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SALT INTAKE AND BLOOD PRESSURE IN AN URBAN DEVELOPING COMMUNITY OF AFRICAN DESCENT

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, for the degree of **Master of Science in Medicine**.

2016

Abstract

Cardiovascular diseases (CVD) often share similar risk factors, and are presently a leading cause of death in South Africa and sub-Sahara Africa. Hypertension (HT) remains the commonest risk factor for strokes and heart attacks. There are several behavioural and environmental contributory factors to the development of HT. In an effort to lessen worldwide prevalence of HT, global strategies have focused on the reduction of salt/sodium (Na^+) intake as a means of lowering BP in populations. In the present study I first validated the 24-hour urine collection method by comparing a one day 24-hour urine sample with six consecutive day 24-hour urine samples and then evaluated the relationship between dietary Na^+ intake and blood pressure (BP) in 629 South Africans of African ancestry. All participants had complete conventional BP measurements, 489 had complete ambulatory BP measurements, and 597 had complete urine collection. The mean age of the participants was 43.9±18.4. The average body mass index (BMI) of the population sample was 29.2±7.9. 37.1% were hypertensive, 23.0% regularly consumed alcohol, 16.9% were tobacco smokers, and 12.2% had diabetes mellitus. The average 24-hour urinary Na^+ excretion rate was 111.2 mmol/day, and K^+ excretion rates were 28.9 mmol/day. There was no significant difference between the one day urine collection and the subsequent six day collection in terms of Na^+ and K^+ excretion. The averages were 109.2 mmol/day and 113.2mmol/day respectively. The Bland-Augment curve showed a bias of -2.4 which is clinically insignificant, suggesting that the two procedures are similar. After correcting for covariates, there was no significant relationship between Na^+ intake and conventional BP {SBP (P=0.49), DBP (P=0.95)}, ambulatory BP {24-hour SBP (P=0.17), DBP (P=0.59); daytime SBP (P=0.15), DBP (P=0.68); night-time SBP (P=0.69), DBP (P=0.25)}. Even when participants were grouped according to age, there was no significant increase in BP in participants whose daily sodium intake was above the threshold of 100mmol/day when compared to participants with normal sodium intake. However there was strong relationship between BP and Na^+/K^+. A multivariate data analysis revealed a strong inverse relationship between urinary Na^+ and

age ($P \leq 0.001$). Plasma renin was related to BP ($P=0.0001$). In conclusion, the present study has demonstrated that in a randomly recruited urban community sample of African ancestry, a single 24-hour urine measurement is sufficient to estimate the levels of Na^+ intake. There is no relationship between both Na^+ and K^+ with BP. However there is a strong association between BP and the sodium-to-potassium ratio indicating that, in this population, the interaction between sodium and potassium ions plays a more important role in the regulation of BP than K^+ and Na^+ alone.

Declaration

I, Franswell Thamsanqa Nyundu declare that this dissertation is my own unaided work, except where stated. It is being submitted for the degree of Master of Science in Medicine in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. The work contained in this dissertation has not been submitted for any degree or examination in this University, or any other University.

Franswell Thamsanqa Nyundu……...…….....on this…….....day of…….2016

I certify that the studies contained in this dissertation have the approval of the Committee for Research in Human Participants of the University of the Witwatersrand, Johannesburg. The ethics approval number is **M150524**

Franswell Thamsanqa Nyundu…...……..……on this…….....Day of…….2016

……	……..
Muzi J. Maseko (Supervisor)	Olebogeng H. Majane (Supervisor)
Date……	Date……

Dedications

This dissertation is dedicated to my mother Ainah Nyundu, my wife Bontle Ayanda Malete and my siblings: Thank you for praying for me, supporting me, encouraging and reassuring me during the writing of this dissertation.

Publications and presentations arising from this dissertation

Data contained in this dissertation has been presented as both oral and poster presentations at the 43rd Meeting of the Physiology Society of Southern Africa held at Khaya iBhubesi Conference Center, Parys, September 2015. The titles of the presentations were:

Thamsanqa Nyundu, Angela J Woodiwiss, Olebogeng HI Majane, Gavin R Norton, Muzi J Maseko. Validation of estimates of salt intake using 24-hour urinary salt excretion in a population of black ancestry.

Muzi J Maseko, Thamsanqa F Nyundu, Harold Majane, Angela Woodiwiss, Gavin Norton. Relationship between dietary sodium intake and blood pressure in a population of black ancestry.

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My contributions to data collection and analysis

I declare that I collected 24-hour urinary excretion data on my own then the anthropometric, blood pressure and sphygmocor data with the assistance of a qualified nurse. Blood and urine samples were sent to an accredited laboratory (CLS, Braamfontein) for analysis. I analysed the data by myself in consultation with my supervisors.

List of abbreviations

ABP	Ambulatory blood pressure
ANP	Atrial Natriuretic Peptide
BP	Blood Pressure
CBP	Conventional Blood Pressure
CIMT	Carotid Intima Media Thickness
CNBP	Central Blood Pressure
CVD	Cardiovascular Disease
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic blood Pressure
ECF	Extracellular Fluid
EHS	European Society of Hypertension
ET	Epidemiological Transition
ET-1	Endothelin-1
HR	Heart Rate
HT	Hypertension
ICF	Intracellular fluid
IDACO	International Database on Ambulatory Blood Pressure in Relation to Cardiovascular Outcomes
K^+	Potassium
LVH	Left Ventricular Hypertrophy
MAP	Mean Arterial Pressure
mg/d	milligram per day
Mmol	millimole
Mmol/l	millimole per litre

Na^+	Sodium
NO	nitric oxide
NS	Nervous System
PP	Pulse Pressure
PWV	Pulse Wave Velocity
RAAS	Renin-Angiotensin-Aldosterone-System
RWTT	Reflected Wave Transit Time
SBP	Systolic Blood Pressure
SOWETO	South Western Township
SSA	sub-Sahara Arica
WCHT	White Coat Hypertension
WHO	World Health Organisation

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Preface

Cardiovascular diseases (CVD), a cluster of conditions that affect the heart and blood vessels which include stroke, heart failure, and peripheral vascular disease, often share similar risk factors, and are presently a leading cause of death in South Africa and sub-Sahara Africa. According to the World Health Organization (WHO), CVD are the number one cause of preventable mortality and morbidity accounting for about 30% of all deaths world-wide with 80% of the total deaths occurring in developing countries. The sudden increase in CVD in low income areas such as those in the SSA is fuelled by the changes in lifestyle associated with urbanisation and westernisation. The burden of CVD in a population results from the frequency of major cardiovascular risk factors, and while these risk factors are diverse, hypertension (HT) remains the commonest risk factor for CVD, particularly in economically developing communities. Causes for HT itself are variable and can be of behavioural or environmental origin.

More importantly, habitual Na^+ intake has continuously been implicated in the development of HT. People of African origin have a higher prevalence of HT, and a prospective study has indicated that South African adults consume an average of 8.1g of salt (3,240 mg Na^+ ≈ 140mmol Na^+) daily as opposed to the WHO's recommended daily intake of 6g/d of salt (2,400mg Na^+ ≈ 104mmol Na^+). Furthermore, South African blacks report adding salt in most cooking than their white counterparts, and that discretionary salt intake is more in blacks than in whites. Therefore, it is possible that Na^+ intake may impact on BP more in people of African ancestry compared to white people. However, up to now, data are inconsistent regarding this matter therefore more studies still need to be done.

Indeed, the management of HT is crucial, but it requires the accurate assessment of net Na^+ balance in the body to establish well-founded relationships, and previously, the reliability of the method used to estimate dietary Na^+ intake in a population of African ancestry living in SOWETO had not been

validated. Therefore the validation of estimates of Na^+ intake is essential for the proper analysis of the relationship between Na^+ intake and BP in this population. Therefore, in the current study I aimed to determine the variation in daily Na^+ intake over a 7 day period and to see if a single 24-hour urine collection provides information on Na^+ intake which accurately reflects the average of a subsequent 6 days of urine collection. Also, to determine the relationship between Na^+ intake and BP and check whether daily Na^+ intake determined from 24-hours of urine collection is more closely associated with ambulatory or office BP. In the first chapter (Introduction), I discuss the general mechanisms responsible for the development of HT, the methods used to monitor BP, and the impact of dietary Na^+ on HT based on literature. In chapter two, I explain the methods used to collect data in this study. In chapter three I display the results obtained from statistical analysis of the data, and in chapter four I discuss the results in contrast to literature and propose possible causes and for the findings. This study will add widely to the existing body of knowledge regarding the continuously debated relationship between Na+ intake and BP.

CHAPTER ONE

INTRODUCTION

1 Introduction

Cardiovascular diseases (CVD), which includes stroke, heart failure, and peripheral vascular disease, often share similar risk factors, and are presently a leading cause of death in South Africa and sub-Sahara Africa (SSA) (MRC Statistics, 2014; Mbewu, 2009). According to the World Health Organization (WHO), CVD are the number one cause of preventable mortality and morbidity accounting for about 30% of all deaths world-wide with 80% of the total deaths occurring in developing countries (Santulli, 2013). Traditionally, infectious diseases and malnutrition have been the primary causes of mortality in SSA with little attention to health problems being focused on CVD (Gaziano *et al*. 2006; Alberts *et al*. 2005). The sudden increase in CVD in low income areas such as those in the SSA is compounded by the element of lacking resources to fight CVD in these areas. Much of this increase in the prevalence of CVD is fuelled by the changes in lifestyle associated with urbanisation and westernisation; a concept initially labelled "the epidemiological transition" (ET) by Omran (1971).

This theory is centred on demographic and socio-economic diversities. Omran's notional perception of ET has largely been used in research because it suggests clarifications for changing cause-of-death patterns corresponding to different stages of transition (Kandala *et al*. 2013). The ET embraces four stages as they appear in Table 1. In summary, the ET is simply defined by a decline in mortality from infections and malnutrition and a rise in non-communicable chronic diseases such as CVD due to a shift in determinants and risk factors for these diseases in a population, especially where there is constant migration from rural to urban areas (Mbewu, 2009). This rapid migration and transition in communities of developing countries in pursuit of economic development propels the dissemination of risk factors such as tobacco use, refined foods, and lifestyle divergence allied with high incidence of CVD (Lim *et al*. 2007; Murray *et al*. 2003).

Table 1.1: Epidemiological Transition			
Phase of epidemiologic transition	**Details from cardiovascular**	**Cardiovascular problems**	**Risk factors**
Age of pestilence and famine	5 - 10	Rheumatic heart disease, infection and deficiency-induced cardiomyopathy	Uncontrolled infection, deficiency conditions
Age of receding pandemics	10 - 35	As above, plus hypertensive heart disease and haemorrhagic stroke	High-salt diet leading to hyper-tension, increased smoking
Age of degenerative and man-made diseases	35 - 55	All forms of stroke, ischaemic heart disease	Atherosclerosis from fatty diets, sedentary lifestyle, smoking
Age of delayed degenerative diseases	Below 50	Stroke and ischaemic hear t disease at older ages	Education and behavioural changes leading to lower levels of risk factors

Mbewu, 2009 based on Omran 1971.

The burden of CVD in a population results from the frequency of major cardiovascular risk factors as shown in Figure 1, and while these risk factors are diverse, hypertension (HT) remains the commonest risk factor for CVD, particularly in economically developing communities (Salako *et al.* 2007; Wiysonge *et al*. 2004).

HT being principally a chronic condition, is characterised by an abnormally raised blood pressure (BP) resulting in end organ damage and is firmly circumscribed to arterial blood pressure. It is usually asymptomatic and those who develop symptoms generally complain of headaches, visual disturbances, nausea, vomiting, and infrequently confusion. Due to its asymptomatic nature, people normally develop complications without knowing it; authenticating this condition a "silent killer" (Mungati *et al*. 2014).

The causes and risk factors for HT itself are interrelated and are based on physiologic states, biological markers or other identifiable factors associated with a change in vascular structure and an increased blood pressure. These causes depict a multifactorial aetiology and because of this, HT is categorised into essential (primary) HT or secondary HT (Kwok *et al*. 2007). When the cause is unknown it is labelled essential HT and usually suggests a genetic link with ancestry. Differently, secondary HT is when the cause is identified such as in the case of chronic kidney disease, adrenal gland disorders, pregnancy or drug induced HT (Price *et al*. 2014; Kaplan, 2007).

Risk factors for HT are characterized as either modifiable or non-modifiable and are often the classical risk factors responsible for cardiovascular clinical endpoint and ultimately CVD mortality and morbidity (Protulipac *et al*. 2015). Non-modifiable risk factors for HT are those practical aspects which cannot be attuned and include conventional risk factors such as age, family history (genetics), gender, and ethnicity.

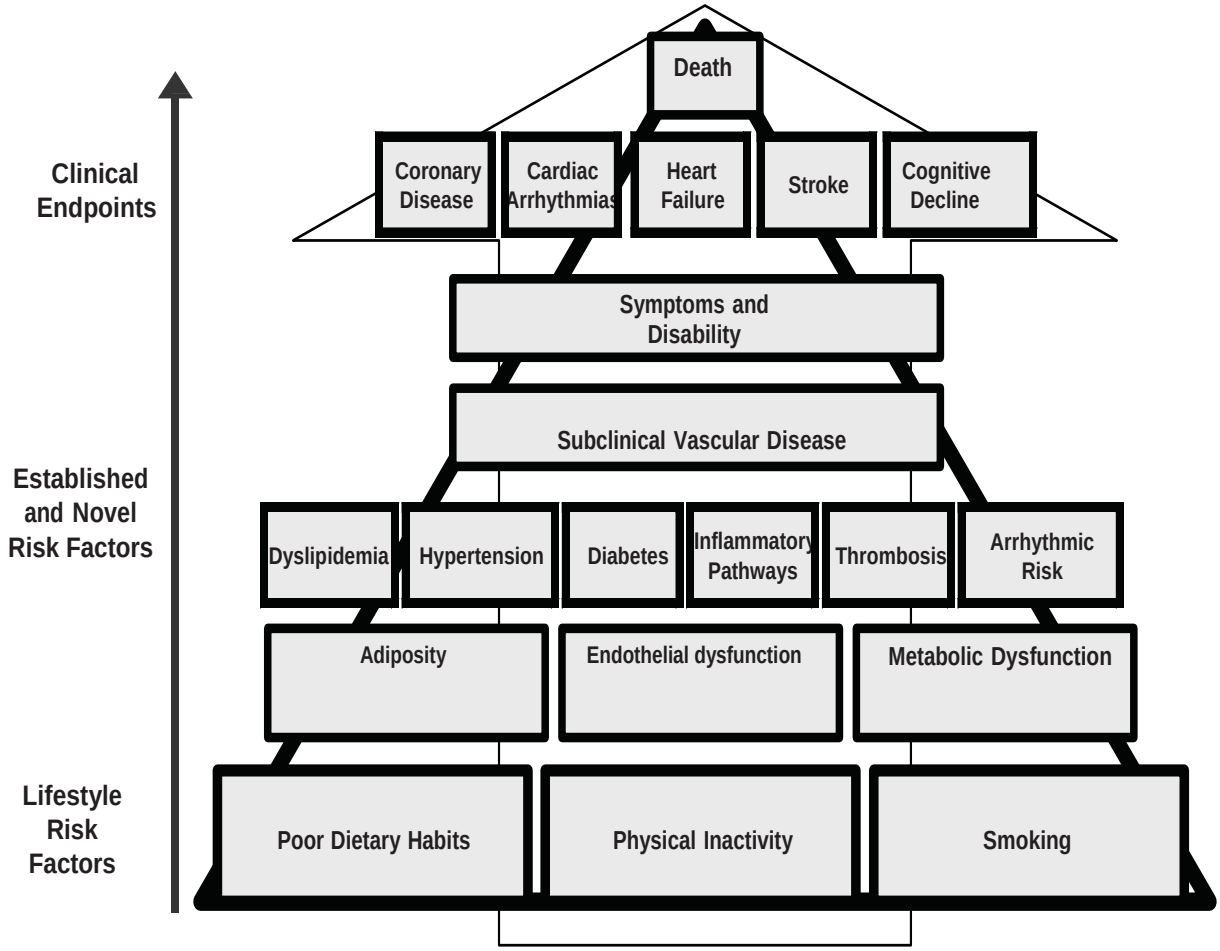


Figure 1.1: The relations of lifestyle, established and novel risk factors, and cardiovascular disease. Assessment and treatment of dyslipidaemia, hypertension, and diabetes are major foci of clinical care, practice guidelines, performance measures, and scientific research. These established cardiovascular risk factors are strongly influenced by lifestyle, including dietary behaviours, physical inactivity, smoking, and adiposity. Adiposity itself is largely a result of poor diet (excess calories) and inactivity. Lifestyle risk factors also influence disease risk via effects on other novel risk factors such as endothelial function, inflammation/oxidative stress, thrombosis/coagulation, arrhythmia, and other pathways. These basic lifestyle habits: poor diet, high Na^+ intake, physical inactivity, and smoking are thus the most proximal risk factors for cardiovascular disease (Mozaffarian *et al*. 2008)

1.1 Risk factors for hypertension

Non-modifiable risk factors include advancement in age, which is recognised in many studies as a major cause of HT (Gamici *et al*. 2009; James *et al*. 2006; Brady *et al*. 2005). It is inescapable and is rightfully regarded as an independent risk factor for the development of cardiovascular disorders (Gamici *et al*. 2009). This biological course is accompanied with a decline and alteration in physiological functions of many organs involved in regulating BP. Furthermore, an enormous amount of proof supports the presence of a considerable genetic component in BP regulation with approximately 20% to 60% of the population variability in blood pressure being genetically determined (Khullar, 2010; Von-Wowern *et al*. 2003). Adding to this, recent studies including the one conducted by Kouremenos *et al* (2014) indicated a successful detection and correlation of genes that are associated with BP regulation and HT. Coupled with age and genetics, gender is also implicated in HT and men are regarded to have higher blood pressure than women through much of life irrespective of race and ethnicity (Sandberg and Ji, 2012). Aditionally, with regards to ethnicity, there are blatant differences in ethnic and racial groups pertaining to HT and its sequelae such as manifestations of CVD. For many years, data from the United States has reliably presented that hypertension is more common, more serious, and develops at an earlier age in African Americans as compared with White Americans (Agyemang and Bophal, 2002).

As mentioned previously, excluding non-modifiable risk factors for HT, there are several other behavioural and environmental contributory factors to the development of HT and in an effort to lessen worldwide prevalence of HT, global strategies and population-based intervention studies including Dietary Approaches to Stop Hypertension (DASH) and the Trial of Hypertension Prevention (TOHP) II have focused on life style changes such as weight loss and particularly the reduction of salt/sodium (Na^+) intake as a means of lowering BP in a population (He and MacGregor, 2010). He *et al* (2012) indicated that Na^+ intake is the major cause of raised BP, therefore reducing its intake would lessen HT. However, life style changes such as salt reduction

should be accompanied by proper understanding of the pathophysiology of HT, and more importantly, the methods used to measure BP and categorise HT should be employed with a clear consideration for their advantages and disadvantages.

1.2 Blood pressure monitoring

1.2.1 Conventional blood pressure monitoring

Due to the multifactorial aetiology of HT, attention should also focus on the methods for assessing HT and its successive cardiovascular outcomes. Traditionally, conventional BP (CBP) measurement has been the standard monitoring tool in practice for many decades (Conen *et al*. 2014). The European Society of Hypertension (EHS, 2013) has published guidelines for ideal office blood pressure measurements techniques. The recommendations of these guidelines are explained in detail in Chapter 2 (Methodology). Since accurate diagnosis of HT is crucial in treating those individuals at high risk for adverse effects (Williams *et al*. 2013), it is essential to understood that BP has a natural circadian rhythm characterised by a nocturnal dip in pressure and a morning surge in pressure that occurs before waking up; a phenomenon not accounted for by conventional BP measurements, thus justifying the considerations, interest and reason for the use of a 24-hour BP monitoring (Mancia and Verdecchia, 2015; Williams *et al*. 2013), the ABP monitoring.

1.2.2 Ambulatory blood pressure measurement

The superior predictive ability of 24-hour ABP monitoring over office measurements is well recognized in both cross-sectional and longitudinal studies because it offers a more precise method of quantifying BP levels in detecting HT (Grossman, 2013). Although both the CBP and ABP monitoring measure peripheral BP in the brachial artery (Rock *et al*. 2014), the ABP monitoring is

deemed superior to CBP because it is able categorise white-coat HT (WCHT), masked HT, daytime and night time BP.

Its major aspect is that it is a reliable tool in predicting target organ damage (Lehmann *et al*. 2013; Hara *et al*. 2011), and it also provides a better prognostic value for cardiovascular events (Weber *et al*. 2012). The sequence of readings obtained during the patient's daily activities affords a superior assessment of the true BP and provides information on BP variability, circadian rhythms, and the effects of environmental and emotional conditions on BP levels (Mancia and Verdecchia, 2015; Grossman, 2013). A sizeable fraction of patients have normal ABP readings, but somehow, their BP readings are elevated when measured in the office; the "WCHT", a term commonly used to denote patients whose BP is only high in the medical or office setting, but is normal when they are going about their daily activity (Dolan *et al*. 2005). Even though WCHT has a comparatively nonthreatening outcome compared to sustained mild hypertension, the adversity of failure to recognize it results in inappropriate prescription and lifelong use of antihypertensive medications (Dolan *et al*. 2004).

On the contrary, patients with masked hypertension have normal office BP but elevated out-of-office BP (Ogedegbe and Agyemang, 2010). The prevalence of masked HT varies considerably as observed in different studies depending on the population characteristics and the presence and absence of antihypertensive treatment. Other factors which may discriminately increase ABP in relation to office BP include increased physical activity, smoking, alcohol use, obesity, and psychological factors such anxiety and stress (Franklin *et al*. 2015; Ogedegbe and Agyemang, 2010). Through its ability to detect changes in BP throughout the day, the ABP monitoring can stratify these risk factors for HT and improve the physician's ability to forecast outcomes even in patients who have masked HT as recorded by ABP machines during diurnal and nocturnal activities (Siti *et al*. 2013).

Circadian variations in physiological processes have provoked awareness and interest concerning the chronological deviations of BP in healthy humans (Larochelle, 2002); basically, BP fluctuates according to the circadian rhythm whereby it decreases during sleep and increases during wakefulness (Mahabala *et al*. 2013). This normal variation in BP is characterised by a 10% to 20% reduction in BP from day to night (Karaagac *et al*. 2014) and subjects who do not exhibit a decrease in BP by this percentage are called "non-dippers", whereas those who display this reduction in BP are referred to as "dippers" (Maseko *et al*. 2006). Some patients show a contradictory rise in BP during the night and are referred to as reverse dippers. Daytime BP does not deliver earnest prognostic accuracy as compared to night-time BP, this is because night-time BP represents basal BP and is a representative of the true BP status of an individual hence in non-dipping BP patterns it can be a useful indicator of HT severity if proven to be connected with other influences and complications (Mahabala *et al*. 2013).

Several studies have revealed that a strong association exist between a non-dipping BP profile and increased Na^+ intake, target organ damage at the cardiac, cerebrovascular and vascular level (Karaagac *et al*. 2012). In a meta-analysis by Fargart *et al* (1995), it was shown than non-dipping pattern in BP accounts for a considerable degree of Left Ventricular Hypertrophy (LVH), Carotid Intima Media Thickness (CIMT), and microalbuminuria (Maseko *et al*. 2006). Numerous factors seem to be implicated in the circadian fall in BP during sleep and its subsequent rise upon awakening (White, 2007; Kotsis *et al*. 2005; Seo and Oh, 2002), these changes are mediated by hemodynamic parameters, neuro-hormonal factors, and electrolytes such as Na^+.

Furthermore, this nocturnal decline in BP relating to the BP circadian rhythm is reported to be affected by Na^+ intake in hypertensives (Koroboki *et al*. 2012; White, 2007). Therefore, in order to manage these patients who are hypertensive, the quantification of the total cardiovascular risk profile requires the inclusion of subclinical target organ damage as a quantifiable measure through none invasive measurement of the central aortic BP (Agabiti-Rosei *et al*. 2007).

1.2.3 Central blood pressure

In addition to the use of ABP monitoring, emerging data from epidemiological studies and clinical observations supports that central BP (CNBP) may be more relevant than brachial BP measurements in predicting cardiovascular events and target organ damage (Ding *et al*. 2011). This is because CNBP are pathophysiolocally more significant than peripheral (brachial) pressures in the pathogenesis of CVD (Agabiti-Rosei *et al*. 2007). Moreover, CNBP is exerted on the heart, brain and kidneys, therefore may be way different than the pressures measured at the arm, particularly in the elderly and hypertensive patients and due to exposure of these organs, cardiovascular risk should relate better to CNBP (McEniery, 2009).

Pressures in the large elastic aorta and carotid arteries causes degenerative changes which are fast-tracked by aging and salt dependant HT. In contrast, peripheral arteries such as the brachial and radial arteries are more muscular than elastic, hence they are less influenced by these degenerative changes caused by salt dependant HT (Avolio *et al*. 1983). It is for this reason that aortic CNBP are said to be a better surrogate marker for CVD (Rosenwasser *et al*. 2014). The left ventricle is subjected to aortic systolic pressure, the pressure wave generated by this ventricle travels though the arterial tree and is reflected at numerous peripheral sites, mostly at resistance vasculature, the small muscular arteries and arterioles (Agabiti-Rosei *et al*. 2007).

As a result, the pressure waveform recorded at any site in the arterial tree reflect the entirety of the forward travelling waveform generated by the left ventricular ejection and the backward waveform of the incident wave redirected at peripheral sites (Kuzeytemiz *et al*. 2013; Agabiti-Rosei *et al*. 2007). If and when the large channel arteries are healthy and compliant, the reflected wave merges with the incident in the proximal aorta during diastole and thus augments the diastolic BP and helping with coronary perfusion (McEniery, 2009; Agabiti-Rosei *et al*. 2007).

On the contrary, when the arteries are stiff, Pulse Wave Velocity (PWV) escalates, speeding the incident and reflected waves, and by so doing, the reflected waves merges with the incident wave in systole and augments aortic systolic rather than the diastolic pressure which consequently increases the ventricular afterload and compromises normal ventricular relaxation and coronary perfusion (Krogager *et al*. 2014; Rosewasser *et al*. 2014; Agabiti-Rosei *et al*. 2007). Unlike in brachial pressure, the variation in the manner in which central aortic pressure waves generated during systole are reflected back to the aorta at erratic velocities is dependent on arterial stiffness (McEniery, 2009). Particularly, in hypertensive patients, the pressure waves are prematurely reflected, and as a result their return to the aorta is simultaneous with the generation of the next pressure wave, hence the outcome is an increase in the CNBP (Rosewasser *et al*. 2014).

1.3 Salt intake, arterial stiffness and target organ damage

Notably, more than a few studies have revealed that HT and its causes such high Na^+ intake increases arterial stiffness in all ages (Kuzeytemiz *et al*. 2013), and in addition, when coupled with other cardiovascular risk factors such as hypercholesterolemia, smoking, and diabetes, the incidence of arterial stiffness is fuelled even further, and can have differential consequences on both the brachial BP, CNBP and eventually target organ damage (Krogager *et al*. 2014; McEniery, 2009).

Arterial stiffness assessed by measuring the PWV has been shown to predict cardiovascular outcomes such as target organ damage independently of other known risk factors in the general population. The carotid-femoral PWV is denoted the gold standard of evaluation (Chen *et al*. 2015; Hansen *et al*. 2006). The value of PWV in determining arterial stiffness is evident in cardiovascular risk stratification (Hamatener *et al*. 2013). Arterial stiffening progresses from a number of multifarious interactions among steady and ongoing changes involving structural and cellular

elements of the vessel wall and these vascular adjustments are subject to hemodynamic and other factors such as hormones and salt regulation (Zieman *et al*. 2005) as seen in Figure 3.

Because increased arterial stiffness is accompanied with structural changes mainly in the medial layer of the arteries, and because it is largely instigated by degeneration of the elastic fibre it affects the regulation of BP in these arteries. Large artery stiffening promotes target organ because of its effect on pressure and flow pulsitility, therefore stiffening of these arteries is important not only as a surrogate marker of cardiovascular risk, but also because of the pathophysiological consequences (Jain *et al*. 2014). Zieman *et al* (2005) demonstrated that dietary Na^+ together with an increase in age amplifies stiffness of blood vessels, however low-sodium diets in older adults improves arterial compliance. Similarly, Na^+ stimulates vascular smooth muscle tone and modifies vascular wall composition; there is obviously a noticeable increase in the medial layer observed with vascular smooth muscle hypertrophy and extensive collagen and elastin production (Bragulat *et al*. 2001). It is also suggested that Na^+ interacts with specific genes involved in the regulation of BP. By reducing the production of NO by the NOS, Na^+ impairs endothelial function (Kaplan, 2007). Coupled with other factors, Na^+ promotes arterial stiffening and hence it is highly implicated in the pathogenesis of HT and target organ damage.

It is therefore important to understand how levels dietary Na^+ intake could impact on peripheral BP and CNBP, a relationship that could not be established in a community of African Ancestry living in the South West Township (SOWETO) of Gauteng, South Africa (Michel *et al* 2012, 2014, Scott *et al* 2011, Millen *et al* 2013). As revealed that both the CNBP and ABP monitoring offers appropriate prognostic values associated with target organ damage and arterial stiffness, some data suggests that CNBP can be decreased and that non-dipper nocturnal BP can be changed to a dipper pattern through salt restrictions (White, 2007; Uzu *et al*. 1997) which further supports the involvement of Na^+ in the pathogenesis of HT.

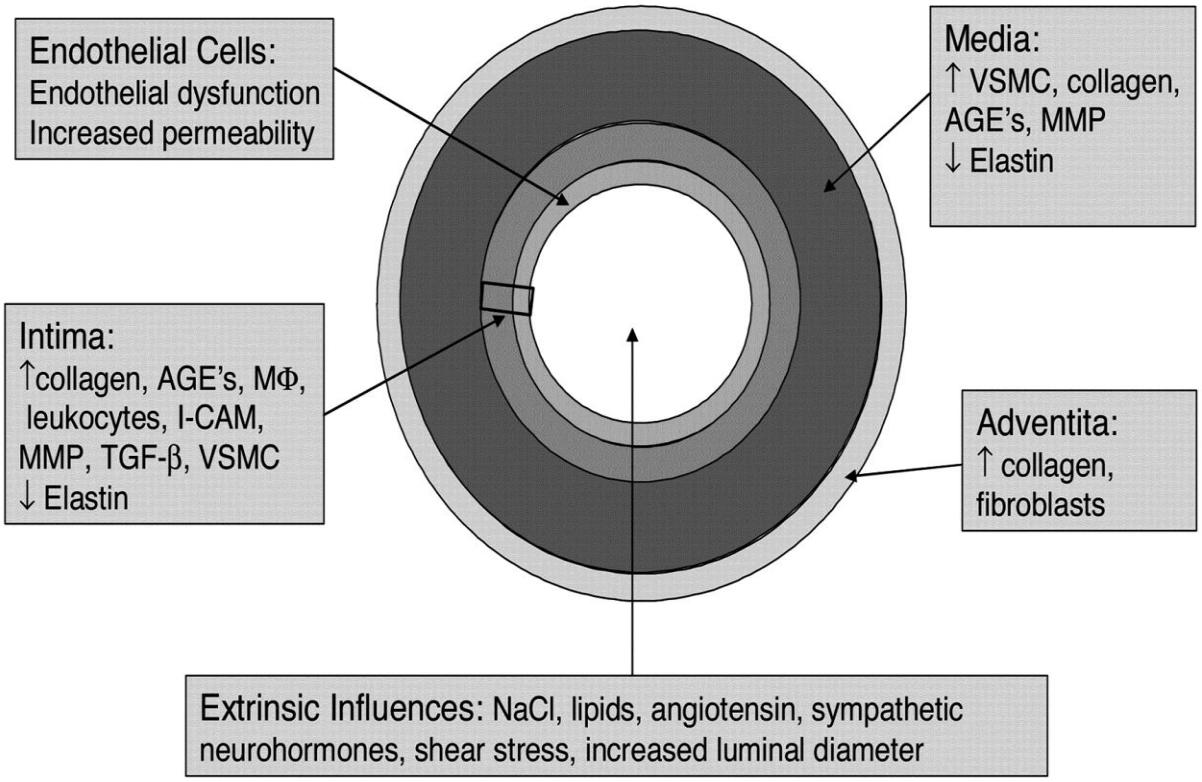


Figure 1.2: Summary of multiple causes and locations of arterial stiffness. (Zieman *et al*. 2005)

1.4 Dietary sodium

1.4.1 Patterns of sodium intake

Worldwide, the regular salt intake per adult is reported to be around 10 g/d (4000 mg/d Na^+ ≈ 174 mmol Na^+), with higher intakes occurring in Asia (Campbell *et al*. 2014). Ostensibly, South African adults consume an average of 8.1g of salt (3,240 mg Na^+ ≈ 140mmol Na^+) daily as opposed to the WHO's recommended daily intake of 6g/d of salt (2,400mg Na^+ ≈ 104mmol Na^+) (Bertram *et al*. 2012). Essentially, in this population, considerable amounts of Na^+ intake emanates from non-discretionary intake with the highest proportion being from bread, and other contributions coming from margarine, soup mixes and gravies. Charlton *et al* (2005) conveyed that more South African blacks reported adding salt in most cooking than their white counterparts.

Furthermore, inconsistency did occur between self-reported dietary Na^+ intake and urinary excretion of Na^+ in blacks as compared to whites which additionally suggested that discretionary salt intake (i.e. salt added to food at the table and in cooking) was more in blacks than in whites. As elaborated earlier, it is not unusual that blood pressure values with regard to salt intake are dissimilar in blacks and whites more especially because of salt sensitivity and irregularities in the physiological control of Na^+ in the body.

1.4.2 Physiology of sodium regulation

Sodium is an essential nutrient necessary to maintain physiological homeostasis by its central role in sustaining intravascular, extracellular, and intracellular volume (Alderman, 2002). It is mostly concentrated in the extracellular fluid (ECF) averaging 140-145mmol/l, as compared to intracellular fluid (ICF) compartments where it averages 10mmol/l (Arcand *et al*. 2009). In addition to its involvement in the transport of molecules across cell membranes, it also helps in maintaining the electrochemical gradients via the Na/K ATPase pump (Arcand *et al*. 2009).

Sodium ions accounts for nearly 90% of positively charged ions in the ECF, and because of its charge properties, precise regulation of this ion is required for impulse conduction along nerve cells, muscle fibre contraction, and maintenance of cell membrane potential. By its control of ECF, Na^+ regulates blood plasma, blood volume and consequently BP (Alderman, 2002).

About 95% of ingested Na^+ is absorbed in the small and large intestines of the gastrointestinal tract irrespective of the amount that is consumed, and usually this intake is beyond physiological need (Elliot and Brown, 2007). Most of the ingested Na^+ is excreted via the kidneys, with varying but literally small amounts lost in sweat, faeces, saliva and less often semen and menstruation (Meneton *et al*. 2005). In standard physiological conditions, renal excretion of sodium is regulated so that balance is maintained between intake and output so as to stabilize ECF volume and maintain BP (Karppanen and Mervaala, 2006).

The body has a number of systems which control BP, but the regulation of BP achieved through controlling Na^+ in ECF is made possible by the kidneys via the "pressure-natriuresis and diuresis mechanism", a model explained by Guyton *et al* (1972). This theory states that as arterial pressure increases beyond normal, the excess pressure increases filtration and causes the kidneys to excrete more water and Na^+ in the urine than are entering the body. Therefore, the ECF and blood volumes decrease. Ultimately, this results in a decreased arterial BP which is caused by a decrease in venous return back to the heart. The converse is true when there is a decrease in arterial BP. In addition to the kidneys' ability to auto regulate Na^+ reabsorption and excretion in the tubules, supplementary mechanism such neuroendocrine feedback mechanisms including natriuretic peptides, and the Renin-Angiotensin-Aldosterone-System (RAAS) are employed to complement the intra-renal ECF/Na^+ regulatory mechanisms (Meneton *et al*. 2005).

The RAAS is the most important and well known hormonal system involved in regulating Na^+ through a cascade of events shown in Figure 3. Although the RAAS offers the most influential

control on the renal handling of Na^+, this Na^+ -retaining, blood pressure raising system is opposed by a system involving the hormone atrial natriuretic peptide (ANP), a hormone that induces excretion of large amounts of Na^+ in the kidneys and causes the lowering of BP (Van Thiel *et al*. 2015; Song *et al*. 2015).

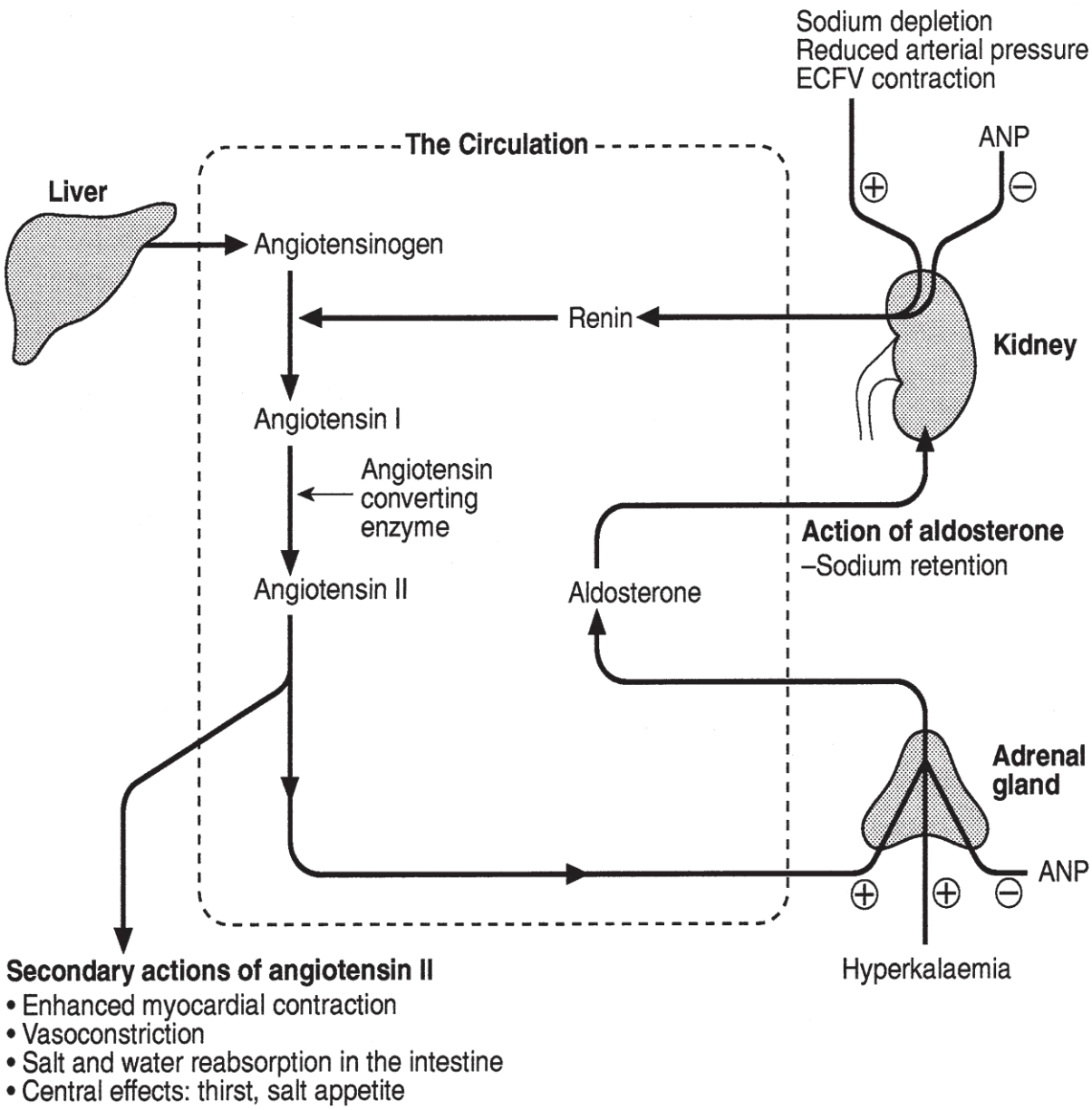


Figure 1.3: The Renin-Angiotensin-Aldosterone-System. ANP (Atrial Natriuretic Peptide), BNP (Brain Natriuretic Peptide). + Stimulatory signal. – Inhibitory signal. (Marshall and Bangert, 2008).

1.4.3 The impact of dietary sodium on hypertension

It is largely documented in many studies that an increased Na^+ intake is associated with an increase in BP, and that reduction in Na^+ is a well-established public health issue (Rhee *et al*. 2014). Even though high Na^+ intake is a concern, the general homeostatic principle states that there is an important physiologic range for intake of all essential nutrients including Na^+. Based on this concept, an intake below the required range for essential nutrients may result in deficiency conditions. On the contrary, if the intake exceeds the physiologic range for prolonged periods, adverse effects may develop as in the case of HT due to high Na^+ intake (Karppanen and Mervaala, 2006).

Indeed, the DASH, controlled clinical trial, provided a clear indication that decreasing Na^+ intake will lower BP in hypertensives (Charlton *et al*. 2005). Although the relationship between BP and an increased Na^+ intake has long been documented, the mechanism of Na^+-dependent HT still remains controversial (Drenjancevic-Peric *et al*. 2011). The modest relationship between 24-hour urinary electrolyte excretion rates and BP has been reported in large epidemiological studies including the Intersalt Study (Intersalt Cooperative Research Group, 1988) and the Scottish Heart Study (Smith *et al* 1988) and the lack of relationship between salt intake and BP in epidemiological studies in Africa (Hoosen *et al* 1985, Charlton *et al* 2005).

1.4.4 Impact of dietary sodium on salt sensitive population

For many years, data has reliably presented that salt dependant hypertension is more common, more serious, and develops at an earlier age in African Americans as compared with White Americans (Agyemang and Bophal, 2002).

Similarly in Europe, people of African descent compared to those of other ethnic origin have higher mortality rates relating to HT (Jones and Hall, 2006; Agyemang and Bophal, 2002). Also, when comparing people of African descent to descendants of other ethnic origin, Africans tend to show less "dipping" in blood pressure at night, a status strongly associated with higher rates of vascular disease (Jones and Hall, 2006). Regardless of past and recent research debates, it still remains an epidemiological puzzle to explain the increased risk of HT experienced by people of African ancestry when likened to other ethnic groups. A number research papers counting Sowers *et al*. (1988) have long proposed that BP in African lineage is heightened by the incidence of high "salt sensitivity" as compared to their ethnic counterparts, though the mechanism responsible for the increased incidence of salt sensitivity in blacks is poorly understood. Although inevitable as explained previously that Na^+ is necessary in the human body, literature shows that habitual intake of dietary Na^+ predisposes individuals to HT (Zhang *et al*. 2013). This is particularly true in the case of salt-sensitive hypertensives dwelling in urbanised areas with a relatively high Na^+ consumption (Rassler, 2010).

1.4.5 Nutrient interaction: The sodium-to-potassium ratio

It has been recognised for a long time that high Na^+ intake is associated with an increase in blood pressure especially in salt sensitive individuals, however the mechanism by which an increase in Na^+ intake leads to the development of salt-dependent HT is poorly understood and still remains a topic of constant debate (Drenjancevic-Peric *et al*. 2011). Nonetheless, studies suggest that the mechanisms responsible for the development of HT due to Na^+ could involve other factors such as low K^+ intake (Adregue & Madias, 2014). Other data suggests that the ratio of Na^+/K^+ is more crucial in BP regulation (Alderman, 2002), and an imbalance in this ratio; as in the case of high levels of Na^+ and low levels of K^+ intake could be associated with high BP and a greater risk for

CVD (Zhang *et al*. 2013). Other studies have long suggested that high K^+ intake reduces BP significantly (Houstin, 2011), however it now appears that the ratio of Na^+/K^+ could be much more important than either sodium or potassium alone.

1.4.6 Reduction of dietary Na^+ intake

Owing to global trends in Na^+ consumption, advocates of salt reduction have conversed a strong consensus that reduction in dietary Na^+ could save lives, health care resources, and ultimately save money (Campbell *et al*. 2015). A number of randomized controlled trials indicate that non-pharmacological tactics, predominantly the decrease Na^+ intake will lower BP in hypertensives and thus become a means to reduce CVD mortality and morbidity (Charlton *et al*. 2005). These encouraging studies include the already mentioned DASH randomized controlled trial, TOHP II trial, and other meta-analysis such as the DASH-sodium trial, the TONE trial, the PREMIER clinical trial to mention a few (Tejada *et al*. 2006).

As a matter of fact, even most health guidelines and the WHO supports the reduction of Na^+ intake to less than 100 mmol/L. In a meta-analysis by Law *et al* (1991), it was reported that a decrease in salt intake of about 50 mmol/day results in BP fall of an average 5 mmHg in both systolic and diastolic values. The DASH-sodium trial is unavoidably the most considerable study because it is the largest intervention trial. It randomised 412 individuals to either a normal diet or a DASH diet (high in fruits and vegetables and low fat dairy products) and placed in them in three categories of salt intake of roughly 9, 6, and 3g/d and continued for 30 days.

There was significant decrease in BP due to salt restriction from 140 to 110 mmol/day, adding to this, BP reduction was seen with a decrease in salt intake from 100 to 65 mmol/day which was associated with a fall in BP of 6.7 mmHg systolic and 3.5 mmHg diastolic BP respectively, but this was also influenced by the DASH diet itself (Karpppanen and Mervaala, 2006; O'Shaughnessy, 2006). Likewise, the TOHP II also showed that Na^+ reduction of 100 mmol/day with or without weight loss was associated with a reduced risk of hypertension by 20% (O'Shaughnessy, 2006; Kumanyika *et al*. 2005).

Even though there is extensive epidemiological data which convincingly demonstrate a relationship between Na^+ intake and hypertension, there is still some reproach to this relationship. Statistical significance of these surveys have principally depended on maverick populations where the consumption of Na^+ was either too high or too little. Also, no BP-Na^+ relationship was detected in the majority of populations whose consumption was between the extreme lower and upper limits (Weinberger, 1999).

These disparities are what drives the constant debate known as "The Salt Saga" which is propelled by different views on the role of Na^+ in the pathogenesis of HT (Logan, 2002). Some scholars are convicted to the idea that the average BP value in a population is largely determined by the average Na^+ consumption (Logan, 2002; Weinberger, 1999), and other contradictory perceptions suggest that there is an individual variation with regard to dietary Na^+ vulnerability where some individuals become hypertensive while others remain normotensive with the same levels of salt intake further advising that others are "salt sensitive" while others are "salt resistant" (He *et al*. 2010; Meneton *et al*. 2005). Despite long-standing reports advocating for a reduction in dietary Na^+ intake, recent studies have raised queries regarding potential adverse effects associated with low Na^+ intake importantly on cardiovascular outcomes and related mortality (Oparil, 2014).

However O'Donnell *et al* (2014) revealed that low Na^+ was associated with increased risk for CVD and therefore a targeted approach to Na^+ restriction would be more rational than a population wide approach which assumes that everyone consumes too much Na^+. Appropriate methodology is required to collect high-quality evidence in order to determine the risk and benefits of low Na^+ intake, hence the methods for estimating Na^+ intake are important.

1.4.7 Estimating daily Na^+ intake

For obvious health reasons, the evaluation and management of HT is crucial, and it requires the accurate assessment of net Na^+ balance in the body (Rhee *et al*. 2014). There are several methods for evaluating Na^+ intake such as dietary recall, a food frequency questionnaire (FFQ), spot urine collection, and 24-hour urine collection for the determination of 24-hour Na^+ excretion. The measurement of a 24-hour urinary Na^+ excretion is considered the "gold standard" method for estimating Na^+ intake in a population (Zhang *et al*. 2014; Ortega *et al*. 2010).

Other methods such as the use of dietary recall and FFQ have numerous flaws and are often accompanied with under reporting of Na^+ intake from participants, for example; accurate reporting of dietary recall requires a high degree of motivation and literacy from the participants and enthusiasm from the researcher (Charlton *et al*. 2005; McLean, 2014). Low income countries do not have resources for nutritional monitoring of intake (Bentley, 2006). Intake can be underrated due to difficulty in quantifying discretionary Na^+ intake (McLean, 2014). Casual spot urine specimens may be useful as alternative methods (Brown *et al*. 2013); however, Ji *et al* (2012) indicated that the validity and reliability of this method is uncertain. Furthermore he stated that extensive research, comprising the use of appropriate study design and statistical testing is essential to conclude on the effectiveness of this method.

Based on frequently encountered errors attributable to other methods of assessment, the use of a 24-hour urine collection for estimating Na^+ intake in individuals and in a population remains a preferred method of measurement. It appropriately characterises each patients' mean urinary Na^+ output as an index of intake; it is also used to confirm the efficacy of other assessment methods of Na^+ intake (McLean, 2014; Rhee *et al*. 2014). Expectedly, this method assumes that in a state of Na^+ balance, urinary Na^+ excretion equals Na^+ intake.

This technique of measurements has therefore been used to assess relationships of Na^+ intake with BP levels. However, this approach assumes that the human diet varies little in Na^+ content from day to day. In reality, daily variations in Na^+ intake may occur depending on desire and food availability (Luft *et al*. 1982). Indeed, the variable relations noted to occur between urinary electrolyte excretions rates and BP in previous large epidemiological studies including the Intersalt Study (Intersalt Cooperative Research Group, 1988) and the Scottish Heart Study (Smith *et al* 1988) and the lack of relationship between salt intake and BP in epidemiological studies in Africa (Hoosen *et al* 1985) may also reflect the assumption that the human diet varies little in Na^+ content from day to day. To avoid this assumption, a 7-day urine collection accounts for daily variations in the intake of Na^+. Nevertheless, few studies are available where the intake of Na^+ has been determined from 7-day consecutive urine collections (Liu *et al*. 1979). Importantly, whether the weak relations between Na^+ intake, determined from 24-hour urine Na^+ excretion, and BP are accounted for by the assumption that the human diet varies little in Na^+ content from day to day, has not been determined because there is no data available for a 24-hour urine collection carried out for 7-days consecutive (Liu *et al*. 1979). Henceforth, in an attempt to decrease the prevalence of HT, accurate BP measurements supplemented by accurate dietary data such as a 24-hour urine collection carried out for prolonged periods (i.e. 7-consecutive days) is essential.

1.4.8 Objectives

The objectives of the present study were therefore to determine:

1) the variation in daily Na^+ intake over a 7-day period and to see whether a single 24-hour urine collection provides information on Na^+ intake which accurately reflects the average of a subsequent 6 days of urine collection.

2) the relationship between Na^+ intake and blood pressure and check whether daily Na^+ intake determined from 24-hours urine collection is more closely associated with ambulatory, central or office BP.

CHAPTER TWO

METHODS

2 Methods

2.1 Study participants

The study was approved by the University of the Witwatersrand Committee for Research in Human Subjects (approval number: M15-05-24) and forms part of the ongoing African Project on Genes in Hypertension and the study design has been described in brief in publications (Shiburi *et al*, 2006; Maseko *et al*. 2006). South Africans of black African ancestry from nuclear families of the predominant chiefdoms (tribes) of Nguni, Sotho and Venda with common ancestral backgrounds were randomly recruited from a metropolitan area of Johannesburg (SOWETO). Nuclear families were recruited if at least one or two offspring of at least 17 years of age and one or both parents were available for examination. There was no upper age limit for the participants; a lower age limit was included as BP rapidly increases with age below this value. Six hundred and twenty-nine (629) South Africans of black African ancestry randomly recruited for the measurement of conventional, ambulatory, and central blood pressures and all other anthropometric measurements. Of the 629 South Africans of black African ancestry randomly recruited for the measurement of BP, all of them had complete conventional BP measurements, 489 had complete ABP measurements, and 597 had complete urine collection.

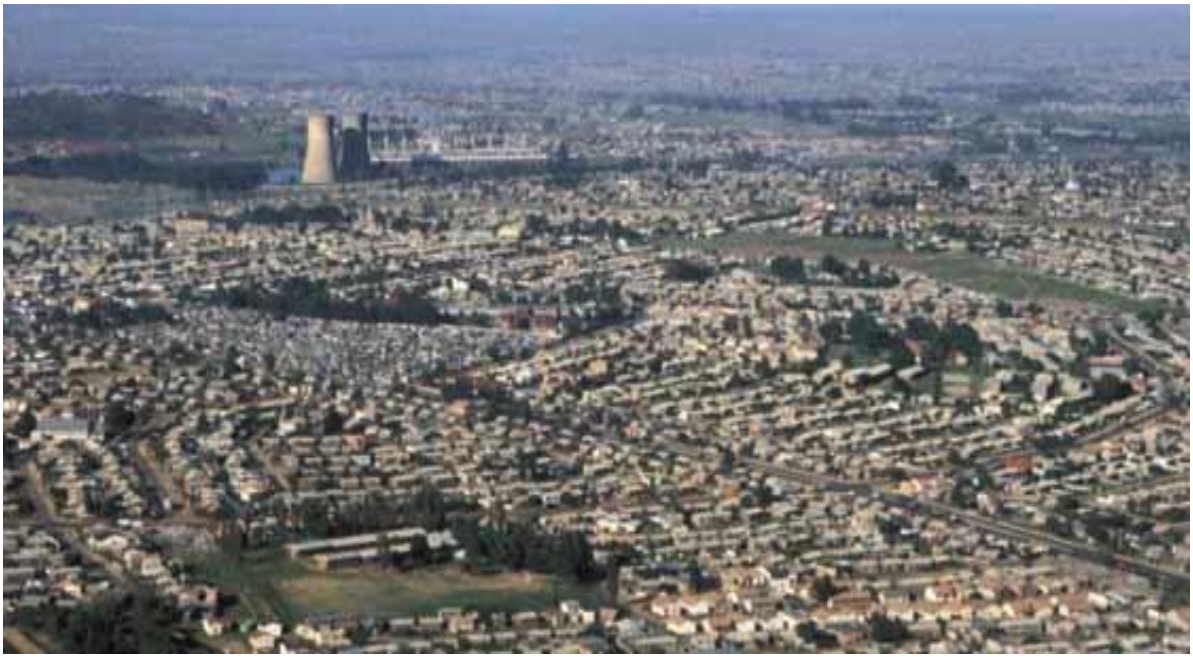


Figure 2.1. Examples of ceremonial residences where people were recruited for this study.

2.2 Clinical, demographic and anthropometric measurements

Subjects completed a standard questionnaire. In order to avoid translational errors, the questionnaire was not translated into an African language, but study assistants familiar with all languages spoken in these townships and who either previously lived in SOWETO or currently reside in SOWETO assisted with the completion of each questionnaire. Only same sex assistants were used to assist each family member with the completion of the questionnaire. Assistance was only provided when requested. The majority of subjects were reasonably proficient in English. Study assistants first visited homes of subjects that agreed to participate in the study in order to develop a trusting relationship. The questionnaire was only completed at a subsequent clinic visit and then ambiguities checked by performing a follow-up home visit. If family members were absent at follow-up home visits, data was checked with them personally via telephonic conversations whenever possible.

Ambiguities in answers to the questionnaire were detected by an independent observer prior to the second home visit. The questionnaire requested specific answers to date of birth, gender, previous medical history, the presence of hypertension, diabetes mellitus and kidney disease, prior and current drug therapy (analgesic use included), prior and current occupation, level of education, smoking status (including the number of cigarettes smoked in the past and at the present time), daily alcohol consumption (beer, traditional beer or other forms of alcohol and the daily quantity), caffeine consumption (number of cups of tea or coffee and whether they are decaffeinated and the number of fizzy or cola drinks a day), exercise frequency and family history of hypertension and cardiovascular events. For females, menstrual history, history of pregnancies and oral contraceptive use was evaluated. Most of the questions simply required a “Yes”-“No” answer, but understanding was assessed by requesting some short answers.

If subjects were unable to provide the name of medication taken these were obtained on the second home visit. Although a crude assessment of SE status (SES) was calculated from the combined levels of education, present occupations and annual income, these data have not been analyzed at this point as an appropriate score has not been derived.

2.3 Conventional blood pressure measurements

Trained nurse/technician observers measured BP using a standard mercury sphygmomanometer during two home visits and a clinic visit. These were not doctors as they are perceived by the community as being in positions of authority and hence may elicit "white-coat" effects. After being trained in the procedure, including being shown the pitfalls of BP measurement (positioning of the cuff, positioning of the arm, first estimating systolic BP using a radial pulse measure in order to avoid increasing cuff pressures too high, detecting auscultatory gaps, releasing valve pressure at the correct speed, using the correct cuff size, etc.), assistants had to demonstrate an ability to perform the procedure on 20 subjects. The study assistants were then tested on their ability to measure BP in two ways. First they were asked to measure BP on a separate group of 20 subjects including patients with hypertension and their readings had to be within 4 mm Hg of an experienced investigator's readings obtained with a stethoscope with two ear pieces. Second, study assistants were asked to watch a video showing a simulated mercury column with Korotkoff sounds where observers were tested on their ability to detect phase I and V sounds under different circumstances including in the presence of a wide auscultatory gap and where phase V Korotkoff was taken as a "muffling" rather than a "disappearance" of sounds (Blood Pressure Measurement, British Medical Journal, BMA House London). To qualify as observers all readings (n= 20) had to be within 4 mm Hg of the reference standard. Assistants who failed on the assessment the first time were given more time to practice on subjects and then asked to repeat the tests.

Home visits were conducted 3-to-4 weeks apart and the clinic visit occurred between the two home visits. A standard cuff with a 12-24 cm inflatable bladder was used, but if upper arm circumference exceeded 31 cm, larger cuffs with a 15-35 cm inflatable bladder were used. After 10 minutes of rest in the seating position, five consecutive BP readings were taken 30 to 60 seconds apart with the subject in a sitting position, followed by a pulse rate count. The cuff was deflated at approximately 2 mm Hg per second and phase I (systolic) and phase V (diastolic) BP recorded to the nearest 2 mm Hg according to the recommendations of the European Society of Hypertension (EHS, 2013). The mean of the five measurements taken at the first home visit was recorded as the home BP. Between the two home visits subjects were invited the School of Physiology Hypertension Clinic where a nurse, following the same procedure as the first home visit, measured the subject's blood pressure under comfortable surroundings. The average of the five readings was taken as the office BP. In the present study quality control of conventional BP assessments was assessed as previously described (Kuznetsova *et al*. 2002). A diagnosis of hypertension was made if subjects were receiving antihypertensive therapy and/or if the average of the mean values for the home and clinic readings was 140/90 mm Hg.

2.4 Anthropometric measurements

Body height, weight, waist and hip circumference and triceps and subscapular skin-fold thickness (Harpenden Skinfold Calliper, Bedfordshire, UK) were measured during the clinic visit by a trained observer. Height and weight were measured with the participants standing and wearing indoor clothes with no shoes. Waist circumference and hip circumference were measured according to conventional techniques (World Health Organization, 2003). Body mass index was calculated as weight in kilograms divided by the square of height in meters and waist-to-hip ratio calculated as an index of central obesity.





Figure 2.2. Hypertension clinic in the School of Physiology. Bottom picture shows reception area and the top picture is the general clinical room.

2.5 Blood sampling

Blood samples were obtained on the day of the clinic visit and sent to the NHLS to perform a full blood count and differential count, to measure urea, creatinine and electrolyte concentrations, to assess liver function (from alanine transaminase, aspartate transaminase, gamma gluteryl transaminase, alkaline phosphatase, albumin, total protein and plasma albumin, total bilirubin, and conjugated and unconjugated bilirubin concentrations) and plasma urate concentrations, to obtain a lipid profile (total cholesterol, low density lipoprotein cholesterol concentrations, high density lipoprotein cholesterol concentrations and triglyceride concentrations), a blood glucose measurement, percentage glycated haemoglobind (HbA1c) and a follicle stimulating hormone concentration in females. These data were used to identify medical conditions, syndromes that may affect BP and to confirm menopausal status in females.

A "spot" urine analysis was also performed to screen for major clinical conditions, such as diabetes mellitus and renal pathology. The NHLS was utilized for blood measurements to ensure reproducibility and reliability as these laboratories have been accredited as fulfilling all criteria of "good laboratory practice" (GLP). In those subjects without a prior history of diabetes mellitus, an HbA1c rather than a fasting blood glucose concentration was utilized in the APOGH study to assess blood glucose control.

2.6 Urinary sodium and potassium excretion

Timed urine samples were obtained over at least a 24-hour period after discarding urine obtained immediately prior to the collection period. Urine Na^+, K^+, and creatinine concentrations were measured by the National Health Laboratories (NHLS) accredited for these measurements.

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 (mmol) was >3.5 and <35 for males and >3.5 and <30 for females. Samples with urine volumes <300 mls/day were also assumed to be incomplete urine collections. These approaches are standard approaches and have been published on numerous occasions by other groups (Kuznetsova *et al*. 2004).

2.7 Validation of estimates of urinary sodium and potassium excretion

Urine samples were collected from the participants over a 24-hour period for seven consecutive days for each participant to validate if the method of estimating Na^+. Each participant was issued a urine collection bottle and was advised to discard the urine sample into the bottle in the morning and record the time. The urine bottles were then collected from each participant the following morning. A new bottle was issued to the participant on each consecutive day and this was continued for 7-days in order to determine the average excretion of Na^+ and K^+ in the urine over a 24-hour period. Urine was analysed by the NHLS as explained above.

2.8 Ambulatory blood pressure measurements

On the same day as 24-hour urine samples were obtained, 24-hour ambulatory BP monitoring was performed using oscillometric monitors (SpaceLabs, model 90207) (see Figure 6).

Ambulatory monitors were calibrated monthly against a mercury manometer. The non-dominant arm was selected for BP monitoring. The size of the cuff was the same as that which was used for conventional BP measurements. The monitors were 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. Subjects kept a diary card for the duration of the recordings to note the time of going to bed in the evening and getting up in the morning. The times when going to bed and getting out of bed in the morning were used to determine the awake and sleep periods of the day. Subjects were also asked to record the time when taking medication in those receiving medication and any times when they either smoked or took caffeine-containing or alcoholic beverages. Subjects were asked to pursue their normal daily activities and to keep the cuff arm steady during measurements. From the subject's diary card data we determined the awake and asleep periods. On average the subjects went to bed at 19:00 h and woke up at 05:00 h.

Considering these patterns of daily activities the daytime and night-time intervals were defined as time intervals ranging from 09:00 h to 19:00 h and from 23:00 h to 05:00 h respectively. These fixed clock-time intervals (Thijs *et al*. 1992) were defined in order to eliminate the transition periods (evening and morning) during which BP changes rapidly in most subjects. On completion of the recording, the data were transferred to a computer for analysis. Intra-individual means of the ambulatory measurements were weighted by the time-interval between successive recordings (Thijs *et al*. 1992). Participants enrolled in the study whose ambulatory BP measurements did not meet the pre-specified quality criteria (more than 20 hours of recordings and more than 10 and 5 readings for the computation of daytime and night-time means respectively) were discarded from the analysis. Ambulatory BP data were expressed as 24-hour average systolic and diastolic BP, the percentage decrease in BP at night (mean day-mean night/ mean day x 100), the difference between day and night BP and the ratio of day-to-night BP.



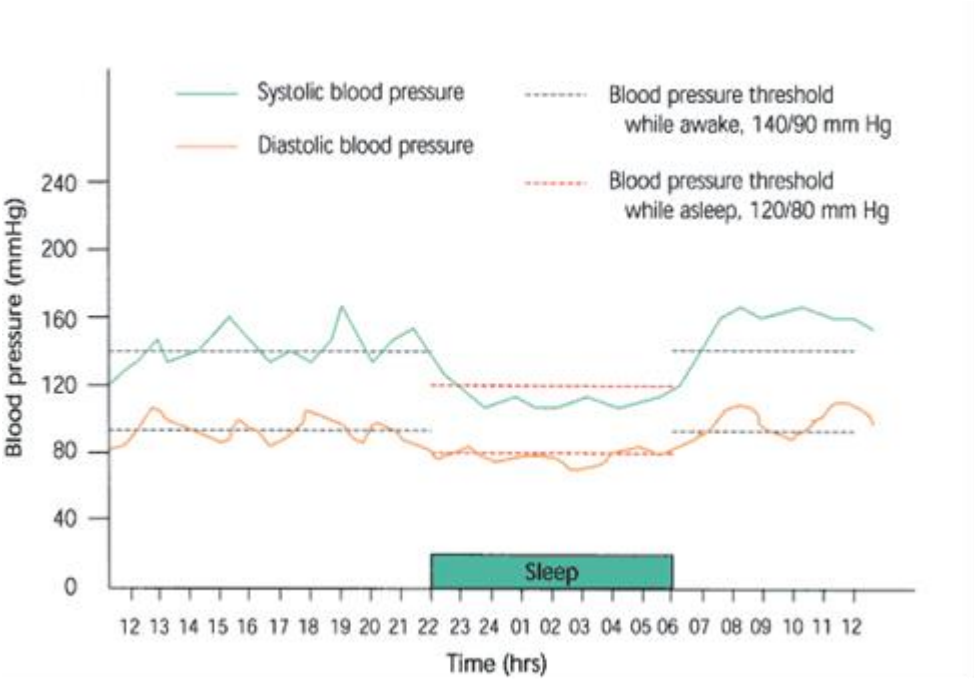


Figure 2.3. Spacelabs model 90207 ambulatory BP monitor (upper panel) and representative data obtained for analysis purposes (lower panel).

2.9 Pulse wave analysis

Central BP, Pulse Pressure (PP), aortic PWV and AIc were estimated using techniques previously described (Shiburi *et al*. 2006). To determine central pressures and the pressure components, after participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm) pulse were recorded by applanation tonometry during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 6.21 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded.

The pulse wave was calibrated by manual measurement (auscultation) of BP taken immediately before the recordings. From a validated inbuilt transfer function an aortic waveform was generated from which central systolic, diastolic and mean arterial BP were derived. The magnitude of the forward pressure component (P1) was determined as the difference between the inflection point at the end of the first systolic shoulder and central diastolic BP (i.e. the height of the first systolic shoulder).

The magnitude of the augmented pressure wave (AP) was determined as the difference between central systolic BP and the inflection point at the end of the first systolic shoulder. Central PP (PPc) was calculated as the difference between central systolic BP and central diastolic BP and mean arterial pressure (MAP) was calculated as [central diastolic BP + 1/3(central PP)]. Although applanation tonometry at the carotid artery is the most accurate non-invasive assessment of the forward and augmented pressures, carotid tonometry cannot be reliably applied in obesity (Laurent *et al*. 2007).

Considering the likelihood of obesity in the study participants, I therefore assessed the pressure components of PPc using radial tonometry. Central AIc was determined as the augmented pressure wave/PP, expressed as a percentage. The reflected wave transit time (RWTT) was determined from the beginning of the incident wave to the end of the first systolic shoulder (i.e., duration of the first systolic shoulder) (Mitchell *et al*. 2004) Aortic PWV was measured from sequential waveform measurements at carotid and femoral sites as previously described (Shiburi *et al*. 2006).

The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Pulse wave transit time i.e. the time it takes the pulse wave to travel from the carotid to the femoral site, was determined as the difference between the times taken to generate the femoral and carotid pulse waveforms. To assess the differences in time of the generation of the femoral and carotid pulse waveforms, a single lead electrocardiogram was performed concurrently with pulse waveform sampling. Aortic PWV was calculated as distance (meters) divided by transit time (seconds).

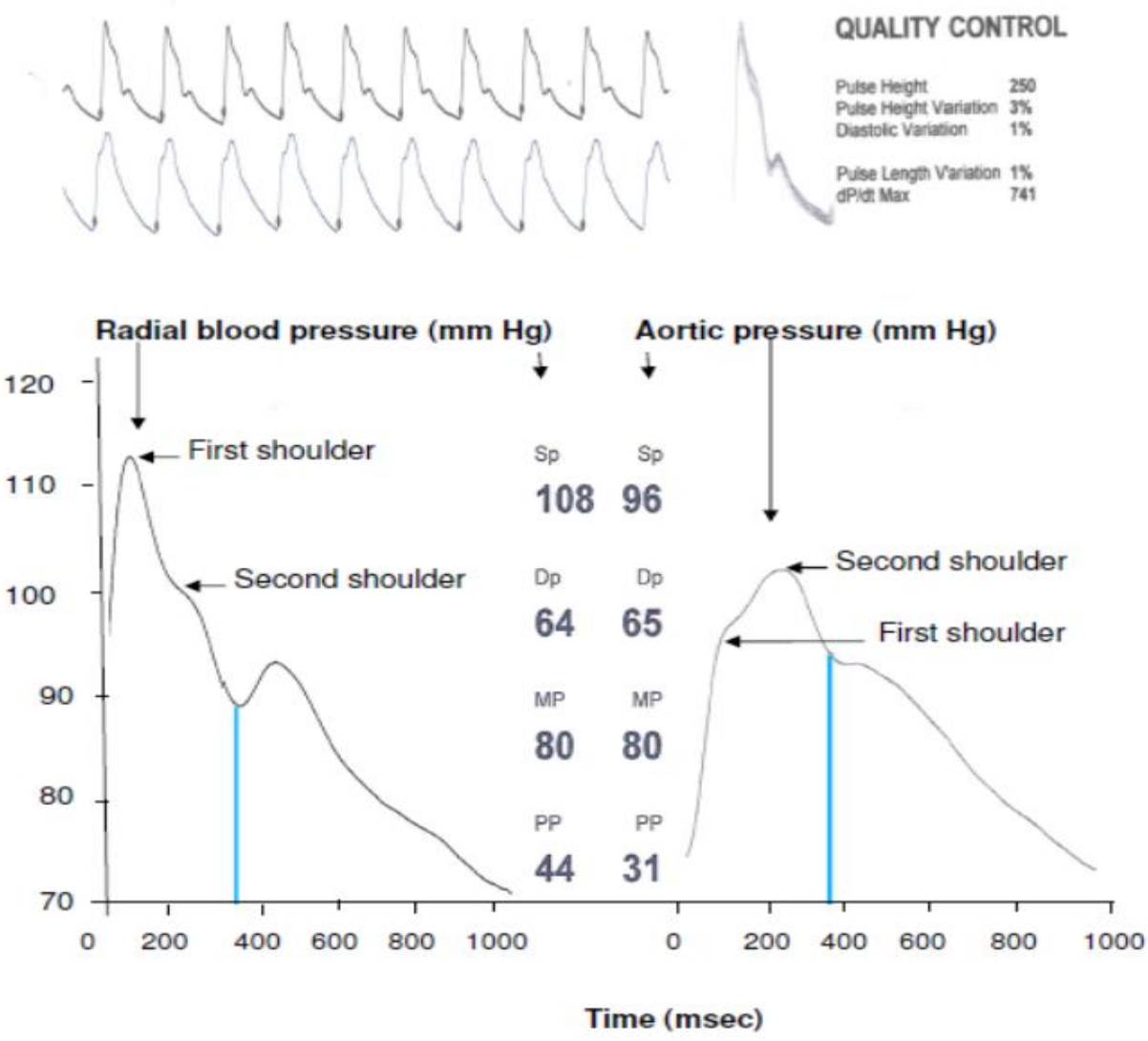


Figure 2.4: Example of a pulse wave recording obtained to determine central haemodynamics. The figure shows the radial artery pulse wave obtained from applanation tonometry (lower left panel) and the aortic pulse wave derived from a population-based transfer function built into the software (lower right panel). The first and second systolic shoulders are identified. See text for a further description. Quality control assessments are shown in the top panel. Sp, systolic blood pressure (BP); Dp, diastolic BP; MP, mean arterial pressure; PP, pulse pressure.



Figure 2.5: SphygmoCor device coupled to an applanation tonometer used to determine central (aortic) haemodynamics and aortic PWV.

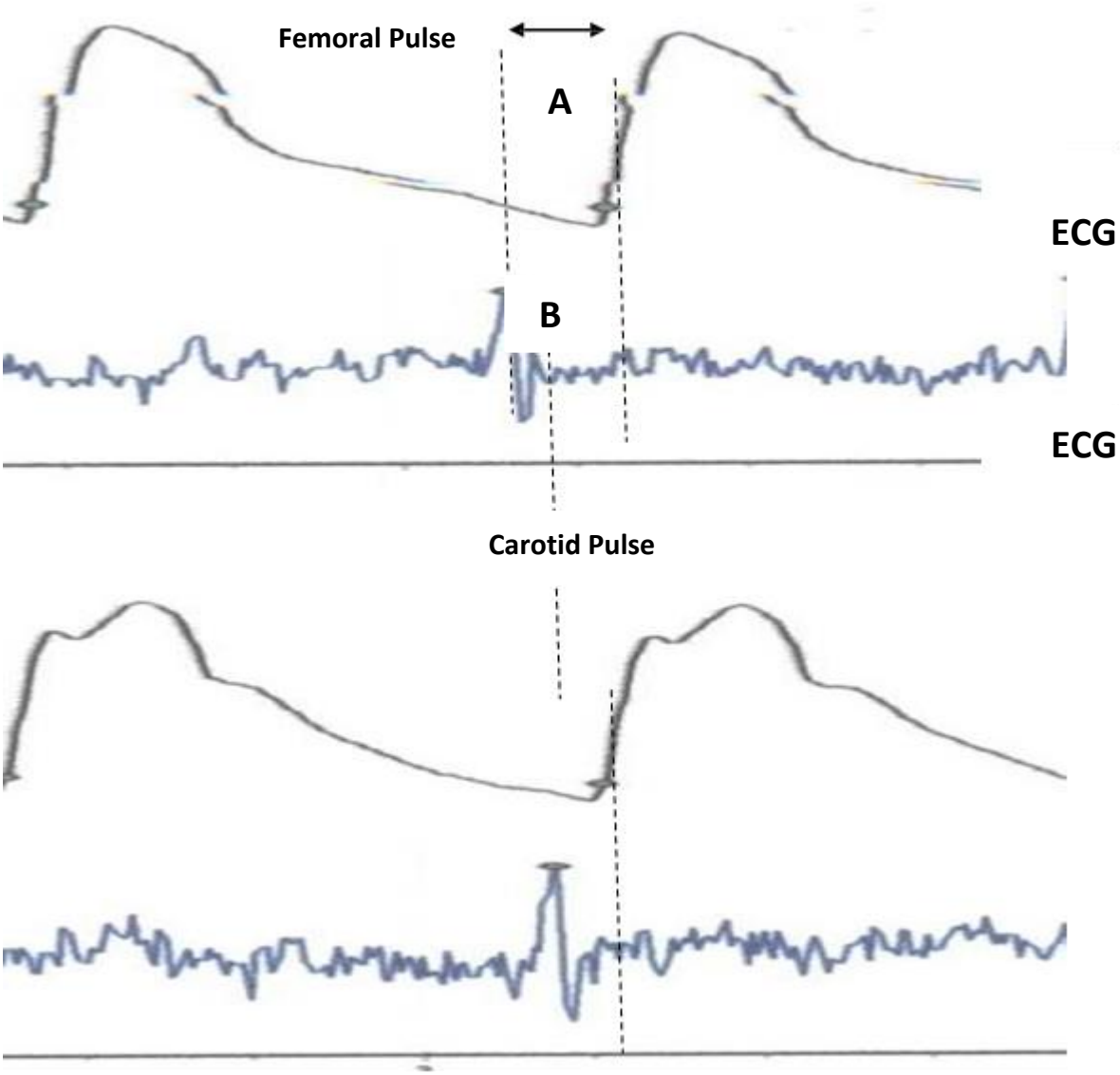


Figure 2.6: Examples of femoral and carotid artery pulse waves obtained using applanation tonometry from the same participants. Together with simultaneous electrocardiographic (ECG) recordings aortic PWV (PWV) is calculated. The arrows indicate the time between electrical events and the arterial pressure changes in the carotid and femoral arteries used to calculate PWV. See text for a further description.

2.10 Definitions and analysis

Definitions for hypertension from auscultatory measurements (≥140 mmHg systolic BP or 90 mmHg diastolic BP) and ambulatory BP (≥130 mmHg systolic BP or 80 mmHg diastolic BP) values were based on published guidelines (World Health Organization; Guidelines Committee of the European Society of Hypertension, 2013; O'Brien *et al*. 2003). The presence of hypertension was identified if subjects were either receiving antihypertensive medication, or had an elevated auscultatory BP value obtained in a clinic environment. Overweight and obesity were defined using body mass index thresholds of between 25-29.9 kg/m^2 for overweight and ≥30 kg/m^2 for obesity.

Database management and statistical analyses were performed with SAS software, version 9.4 (The SAS Institute Inc., Cary, North Carolina, USA). Data from individual subjects were averaged and expressed as mean SD. The X^2-statistic was used to compare proportions. Comparisons between normotensives, hypertensives aware of their condition and hypertensives unaware of their condition and not receiving treatment for it were made using an ANOVA when no adjustments for confounding variables were required and multivariate regression analysis when adjustments were required.

To determine whether awareness and treatment of hypertension was an independent determinant of electrolyte intake, covariates included in the regression model were age, gender, BMI, tobacco and alcohol intake, the class of antihypertensive agent employed and the presence or absence of diabetes mellitus. To identify the predictors of 24-hour ambulatory BP, stepwise multivariate adjusted regression analysis was performed with age, gender, antihypertensive medication, the presence or absence of diabetes mellitus or abnormal blood glucose control, indices of adiposity, 24-hour electrolyte excretion rates and urine Na^+:K^+ ratio included in the regression model.

Probability values were obtained with further adjustments for non-independence of family members (mixed model as outlined in the SAS package).To identify the predictors of indices of nocturnal decreases in BP, stepwise regression analysis was performed with age, gender, the presence or absence of hypertension, antihypertensive medication, the presence or absence of diabetes mellitus or abnormal blood glucose control, indices of adiposity, 24-hour electrolyte excretion rates and urine Na^+:K^+ ratio used in the regression model. Again, probability values were obtained with further adjustments for non-independence of family members (mixed model as outlined in the SAS package). Importantly, adjustments for age, gender, the presence or absence of hypertension, the presence or absence of antihypertensive medication, the presence or absence of diabetes mellitus or abnormal blood glucose control, and adjustments for non-independence of family members were made in all regression analyses, whereas. BMI, waist circumference, waist-to-hip ratio and skin-fold thickness were assessed in separate models from each other, and electrolyte excretion rates were assessed in models separate from those with urine Na^+:K^+ ratios.

CHAPTER THREE

RESULTS

Table 3.1 gives a description of the demographic, anthropometric, and general clinical characteristics of the participants in the present study. These characteristics include age, BMI, blood pressure, urinary Na^+ and K^+ excretion rates, alcohol consumption, smoking and diabetes status. The mean age of participants was 43.9±18.4 with 64% of participants being women. The mean BMI of the group was 29.2±7.9, and 67% of participants were either overweight (24%) or obese (43%). Thirty-seven percent (37.1%) of the sample population was hypertensive, 23.0% consumed alcohol regularly, 16.9% smoked, and 12.2% had diabetes mellitus or an HbA_{1C} >6.1%. Table 3.2 gives the haemodynamic characteristics which include the clinic and home blood pressure value of the participants. The mean and standard deviation is recorded for all home and office blood pressure parameters. Both systolic and diastolic blood pressure measurements for the average of the 24-hour ABP, daytime ABP, night time ABP, central blood pressure, pulse pressure and conventional blood pressures are shown in Table 3.2. Moreover, Daytime systolic and conventional systolic BP are higher than normal BP values.

Table 3.1: Characteristics of the study population

Sample Number	629
Age (years)	43.9 ± 18.4
Female %	64
BMI Females	31.5 ± 8.2
BMI Males	24.9 ± 5.3
Whr	0.84 ± 0.09
% Hypertensive	37.1
% Diabetic	12.2
Regular alcohol (% subjects)	23.0
Regular tobacco (% subjects)	16.9

BMI, body mass index; ± values are the standard deviation; whr, waist-to-hip ratio

Table 3.2: Heamodynamic characteristics

Index	Mean	SD	P5	P50	P95
24 hour SBP	118.2	14.4	99.9	115.0	146.7
24 hour DBP	72.2	9.4	58.8	71.3	90.5
Daytime SBP	122.8	14.2	103.9	119.9	152.2
Daytime DBP	77.4	9.6	64.3	76.0	96.2
Night-time SBP	110.9	16.7	90.1	106.8	145.5
Night-time DBP	64.4	11.0	49.6	62.7	84.4
SBPC	131.1	22.7	102.4	127.6	175.2
DBPC	84.7	12.4	68.4	83.2	106.4
C_SP	122.5	23.5			
C_DP	85.5	12.8	68.0	84.0	109.0
C_PP	37.0	16.2	18.7	34.1	66.9
P_PP	46.3	17.4	26.0	44.0	78.0
PPC	46.3	15.8	27.2	42.8	78.4

SBP, systolic blood pressure; DBP, diastolic blood pressure; SBPC, conventional systolic pressure; DBPC, conventional systolic blood pressure; C_SP, central systolic pressure; C_DP, central diastolic pressure; C_PP, central pulse pressure; P_PP, peripheral pulse pressure; PPC, conventional pulse pressure.

Table 3.3 gives the electrolyte excretion rates, urine electrolyte concentration, blood electrolyte concentration, and the creatinine clearance values of the participants. The average 24-hour urinary Na^+ excretion rate was 111.2 mmol/day, well above the RDA for Na^+ intake of 100 mmol/day.The 24-hour urinary K^+ excretion rates were 28.9 mmol/day way lesser than the RDA intake for K^+ of 120 mmo/day. No major differences were noted in urine volumes between the participants. On multivariate analysis, a younger age was the only independent determinant of 24-hour urinary Na^+ excretion. Data also shows serum values of creatinine, magnesium, glucose and glycated hemoglobin.

Table 3.4 shows how renin levels influence changes in blood pressure. Renin was divided into tertials and correlated with blood pressure. The relationship between renin and each blood parameter i.e. systolic and diastolic of 24-hour ABP, daytime ABP, night time ABP, conventional BP was studied as shown in Table 3.4. Table 3.5 shows analysis of correlation between blood pressure and urinary Na^+ excretion rate. Although daytime ABP appears to be greater than nighttime ABP in the participants due to a decline in BP that occurs at night, none of the blood pressure parameters (systolic and diastolic) i.e. 24-hour ABP, Daytime ABP, night time ABP, conventional BP and central blood pressure was associated with Na^+ intake independent of age. Aldosterone, renin, and renin ratio versus blood pressure was correlated and appears in Table 3.6. The ratio of aldosterone to renin (ARR) was significantly associated with different blood pressure parameters independent of age.

Table 3.3: Electrolytes values

Index	Mean	SD	P5	P50	P95
Bsug	5.4	2.7	3.6	4.7	9.0
SCRT	77.5	34.1	50	7	105
SMG	0.8	0.1	0.7	0.8	0.9
Crtc (CRT)	7.9	5.8	1.0	7.3	17.9
Crt	10.8	11.2	1.2	7.6	31.2
K^+	28.9	21.8	5.0	24.3	69.2
Na^+	111.2	83.2	22.0	94.3	237.6
Nac	88.8	58.3	19	77	204
Nak	4.4	2.4	1.8	3.9	9.2
crcl	2.3	3.2	0.2	1.4	7.6
Ghb	6.1	1.4	5.3	5.8	8.4

Bsug (mmol/l), blood glucose; scrt (µmol/l), serum creatinine; smg (mmol/l), serum magnesium; Crct (mmol/l), urine creatinine concentration; K^+ (mmol/l), potassium; Na^+ (mmol/l), sodium; Nac, urine sodium concentration; Nak, sodium-potassium ratio; crcl, creatinine clearance; Ghb (g/dl), glycated haemoglobin.

Table 3.4 Tertials of renin

	RENIN LEVELS		
	< 5.7	5.7 - 13.5	13.5 - 33
BLOOD PRESSURE			
SBP24	123 ± 16.8	116.9 ± 13.5	114.7 ± 13.4
DBP24	75.9 ± 11.4	72.2 ± 9.0	70.2 ± 9.5
SBPN	116.5 ± 19.5	109.7 ± 15.3	107.6 ± 10.8
DBPN	68.4 ± 12.8	64.4 ± 10.9	62.4 ± 10.8
SBPD	126.7 ±16.4	121.3 ± 13.3	119.3 ± 13.4
DBPD	80.6 ± 11.6	77.2 ± 8.9	75.4 ± 9.8
SBPC	136.4 ± 23.3	127.3 ± 22.1	124.2 ± 19.3
DBPC	87.7 ± 12.6	83.1 ± 12.6	81.6 ± 11.1

SBP24, 24-hour systolic blood pressure; DBP24, 24-hour diastolic blood pressure; SBPN, night-time systolic blood pressure; DBPN, night-time diastolic blood pressure; SBPD, daytime systolic blood pressure; DBPD, daytime diastolic blood pressure; SBPC, conventional systolic pressure; DBPC, conventional systolic blood pressure.

Table 3.5: Relationship between blood pressure and urinary sodium and potassium

Blood Pressure	Urinary Na^+	Urinary K^+	Urinary Na^+/K^+
SBP24	0.1715	0.2991	0.0004
DBP24	0.5950	0.2807	0.1259
SBPN	0.6984	0.3640	0.0011
DBPN	0.2516	0.3563	0.2658
SBPD	0.1581	0.2806	0.0011
DBPD	0.6828	0.3006	0.2490
SBPC	0.4161	0.2405	0.0040
DBPC	0.5890	0.3872	0.1732

SBP24, 24-hour systolic blood pressure; DBP24, 24-hour diastolic blood pressure; SBPN, night-time systolic blood pressure; DBPN, night-time diastolic blood pressure; SBPD, daytime systolic blood pressure; DBPD, daytime diastolic blood pressure; SBPC, conventional systolic pressure; DBPC, conventional systolic blood pressure

Table 3.6: Aldosterone, renin, and renin ratio versus blood pressure

	RENIN		ALDOSTERONE		ARR	
	r	p	r	p	r	p
SBP24	0.08	0.0294	0.10	0.2804	0.26	<0.0001
DBP24	0.10	0.0122	0.07	0.4440	0.27	<0.0001
SBPN	0.07	0.0393	0.09	0.6938	0.26	<0.0001
DBPN	0.09	0.0145	0.06	0.9262	0.26	<0.0001
SBPD	0.08	0.0317	0.09	0.3502	0.23	<0.0001
DBPD	0.08	0.0164	0.07	0.3818	0.23	<0.0001
SBPC	0.10	0.0131	0.08	0.3163	0.26	<0.0001
DBPC	0.10	0.0002	0.10	0.7460	0.23	<0.0001

ARR, Aldosterone renin ratio; SBP24, 24-hour systolic blood pressure; DBP24, 24-hour diastolic blood pressure; SBPN, night-time systolic blood pressure; DBPN, night-time diastolic blood pressure; SBPD, daytime systolic blood pressure; DBPD, daytime diastolic blood pressure; SBPC, conventional systolic pressure; DBPC, conventional systolic blood pressure.

Figure 3.1 shows the daily variations in Na^+ excretion as an index of intake. The figure shows that there is little change from one day to the next throughout the seven consecutive days with regards to Na^+ intake. It also clearly shows that the 24-hour urinary Na^+ excretion measurements can be collected at any day of the week and is not dependant on certain days of the week. Figure 3.2 illustrates that the first day of urinary Na^+ collection is similar to the average of the subsequent six days collection and that the first day collection is as good as the average of the subsequent six days. Figure 3.4 and figure 3.5 depict similar findings to figure 3.1 and figure 3.2 respectively with the exception that the results displayed in figure 3.4 and figure 3.5 are for 24-hour urinary excretion of K^+. The figures show that there is little variation in the intake of K^+ in the population. Figure 3.3 is the Bland-Altman analysis that compares the difference in daily variation and the average change, the data shows that there is little difference between the daily averages which shows that daily variation in Na^+ intake is minimal.

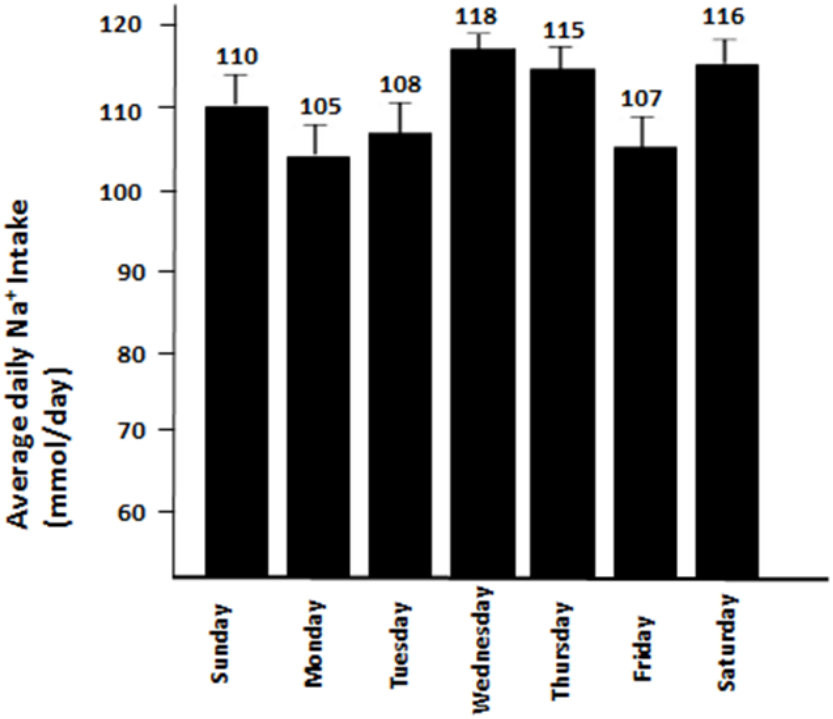


Figure 3.1: Daily Variations in Na^+ Intake
Average values of Sodium intake on different days of the week. Na^+, Sodium

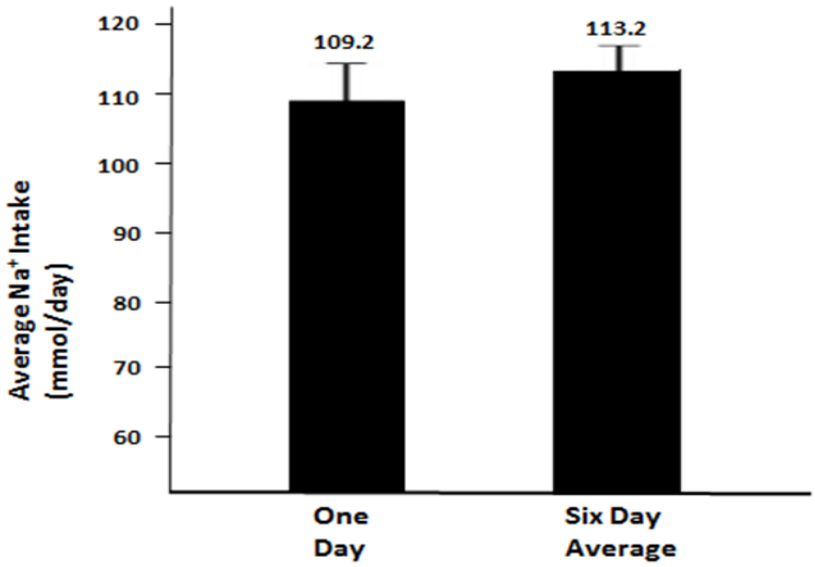


Figure 3.2: One day versus six day average Na^+ intake
Mean values of Na^+ intake based on one day versus the average of six days intake.

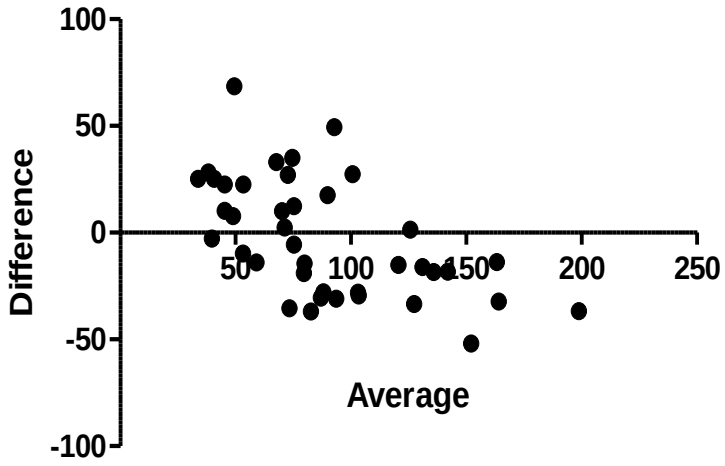


Figure 3.3: Difference versus average: Bland Altman data
Average levels of Na^+ intake versus daily variations in Na^+ intake.

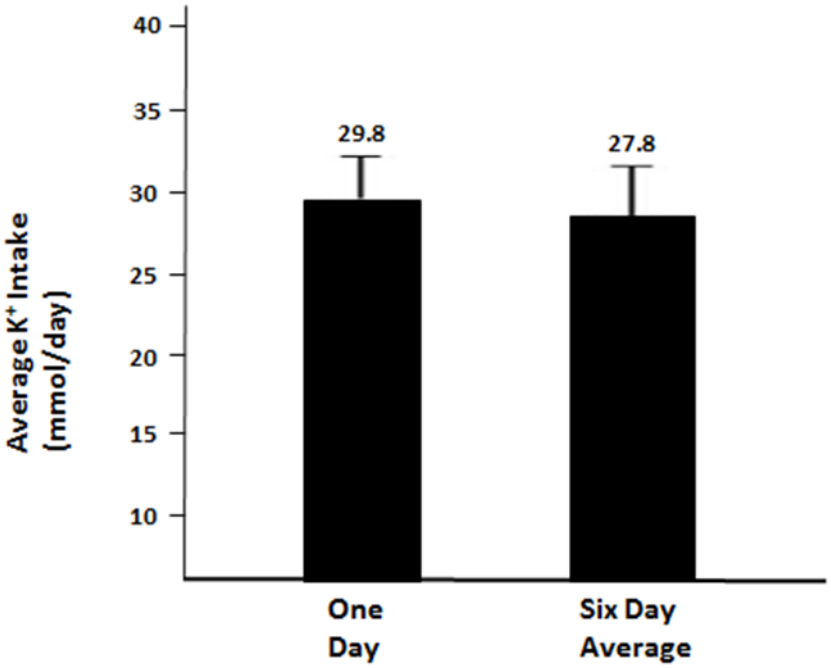


Figure 3.4: One day versus six day average K^+ intake
Mean values of K^+ intake based on one day versus the average of six days intake.

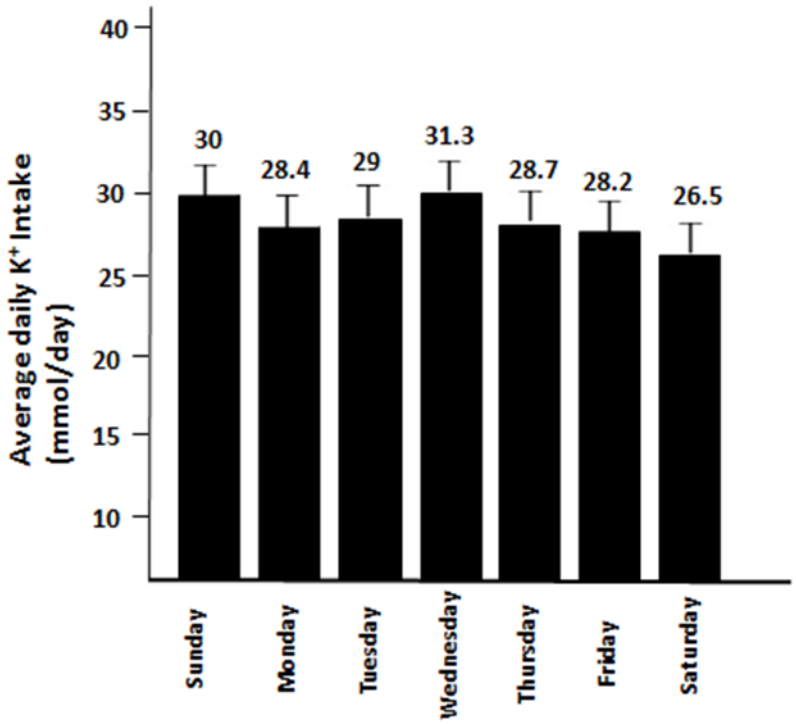


Figure 3.5: Daily variations in K^+ intake
Average values of Potassium intake on different days of the week. K^+, Potassium

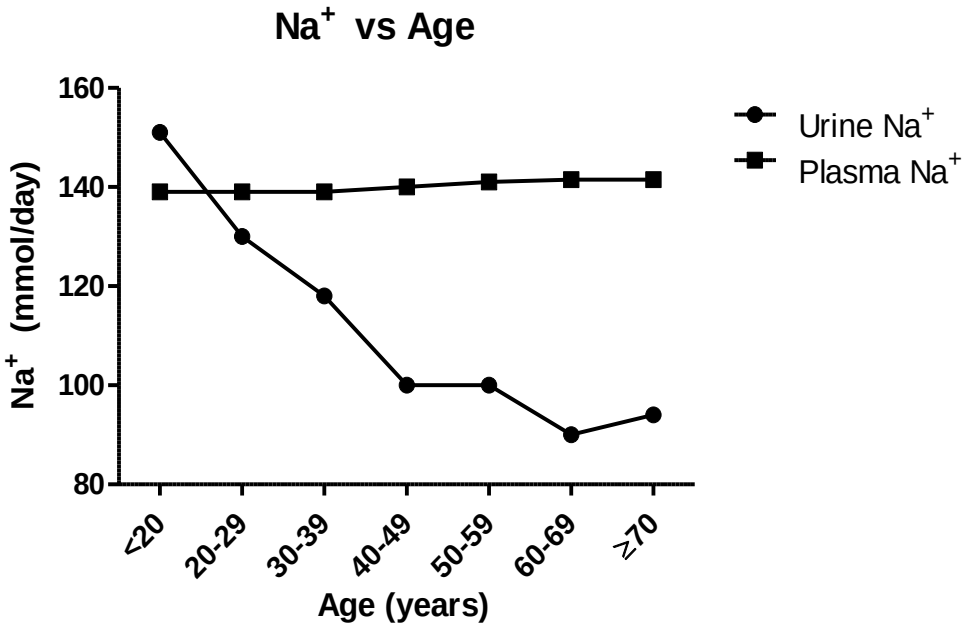


Figure 3.6: Relationship between Age and plasma sodium and urinary Sodium excretion

Figure 3.6 shows the relationship between Age and concentrations of Na^+ i.e. plasma Na^+ and urinary Na^+ excretion. Data obtained from the subjects show that plasma Na^+ remains fairly constant even with an increase in age. However, data shows that urinary Na^+ values in subjects with appropriate urine volumes decreases with an increase in age independent of gender and hypertensive status. Figure 3.7 summarises the results of stepwise regression analysis for relationships between upper limits and lower limits of salt intake versus blood pressure in participants when categorised into different age groups. The figure shows the relationships between age and systolic and diastolic BP i.e. 24-hour ABP, daytime ABP, night time ABP, conventional BP.

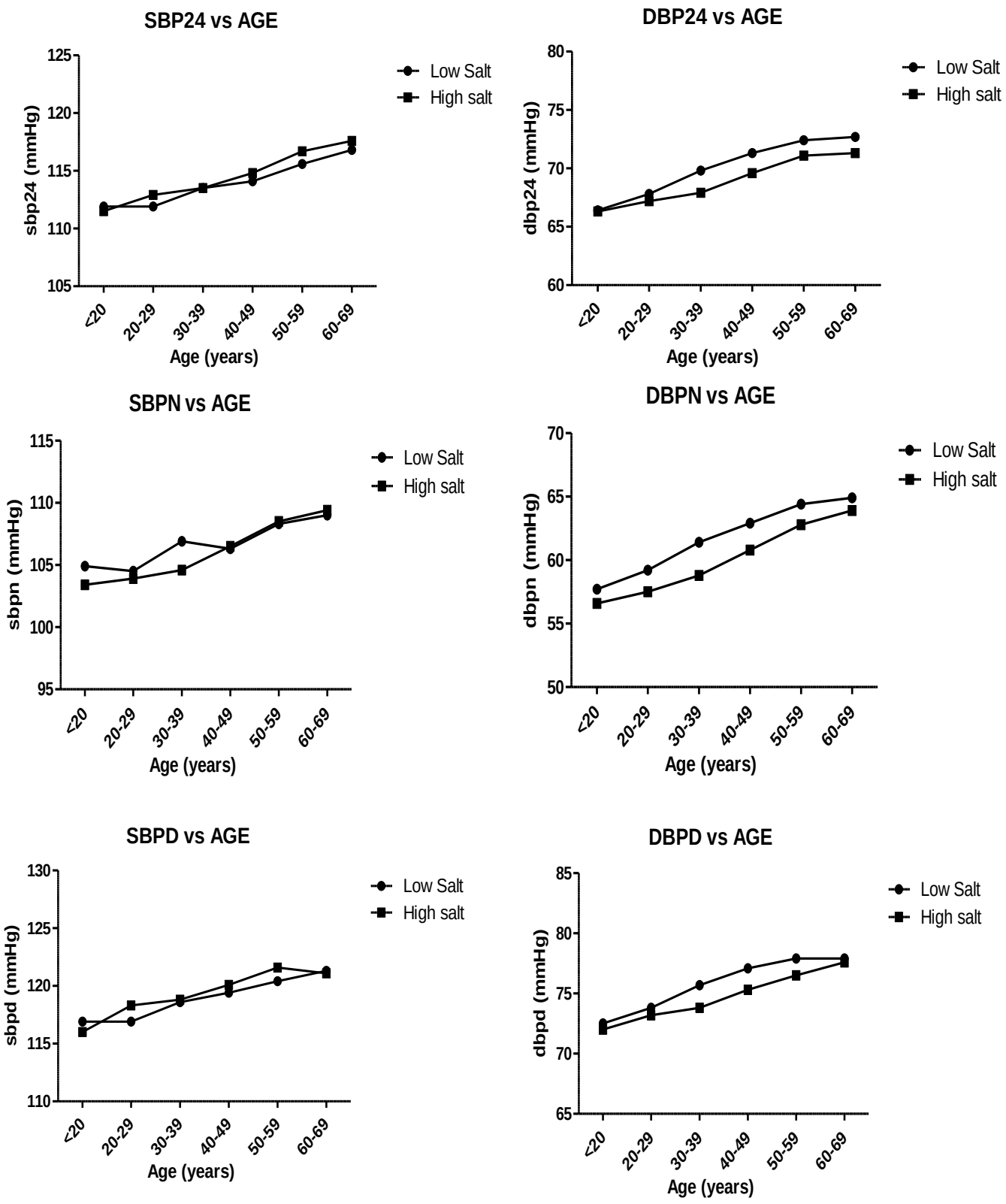


Figure 3.7: Upper limits and lower limits of salt intake versus age and blood pressure i.e SBP24; DBP24; DBPN; SBPN; SBPD;DBPN.

CHAPTER FOUR

DISCUSSION

A number of studies have reported on the relationship between salt intake and blood pressure, however these results are contradictory. Some show a positive relationship between BP and sodium while others have shown no association. The reason for these discrepancies could be methodological problems arising due to the daily variations in sodium intake. This raises a question whether the 24-hour urine collection is a true representation of daily sodium intake. Hence in this study I first validate the 24-hour urine collection method before looking at the relationship between BP and 24-hour urine sodium and potassium excretion.

4.1 Validation of the 24-hour urine collection method

Previous studies have used the 24-hour urine collection method to quantify the levels on dietary Na^+ intake (Ortega *et al*. 2010; Mente *et al*. 2014; Rhee *et al*. 2014; Michel *et al* 2014; Zhang *et al*. 2013; Scott *et al* 2011; Ji *et al*.2012; Millen *et al* 2013; Maseko *et al*. 2006). However, none of these studies have validated the 24-hour urinary excretion method. To my knowledge, other than a study conducted by Liu *et al* (1979) in a group of school children, there are no studies or data available regarding the use of the 24-hour urine collection method for prolonged periods of at least seven days in this population or any other adult population to check whether the levels of estimates of Na^+ intake can be reproducible over several consecutive days. In the above mentioned studies (Michel *et al*. 2014; Scott *et al* 2011; Millen *et al* 2013) there were weak or no relationships between Na^+ intake and BP. It is uncertain whether this is a true reflection because there could be daily variations in Na^+ and K^+ intake.

In order to credibly draw a solid assumption of the relationship between Na^+ intake and BP in a population of African Ancestry, I assessed if a once-off 24-hour urine sample closely approximates daily Na^+ intake over six consecutive days (methods described in Chapter 2). On analysis, I compared the levels of Na^+ and K^+ excretion between a 24-hour urine sample collected on the first day and the subsequent six days collection, and thereafter I compared the two methods.

Our data revealed that there was no major difference in Na^+ and K^+ intake from one day to the next. For Na^+ intake, the average daily variation in the group was 3.2 mmol, and for K^+ the average daily variation in intake was 2.1 mmol. Even where the sum of the consecutive six days collection was averaged (6 days average Na^+ = 113.1 mmol; 6 days average K^+ = 27.8 mmol), it did not vary significantly from the first day of urine collection (109.2 mmol Na^+; 29.8 mmol K^+). When testing for deviations for individual levels of daily Na^+ excretion, data revealed that there was little variation in Na^+ intake for each individual on a day-to-day basis; this was consistent to the report by Liu *et al* (1979). Although conducted for seven consecutive days and individuals had a relatively stable Na^+ intake, his results (Liu *et al* .1979) cannot be a representative of a population because it was conducted in a group of school children, contrary to my study where participants were randomly recruited. I then compared dietary sodium intake on the weekend with the other days of the week. Hypothetically, based on the social lifestyle of this community, it would have been expected that urinary excretion of the ions in question (Na^+ and K^+) would have been higher on days falling on the weekend, particularly Na^+. The reason being that majority of this community attends social gatherings on weekends where they feast on different type of foods. However, our data showed that the levels of urinary excretions of Na^+ and K^+ were similar throughout the week.

To further confirm that a single 24-hour urine collection sample closely approximates urine samples collected over a period of six subsequent days, I used the Bland-Augment plot to compare the two methods i.e. one day 24-hour urine collection versus six subsequent days 24-hour urine collection. The Bland-Augment plot yielded a clinically insignificant low bias (discrepancy) of -2.4. This further proved that the two methods are similar. Based on the above evidence it can be concluded that the use of a single 24-hour urine sample provides as accurate estimate of dietary Na^+ intake in a population of African ancestry.

4.2 Relationship between urinary Na^+ excretions and conventional blood pressure

Sodium intake is a modifiable factor that has previously been reported to increase BP (Stocker *et al*. 2010; Hajjar *et al*. 2001; Farquhar *et al*. 2015; Reddy *et al*. 2015; Zhang *et al*. 2013; du Cailar *et al*. 2004; Rhee *et al*. 2014; Karppanen and Mervaala, 2006; Buyck *et al*. 2009). Having confirmed that Na^+ excretions rates vary little from day-to-day in this population, I then analysed the relationship between Na^+ intake and conventional blood pressure. In the present study of a randomly selected community sample of African ancestry, there was no independent relationship between Na^+ intake and conventional SBP (P=0.47 and conventional DBP (P=0.84) after adjustments for confounding factors. The diffident relationship between Na^+ intake and BP in the group is consistent with reports from previous large epidemiological studies including the Intersalt, the Scottish Heart Study (Intersalt Cooperative Research Group, 1988; Smith *et al*. 1988) and other epidemiological studies in populations of Africa ancestry (Hoosen *et al*. 1985; Charlton *et al*. 2005). This is indeed in disparity to a number of studies which show relationships between an index of Na^+ intake with both SBP and DBP (O'Donnel *et al*, 2014).

However I could not draw a conclusion that there is no relationship between BP and dietary Na^+ based on the conventional BP measurement because using the conventional BP technique to measure BP has a number of limitations. Conventional BP does not account for the circadian rhythms characterized by daytime and night-time BP or the overestimation of BP in people with white coat hypertension. Furthermore conventional BP readings are dependent on the observer therefore I could have overestimated BP values. This is confirmed by the clinically significant difference between the conventional BP (131mmHg/72mmHg) and ambulatory BP (118mmHg/72mmHg) of this population. It was therefore important that I look at the relationship between ambulatory BP and Na^+ before coming to any conclusion.

4.3 Relationship between dietary sodium and ambulatory blood pressure

A stepwise multi regression analysis showed that there is no independent relationship between ABP measurements with Na^+, regardless if it was 24-hour SBP (P=0.17), DBP (P=0.59); daytime SBP (P=0.15), DBP (P=0.68); or night-time SBP (P=0.69), DBP (P=0.25). The findings of the present study are consistent with studies done in other population groups (Shin *et al*. 2014; Staessen *et al*. 1993). However in these studies they were able to show a positive correlation between night-time BP and Na^+ excretion. Because Na^+ is not excreted at the same rate throughout the day, it appears that overnight excretion rate is more positively associated with night time SBP (partial r = 0.29). The explanation to this relationship is understood to be the pressure natriuresis mechanism. Blood pressure is normally low at night compared to daytime. However, if Na^+ is retained during the day, as in the case of people of African ancestry (Hall, 1990; Palacios *et al*. 2004; Harshfield, 2009; Dusta, 1989; Frisancho *et al*. 1984; Dressler, 1990), it may be excreted more during the night by elevating night-time BP levels to encourage the pressure-natriuresis mechanism and this results in non-dipping. That is why individuals who have a tendency to retain Na^+, and those who are salt sensitive are generally non-dippers. In our population sample nocturnal BP dipping is less than 10%. This indicates that nocturnal BP dipping is attenuated. It is therefore possible that in our population sample night-time Na^+ excretion is favored as indicated by the reduced BP dipping at night. Therefore the differences observed between our studies and these studies is that we did not separated daytime urine from nighttime urine samples. In future studies night time urine will be collected separately from daytime in order to establish if there is an independent relationship between dietary sodium and night-time BP in this population. Other factors that could account for the differences in our study with regard to the relationship between BP and dietary Na^+ owing to the differences in composition of the study groups pertaining to age, gender, and race.

4.4 The impact of age on BP and Na^+ excretion.

In our population sample we observed a positive relationship between age and BP. This is consistent with many other studies that have been conducted in the past. Interestingly we observed a negative association between age and urinary Na^+ excretion ($P \leq 0.001$). The possible explanation is that age is a biological progression associated with structural adjustments in body organs and a decline in physiological functions (Gamici *et al*. 2009). Earlier studies have observed that, as with many other organs, there are structural changes that occur in the kidneys such as the reduction in the number of functional nephrons accompanied with progressive glomerulosclerosis which ultimately influences the kidney's ability to excrete Na^+, all of which are associated with the process of aging (Luft et al. 1980; Kasiske, 1987; Berglund, 1983; Meneton et al. 2005). Expectedly, due to the decline in physiological functions associated with age, levels of urinary Na^+ were predicted to decrease with an increase in age based on the understanding that human kidneys typically have a weakened ability to excrete Na^+ (Luft et al. 1980; Kasiske, 1987; Berglund, 1983; Meneton et al. 2005). To my knowledge, this is the first study in this population to show that as age progresses, there is a fall in urinary Na^+ excretion. The finding of the present study is supported by reports that there is a hastening fall in glomerular filtration rate (GRF) associated with age, and this fall begins around the ages of 30 years (Mimran and Ristein, 1992), as described, the average age of this population was well above 30 years age (43.9 years). Although there is a noticeable decline in urinary Na^+ excretion with an increase in age, this does not necessarily suggest that there is a decline in Na^+ consumption in the elderly group of this population. It simply explains the heightened capacity to retain Na^+ in the kidneys of the elderly as explained (Luft *et al*. 1980; Kasiske, 1987; Mimran and Ristein, 1992; Berglund, 1983; Meneton *et al*. 2005). It was also demonstrated (Luft *et al*. 1980) that after sodium load, normal subjects over the age 40 years excrete less sodium than those under 40 years.

With the understanding of the impact of age on N^+ excretion, we divided our population into age groups to assess the impact of Na^+ on age related increases in 24-hour, daytime and night-time BP for both systolic and diastolic BP. After adjusting for covariates, there was no difference in BP between participants whose daily salt intake was above the daily threshold and those whose salt intake was within the normal range across the adult life span. This further confirmed our findings that neither conventional nor ambulatory BP are associated with salt intake in black South Africans and supports the body of knowledge which shows no relationship between dietary Na^+ intake and blood pressure.

4.5. Relationship between the sodium-to-potassium ratio and blood pressure.

Although my study shows no relationship between Na^+ and BP, there is enough body of knowledge that supports the positive association between Na^+ that cannot be ignored. I then hypothesised that since Na^+ does not exist in isolation in body fluids but constantly interacts with other ions; its relation to blood pressure may not be independent of other body electrolytes in this population. We then focused on the sodium-to-potassium ratio (Na^+/K^+) and investigated its relationship to BP independent of other confounders. There are studies conducted in other population groups that have provided evidence to suggests that the ratio of Na^+/K^+ could be responsible for BP regulation (Alderman, 2002), and an imbalance in this ratio; as in the case of high levels of Na^+ and low levels of K^+ intake could be associated with high BP and a greater risk for CVD (Zhang *et al*. 2013). In previous discussions, we have provided suggestions which propose mechanisms responsible for an increased BP in groups of African Ancestry. However, some studies suggest that the mechanisms involved are much more complex and other multiple interconnected factors such as increase in K^+ intake may improve BP (Adregue and Madias, 2014).

Before looking at the relationship between BP and Na^+/K^+, I first assessed the association between BP and K^+ in this population. I investigated whether daily K^+ intake determined from 24-hour urine collection is associated with BP. Although the patterns of dietary K^+ intake were previously explored in this urban, developing community of SOWETO (Maseko *et al*. 2006), I also investigated daily potassium intake in this population and my findings are consistent with data provided (Maseko *et al*. 2006), which specifies that persons living in urban, developing community of SOWETO have 24-hour urinary K^+ excretion rates of 28.9 mmol/day which are less than the RDA of 120 mmol/day. However it should be mentioned that when dietary K^+ is assessed from 24-hour urine excretion, it can be underestimated because unlike Na^+, a considerable amount of the total K^+ intake is excreted through defecation instead of micturition, and people of African Ancestry have low urinary K^+ excretion as compared to whites even when fed the same diet (Turban *et al*. 2008); further stressing that the lower urinary K^+ excretion observed in groups of African Ancestry may reflect more than differences in intake. Also, the ratio of fecal to urinary K^+ is said to be higher in groups of African Ancestry (Turban *et al*. 2008; Barlow *et al*. 1986; Voors *et al*. 1983). Even with the evidence that urinary K^+ could be reduced by one-third as described in earlier studies (Barlow *et al*. 1986), participants would have potassium intake below the RDA of 120 mmol/day, even if that one-third was to be added to the recorded values.

In the present study, our findings indicate that there is an association between urinary Na^+/K^+ ratio with 24-hour SBP (P=0.0004); daytime SBP ((P=0.001); night-time SBP (P=0.001); conventional SBP (P=0.0001). However, neither 24-hour urinary Na^+ nor K^+ excretion alone was associated with BP, but the interaction of Na^+/K^+ influenced BP. This data is consistent with earlier studies which showed an association between urinary Na^+/K^+ ratio and BP (Intersalt Cooperative Research Group, 1988, Smith *et al*. 1988). Outcomes described by the decrease in urinary K^+ excretion on a high Na^+ diet in salt-sensitive individuals (Price *et al*. 2002; Scott *et al*. 2011).

The diminished urinary K^+ excretion may be a result of a heightened activity of the Na-K-2Cl co-transporter in the ascending limb of the renal tubule, hence Na^+ excretion is somewhat associated with an increased BP in individuals with a decreased K^+ excretion demonstrating the presence of an active Na-K-2Cl co-transporter in response to Na^+ intake (Aviv *et al*. 2004). Additionally, diets containing excessive amounts of Na^+ generally have a low K^+ content, an effect that may also be reflected in higher urinary Na^+/K^+ values. Although previously reported that dietary K^+ is associated with a decrease in BP (Alderman, 2002; Adregue and Madias, 2014; Houston, 2011; Geleijnse *et al*. 2003; Appel, 2009) findings of the present study are less consistent with this data; no associations were observed between the index of K^+ intake alone with indices of BP.

The probable explanation concerning the discrepancies in these reports could either be that K^+ absorption in the gastrointestinal tract is reduced as seen in similar populations of African Ancestry (Turban *et al*. 2008) or that indeed this population living in SOWETO consumes less K^+ containing foods (Maseko *et al*. 2006), therefore the lack of relations could be that K^+ may have been too low to identify any meaningful effect in a similar manner observed in other studies (Smith *et al*. 1985). These findings further support previous studies (Krishna *et al*. 1989; Morris *et al*. 1999) backing that a mutual effect of dietary Na^+ and K^+ is greater than either alone in BP. It also appears that a diminished effectiveness of K^+ in lowering BP is observed even when accompanied by a low Na^+ as seen in larger trials such as the DASH study. Since this population has an attenuated night-time BP (Maseko *et al*. 2011; Maseko *et al*. 2013), and a relatively low K^+ intake as estimated from urinary K^+ excretion, the non-dipping BP status could be credited to low levels of K^+. Findings of the present study with regards to the importance of the Na^+/K^+ ratio instead of either alone in regulating BP is strongly supported by outcomes (Wilson *et al*. 1999; Krishna *et al*. 1989; Weinberger *et al*. 1982) which propose that changes in dietary K^+ alter Na^+ balance, wherein K^+ restriction leads to Na^+ retention, whereas K^+ supplementation offers a greater natriuresis.

Notably, data from the present study revealed associations between Na^+/K^+ ratio only with systolic ABP measurements (24-hour SBP, daytime SBP, night-time SBP) but no relations with any diastolic pressures. Similarly, these relations were observed for Na^+/ K^+ ratio with conventional BP measurement. The lack of relations between Na^+/K^+ ratio and diastolic pressures in this population could be explained based on findings previously noted in our group were the relationship between urinary Na^+/K^+ and BP were stronger for PP than DBP (Redelinghuys *et al*. 2010), consistent with findings (Sesso *et al*. 2000) that SBP and PP are more associated with an increased BP, an incidence of CVD and that when considering SBP, DBP, MAP, and PP, both MAP and DBP lose a predictive value for HT and CVD in general as people age (Sesso *et al*. 2000).

4.6 Renin activity and salt sensitivity.

The decline in Na^+ excretion discussed previously appears not be an issue only in the elderly, however this overall deterioration in GRF is more marked in people of African ancestry (Mimran and Ristein, 1992; Meneton *et al*. 2005). Previous studies have demonstrated that Na^+ retention is a problem in populations of African Ancestry (Hall, 1990; Palacios *et al*. 2004; Harshfield, 2009; Dusta, 1989; Frisancho *et al*. 1984; Dressler, 1990). Additionally, existing evidence on clinical and experimental studies have shown that people of African Ancestry, having a higher Na^+ retention also have increased BP (Luft *et al*. 1982).

There is now extensive evidence to suggest that Na^+ retention in populations of African Ancestry is a major pathophysiological mechanism responsible for an elevated BP, it also appears that the very same mechanism has been proposed to cause a suppression of renin and aldosterone secretion (Price and Fisher, 2003). Considering the average low levels of renin in the present study (37.4±77.9), it is consistent with a large body of evidence which reports that people of African Ancestry are classically considered a “low renin” race (Chrysant *et al*. 1979; Rayner and Ramesar, 2015; Rayner

et al. 2001; Sagnella, 2001; Williams *et al*. 2014; Wali and Wei, 1999; Campese, 1996; Aksut and Karimkhani, 2014; Kola *et al*. 2009).

Initially exposed by Helmer and Judson (1968) that people of African origin generally have low renin as compared to whites, it is now widely accepted through numerous literatures (Chrysant *et al*. 1979; Rayner and Ramesar, 2015; Rayner *et al*. 2001; Sagnella, 2001; Williams *et al*. 2014; Wali and Wei, 1999; Campese, 1996; Aksut and Karimkhani, 2014; Kola *et al*. 2009). Although in the present study of a population of African ancestry we did not compare levels of renin between races, we still observed low renin levels (37.4±77.9). This observation of low renin activity in this population of African Ancestry is supported by assertions from previous studies in this particular population (Scott *et al*. 2011; Michel *et al*. 2012). Moreover, our analysis showed that decreasing tertials of renin were associated with an increase in BP. Furthermore, this association is characteristic between normotensives and hypertensives in the group, an observation in keeping with earlier studies (Mitas *et al*. 1979; Leary and Asmal, 1975). The low-renin high-BP relationship in the present study attests that our study population like population of African ancestry is "salt sensitive".

High-Na^+, low-K^+ diets suppress renin release in salt sensitive individuals (Fisher *et al*. 1999). Thus, these findings further suggest that the decrease in renin and heightened Na^+ retention may indeed play a significant part in promoting Na^+ effects on BP in salt sensitive individuals. Therefore, the low renin observed in the present study explains the prevalence of increased BP in this population. Certainly, with more unanimity now than previously, research suggest that the low renin may be ascribed to genetic variation across racial lines (Sagnella, 2001). Indeed, though not investigated in the present study because it was previously studied in our group (Tiago *et al*. 2002; Woodiwiss *et al*. 2006). The low renin levels are attributed to genetic influences.

These influences are demonstrated to occur in salt sensitive populations and may be triggered by Na^+ effects on interactions with specific genes in groups of African Ancestry, and these connections are consistent with results reported in other populations of African Ancestry (Campese, 1996; Brewster and Seedat, 2013). These interactions indicate that Na^+ sensitivity is similarly established in normotensives and hypertensives of African Ancestry, data that is supported by findings in earlier studies (Sowers *et al*. 1988), further proposing common genetic determinants in groups of African Ancestry. The present study has indicated that BP increases with low levels of renin, and the findings may suggest that the association between renin concentration and BP could possibly involve an interaction between BP and Na^+ intake, however the relationship between BP and Na^+ in this population remains controversial and further research is required in this regard.

4.7 Sodium intake and arterial stiffness

Even though Na^+ I could not show any relationship between dietary Na^+ intake and BP, sodium could increase BP indirectly by increasing arterial stiffness. I therefore analyzed the association between Na^+ intake and PWV (an index of arterial stiffness). After adjusting for covariates (including age) there was a strong relationship between PWV and Na^+ intake (P=0.002). This finding is supported by a report which has demonstrated that dietary Na^+ together with an increase in age amplifies stiffness of blood vessels, and that low-sodium diet in older adults improves arterial compliance (Zieman *et al*. 2005). The proposed mechanism relating the increase in PWV and age which are both associated with a decrease in urinary Na^+ excretion in the group is that; age is accompanied with structural changes mainly in the medial layer of the arteries and this promotes arterial stiffening (an increase in PWV), consequently this affects the target organ (kidneys) because of its effect on pressure and flow pulsitility (Jain *et al*. 2014; Zieman *et al*. 2005).

The inability to excrete Na^+ as people age is an overall resultant effect of this mechanism coupled with the structural changes that occur within the kidney itself. Therefore in this population, Na^+ is directly related to arterial stiffness independent of age. Since arterial stiffness is strongly associated with BP; it is possible that associations between Na^+ and BP are strongly influenced by arterial stiffness.

4.8 Conclusion

In conclusion, the present study has demonstrated that in a random recruited urban community sample of African ancestry, the use of a single 24-hour urine sample provides as accurate an estimate of dietary Na^+ and K^+ as that estimated from a longer period of urine collection, therefore a single 24-hour urine measurement is sufficient to estimate the levels of Na^+ intake. Also, in this community of African ancestry, Na^+ and K^+ intake could not be predicted to be higher on certain days of the week and did not depend on which day of the week the urinary Na^+ excretion measurement was taken. Although high Na^+ intake and low K^+ intake (indexed by 24-hour urine electrolyte excretion rates) characterises this community, none of these factors alone is associated with conventional and ambulatory BP measurements. Even when we considered the effects of age on 24-hour excretion and divided the population according to age groups, there was no significant difference in BP between people whose sodium consumption was above the threshold of 100mmol/day and those whose sodium consumption was within normal range. Interestingly the Na^+/K^+ was associated with BP indicating that this ratio is more important in regulating BP in this population than either K^+ or Na^+ alone. This means in this population, advocating for a low sodium diet is not sufficient, but consumption of fruits and vegetables which are rich in potassium should be promoted. This could lead to the effective control of BP in this community easing the burden of patient care on the resource limited health care system.

4.7 Possible study limitations and future perspective

It should be mentioned that in the present study a number of potential limitations were noted and resolutions to these limitations were modelled. As with a number of research studies of this nature, studies where participants are randomly recruited, more women than men participate as is the case in the present study, this is mainly due to that women in settings of urban communities in South Africa are usually available and as compared to men most of them are not employed. Also, men have a negative perception and stigmatisation with issues pertaining to human medical research while women often view participation as means of free medical screening. However, the imbalance in number of males and females could demonstrate bias in the data. None the less, gender was included as a covariate in regression analysis. Often times, age does influence outcomes in research studies, hence it in the present study it was important that age be included in the regression analysis and its effects be studied in different groups. The present study was cross-sectional, hence I did not make interventions to participant's dietary patterns with regards to Na^+ and K^+ intake. Therefore future studies in this population group should focus on dietary interventions such as reductions in Na^+ intake and increased K^+ intake to observe if dietary changes could play a role in decreasing BP particularly in this population as reported in other studies (Geleijnse *et al*. 2003; Appel, 2009; Drenjancevic-Peric *et al*. 2011; Gu *et al*. 2008; He and MacGregor, 2010). Although in the present study it was demonstrated that there is little variation of Na^+ and K^+ intake from day to day, future studies should also investigate whether spot urine can reproduce the same results as that obtained from 24-hour urine collection in this population. Future studies should also separate daytime urine from night-time urine sample to study the effects of each on BP independently.

CHAPTER FIVE

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Appendix 1

R14/49 Mr Nyundu Franswell

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150524

NAME: (Principal Investigator)	Mr Nyundu Franswell
DEPARTMENT:	School of Physiology
PROJECT TITLE:	Validationof Estimates of Salt Intake Using 24-Hour Urinary Salt Excretion in a Population of Ancestry
DATE CONSIDERED:	29 May 2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr. M.J. Maseko
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	06/07/2015

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 Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES