anthropometric measurements

Anthropometric measurements including waist circumference, hip circumference, weight and height were taken for the determination of BMI and body fat distribution. Anthropometric measurements were taken using the International Standards for Anthropometric Assessment (Marfell-Jones *et al*., 2006).

2.5.1. Weight measurement

Weight was measured using a calibrated electronic scale. Participants were provided with light robes and were advised to remove shoes and accessories from the hair. The scale was reset at zero and the participant was instructed to stand on the centre of the scale without support, with weight distributed evenly on both feet. Height was taken using a stadiometer (Leicester portable Stadiometer).

2.5.2. Height measurement

Height was measured as the distance between the transverse planes of the vertex and the inferior aspects of the feet. The participant was instructed to stand perpendicular to the wall of the stadiometer with the heels closed together and buttocks and upper part of back touching the stadiometer. The measurer positioned the head of the participant in the Frankfort plane (the lower edge of the eye socket is in line with the notch superior to the tragus of the ear) with the body standing upright. The participant was instructed to take a deep breath as the measurement was being taken to get maximum height. Height was recorded to the nearest 0.1cm.

2.5.3. Determination of body mass index (BMI)

Body mass index (BMI) was calculated as weight in kilograms (kg) divided by the square of height in meters. Participants were considered as being overweight if their body mass index was $\geq$25 kg/m^2 and obese if their body mass index was $\geq$ 30 kg/m^2 (Peer et al., 2015; Seedat and Rayner., 2012; WHO 1995).

2.5.4 Measurement of waist circumference

Girths were measured using a Lufkin Executive thin line steel anthropometric measuring tape. Waist circumference was measured at the narrowest point between the lower costal (10^{th} rib) border and the top of the iliac crest, perpendicular to the long axis of the trunk (Marfell-Jones *et al*., 2006). The participant was instructed to stand in a position where the arms were folded across the thorax. The measurer used the cross-hand technique to position the tape and at the end of a normal expiration, the measurement was recorded. The criteria by Han and colleagues (1995) was used to determine visceral adiposity. Waist circumference ≥94cm for men and ≥80 cm for women (Peer et al., 2015; Han et al., 1995).

2.5.5. Measurement of hip circumference

Hip circumference was measured at the circumference of the buttocks at the point of increased posterior protuberance. The participant was instructed to stand in a position where the arms were folded across the thorax with feet closed together and the hip muscles relaxed. Again, the measurer used the cross-hand technique to position the tape and HC was recorded. Waist-to-hip ratio (WHR) was determined by dividing waist circumference by hip circumference.

2.6. Conventional (office) blood pressure measurements

Blood pressure readings were taken using a standard mercury sphygmomanometer. The participants were seated and asked to rest for five minutes. The observer measured the participant's sitting BP five times consecutively in the sitting position with one-minute intervals. An average of the five readings was then determined for accuracy.

The average five measurements were recorded as the final brachial BP reading. Standard cuffs with an inflatable bladder with a length of 22cm and a width of 12cm was used unless the arm circumference exceeded 31cm, where larger cuffs with a 31x15cm bladder were used. The cuff was deflated at approximately 2mmHg/s. Phase I and Phase V systolic and diastolic Korotkoff sounds were obtained, respectively. Blood pressure was determined to the nearest 2mmHg (Stergiou *et al*., 2018; Scott *et al*., 2011). Participants were classified as hypertensive according to the Guidelines Committee of the European Society of Hypertension, 2018 wherein if the

mean readings were ≥140/90 for systolic and diastolic BP and/or if they were already taking antihypertensive therapy (McCormack *et al*., 2019; Scott *et al*., 2011).

2.7. Ambulatory blood pressure measurements

Twenty four-hour ambulatory BP (ABP) monitoring was performed using oscillometric monitors. Standard cuffs with an inflatable bladder with a length of 22cm and a width of 12cm was used unless the arm circumference exceeded 31cm, where larger cuffs with a 31x15cm bladder were used (figure 2.1). The monitors are programmed to measure BP at 15-minute intervals from 06:00 to 22:00 and then 30-minute intervals from 22:00 to 06:00. Participants kept a diary card for the duration of the recordings to note the time of going to bed in the evening and time of waking up in the morning. These times were used to determine the wake and sleeping periods (Parati *et al*., 2014; Scott *et al*., 2011). Participants also recorded the time when they took medication, smoked, drank caffeine, or consumed alcohol depending on which ever was applicable to each participant. On completion of the recordings, data was transferred to a computer for analysis. ABP data was expressed as 24-hour average SBP and DBP, the percentage decrease in BP at night (mean-day mean-night/mean-dayx100), the difference between day and night BP and the ratio of day-to-night BP.

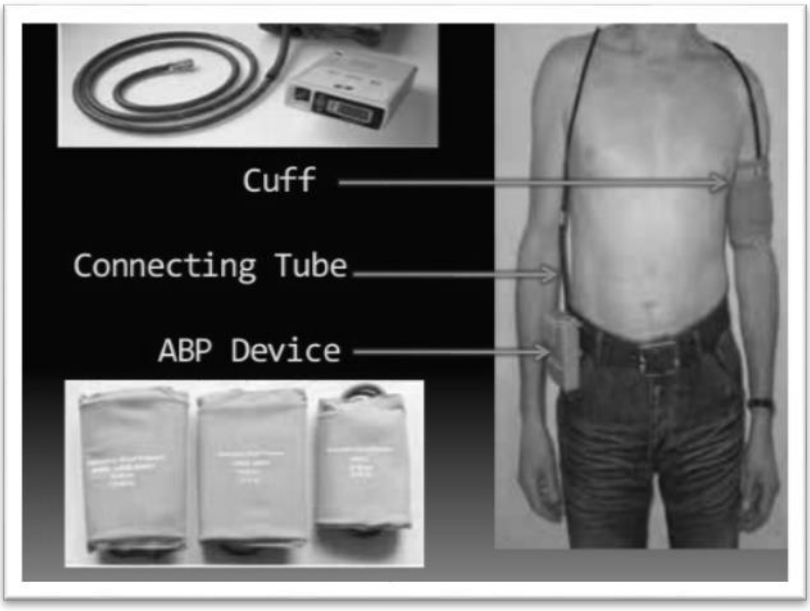


Figure 2.1**.** Spacelabs 90207 (Spacelabs Inc., Redmond, Washington, USA) monitor and illustration of fitting to the participant.

2.8. Twenty-four-hour urine collection

Urine samples were collected over a 24-hour period. Each participant was provided a urine bottle (Figure 2.2A) and was advised to collect the urine sample into a platic beaker (Figure 2.2 B) and pour into the bottle. The urine bottles were then collected from each participant the following morning. Urine was stored in urine storage tubes (Figure 2.2C) and picked up by staff from Clinical Laboratory Services (CLS) at the National Health Laboratory Service (NHLS) South Africa for analysis within 7 days. Twenty-four-hour urine Na^+ and K^+ excretion rates were calculated from the product of urine volume and urine electrolyte concentration. Creatinine clearance was determined from the product of urine volume and urine creatinine concentrations/plasma creatinine concentration. The quality of urine samples was determined by constructing regression relations between 24-hour urine creatinine and body weight and 24-hour urine volume and age in gender-specific groups. Based upon the 95% confidence intervals for each group, a 24-hour urine sample was considered acceptable if 24-hour urine creatinine was >3.5 mmol and <35 mmol for males and >3.5 mmol and <30 mmol for females. Samples with urine volumes <300 ml/day were also assumed to be incomplete urine collections. These are standard approaches and have been published by previous studies (Bawa-Allah *et al*., 2019; Maseko *et al*., 2018; Maseko *et al* 2006).

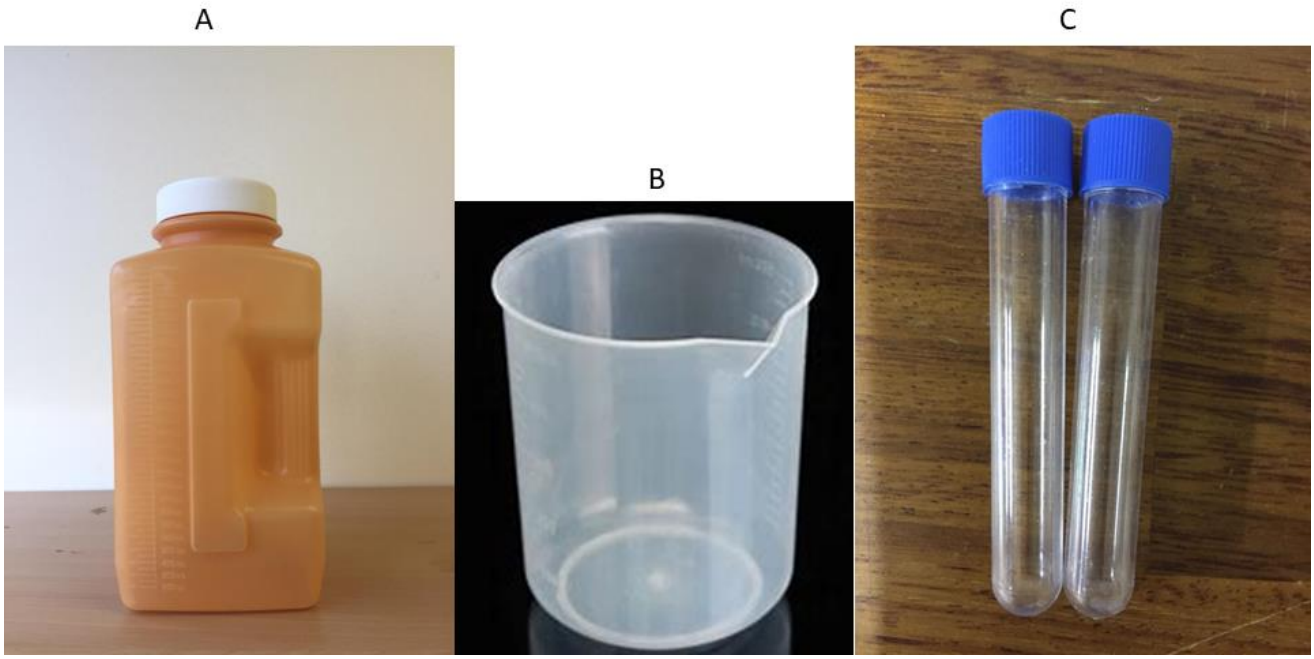


Figure 2.2. Urine collection materials. Panel **A** shows a 24-hour urine collection bottle; **B** represents a beaker while **C** represents urine sample storage tubes.

2.9. Blood sample collection

Venous blood was collected by a registered nurse. Whole blood was collected in Vacutainer® tubes containing no anticoagulant. The samples were allowed to clot. Samples were stored at room temperature until complete clotting and then they were centrifuged at 3000 rpm for ten minutes, to allow separation of serum from whole blood. Within two hours after centrifugation, 500 µL of cell-free sample was transferred to an Eppendorf tube. The tube was tightly closed immediately. Samples were then stored in a bio-freezer at -80°C until further analysis. Plasma glucose was measured from venous blood collected in BD Vacutainer® tubes containing K+ oxalate/Na+ fluoride. Serum and plasma samples were centrifuged for 20 minutes at 3000 rpm; where after serum samples were stored at –80ºC until analysis. Samples were then picked up by staff of CLS (Clinical Laboratory Services) at the National Health Laboratory Service (NHLS) South Africa for analysis. All samples were takem to the laboratory within 7 days of blood collection.

Blood samples were collected to determine levels of aldosterone, renin and serum lipid profile. Full blood count was performed to measure haemoglobin, haematocrit, white cell count, and red cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell indices, platelet count, and mean platelet volume (MPV) and red cell distribution. Chemical pathology tests were done to measure urea, creatinine and electrolytes (Na^+ and K^+). Liver function tests were done to measure total protein, albumin, total bilirubin, conjugated bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT). Finally, the lipid profiles were run to test for total cholesterol (TC), high density lipoprotein cholesterol (HDLc) and low-density lipoprotein cholesterol (LDLc). All these tests were done to validate information recorded in the participant questionnaire and to confirm any pre-existing medical conditions. Diabetes mellitus was determined by percentage glycated haemoglobin (HBA1c).

2.10. Carotid-Femoral Pulse Wave Velocity (CFPWV) measurement

Carotid-Femoral PWV measurements were conducted to determine the level of arterial stiffness.

Shiburi et al., 2006, have previously described the method. The pulse waves were calibrated manually (auscultation) by measuring BP immediately before the recordings. Participants rested for 15 minutes in the supine position. The carotid and femoral waveforms were recorded by applanation tonometry at the participants' dominant arm (figure 2.3 b).

A high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) was used, interfaced with a SphygmoCor software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia); version 9.0 (figure 2.3 a). The aortic pulse wave was calculated using a validated generalised transfer function. The radial (peripheral arterial) Al (Alp) was defined as the ratio of the second to the first peak of the pressure wave expressed in percentage. Distances from the suprasternal notch to the carotid sampling site (labelled as distance A), and from the suprasternal notch to the femoral artery (Distance B) were measured. The pulse transmit time was determined from the average of 10 consecutive beats (the mean time difference between sites A and B). Lastly, aortic PWV was determined as the ratio of the distance in metres to the transit time in seconds. Arterial stiffness was determined using the augmentation index corrected for heart rate (HR). A trained technician who was unaware of the medical history of the participants conducted all the measurements.

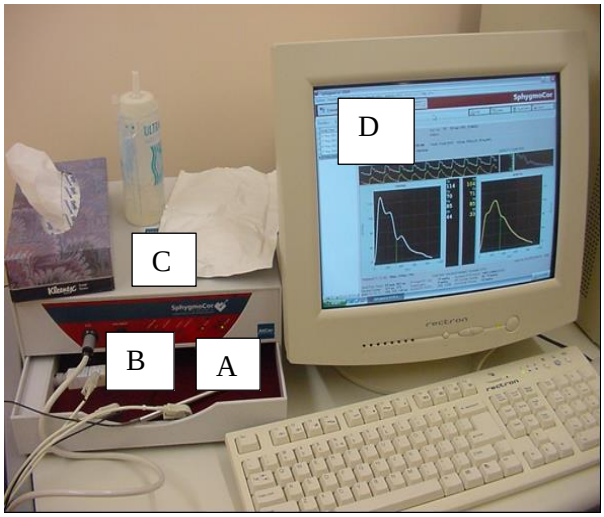


Figure 2.3 a

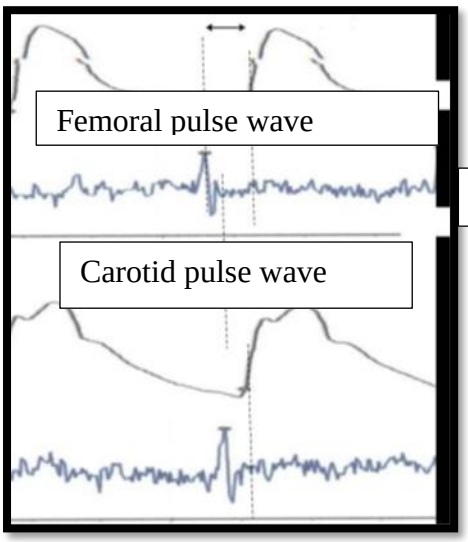


Figure 2.3 b

Figure 2.3. Sphygmocor device setup. Figure 2.3 a: Computer analysis of PWV coupled with ECG (Majane, 2009). **A**-Applanation tonometer used to detect PWV; **B**- ECG electrodes to determine timing of pulse waves; **C**- SphygmoCor device used to amplify pulse waves; **D**-Computer showing PWV analysis. Figure 2.3b: Analysis of femoral and carotid PWV recorded by applanation tonometry coupled with ECG (Majane, 2009).

2.11. Statistical analysis

All data were analyzed using the SAS software version 9.3 (The SAS Institute Inc., Cary, North Carolina, USA).

The five readings obtained from the conventional BP measurements in all chapters were averaged to obtain a single systolic and diastolic BP reading. Ambulatory BP data are expressed as 24-hour, daytime and nighttime average systolic and diastolic pressures.

The distribution of data was tested for normality Shapiro-Wilk statistic and $\chi 2$ for categorical variables. Data distributed normally was presented as mean (standard deviation). Descriptive statistics were presented as means and standard deviation (±SD). ANOVA (Analysis of variance) was used when no adjustments for confounding variables were required. Comparisons between increased and normal BMI groups as well as differences in high and low salt groups were done using student t-test. Multivariate regression analyses were used to determine the relationship between 24-hour urinary Na^+ excretion and BP. The coefficient of determination (r^2) was determined by correlating a 24-hour urinary sodium excretion with BP. A p-value of less than 0.05 was considered statistically significant.

CHAPTER 3

Obesity masks the relationship between urinary sodium excretion and 24-hour blood pressure in people of African ancestry: the impact of obesity on the relationship between sodium and 24-hour blood pressure.

A modified version of this chapter has been published in the

Cardiovascular Journal of Africa.

Maseko, M., Mashao, M., Bawa-Allah, A., Phukubje, E., Mlambo, B., and Nyundu, T.F. (2018). Obesity masks the relationship between dietary salt intake and blood pressure in people of African ancestry: the impact of obesity on the relationship between Na+ and blood pressure. *Cardiovasc J Africa*, 2018, **29**: 172–176.

3.1. Introduction

A number of studies have associated an increased dietary sodium (Na^+) intake and obesity with hypertension and target-organ damage (Mitchel *et al*., 2012). In an effort to lessen the worldwide prevalence of hypertension, global strategies and population-based intervention studies, including Dietary Approaches to Stop Hypertension (DASH), have focused particularly on reduction of Na+ intake as a means of lowering blood pressure (BP) in a population (Charlton et al., 2005; He and MacGregor, 2010; Davy et al., 2015). This could be of benefit to people of African descent because a number of studies have shown that the incidence of hypertension is increasing in African communities (STATSSA, 2018). However, there is a gap in our knowledge on the role of dietary sodium on blood pressure in this community. Even though previous studies conducted in this population have revealed a high prevalence of hypertension (Peer et al., 2013; Ntuli et al., 2015), the relationship between dietary salt intake and BP is still not well understood because studies have revealed contradictory findings on this relationship.

Previous studies conducted in this population have revealed a high prevalence of HT (Ntuli *et al*., 2015; Peer *et al*., 2013) and recommendations to reduce dietary salt intake could therefore be of great benefit to this community. Contradictory findings on the relationship between dietary salt intake and BP have been addressed in some studies (Oparil, 2014; O'Donnell *et al*., 2013). Some studies showed a modest association between salt intake and BP (Intersalt Cooperative research group, 1988) while others showed no associations (Charlton *et al*., 2005; Hoosen *et al*., 1985). In one study, the investigators showed an association between BP and the sodium: potassium ratio (Na^+: K^+), which is also an index of dietary Na^+ intake, but they could not show any significant direct relationship between BP and dietary Na^+ (Redelinghuys *et al*., 2010).

The contradictory findings of the above studies mentioned, are indicative of the complex relationship between BP and Na^+. In this population, the complexity of this relationship could be ascribed to the high prevalence of obesity (Smyth *et al*., 2013) as BMI has shown to have a direct association with BP (Correira-Costa *et al*., 2016; Grimes *et al*., 2016; Yi *et al*., 2014; Gelber *et al*., 2007). The possibility exists that obesity could mask the relationship between BP and dietary Na^+ intake in this population. The biggest contributor to the masking effect of obesity could be due to the high prevalence of overweight/obese people.

3.2 Aim

The aim of the present study was to determine whether obesity is associated with the relationship between dietary salt intake and blood pressure.

3.3 Objectives

3.3.1 The objective of this cross-sectional population study was to determine the relationship between 24-hour urinary sodium excretion and 24-hour ambulatory BP.

3.3.2 To investigate the possible impact of an increased BMI on the relationship between 24-hour urinary sodium excretion and BP.

3.4. Methods

3.4.1. Study Population

Details about the study population are described in chapter 2. Participants were stratified according to BMI status, according to the WHO standards. Of the 750 participants, 547 (345 females and 202 males) fulfilled the quality control requirements as stipulated in chapter 2 and previous publications (Maseko *et al*., 2006, Redelinghuys *et al*., 2010).

3.4.2. Procedures

Details about ambulatory blood pressure measurements, conventional blood pressure measurements, and anthropometric measurements are provided in the methods chapter (chapter 2) of this thesis.

3.4.3. Statistical analysis

All data were analyzed using the SAS software version 9.3 (The SAS Institute Inc., Cary, North Carolina, USA). The mean of the five readings obtained from the conventional BP measurements was determined to get average systolic and diastolic BP values. Ambulatory BP data were also expressed as averages. Data were expressed as means ± standard deviation (SD) for continuous variables. Categorical variables are expressed as absolute or relative frequencies or as percentages. Test for normality of continuous variables was assessed using Shapiro-Wilk's statistic or χ^2 test for categorical variables. Simple regression was used to determine the relationship between ambulatory blood pressure parameters and age. Adjustments were made for age, smoking, alcohol intake, diabetes and hypertension. A p-value of less than 0.05 was considered statistically significant. A p-value <0.05 was considered as statistically significant.

3.5. Results

3.5.1. General characteristics of the participants

Table 3.1 gives a description of the demographic, anthropometric, haemodynamic and general clinical characteristics of the participants in this study. They were divided into three groups: total sample, males and females. Each group was subdivided into 'All', 'Normal weight' and 'overweight/obese'. There were no underweight participants in this study. The characteristics include age, BMI, 24-hour ambulatory BP, 24-hour urinary Na^+ excretion rates, alcohol consumption, smoking, diabetes status and hormone concentrations. The mean age of the total population sample was 45.3± 18.5 years. There was no age difference between males and females (men 45.5 ± 19.9 and females 45.1 ± 17.7 years) with 63% of participants being females. The mean BMI of the group was 29.1 ± 7.8. When BMI was determined according to gender, more females were in the overweight/obese category (75%) compared to males (47%). Thirty-five per cent (35%) of the total sample population was hypertensive, 24% consumed alcohol regularly, 15% smoked, and 14% had diabetes mellitus or an HbA1c > 6.5% (Gillet *et al*., 2009). There was no significant difference in urinary Na^+ excretion between the normal and overweight/obese subgroups in the total sample and in male groups; however, the

overweight/ obese females had significantly lower urinary Na+ excretion rates when compared with the normal weight female subgroup. Both insulin and leptin levels were significantly higher in the overweight/obese individuals compared to the normal weight participants. Gender differences were observed in leptin concentrations. Compared to males, leptin concentrations were significantly higher in females (40.5±28.1).

Table 3.1. Demographics and clinical characteristics of the total study population according to BMI

	Total sample			Males			Females		
	All	Normal[a]	Over[b]/Obese[c]	All	Normal[a]	Over[b]/Obese[c]	All	Normal[a]	Overb/Obesec
N	547	193	354	202	106	96	345	87	258
Age (years)	45.3 ± 18.5	36.3 ± 18.3	50.4 ± 15.2	45.5 ± 19.9	38.2 ± 18.9	53.5 ± 18.0	45.1 ± 17.7	33.7 ± 17.4	48.9 ± 16.1
BMI (kg/m2)	29.1 ± 7.8	21.6 ± 2.1	34.4 ± 1.4*	24.9 ± 5.0	21.2 ± 2.0	29.1 ± 4.1*	31.5 ± 8.1	21.9 ± 2.2	34.6±6.7*
Hypertensive (%)	35.1	17.6	43.8	24.4	19.8	30.2	39.3	14.9	47.5
Diabetic (%)	14.3	7.2	16.9	12.9	9.4	16.8	13.9	4.6	16.9
Alcohol intake (%)	23.6	33.1	18.0	41.8	46.7	36.8	12.7	17.2	11.1
Smokers (%)	15.2	26.4	9.1	33.8	43.4	23.0	4.3	5.7	3.9
Na+ (mmol/day)	105.6 ± 78.4	108.9 ± 89.8	103.8 ± 72.1	106.5 ± 74.7	115.7 ± 71.1	98.5 ± 77.9	105.0 ± 80.55	121.5 ± 99.4	99.5±68.6*
HbA1c (%)	6.2 ± 1.5	5.8 ± 1.0 6.	6.3 ± 1.6	6.1 ± 1.7	5.8 ± 1.4	6.5 ± 1.9	6.2 ± 1.3	5.7 ± 0.3	6.3 ± 1.5
Renin (ng/dl)	36.4 ± 73.8	35.9 ± 75.8	34.8 ± 35.7	35.2 ± 52.2	33.1 ± 51.6	37.5 ± 53.0	37.0 ± 84.1	39.5 ± 98.4	31.0 ± 5.0
Insulin (mmol/l)	14.4 ± 16.6	10.8 ± 13.8	16.8 ± 18.9*	13.8 ± 19.3	9.8 ± 13.3	18.9 ± 23.1*	14.8 ± 14.8	13.1 ± 14.1	15.5 ± 15.0
Leptin (ng/ml)	24.2 ± 25.3	9.9 ± 12.4	43.0 ± 29.4*	8.5 ± 8.7	4.3 ± 6.9	12.2 ± 8.4*	35.0 ± 27.2	17.6 ± 13.9	40.5 ± 28.1*

BMI, body mass index; HbA1c, glycated haemoglobin; Na+, 24-hour urinary Na+ excretion rate.

[a]Normal BMI is defined as < 25 kg/m^2, [b]overweight is defined as BMI ≥ 25 < 30 kg/m^2 and obese is defined as BMI ≥ 30 kg/m^2. Data is presented as mean ± SD or number (%). * P value < 0.05 depicts significant BMI difference.

3.5.2. Haemodynamic measurements in participants with complete ambulatory blood pressure monitoring.

In all participants who had complete ambulatory BP monitoring, 24-hour ambulatotry BP averaged 118 mm Hg systolic and 72 mm Hg diastolic. Blood pressure was significantly higher in overweight/obese group (121.6±16.1/74.4±10.6) vs normal BMI (114.9±12.3/71.3±9.1). Females' BP averaged 116.9 mm Hg systolic and 71.9 mm Hg diastolic. Moreover, BP was significantly higher in the overweight/obese group (116.6±15.1/71.3±9.9) compared to normal BMI participants (109.6±9.8/68.4±7.3). Males' BP was significantly higher in the overweight/obese group (125.4±15.7/66.8±12.6) compared to normal BMI participants (119.8±12.15/63.2±11.1). These data are pesented in table 3.2

Table 3.2 Haemodynamic parameters in participants with complete ambulatory blood pressure measurements.

	All participants	Normal[a] BMI	Overweight[b]/Obese[c]
24-Hour ambulatory blood pressure (mm Hg)			
SystolicBP (min-max)	118.6±14.9 (88.23-180.12)	114.9±12.3 (88.23-167.73)	121.6±16.1 (94.16-180.12) *
Diastolic BP (min-max)	72.2±8.5 (52.93-116.22)	71.3±9.1 (55.14-102.52)	74.4±10.6 (52.93-116.22) *
Females' 24-Hour ambulatory blood pressure (mmHg)			
Systolic BP (min-max)	116.9±98.1 (88.23-180.12)	109.6±9.8 (88.23-143.70)	116.6±15.1 (100.16-168.555) *
Diastolic BP (min-max)	71.9 ±9.9 (52.93-116.22)	68.4±7.3 (55.14-87.95)	71.3±9.9* (57.27-110.40)
Males' 24-Hour ambulatory blood pressure (mmHg)			
Systolic BP (min-max)	123.2±18.1 (99.27-168.55)	119.8±12.15 (99.27-167.73)	125.4±15.7 (94.16-180.12) *
Diastolic BP (min-max)	64.4±11.6 (56.56-110.41)	63.2±11.1 (56.56-102.53)	66.8±12.6 (52.93-116.22) *

BMI, body mass index; BP, Blood Pressure. [a]Normal BMI is defined as < 25 kg/m^2, [b]overweight is defined as BMI ≥ 25 < 30 kg/m^2 and obese is defined as BMI ≥ 30 kg/m^2. Data is presented as mean ± SD. * P value ≤ 0.05 vs normal BMI. min-max = minimum and maximum values

3.5.3. Relationship between 24-hour urinary sodium excretion and 24-hour ambulatory BP stratified according to gender and BMI status.

In table 3.3, there was no significant relationship between 24-hour Na^+ excretion and BP in the total sample but when participants were stratified according to BMI status, the relationship reached statistical significance in the normal weight individuals (p=0.01, SBP; p=0.019, DBP) even though it remained insignificant in the overweight/obese participants (p=0.24, SBP; p=0.25, DBP). When participants were divided according to gender, there was a significant relationship in urinary Na^+ excretion and BP in the total sample and in the normal weight males but not in overweight males. There was no relationship between 24-hour urine excretion and BP irrespective of BMI status observed in the female group of individuals.

Table 3.3 Relationship between 24-hour urinary sodium excretion and 24-hour ambulatory BP stratified according to gender and BMI status

	All participants			Normal[a] BMI			Overweight[b]/Obese[c]		
	Partial r^2	95% CI	P value	Partial r^2	95% CI	P value	Partial r^2	95% CI	P Value
Total population									
SBP24 (mm Hg)	0.08	-0.01 to 0.16	0.0872	0.11	0.02 to 0.19	0.0146*	0.06	-0.04 to 0.17	0.2448
DBP24 (mm Hg)	0.06	0.02 to 0.14	0.1469	0.10	0.01 to 0.18	0.0193*	0.07	-0.04 to 0.17	0.2551
Females' 24-Hour ambulatory blood pressure (mm Hg)									
SBP24 (mm Hg)	0.05	-0.06 to 0.15	0.4095	0.07	-0.14 to 0.29	0.5016	0.08	-0.05 to 0.21	0.2058
DBP24 (mm Hg)	0.07	0.03 to 0.18	0.1747	0.06	-0.14 to 0.29	0.5016	0.09	-0.02 to 0.22	0.1281
Male's 24-Hour ambulatory blood pressure (mm Hg)									
SBP24 (mm Hg)	0.23	0.02 to 0.40	0.0252*	0.25	0.05 to 0.43	0.0122*	0.01	-0.2 to 0.19	0.9091
DBP24 (mm Hg)	0.21	0.02 to 0.40	0.0299*	0.29	0.10 to 0.47	0.0030*	0.01	-0.2 to 0.21	0.9751

CI, confidence intervals; SBP24, 24-hour SBP; DBP24, 24-hour DBP. [a]Normal BMI is defined as < 25 kg/m^2, boverweight is defined as BMI ≥ 25 <30 kg/m^2 and cobese is defined as BMI ≥ 30 kg/m^2. Adjustments were made for age, gender (in the total population), BMI (as a continuous variable), hypertension, diabetes, smoking and alcohol intake. * P<0.05 and **P<0.01 vs normal BMI

3.5.4. Comparison of the slopes of the relationship between 24–hour urinary sodium excretion and BP in the total population.

When participants were divided according to gender, there was a significant association between urinary sodium excretion rate and BP in the total sample of males and in the normal–weight males but not in overweight men. In females, there was no relationship between 24–hour urine excretion and BP, irrespective of BMI status. Figure 3.1 compares the slopes of the relationship between 24–hour urinary sodium excretion and 24-hour ambulatory BP in the total population. The slope (β–coefficient) of the normal–BMI participants was significantly higher than that of the overweight/obese group.

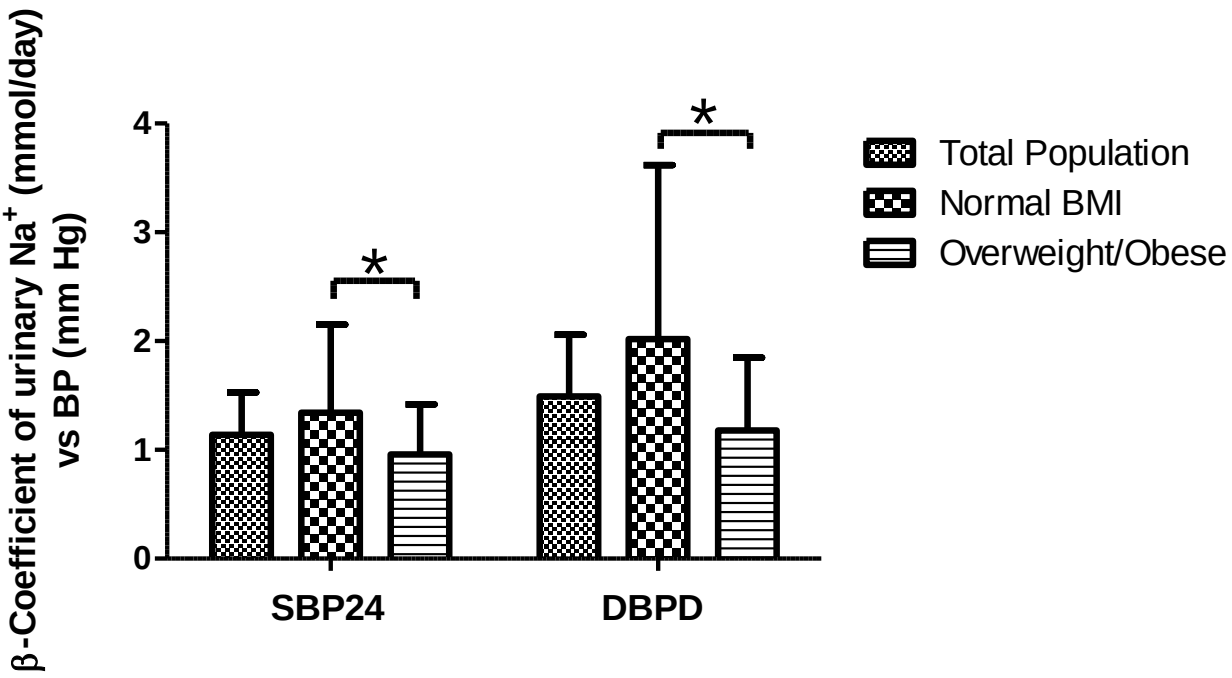


Figure 3.1: Multi–adjusted slopes (β–coefficients) of 24–hour urinary sodium excretion versus 24–hour systolic and diastolic blood pressure in the total sample, normal–weight and overweight/obese participants. Adjustments were made for age, gender, smoking, alcohol intake, diabetes and hypertension. BMI, body mass index; BP, blood pressure; SBP24, 24–hour systolic blood pressure; DBP24, 24–hour systolic blood pressure.

3.6. Discussion

To determine the masking effects of an increased BMI, I first investigated the relationship between 24-hour urinary Na^+ excretion and 24 hour ambulatory BP in the total population sample. Consistent with previous studies in this population (Millen et al., 2013; Mitchel et al., 2015; Davy et al., 2015) and in other population groups (Staessen et al., 1993; Shin et al., 2014) there was no relationship. In spite of the inconclusive results in this population, the relationship between Na^+ level and BP has been well established in a number of studies (Dyer et al., 1994; He et al., 2005; Suckling et al., 2012; Ndanuko et al., 2017), therefore these findings require thorough scrutiny. The limitations of previous studies conducted in this population could be that they did not take into consideration the high prevalence of obesity. So, in order to account for the high incidence of obesity, I stratified participants according to BMI status.

In a multivariate regression analysis, there was a statistically significant relationship between 24-hour urinary Na^+ excretion and both 24-hour ambulatory systolic and diastolic BP. However, Na^+ level was not related to BP in the overweight/obese group. This difference was further demonstrated when the slopes of this relationship were compared between the two groups. The β-coefficients of the normal-BMI participants were significantly higher than those of the overweight/obese groups. This is indicative of a stronger relationship in the normal-weight group compared to the overweight/obese individuals. The differences in the relationship between the two groups are indicative of the masking effects of obesity. These masking effects were further confirmed when the participants were divided according to gender. In males, the relationship was present in the total sample of males and in the lean group but not in the overweight/obese group. In the females, no relationship was observed irrespective of BMI status. The gender differences are due to dissimilarities in the degree of obesity in the two groups. In males the average BMI was 24.9 ± 5.0 kg/m2 and only 47% were overweight or obese. Likewise, the average BMI in females was 31.5 ± 8.1 kg/m2 with 75% of the females in the overweight/obese category. Due to the lower BMI and lower proportion of overweight/obese males, the relationship between Na^+ and BP was not masked. On the other hand, the significantly higher BMI and higher proportion of overweight/obese females resulted in the masking of the relationship in the female group. Since females made up 63% of the total population sample, the masking effects of this female group impacted the whole population sample.

Mechanisms responsible for the masking effects seem to be mediated by leptin. Plasma renin and insulin levels were similar between the different groups, but leptin was significantly higher in the overweight/obese group, especially in females. This could account for the significantly lower urinary Na^+ excretion rate in the overweight/obese females compared to normal-weight females. As explained earlier, females made up 63% of this population and 75% of them were overweight or obese, therefore they were responsible for the significantly lower urinary Na^+ excretion rate (106 mmol/day) in this population compared to other population groups. In a study conducted in 45 countries with a total of 69 011 participants, the average 24-hour urinary Na+ excretion rate was 159.4 mmol/day (Mc Carron et al., 2013). In a Chinese study, the average 24-hour urinary Na+ excretion was 235 mmol/day (Yan et al., 2015), which is more than double that of our population. A low Na^+ excretion rate of 104 mmol/day was observed in the trials of the Hypertension Prevention Collaborative Research Group (TOHP, 1997). However, in this study, the participants were on a low-Na^+ diet. The relatively low urinary Na^+ excretion rate is unique to our population sample and it may account for the leptin-mediated masking effects. The significantly higher leptin concentrations in the overweight/obese groups, especially in females where it was more than three times higher than that of the overweight/obese males, mediated the masking effects. Normally Na+ intake results in a slight increase in BP due to volume expansion. This causes an increased Na+ excretion rate through pressure natriuresis. A steady state is reached where Na^+ intake is equal to Na+ excretion. However, in the presence of high leptin concentrations the steady state is not achieved. Although leptin stimulates the sympathetic nervous system, it also increases nitric oxide concentration (Jerzy, 2011). Therefore, the pressor effects of the sympathetic nervous system are counteracted by the depressor effects of nitric oxide. Consequently, there is no net change in BP. Without an increase in BP, pressure natriuresis is suppressed, resulting in a reduced urinary Na^+ excretion rate. This explains the significantly lower urinary Na^+ excretion rates in overweight/obese females and in the total population sample since females constituted 63% of this sample. The low urinary Na^+ concentration leads to a narrow range of urinary Na+ concentration. As a consequence, the relationship between 24-hour urinary Na^+ excretion and BP is blunted. The masking of a relationship due to low concentrations is called a regression dilution (Yan et al., 2015). To the best of our knowledge, this is the first study to show that increased BMI masks the relationship between 24-hour urinary Na^+ excretion and 24-hour ambulatory BP, and that leptin mediates the mechanisms responsible for the masking effect.

Potential limitations of our study are as follows; firstly, I collected once-off 24-hour urine samples, and this does not account for the daily variation in dietary Na^+ intake. Although 24-hour urinary Na^+ excretion is an acceptable index of dietary Na^+ intake, the once-off 24-hour urine sample does not

take into account the within- and between person Na^+ intake variations. Secondly, the covariates such as alcohol intake and smoking were obtained through a self-reported questionnaire and therefore may not have been accurate. Thirdly, we did not use a dietary recall questionnaire to assess Na+ intake hence we did not use urinary sodium excretion as an index of dietary sodium intake in this study. This could have been a useful tool for assessing the accuracy of 24-hour urinary Na+ excretion as an index of dietary Na^+ intake. Finally, this was a cross-sectional study, therefore conclusions regarding cause and effect must be drawn with caution.

3.7. Conclusions

In conclusion, this study indicates that urinary sodium excretion influenced BP in this population, however this relationship could only be observed in lean participants, since increased BMI masked this relationship. Body mass index only was used as an index of obesity in this thesis and not waist circumference or waist-to-hip ratio. It has been shown in some studies that waist circumference is a better predictor of adverse cardiovascular outcomes than BMI (Lofgren *et al*., 2004); none the less, BMI is widely used in epidemiological studies to define obesity at population level (Mahadevan and Ali, 2016). These data suggest that future studies must take into account the high prevalence of obesity in this population before any conclusions are drawn on the relationship between urinary sodium excretion and BP.

CHAPTER 4

The impact of obesity on the relationship between urinary sodium excretion and arterial stiffness in the African descent population.

4.1 Introduction

Increased arterial stiffness as measured by carotid femoral pulse wave velocity (CFPWV) is an independent predictor of cardiovascular morbidity and mortality (Laurent *et al.,* 2001; Meaume *et al.,* 2001). Stiffening of the arteries occurs as an age-related outcome (Quinn *et al*., 2012). With increasing age, the elasticity of the arteries loses compliance (Franklin, 2005). A decline in elasticity of the arteries results in increased pulse pressure, left ventricular hypertrophy, sub-endothelial ischemia, vessel endothelial dysfunction and cardiac fibrosis (Quinn *et al*., 2012).

Arterial stiffness is one of the signs of vascular ageing and it has been associated with several cardiovascular target organ damage (Lacolley et al., 2002; Benetos *et al.,* 2002; Lebrun *et al.,* 2002). Different studies have postulated that high blood pressure and ageing are the main determinants of arterial stiffness (Avolio *et al.,* 1983; Benetos *et al.,* 2002; Lebrun *et al.,* 2002). Other factors such as obesity and high urinary sodium excretion have been shown to be associated with arterial stiffness (Lacolley et al., 2002; Blacher et al., 1997; Gates et al., 2004; Bagrov et al., 2004; Alecu *et al.,* 2006; Ketel *et al.,* 2010). The reports on the relationship between urinary sodium excretion and arterial stiffness have been conflicting. Some studies have shown an independent association between urinary sodium excretion and arterial stiffness (Han *et al*., 2017; Siriopol *et al*., 2018; Strauss *et al*., 2018; Baldo *et al*., 2019) while another has shown no relationship (Triantafyllou et al., 2018).

Additionally, whether BMI can modify the relationship between urinary sodium excretion and arterial stiffness in this population is currently unknown. Because of the high prevalence of obesity in this population and because I showed that obesity masked the relationship between urinary sodium excretion and BP, I decided to investigate a possible impact of obesity on the relationship between urinary sodium excretion and arterial stiffness. To my knowledge, there are currently no studies conducted in black South Africans to investigate the relationship between Na^+ and both BP and PWV and to determine if a risk factor like obesity could possibly have an impact on this relationship.

I therefore, examined the relationship between urinary Na^+ excretion and PWV stratified by BMI status.

4.2 Aim

To determine if obesity, as defined by an increased BMI is associated with the relationship between urinary sodium excretion and pulse wave velocity.

4.3 Objectives

4.3.1. The objective of this cross-sectional population study was to determine the relationship between 24-hour urinary sodium excretion and pulse wave velocity.

4.3.2 To investigate the possible impact of an increased BMI on the relationship between 24-hour urinary sodium excretion and pulse wave velocity.

4.4 Methods

4.4.1. Study Population

Details about the study population are given in chapter 2 of this thesis. Participants were stratified according to BMI status, according to the WHO standards. Of the 750 participants, 547 (345 females and 202 males) fulfilled the quality control requirements as stipulated in chapter 2 and previous publications (Maseko *et al*., 2006, Redelinghuys *et al*., 2010).

4.4.2. Procedures

Details about ambulatory blood pressure measurements, conventional blood pressure measurements, blood sample collection, anthropometric measurements and measurement of carotid femoral pulse wave velocity (CFPWV) are provided in the methods chapter (chapter 2) of this thesis.

4.4.3 Statistical analysis

Data management and statistical analysis was performed using the SAS software version 9.3 (The SAS Institute Inc., Cary, North Carolina, USA). The distribution of data was tested for normality

Shapiro-Wilk statistic and $\chi 2$ for categorical variables. Descriptive statistics were presented as means and standard deviation (±SD). The coefficient of determination (r^2) was determined by correlating a 24-hour urinary Na+ excretion with BP and PWV. Multi adjusted slopes (β-coefficients) of 24-hour urinary Na+ excretion versus 24-hour systolic and diastolic blood pressure and PWV in the total sample, normal BMI and overweight/obese individuals. Adjustments were made for age, smoking, alcohol intake, diabetes and hypertension. A p-value of less than 0.05 was considered statistically significant.

4.5 Results

4.5.1. Demographic and clinical characteristics

Table 5.1 summarizes demographic, anthropometric, haemodynamic and general clinical characteristics of the participants in the present study, these characteristics include age, BMI, urinary Na+ excretion and PWV. The study comprised of males and females with a mean age of 45.3±18.5 SD. Among the participants that gave consent to partake in the study 547 individuals had all the relevant biochemical and clinical measurements complete. The mean BMI was 31.5±8.5 for females and 24.9±5.0 in males. Thirty-five percent of the sample population was hypertensive, 24% consumed alcohol regularly, 15% smoked, and 14% had diabetes mellitus. There were significant differences for age, BMI, Na^+ and PWV between normal BMI and overweight/obesity individuals.

Table 4.1 Demographic and clinical characteristics according to BMI status and gender.

	All participants	Normal BMI[a]	Overweight[b]/Obese[c]
Number	547	193	354
Age (years)	45.3 ± 18.5	36.3 ± 18.3	50.4±15.2
BMI (kg/m^2)	29.1±7.8	21.6 ± 2.1	34.4±1.4*
BMI females (kg/m^2)	31.5±8.1	21.9±2.2	34.6±6.7*
BMI males (kg/m^2)	24.9±5.0	21.2±2.0	29.1±4.1*
Female (%)	63	25	75
Smokers (%)	15.2	26.4	9.1
Alcohol use (%)	23.6	33.1	18.0
Diabetics (%)	14.3	7.2	16.9
Hypertensive (%)	35.1	17.6	43.8
Na^+	105.6±78.4	108.9±89.8	103.8±72.1
PWV (m/s)	6.5±2.7	5.9±2.3	7.0±3.0*

BMI = body mass index, PWV = pulse wave velocity. Data is presented as mean ± SD or percentage. Na^+, 24-hour urinary Na+ excretion; PWV, pulse wave velocity. aNormal BMI is defined as a BMI < 25kg.m-2. Overweight is defined as BMI ≥ 25 <30 and overweight is defined as BMI ≥ 30

4.5.2. Twenty four-hour Na+ excretion and PWV stratified according to gender and BMI status

Pulse wave velocity and Na^+ were further analysed. Sodium excretion was significantly lower in overweight/Obese females (99.5±68.86) compared to normal BMI females (121.5±99.4). Pulse wave velocity remained significantly higher in the overweight/obese group compared to the normal BMI.

Table 4.2 Twenty four-hour Na+ excretion and PWV stratified according to gender and BMI status.

	All participants	Normal[a] BMI	Overweight[b]/Obese[c]
Total sample	**547**	**193**	**354**
Na^+ (mmol/day)	105.6±78.4	108.9±89.8	103.8±72.1
PWV (m/s)	6.5±2.7	5.9±2.3	7.0±3.0*
Females	**345**	**87**	**258**
Na^+ (mmol/day)	105.0±80.6	121.5±99.4	99.5±68.6*
PWV (m/s)	6.6 ±2.5	5.5±1.5#	7.0±2.6*
Males	**202**	**106**	**96**
Na^+ (mmol/day)	106.5±74.7	115.7±71.1	98.5±77.9
PWV (m/s)	6.9±3.4	6.2±2.9	7.6±3.6*

BMI, body mass index; PWV = pulse wave velocity. [a]Normal BMI is defined as < 25 kg/m^2, [b]overweight is defined as BMI ≥ 25 < 30 kg/m^2 and obese is defined as BMI ≥ 30 kg/m^2. Data is presented as mean ± SD. # $p<0.05$ for PWV between genders. * $p<0.05$ for comparisons between normal BMI and overweight/obese groups.

4.5.3 Relationship between 24-hour Na+ excretion and pulse wave velocity in participants stratified according to BMI status and gender

Table 4.3 shows the relationship between 24-hour urinary Na+ excretion and PWV. After adjusting for age, BMI, smoking, drinking, hypertension and diabetes, a negative relationship was observed between PWV and 24-hour Na^+ excretion in females with normal BMI. There was however no relationship between 24-hour urinary Na^+ excretion and PWV in males irrespective of obesity status.

Table 4.3 Multivariate relationships between 24-hour urinary Na^+ excretion and PWV in participants stratified according to BMI and gender.

	All participants			Normal[a] BMI			Overweight[b]/Obese[c]		
	Partial r2	95% CI	P value	Partial r2	95% CI	P value	Partial r2	95% CI	P value
Total Population									
	-0.07	-0.16 to -0.02	0.1254	-0.18	-0.33 to -0.38	0.0142*	-0.01	-0.12 to 0.11	0.9301
Females									
	-0.15	-0.26 to -0.03	0.0155*	-0.41	-0.59 to- 0.20	0.0002*	-0.06	-0.20 to 0.08	0.3940
Males									
	0.05	-0.10 to 0.19	0.5476	0.01	-0.20 to 0.21	0.9547	-0.07	-0.16 to 0.29	0.5374

Abbreviations: CI, confidence intervals; * significant p value. Adjusted for age, BMI, smoking, drinking, hypertension and diabetes. Normal[a] BMI is defined as a BMI < 25kg.m-2. Overweight is defined as BMI ≥ 25 <30 and overweight is defined as BMI ≥ 30

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4.5.4. Comparison of the slopes of the relationship between 24–hour urinary sodium excretion and pulse wave velocity in the total population

Figure 4.1 compares the slopes of the relationship between 24–hour urinary sodium excretion and PWV in the total population. The slope (β–coefficient) of the normal–BMI participants was significantly higher than that of the overweight/obese group.

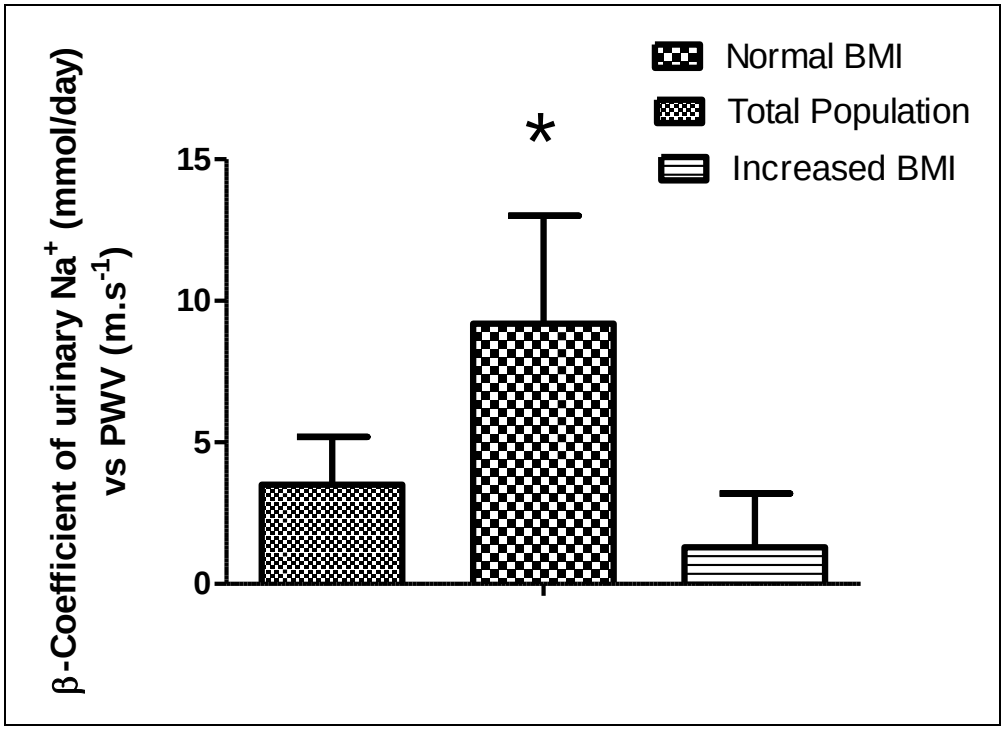


Figure 4.1: Comparison of the slopes (β-coefficients) of the relationship between urinary Na^+ excretion vs PWV in total sample, participants with normal BMI and those with increased BMI. * $p < 0.05$.

4.5.5 Comparison of hormone levels in normal BMI and overweight/Obese groups

Table 4.4 summarizes differences in hormone levels in normal BMI and overweight/Obese groups. There were no significant differences in insulin, renin and aldosterone across all the groups. Aldosterone/Renin ratio was significantly higher in the overweight/obese (27.9±44.6) group compared to normal BMI group (24.6±57.9) in the total population. This effect was also observed in females, however in males the ratio was significantly lower in the overweight/obese group (21.8±31.8) compared to the normal BMI group (30.4±73.7). Leptin was significantly higher in the overweight/obese individuals within all the groups.

Table 4.4 Plasma hormone levels stratified according to gender and BMI status

	Total sample			Males			Females		
	All	Normal	Over/Obese	All	Normal	Over/Obese	All	Normal	Over/Obese
ARR	26.8±49.6	24.6±57.9	27.9±44.6*	26.2±57.3	30.4 ±73.7	21.8±31.8*	27.1±44.6	17.6±28.4	30.2±48.3*
Leptin (ng/mL)	25.3±26.3	10.4±11.9	32.9±28.2*	8.2±9.5	4.4±7.4	11.8±9.8*	35.6 ±27.7	17.5±12.3	41.7±28.8*
Insulin (mmol/L)	13.2±16.2	9.7±12.4	14.9±17.6	12.8±17.9	8.1±10.3	18.1±22.6	13.4±15.3	11.8±14.4	13.9±15.5
Aldo (pg/mL)	195.9±164.3	184.0±156.8	202.0±167.8	198.1±161.2	184.6±161.5	213.3±159.9	194.7±166.2	183.2±151.2	198.2±170.
Renin (ng/mL)	40.9±89.6	44.7±102.8	39.0±81.9	45.7±95.5	47. 5±108.4	43.6±78.2	38.3±86.1	41.1±95.4	37.5 ±83.2

ARR = Aldosterone to Renin ratio, CI=confidence interval. Data is presented as mean ± SD%. * depicts significant difference in hormone levels vs normal BMI and overweight/obese group.

4.6. Discussion

In the present study, I demonstrate in 547 participants of African ancestry that 24-hour urinary sodium excretion (UNa) is an independent predictor of arterial stiffness as measured by carotid femoral pulse wave velocity (cfPWV) in normal weight individuals but not in overweight/obese individuals. We also show that obesity masks the relationship between UNa and cfPWV. In this cohort, normal weight males had a higher level of arterial stiffness as measured by cfPWV when compared with normal weight females.

There have been several studies that corroborate our findings with regards to the relationship between UNa and arterial stiffness. Han et al., 2017 demonstrated a strong relationship between UNa and arterial stiffness as measured using brachial ankle pulse wave velocity in 431 participants. Another study showed similar results (Safar et al., 2000). Additionally, a study by Strauss et al., 2018 showed in 693 black adults that urinary sodium excretion is strongly associated with large artery stiffness. On the other hand, Triantafyllou et al., 2018 failed to demonstrate a relationship between UNa and PWV. The reason for the difference in observation might be that this study was conducted in hypertensive individuals. Another study showed similar results to this one (Du Cailar et al., 2002). This study was however methodologically limited and was carried out in an even smaller population of 49 individuals. When i divided the population into normal weight and overweight/obese groups, the relationship between UNa and PWV was still maintained in the normal weight group but disappeared in the overweight/obese group irrespective of gender. This suggests a masking effect of obesity on this relationship. The mechanism responsible for this masking effect is currently unknown.

I show that the overweight/obese group had a lower urinary sodium excretion when compared with the normal weight group suggesting a relative sodium retention. In this cohort, I believe that this relative sodium retention observed in the overweight/obese group may have been mediated by Leptin. The overweight/obese group has significantly higher levels of leptin when compared with the normal weight group. It has been shown that leptin causes sodium retention (Bravo et al., 2006). The lower UNa in the overweight/obese group may have also been indirectly caused by the elevated BMI. It has been shown that obesity can trigger sodium retention by causing insulin resistance (Rocchini et al., 1989), enhanced activity of the renin-angiotensin system (Tuck et al., 1981) and stimulation of the sympathetic nervous system (Landsberg et al., 1989).

It has been shown that sodium leads to an increase in vascular smooth muscle tone and hypertrophy as well as thickening of the media layer and excessive production of collagen (Gu et al., 1998; Safar et al., 2000; Partovian et al., 1998) all of which contribute to a reduction in arterial compliance. Sodium can also lead to endothelial dysfunction by reducing nitric oxide synthase (NOS) production of nitric oxide, stimulating NOS inhibitor activity and production of reactive oxygen species (ROS) which is a vital contributor to arterial stiffening (Bagrov et al., 2004). A reduction in NOS production and stimulation of ROS activates matrix metalloproteinases, which controls the structural proteins of the extracellular matrix like collagen and elastin (Harvey at al., 2016). Activation of these MMPs leads to the production of excessive amounts of TGF β-1 which leads to fragmentation of elastin fibers and accumulation of collagen fibers reducing the elastin/collagen ratio leading to arterial stiffness (Salvi et al., 2018; Wang et al., 2006).

I show that the overweight/obese group had a higher PWV when compared with the normal weight group in the general population. This trend was still maintained when we divided the population by gender. Our findings are similar to that of Pal et al., 2012 where they found in 26 Caucasian women with an average age of 33 years that obese participants had higher PWV when compared to lean participants. They also observed a strong correlation between obesity and PWV. Another study by Hudson et al., 2017 showed similar results. They reported a positive correlation between obesity and PWV in a cross-sectional study of 174 adolescents between the ages of 12 and 19. This indicates that the relationship between obesity and PWV might be age independent. Different mechanisms have been proposed for obesity related arterial stiffness. Factors such as increased lipolytic activity of visceral adipocytes, increased cytokine levels, increased sympathetic activity, increased insulin, and an imbalance between vasoconstricting and vasodilatory agents have been observed in obese participants and have been shown to play important roles in arterial stiffening (Eckel et al., 2002). In this cohort, leptin may also have been directly responsible for the higher PWV in the obese group. It has been shown that hyperleptinemia is positively associated with central artery stiffness (Kuo et al., 2018). It has also been reported that leptin causes direct sympathetic stimulation, vascular smooth muscle dysregulation and increased oxidative stress; all of which can lead to arterial stiffness (Nasrallah et al., 2013; Oda et al., 2001; Bouloumie et al., 1999). Although I did not investigate the association between obesity and PWV, a higher BMI might have been directly responsible for the observed higher PWV in the overweight/obese group when compared with the normal weight group.

Another factor that might explain the difference in PWV between the two groups is age. In the present study, overweight/obese participants (although not significant) are older than the normal weight participants (50.4±15.2 vs 36.3±18.3). It has been shown that the prevalence of arterial stiffness rises with age (Alghathrif et al., 2013). The walls of large arteries lose distensibility and become thicker with age (Falkow et al., 1993) leading to an increase in PWV. Aging has also been associated with increased sympathetic activation (Barnes et al., 2014) and may lead to inflammation (Zubcevic et al., 2014). Inflammation has been shown to induce arterial stiffness (Johnson et al., 2001).

When I divided the population according to gender, I observed that males had higher PWV when compared to their female counterparts irrespective of BMI. This is consistent with the findings of Kim et al., 2014 where they specifically investigated gender differences in arterial stiffness in a cross-sectional study with 1,588 participants. They reported a higher aortic PWV and pulse pressure amplification in men when compared with women. There have however been controversial findings. A study by Mozos et al., 2017 found no significant gender differences in arterial stiffness. This study however had a smaller population sample of 147 participants hence the results should be considered as statistically weaker than ours and that of Kim et al., 2014. Alghathrif et al., 2013 even showed that males have a sharper increase in arterial stiffness with aging, causing them to have higher PWV when compared with age matched females in their 5^{th} decade. This gender difference in arterial stiffness has been attributed to differences in sex steroids (Ahimastos et al., 2003).

Additionally, I show a higher aldosterone-to-renin ratio in the obese group when compared with the non-obese group in the general population and amongst female participants despite no significant differences in the levels of renin or aldosterone. This suggests a renin-independent increase in aldosterone, is directly induced by obesity. Obesity has been linked to a higher level of aldosterone (Bochud *et al*., 2006). Adipose tissues secrete cytokines such as tumor necrosis factor (TNF) - α and interleukin 6 (IL-6) (Lastra *et al*., 2006) which have been shown to increase aldosterone secretion (Fujita 2010).

The current study is a cross sectional one, hence, cause-effect relationships cannot be established. Other factors like serum lipids which we did not measure in this study may have shed more light on the mechanisms behind obesity related arterial stiffness.

4.7 Conclusion

In a cohort of black Africans, obesity masks the relationship between urinary sodium excretion and PWV. There are gender differences in arterial stiffness in this population. In addition,

urinary sodium excretion is an independent predictor of arterial stiffness in normal weight individuals but not in overweight/obese individuals. Future studies should take into consideration the influence of obesity and gender on arterial stiffness.

CHAPTER 5

An inverse relationship between urinary sodium excretion and conventional blood pressure in normal weight individuals of African ancestry.

5.1 Introduction

Hypertension is the main cause of cardiovascular mortality worldwide (Mensah et al., 2014) with an increasing prevalence in a lot of sub-Saharan countries (Tu et al., 2008; Ataklte et al 2015). With an estimated population of 54 million (STATSSA, 2018), South Africa is profoundly affected by the escalating prevalence of hypertension and its complications (Swanepoel et al., 2016). An estimated 51% of hypertensive individuals and 26 % of normotensives are salt sensitive. Salt sensitive normotensives are attributed to have similar mortality rates as hypertensive individuals (Imaizumi et al., 2016). Black Africans have been shown to be salt sensitive (Scott et al., 2011; Parmer et al., 1994; Falkner et al., 1990; Dustan and Kirk, 1988; Sowers et al., 1988; Weinberger et al., 1982), meaning that they have an abnormal response to salt loading that makes them retain salt. It has been shown that urinary sodium excretion is positively associated with blood pressure (Mente et al., 2014; Jackson et al., 2014). This relationship has been widely supported by several other studies (Santos et al., 2017; Jabonski et al., 2013; Celermayer and Neal., 2013; He et al., 2011; He and MacGregor., 2011; Koga et al., 2008; Frohlich., 2007; Isaacson et al., 1963; Intersalt Cooperative research group, 1988), however it must be noted that there have also been controversial findings suggesting that low salt intakes are associated with hypertension and its cardiovascular complications (Moore, et al, 2017; Oparil, 2014; O'Donnel et al., 2013; Stolarz-Skryzypek et al., 2011; Thomas et al., 2011; Cohen et al., 2008; Alderman et al., 1998; Alderman et al., 1995). A J shaped curve association between sodium intake and cardiovascular disease has been found. Data from a cohort study suggest that estimates of salt (both low and high) are associated with debilitating cardiovascular morbidity. Estimates indicate that high (>7g/day) and low (<3 g/day) sodium excretion are associated with mortality (Lee *et al*., 2015).

A pronounced renal sodium reabsorption in the black African population has been widely reported in a number of studies (Bankir *et al*., 2008; Palaccios *et al*., 2004; Luft *et al*., 1979; Luft *et al*., 1977). It appears to be related to lower plasma renin activity relative to plasma aldosterone concentration. In addition, the "African gene" theory argues that Na^+ retention is a hereditable feature in populations of African descent. This view was first noted by researchers in 1960-70s (Luft *et al*., 1979; Luft *et al*., 1977). They hypothesized that a genetic mutation linked to pressure-natriuresis resulted in salt sensitive hypertension. Therefore, estimation of dietary salt intake using 24-hour urinary sodium excretion might be grossly inaccurate in this population and may not truly reflect the relationship between dietary sodium intake and blood pressure.

To my knowledge, there is no study that has sought to specifically evaluate the accuracy of determing Na^+ intake as an index of urinary Na^+ excretion in relation to BP in black people of African descent. Therefore, in this study, I will statistically determine the relationship between 24-hour urinary Na^+ excretion and conventional systolic and diastolic BP using Prediction bands. This relationship will be used as a measure of renal sodium handling and blood pressure in an African cohort. As I have shown in the previous chapter that obesity can mask the relationship between urinary sodium excretion and blood pressure, I will determine the relationship between urinary sodium excretion and normal blood pressure in normal weight individuals of African ancestry.

5.2. Aim

The aim of this study was to determine the relationship between 24-hour urinary Na^+ excretion and conventional systolic and diastolic BP.

5.3. Objectives

The objective of this cross-sectional population study are as follows:

5.3.1 To determine the relationship between 24-hour urinary sodium excretion and conventional blood pressure in normal BMI individuals of African ancestry.

5.4. Methods

5.4.1. Study Population

Details about the study population are given in chapter 2 of this thesis. Of the 750 participants, I excluded 514 participants who were overweight/obese and who had not fulfilled the quality control requirements as stipulated in chapter 2 as in previous publications (Maseko et al., 2006; Redelinghuys et al., 2010).

5.4.2. Procedures

Details about ambulatory blood pressure measurements, conventional blood pressure measurements, blood sample collection, urine sample collection and anthropometric measurements are provided in the methods chapter (chapter 2) of this thesis.

5.4.3. Statistical analysis

Data management and statistical analysis was performed using the SAS software version 9.3 (The SAS Institute Inc., Cary, North Carolina, USA). The mean of the five readings measured when taking conventional BP was determined as average SBP and DBP. Descriptive statistics were presented as means and standard deviation (±SD). The distribution of data was tested for normality Shapiro-Wilk statistic and $\chi 2$ for categorical variables. Adjusted multiple linear regression analysis was used to determine the relationship between conventional blood pressure and 24-hour urinary Na^+ excretion. A p value <0.05 was considered statistically significant.

5.5. Results

5.5.1. General characteristics of the participants

Table 5.1 summarises demographic, anthropometric, haemodynamic and general clinical characteristics of the participants in the present study. The study population had a mean age of 35.3±18.2 with hypertensive participants significantly older (61±19) than the normotensive group. The mean BMI was 21.5±2.2, moreover BMI was significantly higher in the hypertensive group compared to normotensive individuals. The mean conventional systolic BP was significantly higher in the hypertensive group (142.76) compared to normotensive group (119.71 mm Hg). furthermore, diastolic BP was significantly higher in the hypertensive group (86.99) than in the normotensive group (79.63 mm Hg)). Thirty-two percent of the population consumed alcohol regularly and 26% were smokers. The prevalence of diabetes mellitus was significantly higher in hypertensive individuals compared to normotensive. The mean 24-hour urinary Na+ excretion was 114 mmol/day. Twenty-four-hour Na+ excretion was significantly higher in the normotensive group compared to the hypertensive.

Table 5.1. General and clinical characteristics of the participants

	All participantss	Normotensive	Hypertensive
Number	236	205	31
Age (years)	35.3 ± 18.2	31.4 ± 14.8	60.7±18.5*
BMI ($kg.m^{-2}$)	21.5±2.2	21.3 ± 2.1	22.6±1.8*
Smokers (%)	26.3	23.7	2.5
Alcohol use (%)	31.8	27.5	4.2
Diabetics (%)	4.4	1.7	2.6*
Na^+ (mmol/day)	113.8±92.9	114.7±88.4	107.9±120.2
Systolic BP (mmHg)	122.7±20.12	119.71±17.83	142.76±23.04*
Diastolic BP (mmHg)	80.59±11.38	79.62±10.58	86.99±14.27*

BMI, body mass index, Na^+, sodium. Data is presented as mean ± SD or percentage. * P value <0.05 depicts significant difference between normotensive and hypertensive group.

5.5.2. Comparison of the mean systolic blood pressure and quintiles of 24-Hour urinary sodium excretion

Twenty four-hour urinary Na^+ excretion data was classified into five quintiles. The relationship between urinary Na^+ excretion with SBP and DBP was determined according to the five quintiles. The quintiles were presented as ranges including (<44, 44-68, 69-107, 108-172 and >172 mmol/day). The mean systolic BP was highest at the lowest quintile of urinary Na^+ excretion.

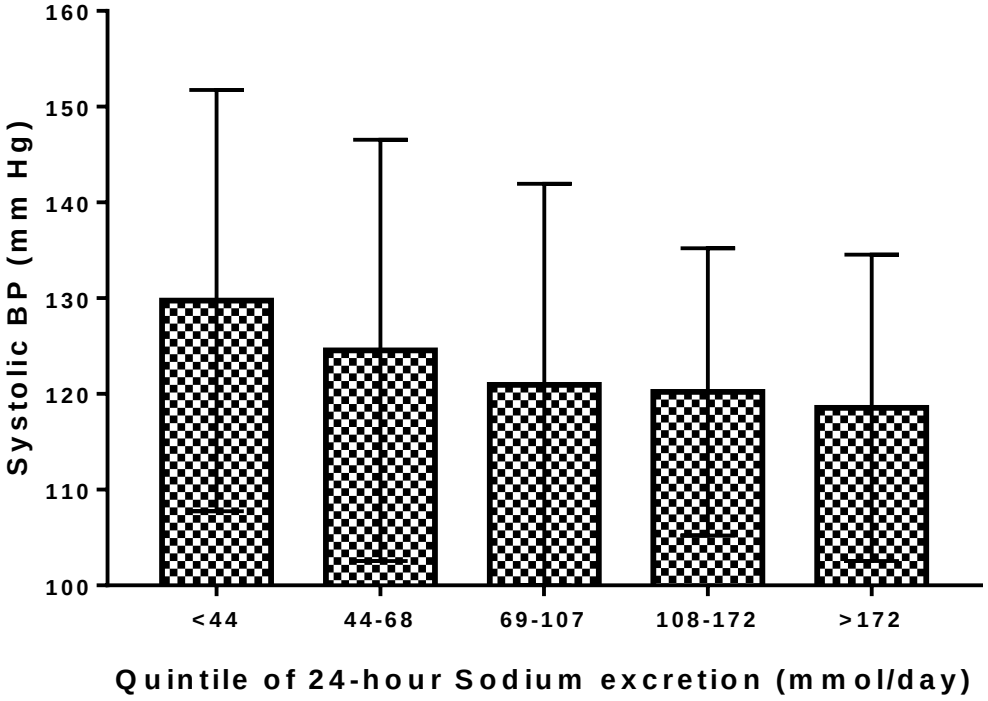


Figure 5.1: Comparison of the mean SBP and quintiles of 24-hour urinary Na^+ excretion, showing an increase in BP relative to low 24-hour urinary sodium excretion.

5.5.3. Relationship between 24-hour urinary sodium excretion and BP.

Multivariate regression shows significant inverse relationship between SBPc (r = 0.03; p=0.0082); DBPc (r =0.03; p=0.02) and 24-hour urinary Na^+ excretion.

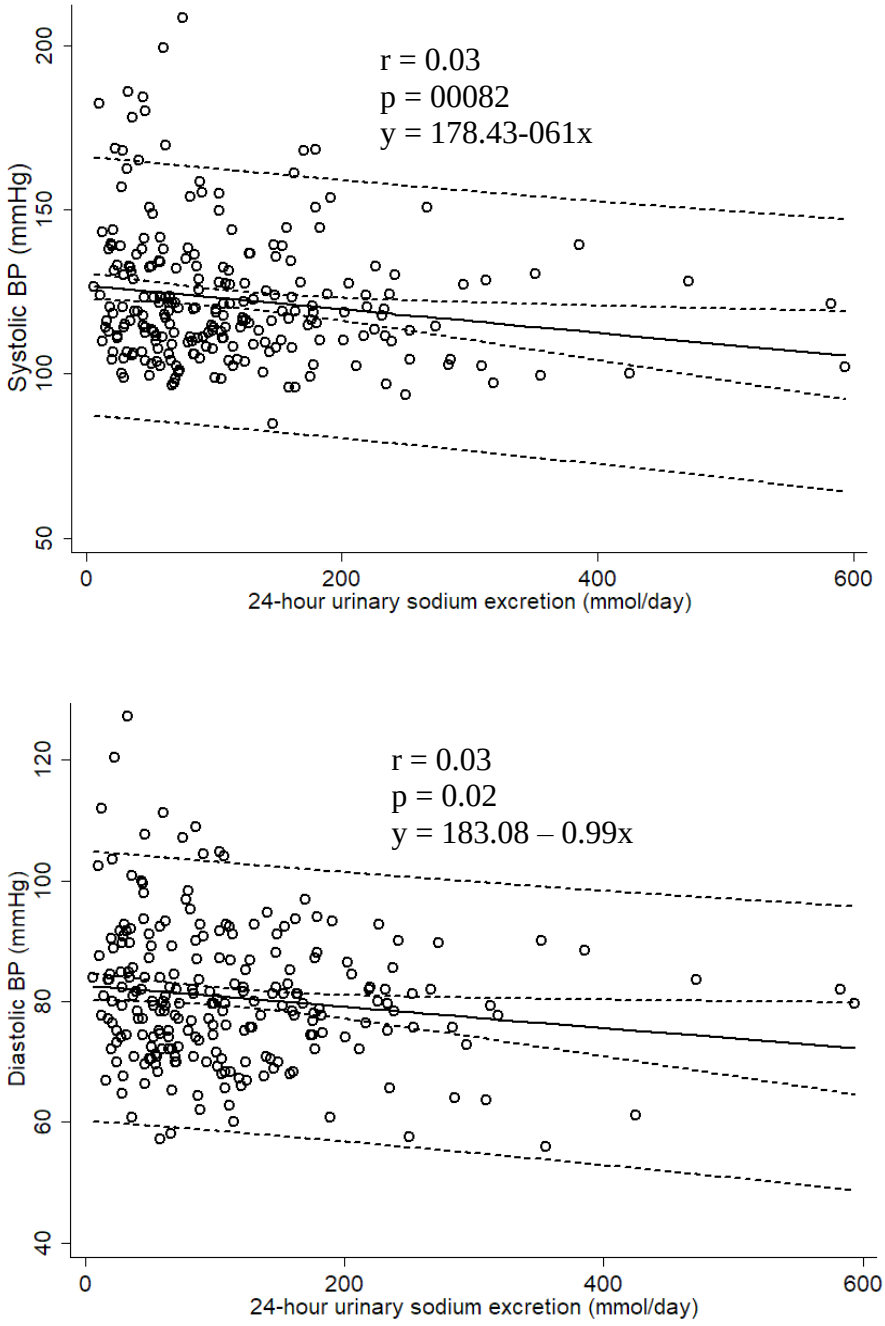


Figure 5.2: Adjusted (age, hypertension, diabetes, smoking, and alcohol intake) multiple linear regression between conventional systolic and diastolic blood pressure and 24-hour urinary Na^+ excretion. Depicted are the regression line, 95% confidence intervals and the 95% prediction bands for mean and individual values of conventional blood pressure and 24-hour urinary Na^+ excretion. The regression lines for systolic blood pressure are depicted in the top panel and diastolic blood pressure, lower panel.

5.5.4. Prevalence of of hypertension according to quintiles of 24-hour urinary Na^+ excretion.

The prevalence of HT was 20%, 15%, 9%, 12% and 11% according the five quintiles respectively.



Figure 5.3. Prevalence of of hypertension according to quintiles of 24-hour urinary Na^+ excretion.

5.6. Discussion

I investigated the relationship between 24-hour urinary sodium excretion (UNa) and conventional blood pressure in 236 normal weight individuals of black African ancestry. In this cross-sectional study, there was an inverse relationship between UNa and BP. Furthermore, there was U shaped relationship between the incidence of hypertension and UNa.

The results show that hypertensive participants are significantly older than their normotensive counterparts. This is not surprising as it has been shown that age is an independent determinant of BP and that BP increases with age (Mozaffanan et al., 2015). Ageing is associated with a progressive decrease in physiologic function in all organ systems (Franceschi at al., 2008). It has been shown that inflammation and the production of reactive oxygen species increase with age (Wei et al., 1992; Ershler et al., 1993; Dinh et al., 2014). The inflammation and oxidative stress have been strongly linked to vascular dysfunction (Briones et al., 2010; Wadley et al., 2013) which directly leads to an increase in systemic vascular resistance and hence blood pressure mainly because of an imbalance between vasodilatory and vasoconstrictor agents (Chrissobolis et al., 2011). Additionally, it has been shown that renal function declines with age, leading to an impairment in the ability to excrete salt load thereby causing salt retention and a consequent high blood pressure (Muhlberg et al., 1999). Since the older hypertensive group in this study has similar UNa compared to the younger normotensive group, it is unlikely that the difference in BP observed between the two groups is due to impaired renal function.

The relationship between UNa and BP has been determined in several studies and have provided varying results. Like the current study observations, Berglund et al., 1976 reported a negative association between UNa and BP, however this negative relationship was found only in 24 hypertensive participants in a cohort of 120 participants. A study by Welsh et al., 2019 reported a direct relationship between UNa and mean arterial pressure in 457 484 participants. Other studies have shown similar results (Lemogoum et al., 2018; Cooper et al., 1980; Oh et al., 2014; Mente et al., 2014; O'Donnell et al., 2014). On the other hand, a study by Moliterno et al., 2018 found no relationship between urinary sodium excretion and blood pressure in 149 participants of European descent. Other studies have shown similar results (Smith et al., 1988; Stamler et al., 2018)

A higher blood pressure has been attributed to a high salt intake as estimated by 24-hour urinary sodium excretion (Mente et al., 2014). Because of the inverse relationship between UNa and BP in our cohort, we suspect that there might be some level of sodium retention. Our cohort is known to be a salt sensitive one (Scott et al., 2011; Falkner et al 1990) and have been shown to retain sodium (Bankir et al., 2008; Palaccios et al., 2004; Luft et al., 1979). Salt sensitive

individuals respond differently to salt loading. During salt loading, the usual response of the sympathetic nervous system is to decrease plasma levels of noradrenaline and increase plasma dopamine levels. Noradrenaline is a known salt retainer while dopamine is known to facilitate sodium excretion (Ely et al., 1997). In salt sensitive individuals however, especially in Africans, this response to salt loading is abnormal and tends to decrease dopamine levels and does not significantly reduce noradrenaline levels leading to salt retention and possibly higher blood pressure (Campese et al., 1982). The above mechanism might be responsible for the inverse relationship seen in this study. It therefore makes it difficult to estimate sodium intake using 24-hour urinary sodium excretion in this cohort.

We also reported a mean daily urinary sodium excretion of 113.8 ±92.9 mmol/day in the general population. This value is much less than the global assessment values of 171.8 mmol/day (Huang et al., 206) and 161.8 mmol/day (Powles et al., 2013) based on 24-hour urinary excretion. This might additionally support our sodium retention theory.

As expected, we reported a reduction in the incidence of hypertension with higher UNa. However, at the 108-172 mmol/day quintile of urinary sodium excretion, the incidence of hypertension increased again producing roughly, a U-shaped curve. This is in complete contrast to studies that investigated the relationship between UNa and blood pressure (Mente et al., 2014; Khan et al., 1988; Jackson et al., 2014). The reversal of the relationship between UNa and the incidence of hypertension beyond 172mmol/day threshold might be due to a homeostatic reflex response to low plasma levels of sodium, secondary to high urinary sodium excretion. Low plasma sodium levels have been shown to trigger the renin angiotensin aldosterone system (RAAS). This leads to an increase in sodium retention and vascular tone which ultimately increases BP (Schweda et al., 2015; Graudal et al., 1998). This mechanism is also observed in salt restriction (Srinivasa et al., 2016). It has been shown that salt retention leads to an increase in BP by causing an expansion of plasma volume (Hamlyn et al., 1986; Villamil et al., 1982). This consequent increase in BP then leads to pressure natriuesis in order to restore normal ECF volume and subsequently reduce BP (Titze et al., 2005). This mechanism is also seen in impaired renal function when the kidneys cannot adequately excrete sodium (Guyton et al., 1969).

This study is a cross-sectional one and precludes conclusions concerning causal relationships. I did not measure potassium excretion in this study, and this might have shed light on the role of potassium in blood pressure regulation in this cohort. I also did not directly measure dietary sodium intake which could have confirmed our theory of sodium retention. In addition, an r-value of 0.03 depicts a weak association despite a significant p-value, hence, conclusions concerning clinical applicability of these results should be done with caution.

5.7 Conclusion

We conclude that in this cohort of black Africans, there is a negative relationship between UNa and BP. This relationship is probably mediated by salt-sensitivity and sodium retention making it difficult to estimate dietary sodium intake using 24-hour urinary sodium excretion. It will be of immense benefit to increase dietary potassium intake in this population as it has been shown that potassium has natriuretic effects (Aburto et al., 2013).

CHAPTER 6

Contextual Narrative and Conclusions

6.1 Introduction

In the chapters 3 to 5 of the present thesis, I provide comprehensive discussion of the findings of my research. In this chapter, I will however give a contextual narrative for the earlier chapters 3 to 5 and then discuss the possible clinical significance of my research as well as its limitations.

Global strategies and population-based intervention studies including Dietary Approaches to Stop Hypertension (DASH) have focused particularly on reduction of salt intake as a means of lowering blood pressure (BP) in a population (Davy *et al*., 2015; He *et al*., 2010; Charlton *et al*., 2005). Although the effects of salt on BP have not been clearly elucidated, nonetheless, its reduction has become a worldwide mandatory approach in preventing and managing hypertension (Karppanen and Mervaala, 2006; Charlton *et al*., 2013; Horn, 2015).

In light of the preceding chapters, a number of studies have associated an increased dietary salt intake with hypertension and target organ damage (Mattes *et al*., 1991; Graudal *et al*., 1998; Sacks *et al*., 2001; Wadner and MacGregor., 2002; Frohlich., 2007; Koga et al., 2008; He and MacGregor., 2011; Appel et al., 2011; Celermayer and Neal., 2013; Jabonski et al., 2013; Santos et al., 2017). On the contrary, other studies have suggested that lower dietary salt intake is associated with CVD mortality (Alderman *et al*., 1995; Alderman *et al*., 1998; Crohen *et al*., 2008; Thomas et al., 2011; Stolarz-Skryzypek *et al*., 2011; Oparil, 2014; Moore, *et al*, 2017). It is evident from these studies that inter-ethnic disparities, environmental and genetic factors exist among the different population groups, that preclude common scientific ground with regards to evaluating the effects of dietary salt intake on BP. In this regard, in populations of African descent, risk factors such as age, gender, salt sensitive hypertension and the high prevalence obesity are evident as common variables that impede direct independent cause and effect associations (Maseko *et al*., 2006, 2011, 2018; Redelinghuys *et al*., 2010; Scott *et al*., 2011; Mitchel *et al*., 2014).

In order to effectively monitor dietary salt intake, different methods that effectively estimate dietary salt intake daily exist. These methods include 24-hour dietary recalls, food frequency questionnaires, spot urine and 24-hour urinary Na^+ excretions (Charlton *et al*., 2005; Ortega *et al*., 2010; Zhang *et al*., 2014). Contradictions in the studies on the effects of dietary salt intake on BP are suggested to be attributed to the varying methods with which dietary salt intake was measured. Although, 24-hour urinary Na^+ is considered the gold standard in estimating Na^+ intake in a population (Ortega *et al*., 2010; Zhang *et al*., 2014) emerging studies are now relying on using estimation and prediction models to determine habitual 24-hour urinary sodium

excretion. This is attributed to variations in reporting of salt intakes in large scale population-based studies.

Diagnosing hypertension through ambulatory blood pressure measurement is recognised as a better method for diagnosing and managing hypertension and its related cardiovascular complications. It is already implemented in some European countries for these purposes (Hinderliter et al., 2013). It is known that BP varies throughout the day hence its quantification over 24 hours provides useful information on the haemodynamic burden and cardiovascular prognosis (Hinderliter et al., 2013). The average ABP is more predictive of target end organ damage and cardiovascular outcomes than conventional BP. However, it is not widely used in routine clinical protocols due to high financial cost. In the present thesis, I highlighted the role of obesity in blunting the relationship between dietary salt intake and BP. Therefore, the following conclusions are deduced from the study findings.

6.2 The impact of obesity on the relationship between urinary sodium excretion and blood pressure.

The findings in the present thesis suggest that in people of African ancestry, obesity masks the relationship between urinary sodium excretion and BP, and therefore, it is imperative to consider stratifying participants according to BMI status whenever drawing associations regarding BP. This study also highlighted that increased BMI is not the only predictor of hypertension and its related CVD.

6.3 The impact of obesity on the relationship between urinary sodium excretion and arterial stiffness in the African descent population.

In the present thesis, I have demonstrated that in addition to the masking effects of obesity on the relationship between 24-Hour urinary sodium excretion and BP in chapter 3, obesity also masked the relationship between 24-hour urinary Na+ excretion and PWV (an index of arterial stiffness) in females. There was however no relationship between urinary sodium excretion and PWV in males. This suggests that the relationship between urinary sodium excretion and PWV might be gender specific and future studies should take into consideration the impact of gender and BMI on arterial stiffness. Results in this chapter also showed that lower urinary sodium

excretion rates translated into increased arterial stiffness in the overweight/obese group. This suggests that sodium rentention in this salt sensitive population might lead to higher arterial stiffness amongst obese indivisuals. The results of this study have serious implications on the impact of sodium on large artery pathology. Moreover, since these relationships are masked by indices of general (BMI) obesity, they pose a serious danger because they are not easily detected.

6.4 An inverse relationship between urinary sodium excretion and conventional blood pressure in the African descent population.

The findings reveal a negative relationship between 24-hour urinary sodium excretion and blood pressure which is in complete contrast to previously established relationships. This negative relationship might be due to salt sensitivity which leads to salt retention upon salt loading. This might mean that, in this known salt sensitive population, a lower rate of urinary sodium excretion might mean more sodium retention which will lead to a higher BP. These results have far reaching implications, as this negative relationship means that urinary sodium excretion cannot be used to estimate dietary sodium intake in this population. Future research is needed to determine the mechanisms that govern the relationship between urinary sodium excretion and blood pressure in this population. Therefore, these findings also suggest that other methods should be used to estimate dietary sodium intake in this population.

6.5 Limitations

The study highlights a few limitations. The sample size is small in epidemiological terms although it is the largest population size for this type of study in the target population. The study is cross-sectional and it precludes conclusions about causal relationships. Statistically gender-based differences were clearly demonstrated; however, the much smaller number of males compared to females might have affected the significance of the findings. This is a general limitation in most clinical studies as males are less likely to attend clinics because the majority of them are sole breadwinners and thus unable to take leave from work (Nell et al., 2015). In addition, the urine collection protocol did not include ‘day-time’ and ‘night- time’ urine; this could have shed more light on the diurnal and nocturnal pattern of urinary electrolyte excretion and its relationship with ambulatory blood pressure parameters. Finally, all studies presented in

the present thesis were conducted in black people of African ancestry, hence, generalization of our findings to other populations might be limited.

6.6 Conclusions

In conclusion, the findings of the present thesis provide evidence to suggest that the relationship between sodium and BP has not been clearly accounted for in this African population due to the high prevalence of obesity modifying this relationship. Moreover, to our knowledge there has not been a study that indicate that obesity is a significant mediator to the relationship between sodium and BP highlighting the novelty of the study. Second, the current findings have highlighted the importance of stratifying participants according to indices of obesity. Furthermore, the findings indicate that salt sensitive hypertension is a common feature in the African population as also indicated in other studies (Scott *et al.*, 2011; Mitchel *et al.*, 2014). It is suggested that individuals in our study population should increase dietary potassium intake as it has been shown that potassium has natriuretic effects (Aburto et al., 2013) and can be of immense benefit in reducing the incidence of hypertension and resultant cardiovascular target organ damage.

MY ROLE IN THIS RESEARCH

This was a collaboratve effort, due to the nature of the study being a large cross sectional population study. None the less, my role in the study was to critically execute the study protocol and ethics application; familiarise and execute as described in chapter 2 (methods) the design and planning of the study including collecting data relevant to my study; to analyse and interprete the data and finally to write the thesis.

APPENDICES

Appendix A

Ethical clearance certificate

R14/49 Miss Mercy Mashao

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170215

NAME: (Principal Investigator)	Miss Mercy Mashao
DEPARTMENT:	School of Physiology Hypertension Clinic
PROJECT TITLE:	The Impact of Dietary Salt Intake on Blood Pressure and Cardiovascular Target Organ Damage
DATE CONSIDERED:	24/02/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Muzi Joseph Maseko
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	09/06/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised 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 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 review February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B

“Turn-it-in” Plagiarism report

0604570k:Mercy_Thesis.docx

ORIGINALITY REPORT

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5	hdl.handle.net Internet Source	2%
6	www.science.gov Internet Source	1%
7	Muzi Maseko, Mercy Mashao, Abdulraheem Bawa-Allah, Edgar Phukubje, Bongubuhle Mlambo, Thamsanqa Nyundu. "Obesity masks the relationship between dietary salt intake and blood pressure in people of African ancestry: the impact of obesity on the relationship between sodium and blood pressure", Cardiovascular	1%

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