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NOCTURNAL NON-DIPPING BLOOD PRESSURE PATTERNS AND CARDIOVASCULAR TARGET ORGAN DAMAGE

Abdulraheem Babalola Bawa-Allah

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

Johannesburg, South Africa
2019

DECLARATION

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

_____

Abdulraheem Babalola Bawa-Allah

Signed on _____ day of _____, 2019 in PARKTOWN

I certify that the studies included in this thesis have the approval of the Human Research Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics clearance number is M17-02-13.

_____

Abdulraheem Babalola Bawa-Allah

Signed on _____ day of _____, 2019 in PARKTOWN

_____ _____

Professor Muzi J. Maseko (Supervisor) Dr Frederic S. Michel (Supervisor)

I dedicate this thesis to my parents, wife and daughter. It’s been a nice run; I appreciate them for their immense support.

ABSTRACT

The advent of ambulatory blood pressure monitoring has provided us with better understanding of the diurnal pattern of blood pressure and its role in physiology and pathology. One of the abnormalities of nocturnal blood pressure behaviour is the nocturnal blood pressure non-dipping pattern which is a failure of the night time blood pressure values to fall by at least 10% of daytime values. This blood pressure pattern is associated with several cardiovascular target organ pathologies.

Although it is thought that black individuals have higher ambulatory blood pressure values when compared to their white counterparts, normal thresholds for ambulatory blood pressure have not been determined in people of African ancestry but in blacks who live in the developed world. Therefore, in this thesis, my first task was to determine normal thresholds for ambulatory blood pressure in people of African ancestry and to establish if their average ambulatory blood pressure values are comparable to those of other ethnic groups. Using 24-hour blood pressure monitors (Spacelabs model 90201), I measured ambulatory blood pressure in a total sample of 530 healthy participants of African ancestry. Results from the present thesis showed normal thresholds for 24-hour blood pressure to be 135/85mmHg, daytime blood pressure to be 140/90mmHg and night-time blood pressure to be 130/80mmHg. This is the first time that ambulatory blood pressure reference values have been determined in this population. The mean ambulatory blood pressure values in our study population are very similar to those measured in Caucasians. These findings contradict data from earlier studies which show that black individuals have higher ambulatory blood pressure values. The uniqueness of the findings of this study is that the study was conducted in people of African descent living in Africa. The determination of thresholds for ambulatory blood pressure is of immense clinical relevance as it provides reference values for the accurate diagnosis of hypertension as it can help expose masked hypertension and identify white-coat hypertension.

Although previous studies have shown that nocturnal blood pressure non-dipping is more prevalent amongst black people, the actual prevalence and possible mechanisms behind nocturnal blood pressure non-dipping pattern in a people of African ancestry has not been determined. Moreover, the impact of dietary salt intake on dipping is currently unknown in this salt sensitive population. In this thesis I was able to show that in 1219 randomly recruited individuals of African ancestry there is a high prevalence of nocturnal blood pressure non-dipping pattern of

52%. This prevalence was higher in females (54%) compared to males (49%). Further analysis of our data indicates that the nocturnal blood pressure non-dipping pattern in this population might be caused by a high plasma aldosterone concentration which causes increased sodium retention and the subsequent increase in nocturnal blood pressure. This was further confirmed by the significant positive relationship between 24-hour urinary sodium excretion and dipping status amongst non-dippers but not amongst dippers indicating that 24-hour urinary sodium excretion is an independent predictor of the nocturnal non-dipping blood pressure pattern.

Arterial stiffness is a known marker of arteriosclerosis and subclinical atherosclerosis and is an independent predictor of future adverse cardiovascular events. A non-dipping pattern of blood pressure is associated with a higher level of arterial stiffness and it is known that arterial stiffness increases with age. Because we reported a high prevalence of non-dipping in chapter 4, we investigated a possible involvement of non-dipping in modifying the relationship between arterial stiffness as measured by carotid femoral pulse wave velocity and its determinants in a population of African ancestry. In this thesis, I was able to show in 796 participants of African ancestry that age, hypertension and diabetes are independent determinants of arterial stiffness. I also show that non-dipping strengthened the relationship between age and arterial stiffness but blunted the relationship between arterial stiffness and diabetes. I show interactions of non-dipping in the relationship between pulse wave velocity and gender. The modification of the relationships between arterial stiffness and its determinants by non-dipping may have been due to masking effects or additive effects. Our results suggest that non-dipping might be an important phenomenon to consider in age related arterial stiffness as well as in gender differences in arterial stiffness.

Gender differences have been reported in age related arterial stiffness and have been attributed to sex steroids. There is however controversy as to which gender exhibits a sharper age-related arterial stiffness. Because we reported a high prevalence of the nocturnal non-dipping blood pressure pattern, interaction of non-dipping with age related increase in pulse wave velocity and interaction of non-dipping with the relationship between pulse wave velocity and gender in chapters 4 and 5, we decided to investigate futher, the role of non-dipping in gender differences in arterial stiffness and age related arterial stiffnes. In this thesis, I have been able to demonstrate in 413 non-dippers (140 males and 273 females) of African ancestry that non-dipper females have lower levels of arterial stiffness and might be at a lower risk of developing adverse cardiovascular outcomes secondary to arterial stiffness as measured using carotid-femoral pulse

wave velocity when compared to non-dipper males. Data from this thesis suggests that this observed difference might be mediated by higher serum levels of high-density lipoprotein cholesterol coulpled with lower serum levels of triglycerides in non-dipper females when compared with non-dipper males. High serum levels of high-density lipoprotein cholesterol and low levels of triglycerides are associated with improved arterial distensibility. The observed differences may also be due to a higher prevalence of diabetes in the non-dipper male group. These observations suggest that serum high density lipoprotein levels and serum triglycerides levels might be important targets for therapy in non-dipping related arterial stiffness.

In conclusion, the data presented in this thesis have filled the gap in knowledge concerning normal thresholds for ambulatory blood pressure values in Africans living in Africa and have advanced our knowledge about the prevalence of the nocturnal non-dipping and possible mechanisms responsible for it in people of African ancestry. It has also advanced our knowledge on the possible mechanisms behind gender differences in non-dipping related arterial stiffness.

PRESENTATIONS

The following presentations have arisen from this work.

Oral presentation:

Oral presentation at the First Conference of Biomedical and Natural Sciences and Therapeutics (CoBNeST) conference, 7-10 October, Spier Estate, Stellenbosch, SA, 2018. Presentation Title: Prevalence of Nocturnal Blood Pressure Non-dipping in People of African Ancestry: The Role of 24-Hour Sodium Excretion.

Poster presentation:

Presented research poster at the Faculty of Health Sciences Biennial Research Day and Post-graduate Expo. 6 September, Wits University Medical Campus, Parktown, Johannesburg, SA, 2018. Poster Title: Twenty-Four Hour Ambulatory Blood Pressure Reference Values in Africans.

PUBLICATION

The following publication has arisen from this work:

Bawa-Allah, A.B., Mashao, M.M., Nyundu, T.F., Phukubje, E.M., Mlambo, B.W., Ngema, M.V., Nkosi, B.G. and Maseko, M.J. (2019). Twenty-four hour ambulatory blood pressure reference values in Africans. Blood Press Monit, 24, 103-109.

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LIST OF ABBREVIATIONS

ABP	ambulatory blood pressure
ABPM	ambulatory blood pressure monitoring
ACE	angiotensin converting enzyme
AGEs	advanced glycation end products
AHI	apnoea hypopnoea index
ANOVA	analysis of variance
ARR	aldosterone to renin ratio
ART	anti-retroviral therapy
BMI	body mass index
BP	blood pressure
BPS	belgian population study
CAD	coronary artery disease
CAVI	cardio-ankle vascular index
CF-PWV	carotid femoral pulse wave velocity
CLS	clinical laboratory services
cm	centimetre
DBP	diastolic blood pressure
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
g	gram
g/day	gram per day
HDLc	high density lipoprotein cholesterol
HIV	human immunodeficiency virus
HR	heart rate
HREC	human research ethics committee
hs-CRP	high-sensitivity c-reactive protein
IL-6	interleukin-6
ISH	isolated systolic hypertension
kg	kilogram
kg/m^2	kilogram per $metre^2$

LDLc	low density lipoprotein c
LVH	left ventricular hypertrophy
m	metre
m/s	metre per second
MAP	mean arterial pressure
ml	millilitre
ml/day	millilitre per day
mmHg	millimetre of mercury
mmol	millimole
mmol/day	millimole per day
Na^+/K^+	urinary sodium to potassium excretion ratio
NaCl	sodium chloride
NDP	nocturnal non-dipping blood pressure pattern
NHLS	national health laboratory service
NOS	nitric oxide synthase
OSA	obstructive sleep apnoea
PAMELA	pressioni arteriole monitorate e loro associazoni
PWV	pulse wave velocity
RAAS	renin angiotensin aldosterone system
RPM	revolutions per minute
SBP	systolic blood pressure
SD	standard deviation
SNS	sympathetic nervous system
TGF-β	transforming growth factor beta
UK^+	twenty-four-hour urinary potassium excretion
UNa^+	twenty-four-hour urinary sodium excretion
VLDL	very low-density lipoprotein
vs	versus
WHR	waist to hip ratio

PREFACE

The use of ambulatory blood pressure monitoring (ABPM) is increasing worldwide in clinical practice as well an in research. It is a superior predictor of adverse cardiovascular events when compared to conventional office blood pressure measurements. An especially important advantage of ABPM is that it provides information on night-time blood pressure values which should normally fall to about 10% of daytime values. Lack of this normal night time fall in BP is termed nocturnal non-dipping blood pressure (NDP). Individuals with this kind of blood pressure profile have been known to be at a higher risk of developing adverse cardiovascular conditions like left ventricular hypertrophy, arterial stiffness, microalbuminuria, congestive heart failure amongst others.

Reference values for normal ambulatory blood pressure monitoring in black Africans of African ancestry has not been determined. Because ABPM use is on the increase globally, it is of immense diagnostic and prognostic importance to determine reference values in this population and not just use reference values from other population studies. Using reference values from other populations might not be appropriate, due to psychosocial and economic differences. Several studies have shown that black individuals are more likely to be non-dippers when compared with other races. However, the actual prevalence of NDP and the mechanism responsible for this kind of BP pattern in Africans has not been established. Arterial stiffness is one of the target organ pathologies associated with NDP and is the target organ effect of focus in this thesis. The determinants of arterial stiffness have however not been determined in this study population. It is known that NDP is associated with higher arterial stiffness but the interaction of gender and age on the relationship between arterial stiffness and NDP has not been studied and I believe it is of immense importance as both are independent predictors of adverse cardiovascular events.

In this thesis, I have been able to determine reference values for normal ambulatory blood pressure values in people of African ancestry. I have also been able to determine the prevalence of NDP in an African population and proposed possible factors responsible for it. I was also able to define the determinants of arterial stiffness as measured by carotid femoral pulse wave velocity in people of African ancestry. Finally, I was able to determine the gender differences in NDP related arterial stifness and age-related arterial stiffness in individuals of African ancestry and proposed possible factors responsible for these differences. The findings of this thesis provide

new insights concerning what is known about the normal 24-hour blood pressure reference values in Africans as well as the prevalence of NDP in the same population. Furthermore, this thesis provides novel observations concerning gender differences in arterial stiffness.

In the chapter 1 of this thesis, I review what is currently known about ambulatory blood pressure monitoring as well as NDP and associated cardiovascular end organ effects. I also gave motivation for carrying out the studies therein. In chapter 2, I give the details of the materials and methodology used during my research. In chapters 3-6, I elucidate each of these studies, their results and inferences thereof. Each of the chapters 3-6 are set up as semi-independent chapters each with its own, introduction, methods (brief), results, discussion and conclusion sections. Chapter 7, which is a summary of the findings in chapters 3-6, highlights the relevance, limitations and novelty of the findings in this thesis.

To buttress this thesis, the data provided in chapter 3 has been published in the Blood Pressure Monitoring Journal (Bawa-Allah et al., 2019)

CHAPTER 1

INTRODUCTION

Current knowledge about the use of ambulatory blood pressure monitoring and the non-dipping blood pressure pattern

1.1 Introduction

The advent and clinical use of ambulatory blood pressure monitoring (ABPM) has revealed many features of the circadian blood pressure rhythm and it has been shown that ABPM has more predictive value when compared to conventional BP measurements in evaluating cardiovascular risk (Verdecchia *et al.,* 1994; Dolan *et al.,* 2005; Clement *et al.,* 2003). An especially important advantage of ABPM over other forms of blood pressure (BP) measurement is that it provides information about night-time blood pressure. Normally, night-time BP values in most people are lower than day-time values and lack of this normal nocturnal fall in BP should be seen as being abnormal (O'Brien *et al.,* 1988).

A non-dipping blood pressure pattern (NDP) can be defined as failure of the night-time BP values to fall by at least 10% of the day-time values (Head *et al.,* 2010). This kind of blood pressure pattern is of clinical relevance because of its close association with cardiovascular target organ damage like carotid intima-media thickening, left ventricular hypertrophy, arterial stiffening, stroke, microalbuminuria and congestive heart failure (Fan *et al.,* 2010, Cicek *et al.,* 2013).

In this introduction to my thesis, I will discuss the technique of ABPM and touch on its advantages and limitations. I will also do a review of literature on what is known about NDP, touching on ethnic prevalence, possible mechanisms leading to NDP and also pathologic consequences of being a non-dipper. One of the pathologies associated with NDP is arterial stiffness and I will also elaborate on it, because it is the target organ damage of focus in this thesis.

1.2 Ambulatory blood pressure monitoring

Ambulatory blood pressure monitoring (ABPM) is a non-invasive, automated portable technology that measures and records blood pressure (BP) over a 24 h period (Pickering *et al.,* 2005). It allows for collection of blood pressure readings several times an hour over a 24-hour period; it was first described over 50 years ago by Kain and colleagues in 1964.

Blood pressure measurements are extremely important for clinicians in the diagnosis and treatment of hypertension (Turner *et al.,* 2013) consequently, choosing the most informative blood assessment protocol has been a topic of great importance. Studies have shown that the

clinical measurement of blood pressure is not a measure of the 'true' blood pressure as it is not as accurate as determining the mean blood pressure over prolonged periods (Pickering *et al.*, 2006).

The readings obtained from ambulatory blood pressure measurement can be collated to yield 24 hour means and grouped into time windows (i.e mean daytime and mean night time values). These blood pressure categorizations obtainable from ambulatory blood pressure monitoring are valuable for the clinical management of hypertension due to their high level of accuracy for diagnosis and prediction of cardiovascular risk (Krakoff *et al.*, 2013).

Ambulatory blood pressure monitoring (ABPM) generates more information than the single reading obtained from clinic measurements by giving a profile of blood pressure variation over a 24-hour period (Liu *et al.*, 2014). It also provides information on how blood pressure throughout this period can be beneficially influenced by antihypertensive therapy (Liu *et al.*, 2014).

1.2.1 Monitors used for ABPM

The recent ambulatory blood pressure monitors are completely automatic and can record blood pressure for up to 24 hours or more with the patients being able to go about their regular daily activities. Most of these monitors employ the oscillometric technique which relies on the analysis of pulse waves determined from the cuff during constricted blood flow. The cuffs are the sensors that determine the pulse waves in this technique, as opposed to the auscultatory method which relies on the detection of audible sounds by observers during constricted blood flow.



Figure 1.1 Ambulatory blood pressure monitor. It consists of a brachial cuff and the blood pressure monitor which is portable and can fit into a person's pocket

1.2.2 Information that can be derived from ABPM

1.2.2.1 Blood pressure variability

It is well known that asides from the nocturnal BP changes, 24-hour BP is typified by brief variations that are especially manifest and recurrent in the daytime period but also happens to a lesser extent during the night-time (Mancia *et al.*, 1997). It has been shown that these variations in blood pressure highly depend on behavioural activities as well as non-behavioural activities that can influence BP changes. The effects of these activities on the heart and blood vessels are mainly mediated by the autonomic nervous system and vasoactive chemicals that may have peripheral or local effects (Mancia *et al.*, 2012). Furthermore, it has been shown that blood pressure variability increases with age and is directly related with mean BP level (Mancia *et al.*, 1983 b). Twenty-four-hour blood pressure variability usually follows a gradual increase from normal BP values to mild or severe hypertension levels irrespective of the BP measuring technique (Mancia *et al.*, 1983 a).

For a long time, this variability in blood pressure has been seen to be a potential risk factor for cardiovascular disease (Fratolla *et al.*, 1993). Long term variability in BP has been recognised as a predictor of stroke and coronary events (Grove *et al.*, 1997). It has also been clearly demonstrated that excessive fluctuations in BP is associated with cardiovascular target organ damage (Su *et al.*, 2005). It is clear from the above discussions that the assessment of blood pressure variability is of pathophysiological and prognostic importance, thereby highlighting a major advantage of ambulatory blood pressure monitoring.

1.2.2.3 The diurnal rhythm of blood pressure

Earlier recording of intra-arterial BP in mobile individuals showed that BP is characterized by a circadian pattern, with BP values reaching a peak in the daytime and a dip normally to a reasonable degree in the night-time (Littler *et al.*, 1972; Mancia *et al.*, 1983b; Bevan *et al.*, 1969).

The intra-arterial blood pressure measurement technique has been replaced by recent non-invasive methods of ambulatory blood pressure monitoring which reproduces the observed diurnal pattern of BP (Pickering *et al.*, 1996; Cowan *et al.*, 1980). The reproducibility of the circadian BP pattern by the non-invasive methods of ABPM demonstrates that the nocturnal dip in BP is not attenuated by inconveniences like device weight, cuff inflations and noise (Villani *et al.*, 1992).

Several factors have been postulated to be responsible for the diurnal variations in BP. Some of these factors will be discussed in the following section.

1.2.2.3.1 Mechanisms behind the diurnal rhythm of blood pressure

The sleep/wake cycle is an important determinant of the diurnal rhythm of BP. The sleep/wake cycle is controlled by different sensory, motor, autonomic, endocrine and cerebral 24-hour rhythms (McGinty *et al.,* 1994). Changing of the diurnal rhythms of melatonin, serotonin, vasoactive intestinal peptide, somatotropin, insulin, arginine vasopressin and steroid hormones are related with night-time sleep, while phasing of the diurnal rhythms of corticotropin releasing hormone, adrenocorticotropic hormone, thyroid releasing hormone, opioids and prostaglandins is associated with early morning arousal (Fabbian *et al.,* 2013). The diurnal variations in the above-mentioned factors are said to be responsible for the diurnal pattern of BP (Smolensky *et al.,* 1976).

Nigh time increase in peripheral blood flow and concomitant shunting of fluid from central to extracellular compartments leads to night time reduction in plasma volume (Finlayson *et al.,* 1964) and consequently BP. This phenomenon is reversed in the morning and awake periods.

Sympathetic nervous system tone is dominant during wakefulness while parasympathetic nervous system tone is dominant during sleep (van de Borne *et al.,* 1994). Plasma norepinephrine and epinephrine and urinary cathecolamine levels are highest in the morning to early afternoon and lowest during night time sleep (Lakatua *et al.,* 1986).

The Renin-Angiotensin-Aldosterone-System (RAAS) regulates 24-hour BP rhythm via mechanisms that control body sodium, water, sympathetic nervous system and vasomotor balance. Activity of the RAAS is usually highest in the morning and lowest in the late evening (Li *et al.,* 2007; Cugini *et al.,* 1988), playing an important role in the diurnal rhythm of BP.

Activity of the RAAS is however controlled by the circadian rhythms of prorenin, plasma renin activity, serum angiotensin converting enzyme (ACE) activity, plasma angiotensin II concentration, aldosterone concentration and tissue angiotensin I receptor expression (Li *et al.,* 2007; Cugini *et al.,* 1988; Kool *et al.,* 1994; Sica *et al.,* 1999).

Endothelial vasodilator, nitric oxide (NO) modulates vascular tone and hence BP. Nitric oxide concentrations show diurnal variation being lowest in the morning sleep/wake transition and highest after about 12-hours (Mheid *et al.,* 2014).

Studying the mechanisms responsible for the diurnal pattern of BP has led clinicians to investigate the value of timing hypertensive medication. Medications like calcium channel blockers, ACE inhibitors and angiotensin receptor blockers have been said to be more efficient at lowering BP and improving 24-hour BP profile towards normal values and also reducing the risk of target organ damage if ingested at bedtime (Hermida *et al.,* 2011; Hermida *et al.,* 2010).

A representation of the diurnal rhythm of blood pressure is shown in figure 1.2.

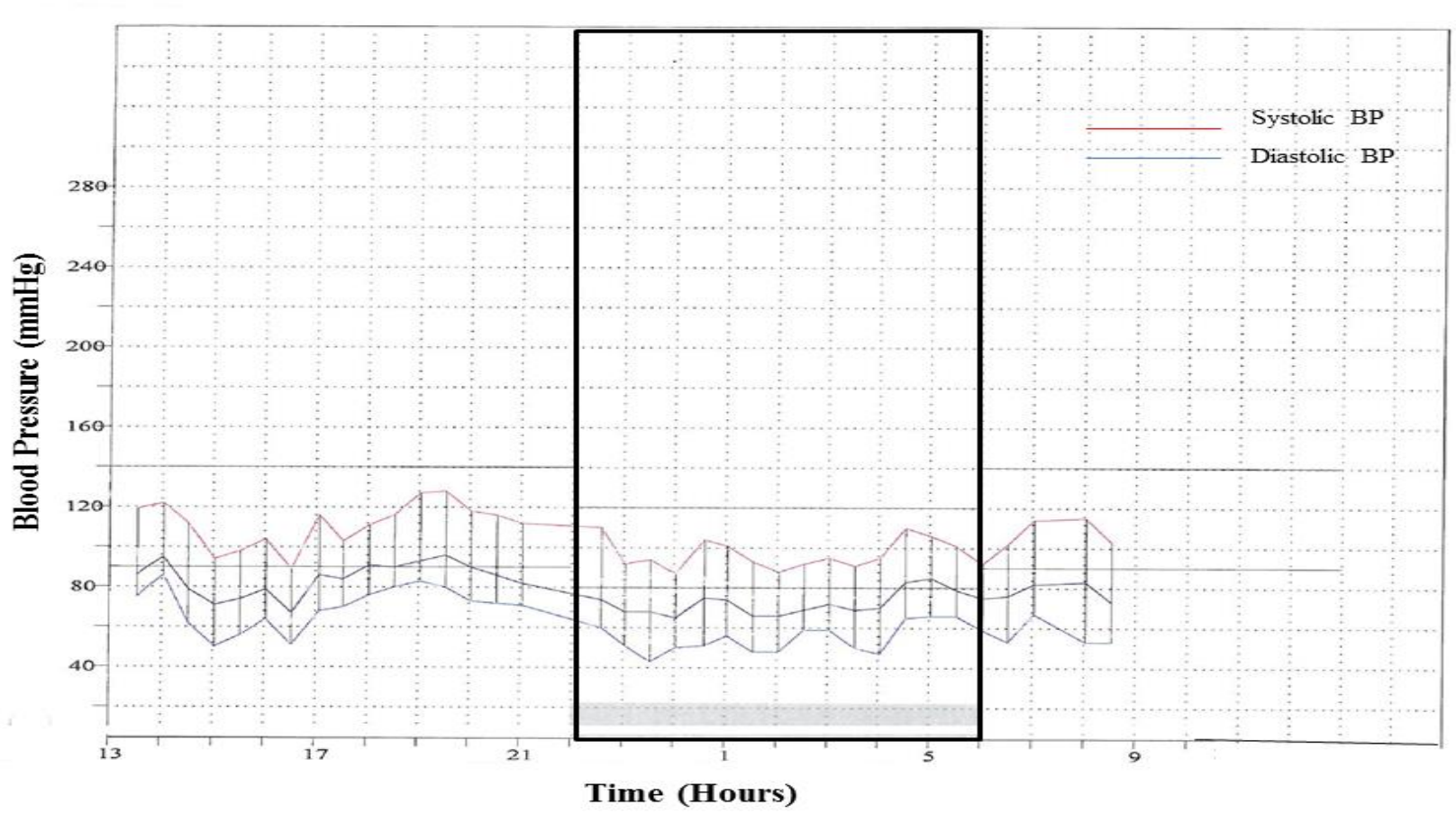


Figure 1.2. Representation of the typical diurnal rhythm of blood pressure. It depicts changes in systolic and diastolic blood pressures over a 24-hour period. The highlighted area represents night time 'asleep' period during which blood pressure 'dips' relative to daytime values. BP, Blood pressure.

1.2.3 Use of ABPM in clinical practice

Ambulatory blood pressure monitoring can be very beneficial in clinical practice depending on if an individual is being treated for hypertension or not (Verdecchia *et al.,* 2007). Ambulatory blood pressure monitoring can help categorize untreated hypertensive patients into different categories of cardiovascular risk. This would otherwise be impossible using conventional BP measurement. Cardiovascular risk level may be low (if the patient shows white coat hypertension) or high (if the patient shows sustained hypertension). This kind of risk assessment is key to determine if the patient should be given antihypertensive medication (Verdecchia *et al.,* 2005a). In patients who are already receiving treatment for hypertension ABPM can be very useful in the tailoring of treatment protocols that can be of great benefit in cases of obvious drug resistance, hypotension and wide discrepancies between in and out-of-office BP values (Verdecchia *et al.,* 2007).

1.2.4 Advantages and uses of ABPM

1.2.4.1 Correct diagnosis of white coat hypertension

White coat hypertension is a phenomenon in which certain persons who are not on anti-hypertensive therapy exhibit high blood pressure in a clinical setting but do not when evaluated using ABPM (Pickering *et al.,* 1988). This phenomenon is said to occur in 15% to 20% of individuals with high office blood pressure readings (Franklin *et al.,* 2013). Ambulatory BP monitoring helps to ensure that white coat hypertension is not misdiagnosed as true hypertension. This misdiagnosis might cause patients to unnecessarily take chronic medications which have financial and debilitating side effect consequences (Lovibond *et al.,* 2011). ABPM prevents unnecessary treatment of white coat hypertension and has been found to be the most cost-effective strategy for the diagnosis of hypertension (Lovibond *et al.,* 2011). As a matter of fact, in the United Kingdom, there are now guidelines that recommend ABPM for all patients before diagnosing hypertension (Bloch *et al.,* 2011).

A study carried out in untreated hypertensive individuals showed that cardiovascular morbidity was much less in individuals with white coat hypertension and was similar when compared with individuals with clinical normotension (Ohkubo *et al.,* 2005). The incidence of target organ damage and other cardiovascular events in individuals with white coat hypertension was also similar to that observed in normotensive subjects (Pierdomenico *et al.,* 2011; Khattar *et al.,* 1998; Mancia *et al.,* 2009).

1.2.4.2 Elimination of white coat effect in resistant hypertension.

The white coat effect can also be observed in patients who are already on anti-hypertensive medication. Some patients with suspected resistant hypertension according to their office blood pressure measurements may have controlled blood pressure when evaluated using ABPM (Brown *et al.,* 2001). Thus, ABPM can be conducted for patients with suspected resistant hypertension before increasing the dose of their medications or adding extra blood pressure lowering drugs (White *et al.,* 2001).

1.2.4.3 Exposing masked hypertension

Masked hypertension is a phenomenon in which certain individuals that are not on any anti-hypertensive therapy show normal blood pressure in a clinical setting but exhibit high blood pressure outside clinic settings. About 15% to 30% of adults have masked hypertension (Peacock *et al.,* 2014) meaning that medical practitioners should expect that about 1 out of 6 or 7 patients with normal in-office BP will have high ambulatory BP values (O'Brien *et al.,* 2013). It has been consistently shown that in patients with masked hypertension there is a higher incidence of metabolic abnormalities like dyslipidemia, impaired glucose tolerance, being overweight and diabetes mellitus (Angeli *et al.,* 2010; Ohkubo *et al.,* 2005). There is also a higher prevalence of subclinical cardiac, vascular and kidney damage and also a higher risk of developing sustained hypertension in individuals with masked hypertension (Bjorklund *et al.,* 2003; Hansen *et al.,*

2006). It has been shown that the incidence of left ventricular mass index and carotid plaque are not quite different between people who have masked hypertension and those who have sustained hypertension (Liu *et al.,* 1999; Sega *et al.,* 2001). The above discussions point to the fact that individuals with masked hypertension might have an underestimated cardiovascular risk (Myers *et al.,* 2005).

1.2.4.4 Monitoring patients with postural hypotension

Postural hypotension is a common occurrence in older individuals; it is a form of low blood pressure that occurs when standing up from sitting or lying down. It is characterized by dizziness while standing for long periods and also episodes of syncope. The BP of patients with postural hypotension depends on their body position. It could be quite high when they are in the supine position especially at night (Sega *et al.,* 2001). Treatment with vasopressor agents and antigravity stockings may cause their blood pressure to go too high or too low respectively hence ABPM is important for assessing optimal BP control in patients with postural hypotension (Verdecchia *et al.,* 2005a).

1.2.5 Reference values for ambulatory blood pressure monitoring

Different large and medium size population studies have determined thresholds for ambulatory blood pressure monitoring for the diagnosis of hypertension. The table on the next page (Table 1) shows ambulatory blood pressure values from the following 5 population studies; Belgian population study (BPS) (Staessen *et al.,* 1996), Ohasama study (Imai *et al.,* 1993), PAMELA study (Mancia *et al.,* 1995), The Jingning population study (Li *et al.,* 2005) and the Taiwan study (Chen-Huan *et al.,* 1995). The values shown in the table have been extracted from these studies and represents 'normotensive' individuals with no history or current diagnosis of hypertension.

Although it has been shown that black individuals have higher ambulatory blood pressure values when compared to other races (Chase et al., 1997), we believe this is not representative of

Africans living in Africa because of lifestyle and psychosocial differences. Notwithstanding, reference values for ambulatory blood pressure in Africans are yet to be determined.

Thresholds for ambulatory BP as recommended by the European Society of Hypertension (ESH) were determined using data from Caucasian, Asian and South American populations (O'Brien et al 2013) without the inclusion of data from black Africans living in Africa. Considering the psychological and socioeconomic uniqueness of this African population, we believe that the inclusion of their ABPM data in the determination of standard reference values for ambulatory blood pressure is of high importance. Hence in the present thesis, I determined reference values for ambulatory blood pressure in an African population.

Table 1.1 Reference values for ambulatory blood pressure monitoring in different population studies

Study	BPS	PAMELA	OHASAMA	Taiwan	JingNing
Number	729	1225	335	720	239
Mean age	50	46	54	51	41
Women	53%	51%	68%	49%	53%
24-h ABP					
Mean	115/70	115/72	116/69	116/73	120/74
SD	8/6	8/6	10/7	10/7	16/10
Daytime					
Mean	121/75	120/77	121/72	118/75	119/78
SD	9/7	9/7	11/7	10/7	9/7
Night-time					
Mean	104/60	105/63	106/61	114/71	105/66
SD	9/7	9/7	11/7	11/7	11/8

ABP= ambulatory blood pressure; BPS= Belgian population study; PAMELA = Pressioni Arteriole Monitorate E Loro Associazoni, Monza, Italy; OHASAMA= survey conducted in Ohasama, Japan. Jingning= study conducted in the Jingning county in China.

1.2.6 Limitations of ABPM

The limitations of ABPM include possible discomfort during the night time, reluctance of some patients to undergo the procedure (especially when it involves repeated measurements) and the limited availability of ABPM in day to day practice (Parati *et al.*, 2014).

A lot of studies that look into the clinical significance of morning blood pressure rise usually face unavoidable difficulties. This is because ABPM provides fewer recordings during the nigh-time hence the true BP values in the time frame immediately before arousal may not be captured. Another reason these types of studies may face difficulties is that since early morning BP surge is progressively much higher than night-time BP values, its prognostic function may be partially or completely masked by a factor like hypertension which reduces the extent of BP surge in the morning leading to possible overestimation of cardiovascular risk (Verdecchia *et al.,* 2012). Most insurance companies are also reluctant to reimburse patients who undergo ABPM, this is of great concern in some countries like the United States of America (Parati *et al.,* 2014). Another limitation to the use of the ABPM is that the equipment is more expensive than the regular cuff blood pressure measurement device. A lot of hypertension guidelines do not contain information on the use of ABPM, this might lead a lot of clinicians to believing that it is not of importance in clinical practice.

1.3 The nocturnal non-dipping blood pressure pattern (NDP)

Nocturnal BP values are usually 15% lower than daytime BP values (Pickering *et al.*, 2001). A non-dipping blood pressure profile (NDP) is defined as failure of the night-time BP values to drop by a minimum of 10% of the day time values (Head *et al.*, 2010). This is equal to a night-to-day BP ratio greater than 0.9. As the level of physical activity affects systolic and diastolic blood pressures differently, it has been proposed that the mean arterial blood pressure should be used as the blood pressure index for the classification of dipping status (Birkenhager *et al.,* 2007).

The fall in nocturnal blood pressure is common in most subjects and an attenuation of the physiological nocturnal decline should be seen as abnormal (Birkenhager *et al.,* 2007). The

concept of non-dipping has been of interest in research due to the clinical relevance of this abnormality and its association with hypertensive target organ damage, increased risk of future cardiovascular events, secondary forms of hypertension, renal function impairment and an imbalance of the autonomic nervous system (Liu *et al.,* 2003; Rahman *et al.,* 2005).

The knowledge about different dipping patterns has been made possible with the advent and clinical application of ambulatory blood pressure monitoring which is discussed in detail in the previous section. A non-dipping blood pressure pattern especially in patients in high blood pressure has been recognized as a risk factor for end-organ damage like left ventricular hypertrophy, stroke, carotid intima-media thickening, congestive heart failure, and microalbuminuria (Fan *et al.,* 2010). These conditions can also be found in normotensive individuals with a non-dipping blood pressure pattern (Hoshide *et al.,* 2003) and it has been shown that a 5% increase in the nocturnal diastolic BP is significantly associated with a 20% increase in cardiovascular mortality risk in normotensive individuals (Ohkubo *et al.,* 2002)

1.3.1 Pathophysiology of NDP: Proposed mechanisms

The mechanisms involved or responsible for the non-dipping blood pressure pattern remain unclear (Pickering *et al.,* 1990) however there have been proposals as to what these mechanisms might be, these are examined in the following sections.

1.3.1.1 Autonomic dysfunction

Non-dipping may be due to systemic and vascular inflammation and endothelial dysfunction (Birkenhager *et al.,* 2007). Nocturnal sympathovagal imbalance has been shown to mediate nocturnal hypertension and non-dipping BP pattern (Kario *et al.,* 2002). A study showed that non-dippers had an attenuated nocturnal fall in catecholamine levels and increased α-1 adrenergic receptor sensitivity to phenylephrine when compared to dippers (Sherwood *et al.,* 2002). Non-dipping could also be due to failure of regulatory mechanisms that control BP (Redon *et al.,* 2008).

1.3.1.2 Renal mechanisms

The non-dipping blood pressure pattern has also been attributed to abnormal renal sodium handling. A large study showed that African participants who had the lowest day-to-night ratios of urinary sodium excretion had significantly higher night time systolic BP and lower systolic BP dipping (Uzu *et al.,* 1996). Studies have also shown that night-time urinary sodium excretion was significantly higher in non-dippers than dippers and there was a significant positive correlation between night-to-day ratios of urinary sodium excretion and mean arterial pressure (MAP) (Uzu *et al.,* 1996), pointing to a possibility that renal sodium handling plays a major role in the development of a non-dipping BP pattern.

A high salt sensitivity of BP is commonly present in non-dippers (Wilson *et al.,* 1999a) and may mediate nocturnal hypertension (Uzu *et al.,* 2006) and hence contribute to the development of NDP. The exact mechanism responsible for the non-dipping blood pressure pattern in black Africans has not been determined. Since this population is known to be a salt sensitive one, I decided to investigate the role of 24-hour urinary sodium excretion in nocturnal non-dipping blood pressure pattern as part of this thesis. A study in Asian patients with essential hypertension also showed that non-dippers on a high sodium diet had a significantly higher night time systolic BP and mean arterial pressure compared to those on a lower sodium diet (Uzu *et al.,* 1997). However, nocturnal urinary sodium excretion is much higher in non-dippers when compared with dippers and this correlated positively with mean arterial pressure (Uzu *et al.,* 1999). Finally, a significant drop in systolic BP and mean arterial pressure and night-time sodium excretion was observed when non-dippers patients were put on a salt restricted diet (Uzu *et al.,* 1999). These observations suggest that putting a patient on a salt restricted diet might lead to better regulation of arterial blood pressure. Salt sensitivity is however more common in the black population which may explain why the non-dipping blood pressure status is more common amongst the black population (Polonia *et al.,* 2014).

It has been established that a non-dipping blood pressure pattern is associated with renal function impairment (Goto *et al.,* 2005), although there is not enough evidence that the non-dipping blood pressure profile can accelerate the progression of renal dysfunction (Barenbrock *et al.,* 1999).

1.3.1.3 Effect of sleep quality and daytime activity

Sleep and inactivity may also explain non-dipping BP. It has been shown that, daytime inactivity and poor sleep quality may lead to abnormal BP decline (O'Shea *et al.,* 2000). People who are inactive during the day and have a poor sleep quality are more likely to develop a non-dipping BP pattern and are more at risk of cardiovascular events (O'Shea et al, 2000). It should however be pointed out that non-dipping also occurs in patients with good sleep quality (Cavelaars *et al.,* 2004). Some researchers have shown a positive correlation between the non-dipping blood pressure profile and an increased nocturnal activity and poor sleep quality (Leary *et al.,* 2000).

It has been suggested that individuals with a higher risk of cardiovascular events may be more likely to be relatively more inactive during the day and also more likely to be diagnosed as non-dippers (O'Shea *et al.,* 2000). Posture and daily activity have also been shown to strongly influence cardiac output and systemic vascular resistance both of which are relatively higher in non-dippers when compared with dippers (Cavelaars *et al.,* 2004).

In a study involving patients with coronary heart disease with an established non-dipping blood pressure pattern, it was discovered that intervention programs like aerobic exercise for 35 minutes a day, 3 times a week significantly improved their nocturnal dipping pattern (Sherwood *et al.,* 2017). This probably means an increased daytime activity may be beneficial to non-dippers and might help achieve a dipping status on the long run.

1.3.1.4 Hemodynamic mechanism

Some hemodynamic changes have also been shown to be associated with the development of NDP. Normally, the dipping pattern comes with a decline in cardiac output and an unchanged or increased vascular resistance (Cavelaars *et al.,* 2000). The night-time fall in cardiac output may be due to a fall in heart rate (HR) with an unchanged stroke volume (Veerman *et al.,* 1995). It has been shown that NDP may be due to a reduced night-time drop in cardiac output and an exaggerated rise in vascular resistance or both (Takakuwa *et al.,* 2001).

1.3.1.5 Hormonal mechanisms

In a Japanese study it was shown that atrial natriuretic peptide and brain natriuretic peptide were significantly higher in non-dippers when compared with dippers (Hoshide *et al.,* 2003). These hormones are vasoactive and might explain their contribution to a blunted nocturnal blood pressure dipping. Individuals with the non-dipping blood pressure pattern have also been found to have high levels of certain hormones including cortisol (Zachareiva *et al.,* 2004), thyroxine (Middeke *et al.,* 1994), parathyroid hormone (Letizia *et al.,* 2005), catecholamine (Coca *et al.,* 1994) and aldosterone (White *et al.,* 1992).

Patients with primary aldosteronism and Cushing's syndrome have been shown to have a higher incidence of the non-dipping blood pressure pattern (Zelinka *et al.,* 2004). These patients have abnormally high levels of aldosterone and cortisol which may induce NDP due to their sodium retaining abilities; this is especially true for aldosterone. Patients with phaeochromocytoma have catecholamine secreting tumours in their adrenal glands these tumours secrete excessive amounts of catecholamine which may mediate a non-dipping blood pressure pattern by elevating sympathetic tone (Zelinka *et al.,* 2004). Because the exact mechanism behind non-dipping BP pattern in Africans living in Africa is currently unknown, I have decided to investigate what role the adrenal cortical hormone aldosterone plays in non-dipping BP pattern in this population.

1.3.2 Racial prevalence of the nocturnal non-dipping blood pressure pattern

The non-dipping blood pressure pattern is found to be more prevalent in one race or ethnic group more than the others (Mayet *et al.,* 1998). Many studies have reported an ethnic variation in ambulatory blood pressure patterns particularly with nocturnal dipping (Wang *et al.,* 2006; Cooper *et al.,* 2009; Sherwood *et al.,* 2003).

High rates of hypertension and high rates of mortality from cardiovascular and cerebrovascular disease are said to be more prevalent amongst the black population (Mayet *et al.,* 1998). The non-dipping blood pressure pattern is also found to be more prevalent amongst the black population, predisposing them to more cardiovascular target organ

damages when compared with other races (Murphy *et al.,* 1991). Although daytime blood pressure is similar between blacks and whites, blacks had a higher nocturnal blood pressure average and a higher incidence of left ventricular mass index (Murphy *et al.,* 1991). Some studies have suggested that the circadian alteration in blood pressure and heart rate in blacks could be genetically dependent (Osei *et al.,* 1996). Factors that have been implicated for these differences include environmental, socio-economic, personality, lifestyle, level of activity, salt intake, salt sensitivity and work stress (Harshfield *et al.,* 1990, James *et al.,* 1991, Shapiro *et al.,* 1996).

In a study by Rodriguez *et al.,* (2013), it was reported that a lower socio-economic status measured by years of educational attainment and income is significantly associated with a non-dipping blood pressure status. This study might suggest that the non-dipping blood pressure pattern might be related to adverse environmental and psychosocial factors which are prevalent in urban Africa (Stepnowsky *et al.,* 2004).

There is a possibility that individuals with lower socioeconomic status are more likely to be frequently exposed to serious stressors and hence experience more sympathetic stimulation. This frequent adrenergic stimulation may also be reflected as a blunted nocturnal ambulatory blood pressure variability which might suggest an altered sympathetic-parasympathetic balance (Sherwood *et al.,* 2002; Sherwood *et al.,* 2011). On the other hand, a higher socio-economic status is associated with positive health outcomes irrespective of disease (Major *et al.,* 2010, Du *et al.,* 2011). This could reflect a better health behaviour profile, reduced psychosocial and physiological effects of stress and also availability of better resources with which to cope with stress (Mujahid *et al.,* 2011). The higher prevalence of the non-dipping blood pressure pattern in blacks could also be due to poor access to health care and obesity (Odoms-Young *et al.,* 2009).

A study has shown that black Hispanics are more prone to developing a non-dipping blood pressure status than white Hispanics (Rodriguez *et al.,* 2013). It has been shown that untreated black hypertensives living in Africa are more likely to be non-dippers when compared with untreated white hypertensives living in Europe after being matched for sex and age (Polonia *et al.,* 2014), hence blacks have a worse cardiovascular prognosis in hypertension than whites. It was observed that Asians have a more blunted drop in nocturnal blood pressure when compared with Europeans (Wang *et al.,* 2012).

According to the above findings, there is no doubt that black Africans have a higher prevalence of the non-dipping blood pressure pattern when compared with other ethnic groups making them more susceptible to adverse cardiovascular events than the other ethnic groups. There has not been any study to determine the prevalence of NDP in an urban developing population of African ancestry that live in Africa, this has however been addressed in this thesis.

1.3.3 Consequences of and conditions associated with the nocturnal non-dipping blood pressure pattern

The non-dipping blood pressure pattern is of clinical and prognostic relevance as it has been observed in several cross sectional and longitudinal studies that the non-dipping blood pressure pattern is associated with increased target organ damage and a greater risk of cardiovascular morbidity when compared with non-dippers (Shimada *et al.,* 1990). There is also evidence suggesting that individuals with the non-dipping blood pressure profile are at a higher risk of cardiac and extra cardiac morbidity (O'Brien *et al.,* 2005).

The cardiovascular events associated with a non-dipping blood pressure pattern include myocardial infarction, unstable angina, stroke, transient cerebral ischemia, symptomatic aorto-iliac occlusive disease, congestive heart failure, left ventricular hypertrophy and sudden cardiac death (O'Brien *et al.,* 2005).

Some of the target organ effects of the non-dipping blood pressure pattern shall be discussed in the following sections.

1.3.3.1 Cardiac structural alterations

Left ventricular hypertrophy has been said to be a strong predictor of cardiovascular complications and death in persons with essential hypertension (Schillachi *et al.,* 1996). A study showed that left ventricular hypertrophy is more common in individuals with a non-dipping blood pressure pattern. The prevalence was however found to be even higher in those

with reproducible non-dipping blood pressure pattern (Cuspidi *et al.,* 2003). In the study by Cuspidi and colleagues, it was observed that both dippers and non-dippers had almost the same 48 hour systolic and diastolic blood pressure average, suggesting that the left ventricular structural changes might be due to the nocturnal non-dipping blood pressure status since the left ventricular structural changes were not observed in dippers (Cuspidi *et al*., 2004). Another study has also shown similar results pointing at a higher prevalence of left ventricular hypertrophy in non-dippers (Salvetti *et al.,* 2001).

It has been shown that individuals with untreated hypertension and classified as non-dippers have significantly greater left ventricular mass and left ventricular indices when compared with dippers despite having similar 48 hour systolic and diastolic blood pressure averages (Mezue *et al.,* 2016). Similar findings have also been observed in individuals with essential hypertension (Anan *et al.,* 2003) and even in non-dipping individuals with normal blood pressure (Hoshide *et al.,* 2003). It was observed in some studies that left ventricular mass and left ventricular mass index increase with increasing nocturnal blood pressure averages suggesting that the cardiac abnormalities are more likely due to an altered diurnal blood pressure rhythm as opposed to a higher average 24-hour blood pressure load (Verdecchia *et al.,* 1995).

An abnormal cardiac geometry is more commonly seen in hypertensive individuals. The more common abnormality is left atrium enlargement (Shigematsu *et al.,* 1998). Individuals with hypertension who have concentric left ventricular hypertrophy have more severe target organ damage and are more prone to having adverse cardiovascular events compared to individuals with other left ventricular geometric patterns (Verdecchia *et al.,* 1995).

Studies have shown a higher incidence of altered pattern of cardiac structure and geometry in persons with a non-dipping blood pressure pattern (Verdecchia *et al.,* 1995). A study showed that hypertensives had significantly larger left atriums, a higher atrial filling fraction and end-diastolic diameter indexes than normotensive individuals (Ferrara *et al.,* 1998). However, hypertensives with a non-dipping blood pressure pattern develop these cardiovascular abnormalities faster than dippers (Ferrara *et al.,* 1998). Alteration in cardiac structure has also been observed in individuals with normal blood pressure who are non-dippers (Hoshide

et al., 2003), they show a higher prevalence of relative wall thickness and concentric hypertrophy compared with dippers, this suggests that normotensive non-dippers may have a poorer cardiovascular prognosis than normotensive dippers (Mezue *et al.,* 2016)

1.3.3.2 Cardiac functional alterations

Research has shown that non-dippers may be at a greater risk of cardiac dysfunction than dippers. It has been shown that they have a higher level of cardiac natriuretic hormones (Nystrom *et al.,* 2005), lower left ventricular ejection fraction (Aydin *et al.,* 2004), higher number of ectopic beats (Rizzo et al, 2000), prolonged QT dispersion (Kohno et al, 1998) and a prolonged QT interval (Passino *et al.,* 2003). Another study has shown that there is an impairment of diastolic and global function of the left and right ventricles in non-dippers when compared with dippers (Ivanovic *et al.*, 2013).

1.3.3.3 Haemodynamic alterations

It has been observed that individuals who are classified as non-dippers have a higher systemic vascular resistance in the night when compared with dippers who show no significant changes in systemic vascular resistance (Takakuwa *et al.,* 2001). These observations might then suggest that a non-dipping blood pressure pattern may be associated with vascular structural alterations (Pierdomenico *et al.,* 1997).

Studies have shown that there is an increase in stroke index in non-dippers when compared with dippers (Takakuwa *et al.,* 2001). This might contribute to the normal nocturnal blood pressure drop. However, there is no significant difference between the cardiac output between dippers and non-dippers (Passino *et al.,* 2003).

1.3.3.4 Carotid artery abnormalities

It has been established that NDP is linked to carotid artery hypertrophy and arteriosclerotic complications (Zanchetti *et al.,* 2002). Thickness of the intima-media of the common carotid artery and the presence of carotid plaques are said to be markers of subclinical arteriosclerosis (Touboul *et al.,* 2005) and hence predictors of future cardiovascular events (Allison *et al.,* 2005). It has been shown in some studies that individuals who have essential hypertension and a non-dipping blood pressure profile have a significantly higher incidence of common carotid artery intima-media thickness (Cuspidi *et al.,* 2001; Pierdomenico *et al.,* 1997) when compared with dippers. It was also discovered that non-dippers have a higher incidence of arteriosclerotic plaques in their carotid arteries when compared with dippers (Salvetti et al, 2001)

1.3.3.5 Cerebrovascular disease

It has been said that hypertension is strongly associated with cerebrovascular damage (Yamamoto *et al.,* 1995); however, hypertensives with a non-dipping blood pressure profile are at a higher risk of stroke (Metoki et al, 2006). Lacunae and paraventricular intensities are also more prevalent in non-dipper hypertensives and normotensives when compared with dippers (Kario *et al.,* 1996). These studies might suggest that advanced cerebrovascular disease may prevent blood pressure from falling at night in order to maintain cerebral perfusion pressure (Shimada et al, 1992). Another study showed no significant difference in cerebral abnormalities between normotensives and hypertensives with a dipping blood pressure pattern (Shimada *et al.,* 1992) suggesting that a dipping blood pressure status might inhibit the development of cerebrovascular abnormalities.

It has also been shown that a non-dipping systolic blood pressure is associated with a higher incidence of white matter lesions (Sander *et al.,* 2000). Non-dippers have also been shown to have a poorer long-term prognosis which includes a higher risk of developing further ischemic lesions and symptomatic ischemic strokes (Yamamoto *et al.,* 1998).

1.3.3.6 Renal damage

The kidney is susceptible to vascular injury in persons with hypertension. Research has shown that microalbuminuria is a strong predictor of cardiovascular morbidity and mortality in non-diabetic hypertensive patients (Klausen *et al.,* 2005). It has been shown that non-dippers have a higher incidence of microalbuminuria (Tsioufis *et al.,* 2002). A cross sectional study showed similar results (Bulatov *et al.,* 2001).

1.3.3.7 Arterial stiffness

Arterial stiffness as a result of a reduced arterial compliance is one of the major signs of vascular aging (Redheuil *et al.,* 2010). A non-dipping blood pressure profile may be associated with premature vascular aging which could increase cardiovascular complications (Cicek *et al.,* 2013). It has however been documented that arterial stiffness is a predictor of all-cause mortality and cardiovascular mortality (Vlachopoulos *et al.,* 2010).

Arterial stiffness can be measured using Carotid-Femoral Pulse Wave Velocity (CF-PWV), a technique that has been embraced as the gold standard for this purpose as it is a well-known predictor of adverse cardiovascular outcome having a better predictive precision than classical cardiovascular risk factors (Laurent *et al.,* 2006). This technique is examined in more details in subsequent sections. Research has suggested that a high pulse wave velocity and impaired vascular smooth muscle tone during the night is associated with a non-dipping nocturnal blood pressure pattern (Cicek *et al.,* 2013).

The cardiovascular target organ damage of focus in this thesis is arterial stiffness, hence I will go into a bit of detail on this topic, citing mechanisms and consequences and possible treatment of arterial stiffness.

1.3.3.7.1 Mechanisms of arterial stiffness

It has been shown that vascular stiffening is due to a complicated interaction between changes which involve cellular and structural components of the vascular wall (Zeiman *et al.,* 2005). These vascular changes can be influenced by haemodynamic forces and by external factors like salt and glucose homeostasis (Wolinsky *et al.,* 1964; Wolinsky *et al.,* 1969). Aging and diseases such as hypertension and diabetes can speed up the vascular changes that cause arterial stiffening in different ways. The effect of aging on arterial stiffness is examined in more details in subsequent sections.

Compliance of the arterial wall depends on relative synergy of its two most important scaffolding proteins collagen and elastin. The homeostasis of these molecules is maintained by a constant process of synthesis and degradation. A distortion of this homeostasis mainly due to an inflammatory process can lead to the production of high amounts of abnormal collagen and very low amounts of elastin leading to arterial stiffness (Johnson *et al.,* 2001). Hypertension can also lead to excessive collagen production (Xu *et al.,* 2000). The above-mentioned changes can lead to substantial increase in intima-medial thickness as one ages (Nagai *et al.,* 1998; O'Leary *et al.,* 1999) and hypertrophy of the vascular smooth muscle layer (Virmani *et al.,* 1991). Stiffened arteries when viewed microscopically show abnormal endothelial cells, high collagen, fractured elastin molecules and different components of inflammation including macrophages, polymorphonuclear cells and cytokines (Lakatta *et al.,* 2003)

Elastin molecules are usually stabilized by cross-linking to form the elastic proteins desmosine and isodesmosine. Distortion of these cross-links lead to weakening of the elastin structure which makes it susceptible to mineralisation by calcium and phosphorus which leads to an increase in arterial stiffness (Watanabe *et al.,* 1996; Spina *et al.,* 1976; Cattell *et al.,* 1996).

At the cellular level, a low expression of nitric oxide and an increased expression of nitric oxide synthase (NOS) inhibitor have been linked to arterial stiffness (d'Alessio *et al.,* 2004;

Lyons *et al.,* 1997; Miyazaki *et al.,* 1999). Reactive oxygen species such as peroxynitrite have also been linked to arterial stiffness (Kruger *et al*., 2012).

Some hormones have been implicated in the development of arterial stiffness. Angiotensin II for example has been shown to cause an increase in collagen formation, vascular hypertrophy, reduction in nitric oxide bioavailability, oxidative stress and a reduction in the synthesis of elastin (Kato *et al.,* 1991; Gibbons *et al.,* 1992. Angiotensin II also stimulates components of the inflammatory response which are known to also contribute to arterial stiffness (Tokimitsu *et al.,* 1994). Aldosterone which is mainly controlled by angiotensin II has also been shown cause arterial stiffness and hence hypertension by causing fibrosis and stimulation of vascular smooth muscle cell hypertrophy (Lacolley *et al.,* 2002; Blacher *et al.,* 1997). Aldosterone increases the production of endothelin-1 which is a known vasoconstrictor, and which also has fibrotic effects on the vascular wall (Park *et al.,* 2001).

It has been shown that arterial stiffness is increased by dietary salt, and a reduction in dietary salt intake can improve arterial compliance (Gates *et al.,* 2004; Bagrov *et al.,* 2004). Salt has been shown to cause an increase in vascular smooth muscle tone and hypertrophy coupled with thickening of the vascular medial layer and excessive production of collagen (Gu *et al.,* 1998; Safar *et al.,* 2000; Partovian *et al.,* 1998). It is said that sodium causes endothelial dysfunction by diminishing NOS production of nitric oxide therefore stimulating NOS inhibitor activity and production of reactive oxygen species which is an important contributor to arterial stiffening (Bagrov *et al.,* 2004). A reduction in NOS production and stimulation of reactive oxygen species activates matrix metalloproteinases (MMPs), which regulate the structural proteins of the extracellular matrix including elastin and collagen (Harvey *et al.,* 2016). Activation of MMPs leads to high levels of transforming growth factor-beta 1 (TGFβ-1) which causes fragmentation of elastin fibres and accumulation of collagen fibres reducing the elastin/collagen ratio therefore, causing arterial stiffness (Salvi *et al.,* 2018a; Wang *et al.,* 2006b). A summary of this mechanism is provided in figure 1.2

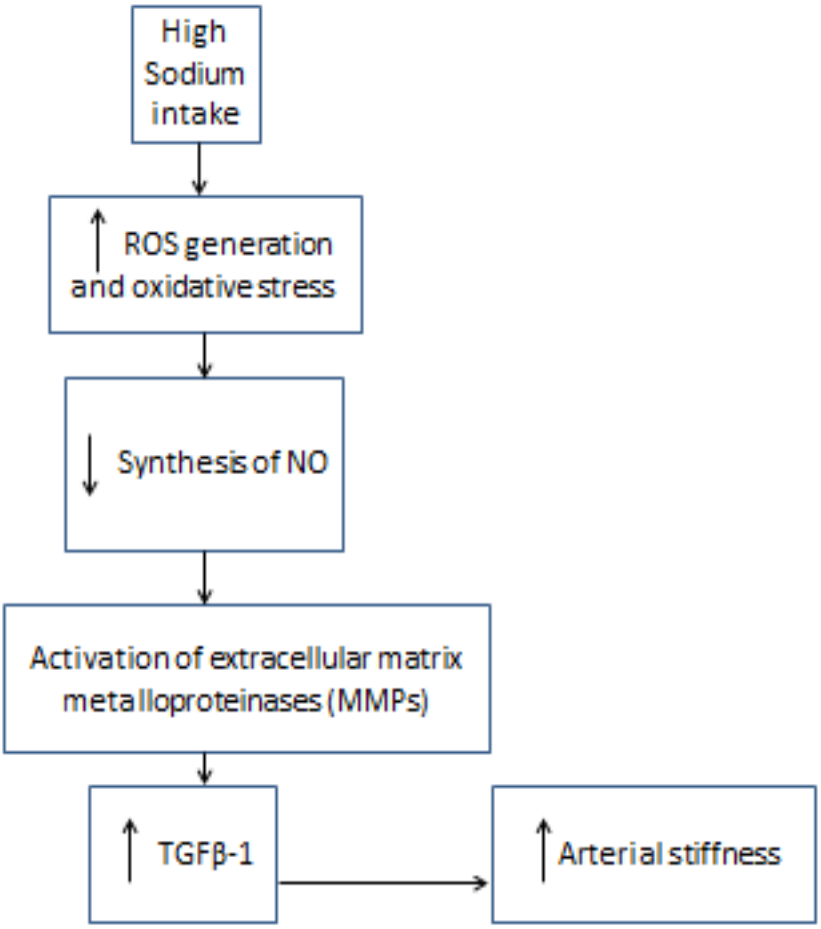


Figure 1.3. Illustration showing the mechanism of salt induced arterial stiffness

Insulin resistance is also another condition that has been shown to cause arterial stiffening (Salomaa *et al.,* 1995; Sutton-Tyrrell *et al.,* 2001). Persistent hyperglycemia and hyperinsulinemia have been shown to increase the activity of the RAAS and the up regulation of type 1 angiotensin II receptor (Nickenig *et al.,* 1998) thereby enhancing vascular smooth muscle cell hypertrophy and fibrosis (Jesmin *et al.,* 2003; Rizzoni *et al.,* 2001). Hyperinsulinemia on its own has also been shown to have proliferative effects on vascular smooth muscle cells (Cusi *et al.,* 2000). A blunted glucose tolerance has been shown to alter the mechanical properties of the tissues in the vascular wall (Brownlee *et al.,* 1988). Increased levels of low-density lipoproteins (LDLs) and free fatty acids as seen in impaired glucose tolerance also leads to further stiffening of the arteries (Steinberg *et al.,* 1997). High amounts of endothelin-1 as well as reduced levels of adiponectin and natriuretic peptides also worsen impaired glucose tolerance induced arterial stiffness (Matsuzawa *et al.,* 2004; Wang *et al.,* 2004).

A summary of the different factors contributing to arterial stiffness is presented in figure 1.4 on the next page.

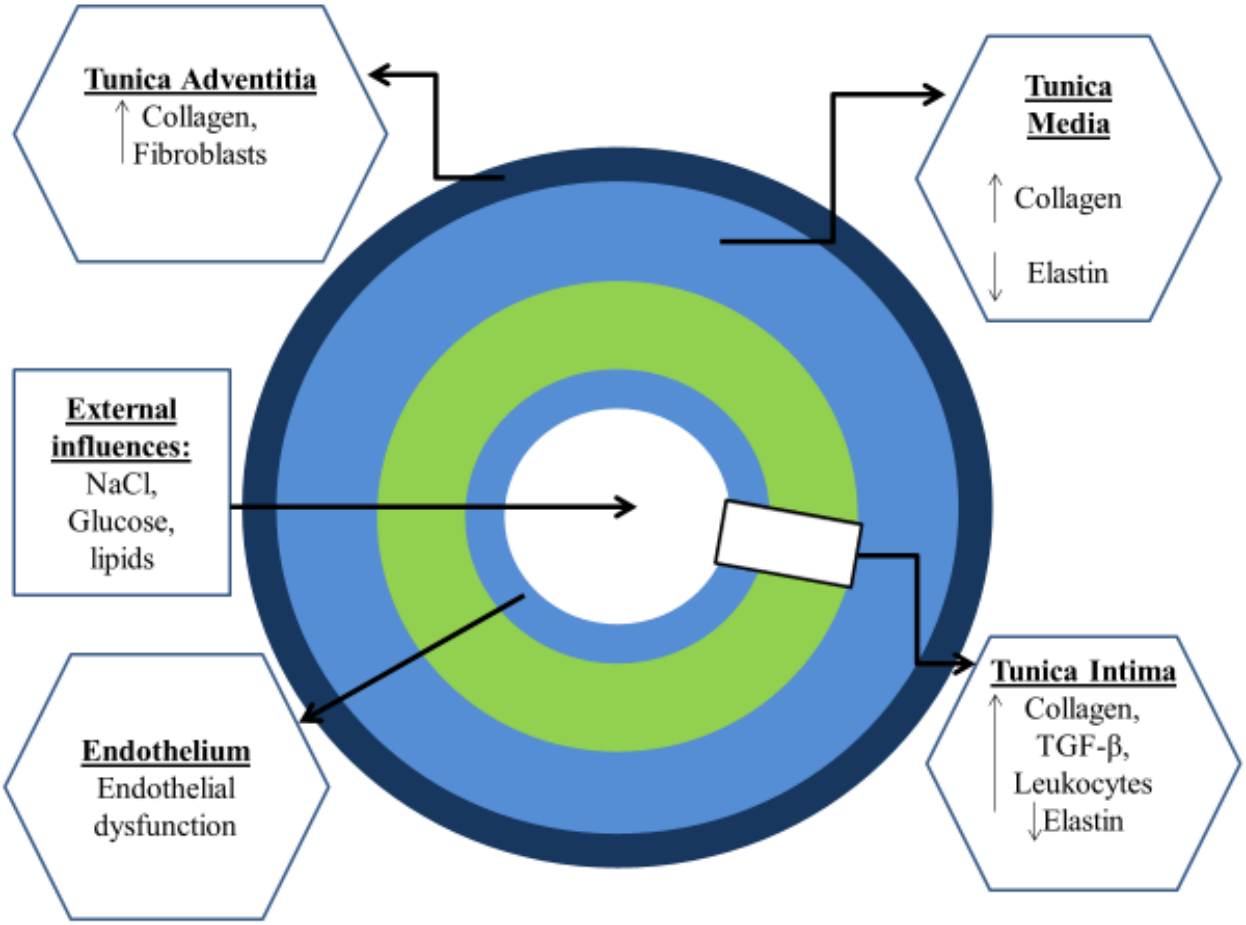


Figure 1.4. Summary of the different causes and localization of arterial stiffness. Figure shows the three layers of a typical artery and the events that accompany arterial stiffness.

1.3.3.7.2 Relationship between kidney disease and arterial stiffness

It has been shown that arterial stiffness increases in individuals with chronic renal insufficiency and is a powerful independent predictor of mortality in this group of patients (Blacher at al., 1999). The hypertension that develops from chronic renal disease leads to an increase in vascular wall stress leading to arterial stiffness. Vascular smooth muscle cell proliferation and an increase in vascular wall collagen content are caused by an increase in activation of the RAAS (Nickenig *et al.,* 1998). Arterial stiffness secondary to chronic renal insufficiency is caused by excessive calcification to the arterial media mainly due to the role of osteoblast-like cells secreting bone matrix protein (Moe *et al.,* 2004; Jono *et al.,* 2000; Block *et al.,* 1998).

1.3.3.7.3 Clinical consequences of arterial stiffening

Elevated pulse pressure and isolated systolic hypertension are the major clinical presentations of arterial stiffness (Dart *et al.,* 2001). An increased pulse wave velocity, isolated systolic hypertension and high pulse pressure leads to a significantly higher risk for myocardial infarctions, strokes, heart failures and overall cardiovascular mortality in older adults (Kostis *et al.,* 2001; Benetos *et al.,* 1997; Meaume *et al.,* 2001).

1.3.3.7.4 Arterial stiffness and target organ damage

Arterial stiffness leads to an increased loading on the left ventricle by increasing pressure pulsatility which subsequently leads to an increase in early systolic load (Chirinos *et al.,* 2012). Arterial stiffness also leads to excessive blood pressure variability by causing an impairment in baroreceptor function (Schillaci *et al.,* 2012; Okada *et al.,* 2011) and hence a reduction in baroreflex sensitivity which can lead to an increased susceptibility of important organs like the brain to episodic fluctuations in blood pressure leading to temporary ischemia

in these important organs because of an impaired baroreflex function (Mitchell *et al.,* 2008). This leads to an inappropriate response to changing blood pressure (Mitchell *et al.,* 2011).

1.3.3.7.5 Can arterial stiffness be treated?

Some life style modifications have been shown to be of benefit in improving arterial distensibility. One of those is increased consumption of dairy products which has been linked to lower carotid femoral pulse wave velocity, pulse pressure and systolic blood pressure (Crichton *et al.,* 2012). The relationship between increased dairy intake and a reduction in the above-mentioned hemodynamic parameters showed a dose-response effect. The authors suggested the potential beneficial effects of the components of milk including calcium, magnesium, potassium and phosphorus. Supplementation with n3 polyunsaturated fatty acids has been shown to improve systemic arterial distensibility probably by reducing the levels of triglycerides and very low-density lipoproteins (VLDLs) (Nestel *et al.,* 2002). A high dietary consumption of isoflavones, which are found in high amounts in soy beans have also been shown to reduce pulse wave velocity (van der Schouw *et al.,* 2002).

Higher urinary sodium-to-potassium excretion ratio has been associated with a reduction in arterial compliance in a cohort of salt sensitive Africans (Redelinghuys *et al.,* 2010). However, in a dietary intervention study, it was observed that potassium supplementation led to a reduction in carotid femoral pulse wave velocity (CFPWV) (He *et al.,* 2010). A low salt diet has also been shown to be beneficial at reducing blood pressure, CFPWV, and oxidative stress (Hummel *et al.,* 2012).

Weight loss has been shown to reduce CFPWV even after adjusting for mean arterial pressure (Dengo *et al.,* 2010). Level of physical activity is said to correlate with arterial stiffness. Individuals who were more active in their earlier years up till adulthood were shown to have a lower level of arterial stiffness when compared with those who did not (van de Laar *et al.,* 2010). Older people who engage in light physical activity have lower pulse wave velocity compared to those who do not (Gando *et al.,* 2010).

It has been shown experimentally that the angiotensin II type 2 receptor agonist 'compound 21' led to a reduction of collagen and fibronectin deposition and a reduction of macrophage infiltration in the walls of the aorta (Rehman *et al.,* 2012) thereby preventing arterial stiffness and fibrosis (Paulis *et al.,* 2012). The angiotensin II type 1 receptor antagonist 'Olmesartan' has also been shown to reduce aortic pulse wave velocity (Paulis *et al.,* 2012).

1.3.3.7.6 Aging and arterial stiffness

It is known that the prevalence of arterial stiffness increases with age (Alghatrif *et al.,* 2013). Aging is described as the age-related reduction in physiological functions that are important for survival and fertility. Cardiovascular aging is very important as it can determine lifespan. The wall of large arteries like the aorta lose distensibility and become thicker as age advances (Folkow *et al.,* 1993) leading to an increase in pulse wave velocity (PWV). This increase in arterial stiffness leads to a reduction in the reservoir function of the conduit arteries closer to the heart leading to an increase in systolic BP and pulse pressure (Greenwald *et al.,* 2007). Age induced hypertension is hence characterized by a much higher systolic BP with no change or sometimes a reduction in diastolic BP, this is known as isolated systolic hypertension (ISH) (McEniery *et al.,* 2009).

Aging is associated with an increased sympathetic activation (Barnes *et al.,* 2014) which may also lead to increased inflammation (Zubcevic *et al.,* 2014). The mechanism of inflammation induced arterial stiffness has been discussed in the preceding sections. It is also known that salt sensitivity and endothelial dysfunction increase with age, both of which contribute to arterial stiffening (Weinberger *et al.,* 1991). Metabolic syndrome has been shown to lead to arterial stiffness and speed up arterial aging (Safar *et al.,* 2006; Sowers *et al.,* 2013)

Gender differences in age related arterial stiffening has been reported with different studies showing contradicting results. A report showed that females show a faster rate of age-related increase in arterial stiffness when compared to males which leads to them having a higher degree of arterial stiffness after menopause compared to age matched males (Nethononda *et al.,* 2015). On the other hand, another study showed that males have a sharper increase in arterial stiffness with aging, causing them to have greater arterial stiffness than age matched females in their fifth decade (Alghatrif *et al.,* 2013). The differences in observation might

have been due to differences in study design and methods of measuring arterial stiffness.In this thesis however, I investigate the gender differences in age related arterial stiffness.

The gender differences in age related arterial stiffness have been attributed to sex steroids. Ovarian hormones have been shown to have vascular protective effects (Clarkson *et al.,* 1994) and the withdrawal of these vascular protective effects in menopause have been said to be responsible for a higher level of arterial stiffness in post menopausal females (Chester *et al.,* 1995). Gender differences in serum lipid profiles have however been shown to be responsible for these gender differences. In this thesis, I investigate the role of serum lipid profile in gender differences in non-dipping induced arterial stiffness and age-related arterial stiffness.

1.3.3.8 Obstructive sleep apnoea (OSA)

Obstructive sleep apnoea is a chronic disorder characterized by total or incomplete collapse of the upper respiratory tract during sleep leading to halting or a reduction in airflow (Guillerminault *et al.,* 1976; Guillerminault *et al.,* 2001). This disorder can be quite common, with some studies showing a prevalence of 50% in males and 20% in females (Arnardottir *et al.,* 2016; Heinzer *et al.,* 2015). The severity of OSA is usually defined by using the apnoeal hypopnoea index (AHI) which is described as the number of obstructive episodes per hour of sleep measured in events per hour (events h^{-1}) (Berry *et al.,* 2012).

Obstructive sleep apnoea has been shown to be an independent risk factor for hypertension and the nocturnal non-dipping blood pressure pattern (NDP) (Parati *et al.,* 2013; Seif *et al.,* 2014) such that a dose-dependent increase in the development of non-dipping hypertension has been described in a 7 year follow up study (Hla *et al.,* 2008). Sleep disordered breathing events that happen during rapid eye movement sleep is especially linked to NDP (Mokhlesi *et al.,* 2015). As mentioned earlier, NDP is associated with a higher risk of adverse cardiovascular events when compared with dippers, it is important to know that these adverse cardiovascular events occur more frequently in individuals with OSA who have NDP even if they are not diagnosed with hypertension (Sasaki *et al.,* 2015).

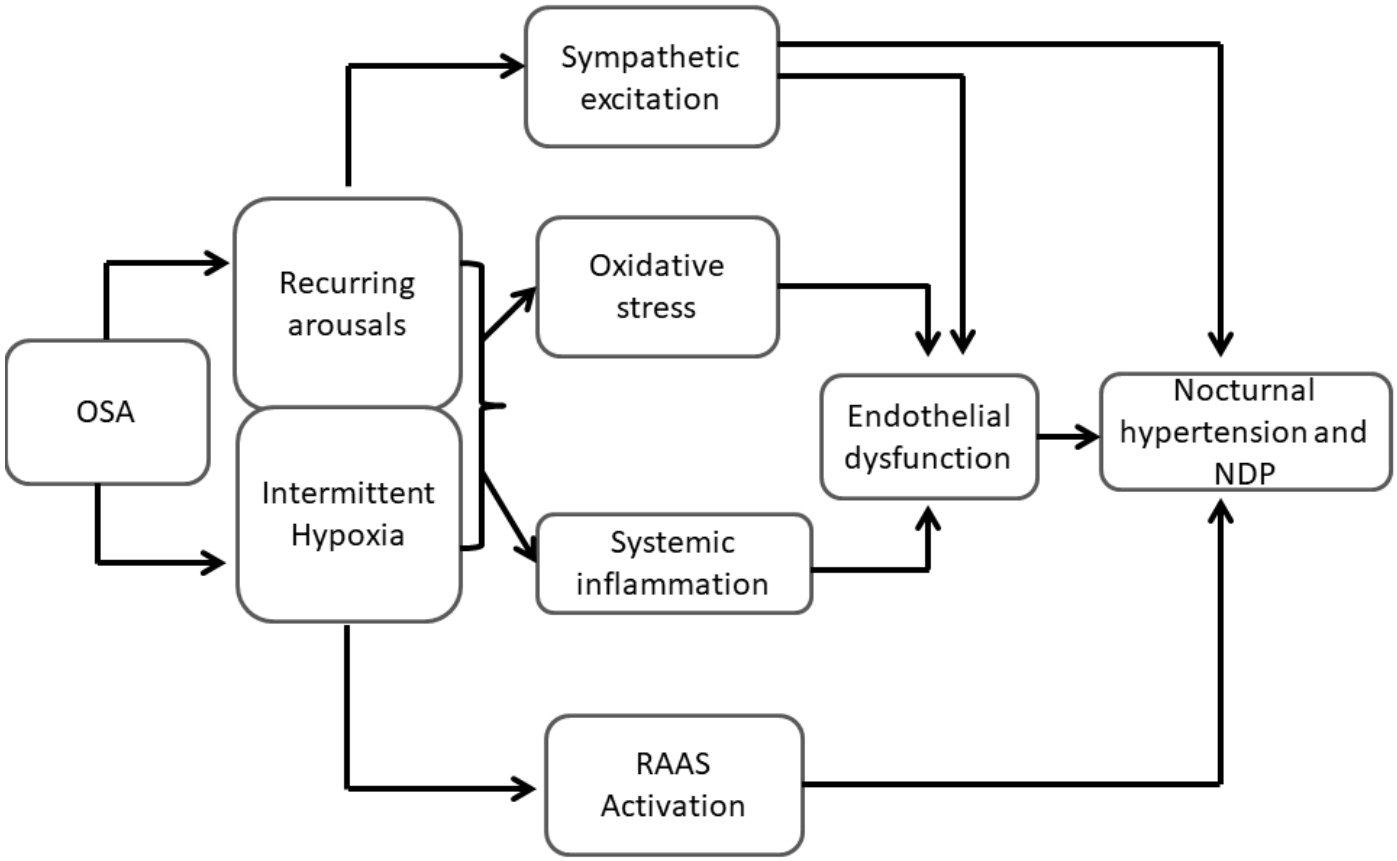


Figure 1.5. Illustration of the proposed relationship between Obstructive Sleep Apnoea (OSA) and the nocturnal non-dipping blood pressure pattern (NDP). RAAS, Renin-Angiotensin Aldosterone System. The explanations for this relationship are given in more details in the following sections.

1.3.4 Reproducibility of the nocturnal non-dipping blood pressure pattern

It is well known that the diagnosis of clinical hypertension is derived from conventional office blood pressure measurements as most data that relates to morbidity and mortality from hypertension are derived from studies that employ conventional office blood pressure values (Staessen *et al.*, 1999). These conventional office BP measurements may not fully take into consideration phenomena like masked hypertension, white coat hypertension or BP dipping status which could be very important causes of morbidity or mortality related to hypertension (Pickering *et al.*, 2006).

The clinical value of NDP depends on its reproducibility from one instant to the other. Sometimes, the dipping status changes when it is measured more than once on different occasions. It was shown in a study that only about 54% of participants who had ABPM and were classified as dippers maintained this status after performing three ABPMs in a space of 6 months (Manning *et al.,* 2000). In another study with 170 participants, 60% of the total participants maintained their dipping status after ABPM was conducted on two different occasions in the space of 1 year. This study was conducted in hypertensive patients who were off medication (Omboni *et al.,* 1998). On the other hand, a more recent study than the above-mentioned ones had a very high level of dipping status reproducibility (Stenehjem *et al.,* 2004). The study had 65 hypertensive patients who underwent repeated ABPM and 88% of them maintained their dipping status. This level of reproducibility was considered as satisfactory. Other studies that had strict daytime activity observation protocols also showed that blood pressure dipping is very reproducible (Gretler *et al.,* 1993; O'Shea *et al.,* 1997).

One of the proposed explanations for this wide variation in ambulatory BP variability is that participants in the study are more likely to behave in similar ways during repeated measurements if there is a specific protocol to follow (as in the daytime activity monitoring study mentioned above) as opposed to just noting daytime activity in a diary (Birkenhager *et al.,* 2007). It has also been proposed that change in body position during sleep from one night while ABPM is being conducted to another study night can influence reproducibility (Netea *et al.,* 2003a). Lying on one's back as compared to lying on the side can lead to a 12-14mmHg difference in BP if the blood pressure cuff is fastened to the upper arm or forearm (Cavelaars *et al.,* 2000; Netea *et al.,* 2003b). Using activity monitoring systems can effectively measure arm position during ABPM and these systems can correct for changes in

BP due to arm and body position and this has been shown to improve reproducibility of dipping status (O'Shea *et al.*, 2000).

1.4 Overall summary

A non-dipping blood pressure pattern is the failure of the nocturnal blood pressure to fall by at least 10% of the daytime values and has been identified to be a risk factor for the development of cardiovascular end organ damage. The study of NDP has been made possible with the use of ambulatory blood pressure monitoring, a technique which was developed more than 50 years ago and enables the measurement of blood pressure values over a 24-hour period.

A non-dipping blood pressure profile has been said to be associated with a poorer cardiovascular prognosis when compared with a dipping blood pressure status. Conditions associated with the non-dipping blood pressure profile include left ventricular hypertrophy, congestive heart failure, carotid intima media thickness, arterial stiffness, microalbuminuria, stroke and renal damage.

The non-dipping blood pressure status has been said to be more prevalent amongst the black population and to a lesser extent in the Asians when compared with Europeans. Factors such as socioeconomic status, lifestyle, genetic and salt sensitivity have been said to be responsible for these differences. These ultimately put the black population in a position of having a higher rate of cardiovascular target organ damage in hypertension than other ethnic groups.

1.5 Aims

The aims of this thesis are as follows:

1. To determine normal reference values for ambulatory blood pressure monitoring in individuals of African ancestry. These data are described in chapter 3 of this thesis and have been accepted for publication in the Blood Pressure Monitoring Journal.

2. To determine the role of urinary sodium excretion and aldosterone in the pathophysiology of the Non-Dipping blood pressure pattern (NDP) in a population of African ancestry. These data have been presented in chapter 4 of this thesis.

3. To define the determinants of arterial stiffness and to investigate the role of NDP in modifying the relationship between arterial stiffness and its determinants.

4. To investigate gender differences in non-dipping related arterial stiffness in a black African population.

CHAPTER 2

Methods

2.1. Introduction

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

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

2.2. Study Population

I randomly recruited 1,219 participants of African ancestry living in the metropolitan area of Johannesburg South Africa. They were recruited by word of mouth either individually, from religious groups, families or local communities. The minimum age of participation was 18 years. Participants gave informed, written consent before the commencement of individual studies. Standard questionnaire was given to participants to record data on medical history, smoking habits, alcohol intake, gender and age. Of the 1,219 participants enrolled for the study, data from 302 participants were not included in statistical analysis because their ambulatory BP data did not meet our pre-specified criteria of 90% or more recordings. Ambulatory BP could not be measured in 121 participants because they were too obese hence, only data from 796 participants were used to draw conclusions. However, in the determination of reference values for ambulatory blood pressure measurement (chapter 3) a healthy population sample was generated. To do this, we further excluded data from a total of 266 participants because they were either hypertensive or had concomitant cardiovascular disease, including coronary heart disease, heart failure or transient ischemic attack. The overall number of participants whose data were included in the statistical analyses for chapter 2 totalled 530.

2.3. Ethics

The studies carried out in the present thesis were approved by the Human Research Ethics Committee HREC (medical) of the University of the Witwatersrand Johannesburg South Africa (HREC approval number M170213). The ethical approval certificate is attached as an appendix to the present thesis. The study was carried out according to the Helsinki declaration on ethical principles for medical research involving human subjects.

2.4. Anthropometric measurements

Height, weight, waist and hip circumference were measured while the participants were in the standing position with no shoes on. Height was expressed in meters (m) while waist and hip circumference were expressed in centimetres (cm).

2.4.1. Weight measurement

Weight was measured using an analogue weighing scale. The scale was placed on a horizontal hard floor surface before the participants stepped on it and the weight was read off accurately by a trained observer. It was ensured that the participants stood in the centre of the platform of the weighing scale and that they do not stand off-centre as this might affect body weight distribution and hence measurement.

2.4.2. Height measurement

Height was measured using a stadiometer, with the participant's head held in the Frankfurt horizontal plane (The top of the external auditory meatus should be level with the inferior margin of the cheekbone with the participant looking straight. It was ensured that the height rule was placed vertically with a heavy stable base. The participants stood with their backs to the height rule with the back of the head, buttocks, calves and heels touching the upright, with their feet together. The headpiece of the stadiometer was then lowered to the top of the head (and to compress the hair if present). Height was then accurately read off the height rule to the nearest millimetre or half centimetre by a trained observer. If the participant was taller than the observer, the observer stood on a platform to ensure proper reading of the height.

2.4.3. Determination of body mass index (BMI)

Body mass index (BMI) was calculated as weight in kilograms (kg) divided by the square of height in meters. Participants were considered as being overweight if their body mass index was $\geq$25 kg/m^2 and obese if their body mass index was $\geq$ 30 kg/m^2.

2.4.4 Measurement of waist circumference

Waist circumference was measured using a measuring tape. It was measured at a level midway between the lower margin of the rib and iliac crest with the tape all around the body in horizontal position. It was ensured that participants stood with their feet fairly close together with their weight equally distributed to each leg. It was also ensured that the tape was held firmly making sure it's in the horizontal position and that it was loose enough to allow the observer place one finger between the measuring tape and the participant's body. Participants were asked to breathe normally, and the reading was taken at the end of gentle exhalation. This was to prevent subjects from contracting their abdominal muscles or from holding their breath.

2.4.5. Measurement of hip circumference

Hip circumference was also measured using a measuring tape. It was measured as the maximal circumference over the buttocks. It was ensured that participants stood with their feet close together with their weight equally distributed to each leg. It was also ensured that the tape was held firmly making sure it's in the horizontal position and that it was loose enough to allow the observer place one finger between the measuring tape and the participant's body. Waist-to-hip ratio (WHR) was determined by dividing waist circumference by hip circumference.

A pictorial representation of the devices used in anthropometry is shown in figure 2.1

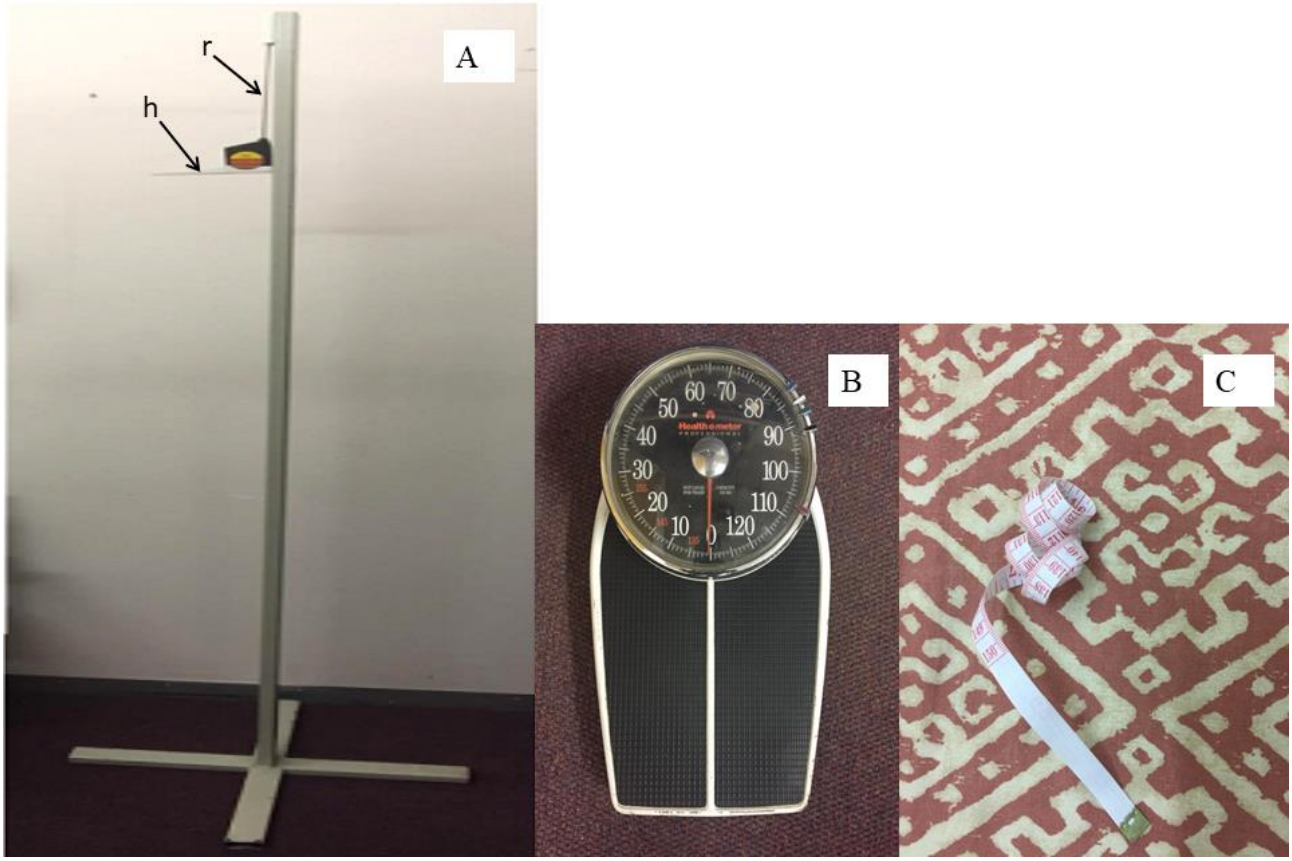


Figure 2.1. Equipment for anthropometry. The panel labelled **A** is the stadiometer while **h** represents the headpiece of the stadiometer and **r** represents the height rule of the stadiometer. Panel **B** shows the weighing scale while panel **C** shows the measuring tape.

2.5. Conventional (office) blood pressure measurements

The Omron HBP-1300 (OMRON Healthcare Europe B.V) professional blood pressure monitor was used to determine conventional office BP (Figure 2.2). In most participants, standard cuffs were used, which had an inflatable bladder with a length of 22 cm and a width of 12 cm. If arm circumference exceeded 31 cm, larger cuffs with a 31 x 15 cm bladder were used.

Brachial blood pressure was measured in each participant by trained observers after the participants have been seated and rested for at least 10 minutes. The observers measured the participants' sitting BP five times consecutively with 1 minute intervals and an average of the 5 readings was determined for accuracy. Participants were considered as being hypertensive if they had an average BP of at least 140 mm Hg systolic or 90 mm Hg diastolic or if they were on antihypertensive medication.

It was ensured that participants sat comfortably, with back supported, legs uncrossed, and upper arm bared and supported at heart level. It was also ensured that the cuff encircled at least 80% of the participant arm circumference. Participants were also instructed to relax and not talk during BP measurements (Pickering *et al.*, 2005). It was also ensured that the room was at room temperature throughout the study.



Figure 2.2. Omron HBP-1300 (OMRON Healthcare Europe B.V) professional blood pressure monitor. This device was used to measure conventional (office BP) in all participants. The part labelled **C** is the brachial cuff, while **M** represents the BP monitor.

2.6. Ambulatory blood pressure measurements

Twenty-four-hour ambulatory BP monitoring was carried out on the participants' typical working day or a day involving their usual activities using a Spacelabs 90207 (Spacelabs Inc., Redmond, Washington, USA) monitor (Figure 2.3). The monitor was programmed to measure BP at 15-minute intervals from 06:00 to 22:00 and then 30-minute intervals from 22:01 to 05:59. Participants were instructed to note the time they go to bed and the time they wake up in the morning in an activity diary which was used to determine 'awake' and 'asleep' periods. Upon completion, data was transferred from the ambulatory BP monitors to a computer for analysis. Ambulatory BP parameters recorded included 24-hour systolic BP (24-hour SBP), 24-hour diastolic BP (24-hour DBP), Day-time systolic BP (Daytime SBP), Daytime diastolic BP (Daytime DBP), Night-time systolic BP (Night-time SBP) and Night-time diastolic BP (Night-time DBP). A recording was considered as successful if at least 90% of valid recordings were obtained.

The entire procedure was explained to the participant including the frequency of inflations and that the device will repeat the measurement in case of a failed measurement. The brachial cuff was attached to the participant's non-dominant arm and they were instructed to keep their arm steady during cuff inflation and to engage in their normal daytime activities in between measurements. They were also asked to keep the monitor attached at night.

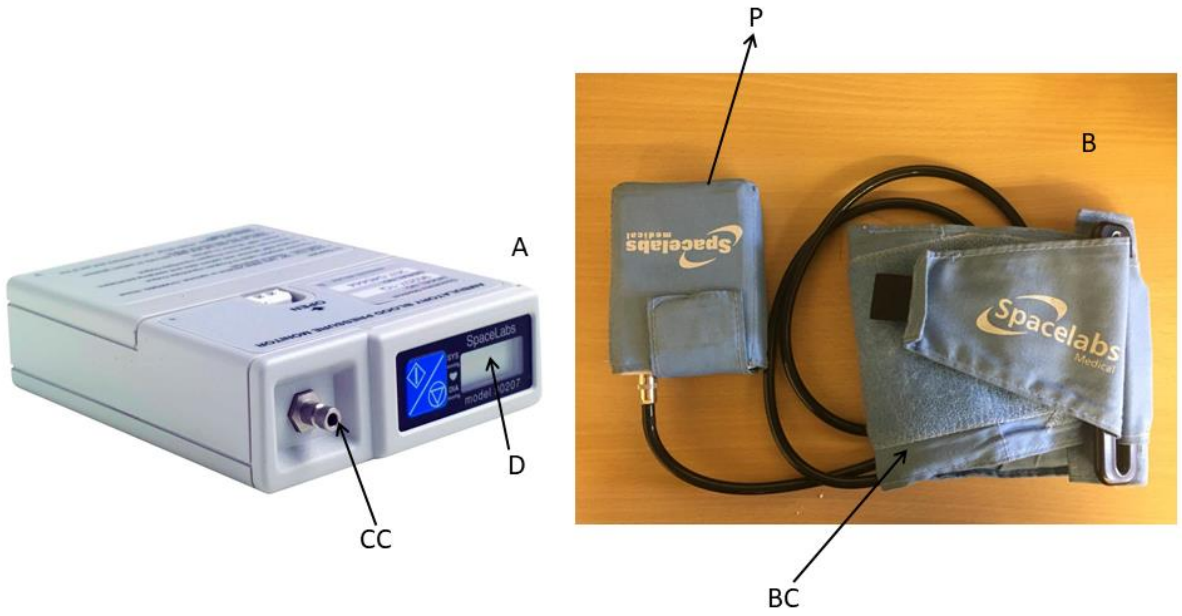


Figure 2.3. Spacelabs 90207 (Spacelabs Inc., Redmond, Washington, USA) monitor.

Panel A shows the monitor with **CC** representing the cuff connector, **D** represents the display where BP measurements are displayed. Panel **B** shows the blood pressure monitor in a pouch **P** and its brachial cuff **BC**

2.7. Twenty-four-hour urine collection

Participants were given urine bottles (figure 2.4 A) to collect their urine over a 24-hour period. They were also provided with plastic beakers (Figure 2.4 B) to catch their urine and subsequently empty the urine into the urine collection bottles. Urine samples were later retrieved from the participants and stored in urine storage tubes (figure 2.4 C); labelled appropriately including date of urine collection and time urine collection started and ended. Urine samples were then frozen in a -80°C freezer and picked up by staff from Clinical Laboratory Services (CLS) at the National Health Laboratory Service (NHLS) South Africa for analysis within 7 days.

The urine samples were collected to estimate 24-hour urinary sodium and potassium excretion (mmol/day). Twenty-four-hour urinary sodium and potassium excretion rate was calculated from the product of urine volume and urine electrolyte 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. A 24-hour urine volume less than 500 ml/day was considered incomplete and was discarded.

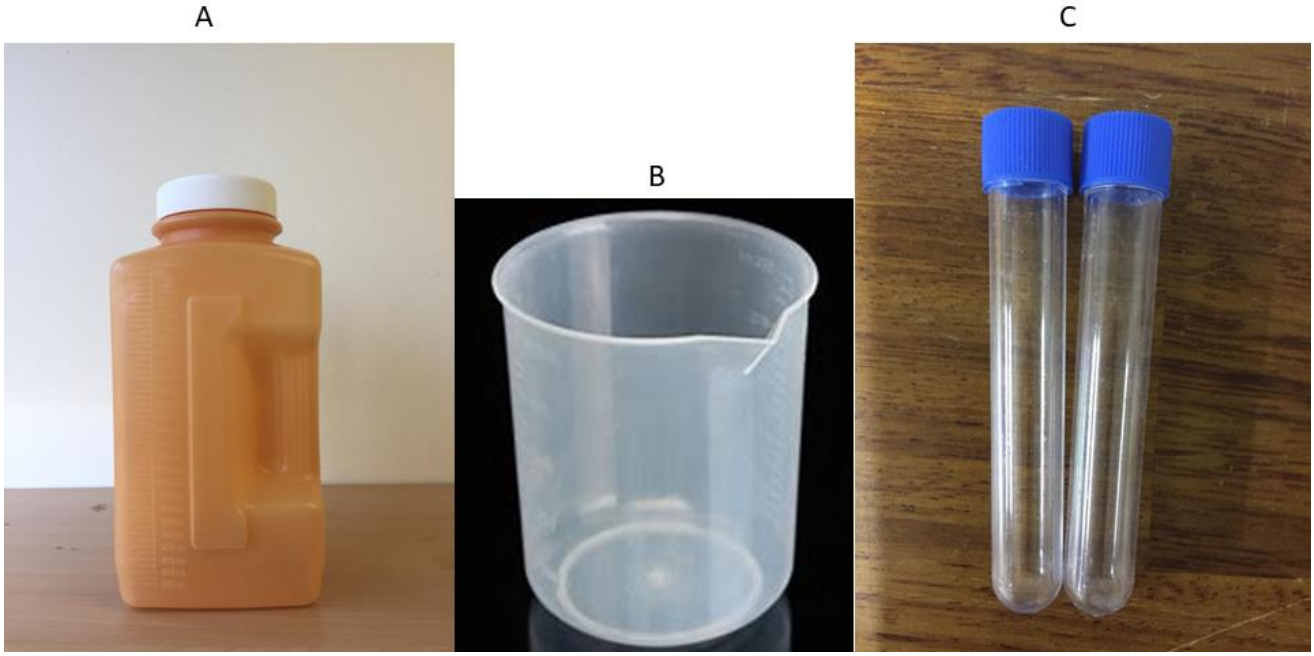


Figure 2.4. Urine collection materials. Panel **A** shows a 24-hour urine collection bottle; **B** represents a beaker while **C** represents urine sample storage tubes.

2.8. Blood sample collection

Venous blood samples were taken from participants by an experienced qualified nurse who is part of our research group. The participants were asked to relax, and blood sample was collected in the sitting position. For plasma analyses, blood samples were collected into 9 ml EDTA Vacuette® (Greiner Bio One International GmbH) blood collection tubes (figure 2.5). Blood samples were spun down in a centrifuge at 2200g for 15 minutes and the plasma aliquoted into properly labeled vials and stored in a -80°C freezer within 1 hour of blood draw. For serum analyses, blood samples were collected into 5ml Vacuette® Serum Gel tubes, gently inverted and allowed to clot for 30 minutes. The sample was then centrifuged at 2200g for 15 minutes and the serum carefully removed with a fine-bore pipette and then stored in properly labelled sterile vials and stored at 8°C before shipment takes place. Samples were then picked up by staff of CLS (Clinical Laboratory Services) at the National Health Laboratory Service (NHLS) South Africa for analysis. All samples were takem to the laboratory within 7 days of blood collection.

Blood samples were collected to determine levels of aldosterone, renin and serum lipid profile

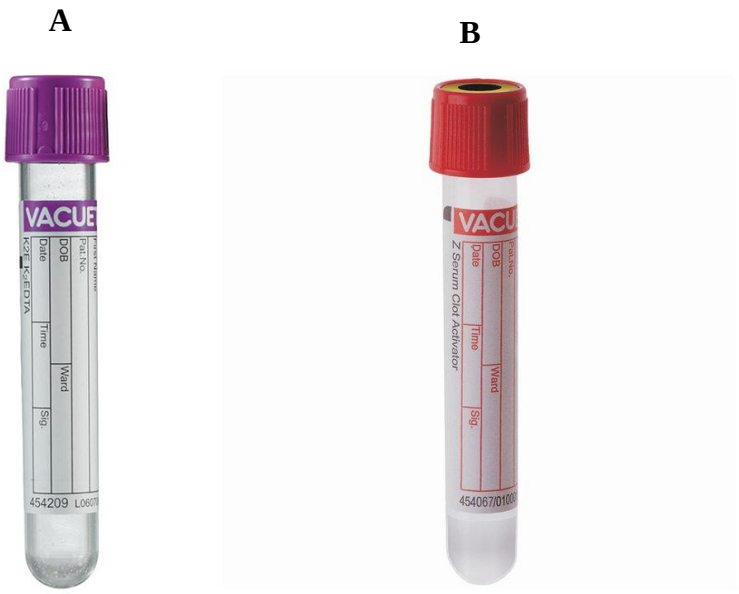


Figure 2.5. Blood sample collection tubes. Panel **A** is the 9.0 ml Vacuette® (Greiner Bio One International GmbH) EDTA blood sample collection tube. Panel **B** is the 9.0 ml Vacuette® Serum Gel tube.

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

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

After resting for about 15 minutes in the supine position, CF-PWV was measured using a high fidelity SPC-301 micromanometer (Millar instruments Inc., Houston, TX) (Figure 2.6) interfaced with a computer running SphygmoCor software version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Pulse pressure waveforms were recorded from the right common carotid artery and the right femoral artery. Pulse wave velocity was calculated automatically by the software as explained below.

Carotid-femoral pulse wave velocity was determined from sequential waveform measurements at carotid and femoral sites using applanation tonometry and SphygmoCor software (Figure 2.6). The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Aortic PWV was calculated as the ratio of the distance to the transit time (m/s).

All measurements were made by a single experienced trained technician unaware of the clinical history of the participants.

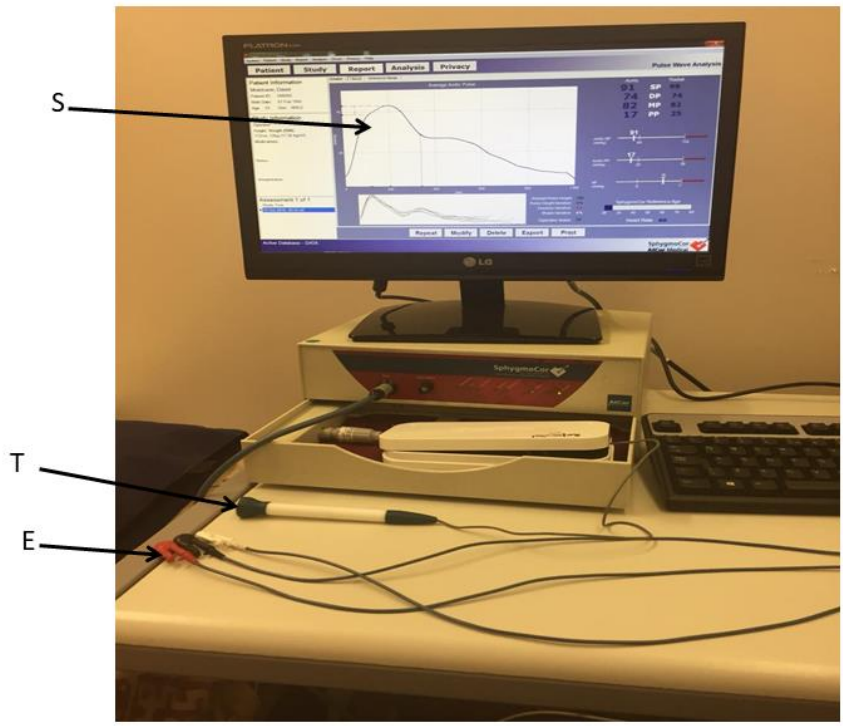


Figure 2.6. Sphygmocor device setup. Figure shows a Sphygmocor device coupled with an applanation tonometer (**T**) which was used to determine carotid femoral pulse wave velocity (CF-PWV). The tonometer was interfaced with a computer running the Sphygmocor software (**S**). The device '**E**' represents electrocardiogram chest leads.

2.10. Classification of Dipper status

Participants were classified as dippers if their nocturnal drop in systolic BP was ≥10% and as non-dippers if they had a nocturnal drop in systolic BP of <10%.

2.11. Statistical analysis

All data were analyzed using STATA (StataCorp LLC Texas USA) data analysis and statistical software version 13.0.

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

Data are expressed as mean ± standard deviation (SD) for continuous variables. Categorical variables are expressed as absolute or relative frequencies or as percentages. Test for normality of continuous variables was assessed using Shapiro-Wilk's statistic or χ^2 test for categorical variables. Comparisons between dipper and non-dipper groups as well as gender groups were done using independent Student's t-test or one-way analysis of variance (ANOVA) (with post hoc Bonferroni tests). Simple regression was used to determine the relationship between ambulatory blood pressure parameters and age in chapter 3. Multiple regression analyses were used to determine association between 24-hour urinary sodium excretion, blood pressure parameters and dipping in chapter 4. Logistic regression analyses were used to determine association between carotid femoral pulse wave velocity and dipping status in chapter 5. A P-value <0.05 was considered as statistically significant.

CHAPTER 3

Twenty-Four Hour Ambulatory Blood Pressure Reference Values in Africans.

The data in this chapter have been published in the Blood Pressure Monitoring Journal

Bawa-Allah, A.B., Mashao, M.M., Nyundu, T.F., Phukubje, E.M., Mlambo, B.W., Ngema, M.V., Nkosi, B.G. and Maseko, M.J. (2019). Twenty-four hour ambulatory blood pressure reference values in Africans. *Blood Press Monit*, 24, 103-109.

3.1. Introduction

Ambulatory blood pressure monitoring employs a non-invasive, portable, automated technology capable of measuring BP for a 24-hour period (Pickering *et al.,* 2005b). This technique was developed over 50 years ago by Kain and colleagues in 1964 (Kain *et al.,* 1964).

Ambulatory blood pressure (ABP) has been shown to be a better predictor of cardiovascular target organ damage and cardiovascular risk than conventional blood pressure (BP) values (Kuznetsova *et al.,* 2000). It also provides more reproducible information than conventional blood pressure measurements (Krakoff *et al.,* 2013). Furthermore, it allows for the diagnosis of masked hypertension, white coat hypertension, isolated nocturnal hypertension and nocturnal BP non-dipping (Peacock *et al.,* 2014; Franklin *et al.,* 2013; Parati *et al.,* 2014; Cuspidi *et al.,* 2001). It has hence been recommended for use in the diagnosis and treatment of hypertension in some countries (Bloch *et al.,* 2011). In the United Kingdom for instance, it has been recommended for use in the diagnosis of hypertension in routine clinical practice. The UK National Clinical Guideline Centre (NCGC) in May 2011 published the guideline for the clinical management of hypertension (NCE 2011). In these guidelines, conventional blood pressure measurements are used to screen for hypertension and if the mean office BP is ≥ 140/90 mmHg, the clinician is instructed to conduct ABPM to verify the diagnosis of hypertension. These recommendations have yielded two main benefits of note. Firstly, it has ensured a more effective diagnosis of hypertension and secondly, it has proven to be cost-effective when long-term costs are put into consideration when compared with the use of office BP measurements alone.

Although ABP reference values have been described in Asians and Europeans, there has not been any study that has specifically determined ABP reference values in people of African descent living in Africa (Parati *et al.,* 2014; Bloch *et al.,* 2011; Imai *et al.,* 1993). Studies that have defined reference values for African people are based on studies conducted in African Americans (Urbina *et al.,* 2008; Chobanian *et al.,* 2003).

Due to socio-economic and environmental differences, studies conducted in African American or black European communities may not be accurately interpreted to be representative of people of African descent living in Africa. A lower socio-economic status

prevalent in Africa has been attributed to a higher nocturnal BP (Rodriguez *et al.,* 2013). It has also been said that people with lower socio-economic status are consistently exposed to serious stressors like poverty, poor access to healthcare, malnutrition (Odoms-Young *et al.,* 2009), low social support and hostility (Anderson *et al.,* 1989). These stressors could lead to more sympathetic stimulation which could lead to higher ambulatory blood pressure (Sherwood *et al.,* 2002; Sherwood *et al.,* 2011).

All of these could potentially account for significant differences in ambulatory blood pressure values between Africans living in Africa and blacks who live in Europe and America or other races. Also, factors like differences in dietary salt intake, dietary potassium intake, physical activity (Ferdinand 2003) and perceived racism (Clark *et al.,* 2003) could account for differences in ABP.

Earlier studies have revealed a high prevalence of hypertension in our study population. Indeed, more than 20% of this population is hypertensive (Steyn *et al.,* 2001). Moreover, one of our earlier studies revealed that there is marked underestimation of BP control when conventional BP was used to define BP control (Maseko *et al.,* 2011). The underestimation of BP control places a very heavy burden on the resource limited South African health care system because it means several people are erroneously classified as resistant hypertensives and therefore put on 3 or more antihypertensive medications unnecessarily. Besides the underestimation of BP control, the high prevalence of white coat hypertension in this population means that several normotensive people are misdiagnosed as hypertensive when conventional BP monitoring is used to diagnose hypertension (Maseko *et al.,* 2013). This creates an urgent need for the development of strategies that will lead to proper diagnosis and effective control of hypertension which is achievable using ambulatory BP monitoring. However, this cannot be achieved unless diagnostic thresholds for ambulatory BP, which has been shown to be far superior to conventional BP in the diagnosis and management of hypertension, are established.

3.2 Aim

The aim of the present study was to establish diagnostic thresholds for ambulatory BP in people of African ancestry living in Africa.

3.3 Objective

The objective of this cross-sectional population study was to determine thresholds for ambulatory BP values in an African population using the ambulatory blood pressure monitoring technique.

3.4. Methods

3.4.1. Study Population

Details about the study population are given in chapter 2 of this thesis. Of the 1,219 participants enrolled for the study, we excluded 423 because their ambulatory BP data did not meet our pre-specified criteria of 90% or more recordings. To generate a healthy population sample, we further excluded a total of 266 participants because they were either hypertensive or also had other cardiovascular diseases, like coronary heart disease, heart failure or stroke. The overall number of participants included in the statistical analyses totalled 530.

3.4.2. Procedures

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

3.4.3. Statistical analysis

All data were analyzed using STATA (StataCorp LLC Texas USA) data analysis and statistical software version 13.0. The mean of the five readings obtained from the

conventional BP measurements was determined to get average systolic and diastolic BP values. Ambulatory BP data were also expressed as averages. Data were expressed as means ± standard deviation (SD) for continuous variables. Categorical variables are expressed as absolute or relative frequencies or as percentages. Test for normality of continuous variables was assessed using Shapiro-Wilk's statistic or χ^2 test for categorical variables. Simple regression was used to determine the relationship between ambulatory blood pressure parameters and age. A P-value <0.05 was considered as statistically significant.

3.5. Results

3.5.1. General characteristics of the participants

The mean age of the total population was 44±18 years. Women had a higher BMI than men (p<0.001). Smoking and alcohol use was different between the genders with more males smoking and drinking alcohol compared to females. The healthy participants were younger (average age 38 years) and had similar BMI (28 kg/m^2) compared to the whole group (29.5kg/m^2). Of the 1 219 participants, 11% of men and 4% of women were smokers and 13% of men and 9% women reported alcohol consumption. Females had higher waist and hip circumference when compared with males in the general population or amongst healthy participants. These data are presented in table 3.1.

Table 3.1. Demographics and clinical characteristics of the total study population and healthy participants

	Total population				Healthy participants			
	All Subjects	Males	Females	P	All Subjects	Males	Females	P
Number	1219	432 (35%)	787 (65%)		530	179 (34%)	351 (66%)	
Age (years)	44 ± 18	45 ± 19	43 ± 17	0.061	38 ± 16	37.9 ± 18	38.5 ± 16	0.175
Weight (kg)	75.4±19.9	73.0±18.3	77.8±18.5	<0.0001	71.2±16.9	71.0±17.1	71.6±17.7	<0.1081
Height (m)	160.8±8.7	168.7±6	156.8±7.1	<0.0001	161.7±8.6	168.4±7.1	157.7±6.6	<0.0001
Waist circ (cm)	89.9±16.3	87.2±14.6	91.2±14.9	<0.0001	6.3±15.1	82.9±14.1	88.4±15.2	<0.0001
Hip circ (cm)	106.2±15.1	99.4±12.3	109.8±14.9	<0.0001	103.5±14.1	98.1±10.9	106.7±14.3	<0.0001
BMI (kg/m^2)	29.5±8.0	25.1 ± 5.7	31.8 ± 8.1	<0.0001	28.0±7.5	24.5 ± 5.9	30.1 ± 7.6	<0.0001
WHR	0.84 ± 0.1	0.86±0.01	0.82±0.1	<0.0001	0.82±0.1	0.85±0.09	0.79±0.1	<0.0001
Smokers (%)	15	11	4		16	13	3	
Alcohol use (%)	22	13	9		23	16	7	
Diabetics (%)	14	14	13					
Hypertensives (%)	37	46	42					

BMI, body mass index; WHR, waist-to-hip ratio; Hip circ, hip circumference; Waist circ, waist circumference. Data is presented as mean ± SD or number (%). A P value ≤ 0.05 depicts significant gender difference.

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

In all healthy participants who had complete ambulatory BP monitoring, 24-hour BP averaged 112 mm Hg systolic and 69 mm Hg diastolic. Conventional office BP averaged 117 mm Hg systolic and 78 mm Hg diastolic. Daytime BP averaged 118mm Hg systolic and 74mm Hg diastolic. Night-time BP averaged 105mm Hg systolic and 61mmHg diastolic. Systolic BP was higher in males than in females (Conventional p=0.0333, 24-hour p=0.0004, daytime p= 0.0012, night-time p=0.0029), diastolic BP was not significantly different between the genders. Nocturnal dipping was normal (≥10%) and there were no significant differences in nocturnal dipping between males and females. The proportion of the study population who were non-dippers was 48.7% and there were more female non-dippers than male non-dippers (51.4% vs 44.3%) (Table 3.2).

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

	All participants	Males	Females	P
Conventional blood pressure (mmHg)				
Systolic BP	117.0±12.0	119.0±10.1	115.8±12.6	0.0333
Diastolic BP	77.8±7.9	79.6±6.8	76.9±8.2	0.0881
24-Hour blood pressure (mmHg)				
Systolic BP	112.0±10.7	115.7±9.9	111.0±10.8	0.0004
Diastolic BP	69.1±7.5	70.2±7.4	68.5±7.0	0.2978
Daytime blood pressure (mmHg)				
Systolic BP	117.6±10.9	120±10.1	115.6±10.8	0.0012
Diastolic BP	74.3 ±7.9	75.0±8.1	73.6±7.8	0.3323
Night-time blood pressure (mmHg)				
Systolic BP	105.3±12.6	107.0±12.9	104.0±12.3	0.0029
Diastolic BP	61.2±9.2	62.0±9.5	60.8±8.7	0.6007
Nocturnal dipping (%)				
Systolic BP	10.0±0.83	11.0±0.80	10.0±0.85	0.7149
Diastolic	17.0±1.04	18.0±1.08	17.0±1.00	0.6475
Non-dippers (%)	48.7	44.3	51.4	

BP, Blood Pressure. Data is presented as mean ± SD. A P value ≤ 0.05 depicts significant gender difference.

3.5.3. Distribution of 24-hour Blood Pressure

3.5.3.1. Distribution of 24-hour systolic BP

In all healthy participants who had complete ambulatory BP monitoring, 24–hour systolic BP were normally distributed (Figure 3.1). The most frequent (20%) 24-hour systolic blood pressure in the total population was 112mmHg.

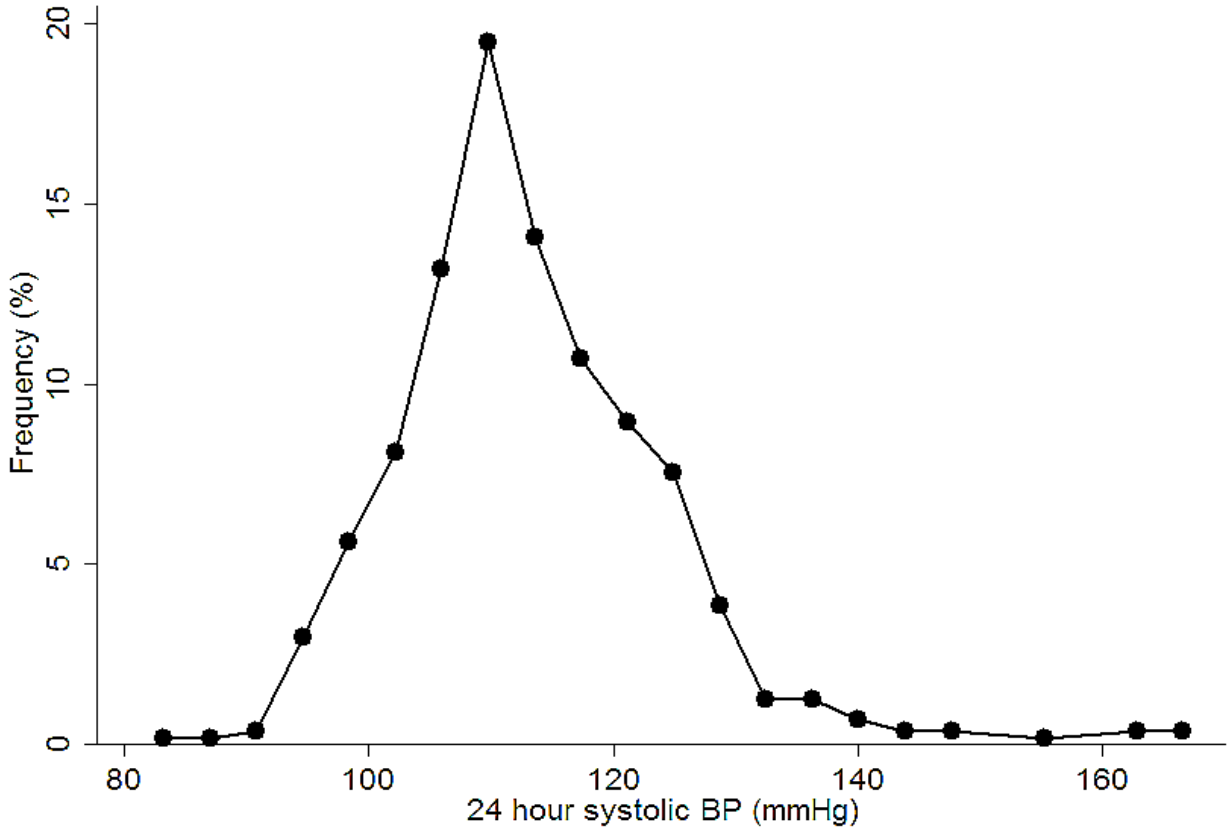


Figure 3.1: Distribution of 24-hour systolic blood pressures in 530 healthy participants with complete ambulatory blood pressure monitoring.

3.5.3.2. Distribution of 24-hour diastolic BP

In all healthy participants who had complete ambulatory BP monitoring, 24–hour diastolic BP were normally distributed (Figure 3.2). The most frequent (14%) 24-hour diastolic blood pressure in the total population was 70mmHg.

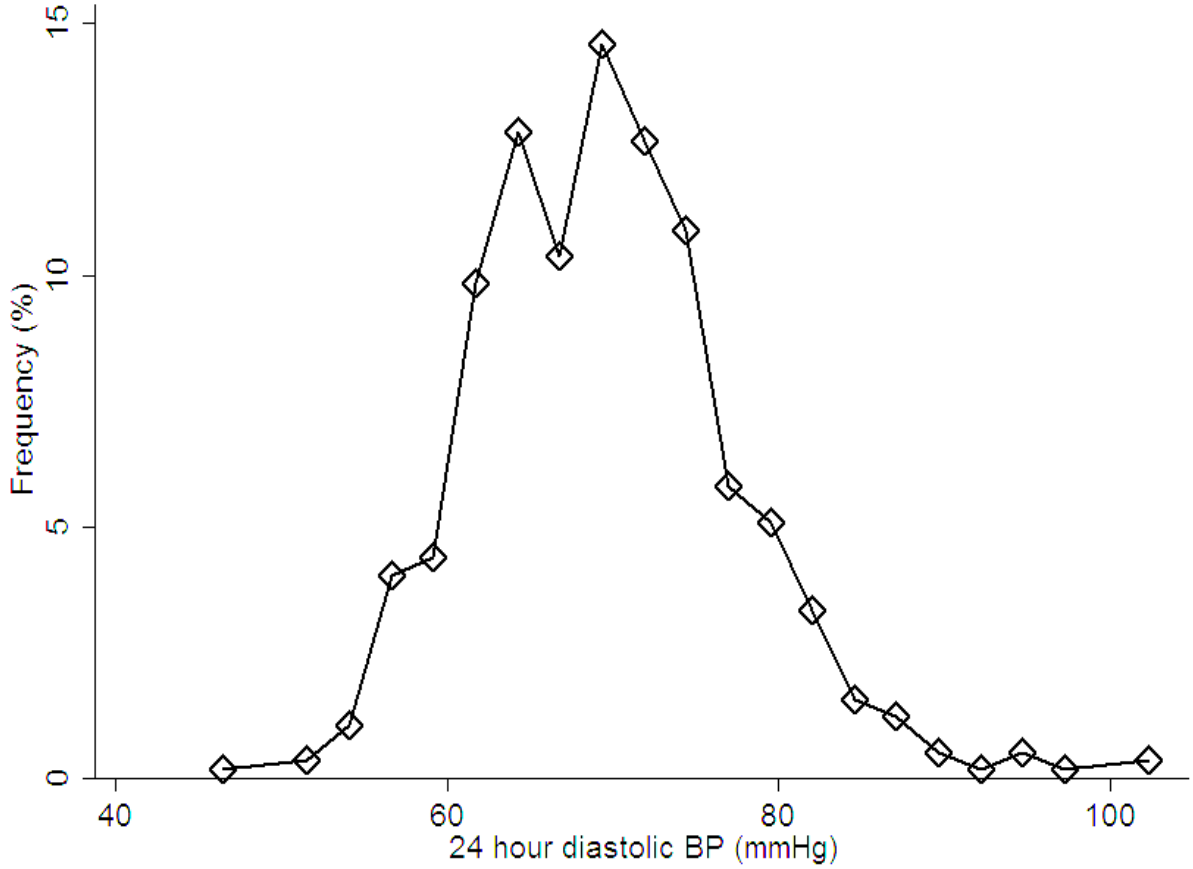


Figure 3.2: Distribution of 24-hour diastolic blood pressures in 530 healthy participants with complete ambulatory blood pressure monitoring.

3.5.4. Proposal for diagnostic thresholds

To determine diagnostic thresholds for hypertension in both males and females, we rounded the 95th prediction band for the approximate mean age of healthy participants (38 years) downwards to the nearest integer or value ending in zero for ambulatory BP.

3.5.4.1. Diagnostic threshold for 24-hour systolic BP

The procedure described above yielded a threshold of 135 mmHg for 24-hour systolic blood pressure (Figure 3.3).

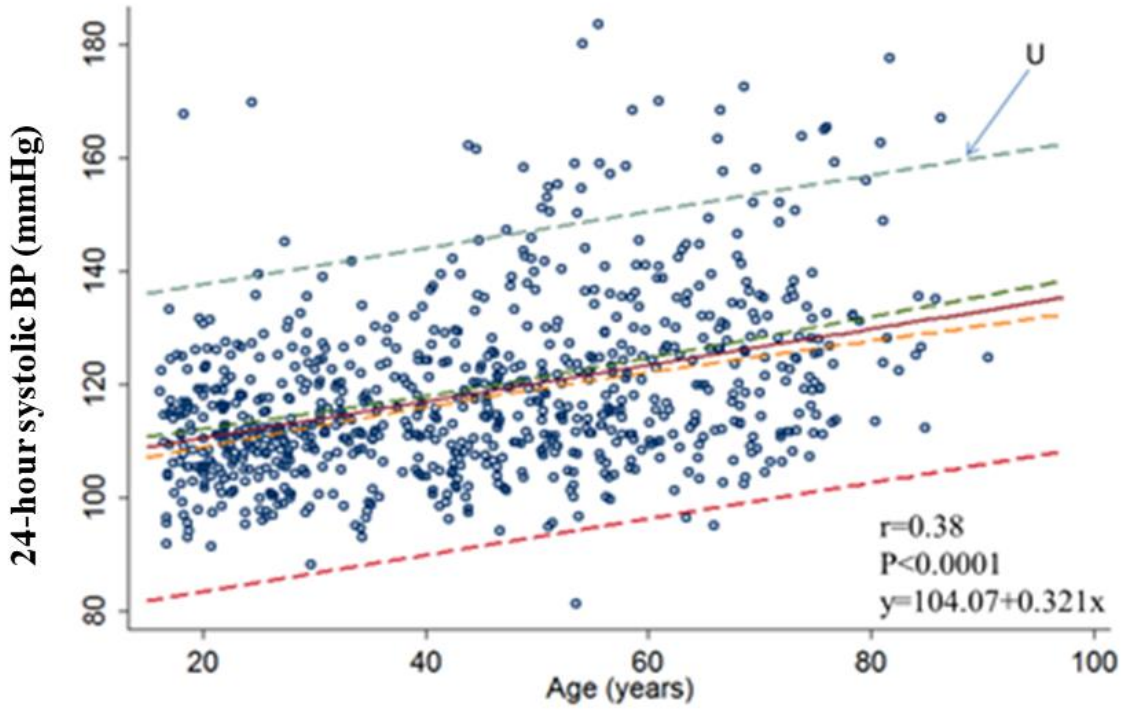


Figure 3.3: Twenty four-hour systolic BP vs age in 530 healthy participants with complete ambulatory blood pressure measurements. This figure shows the regression line, 95% confidence interval and the 95% prediction bands for mean and individual values of the 24-hour systolic blood pressure. The portion labelled **U** represents the movement from normal to elevated values.

3.5.4.2. Diagnostic threshold for 24-hour diastolic BP

The procedure described in section 3.3.4 yielded a threshold of 85 mmHg for 24-hour diastolic blood pressure (Figure 3.4).

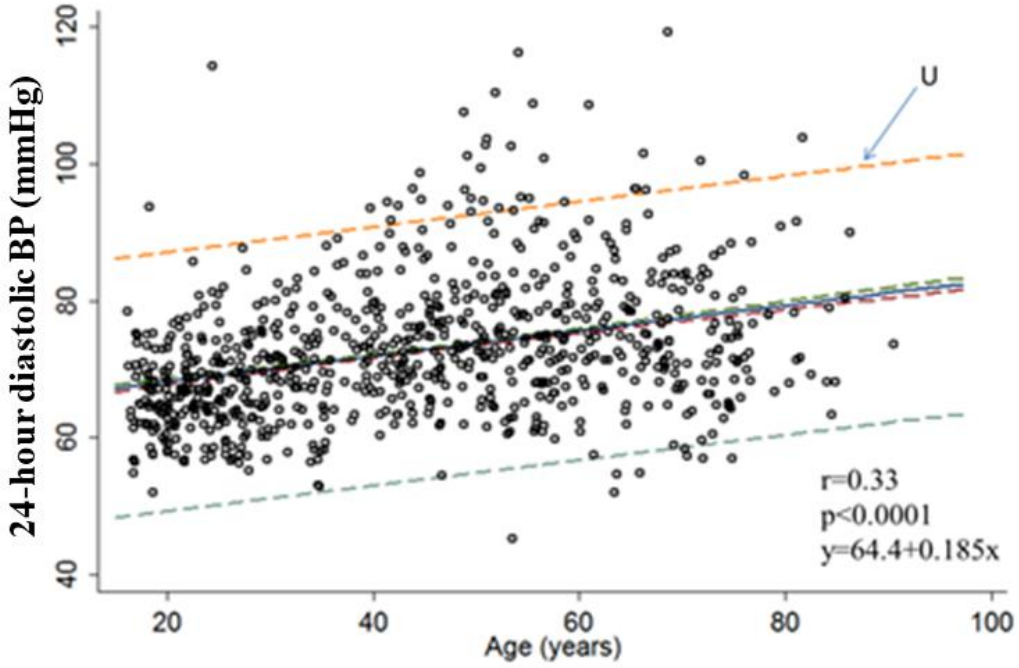


Figure 3.4: Twenty four-hour diastolic BP vs age in 530 healthy participants with complete ambulatory blood pressure measurements. This figure shows the regression line, 95% confidence interval and the 95% prediction bands for mean and individual values of the 24-hour diastolic blood pressure. The portion labelled **U** represents the movement from normal to elevated values.

3.5.4.3. Diagnostic threshold for daytime systolic BP

The procedure described in section 3.3.4 yielded a threshold of 140 mmHg for daytime systolic blood pressure (Figure 3.5).

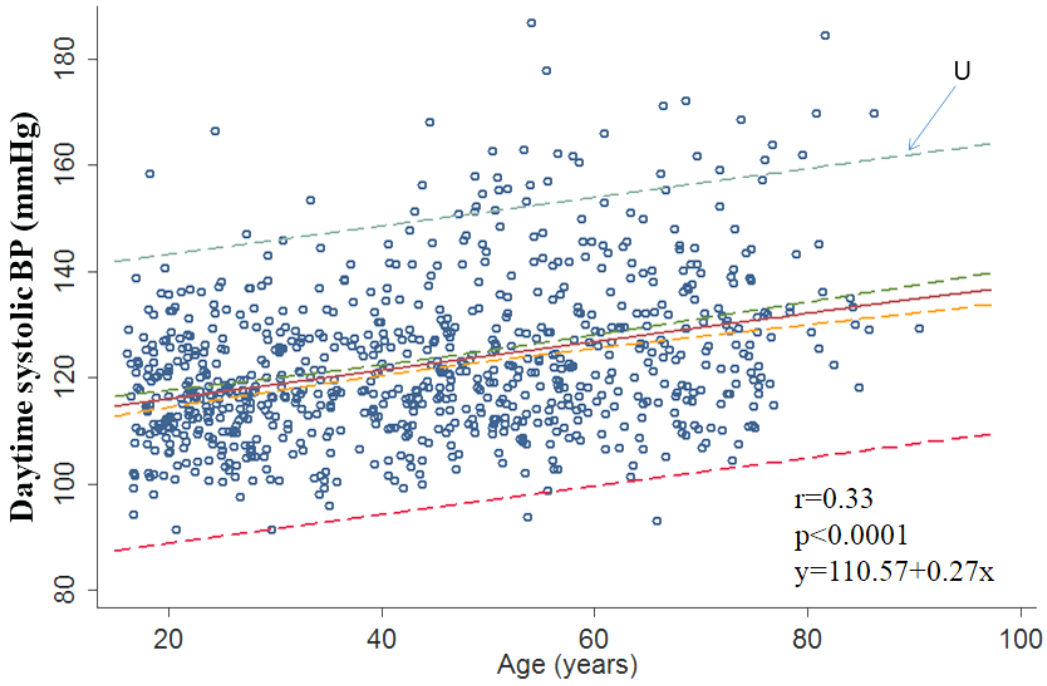


Figure 3.5: Daytime systolic blood pressure vs age in 530 healthy participants with complete ambulatory blood pressure measurements. This figure shows the regression line, the 95% confidence interval and the 95% prediction bands for mean and individual values of the Daytime systolic blood pressure. The portion labelled **U** represents the movement from normal to elevated values.

3.5.4.4. Diagnostic threshold for daytime diastolic BP

The procedure described in section 3.3.4 yielded a threshold of 90 mmHg for daytime diastolic blood pressure. (Figure 3.6).

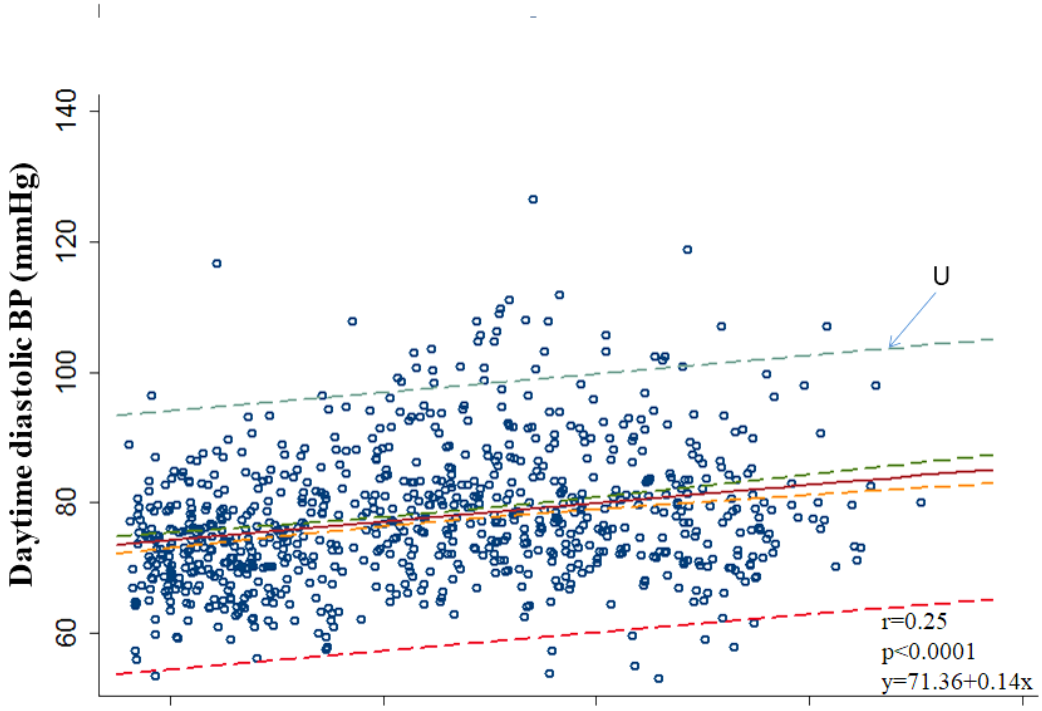


Figure 3.6: Daytime diastolic blood pressure vs age 530 in healthy participants with complete ambulatory blood pressure measurements. This figure shows the regression line, the 95% confidence interval and the 95% prediction bands for mean and individual values of the Daytime diastolic blood pressure. The portion labelled **U** represents the movement from normal to elevated values.

3.5.4.5. Diagnostic threshold for night-time systolic BP

The procedure described in section 3.3.4 yielded a threshold of 130 mmHg for night-time systolic blood pressure. (Figure 3.7).

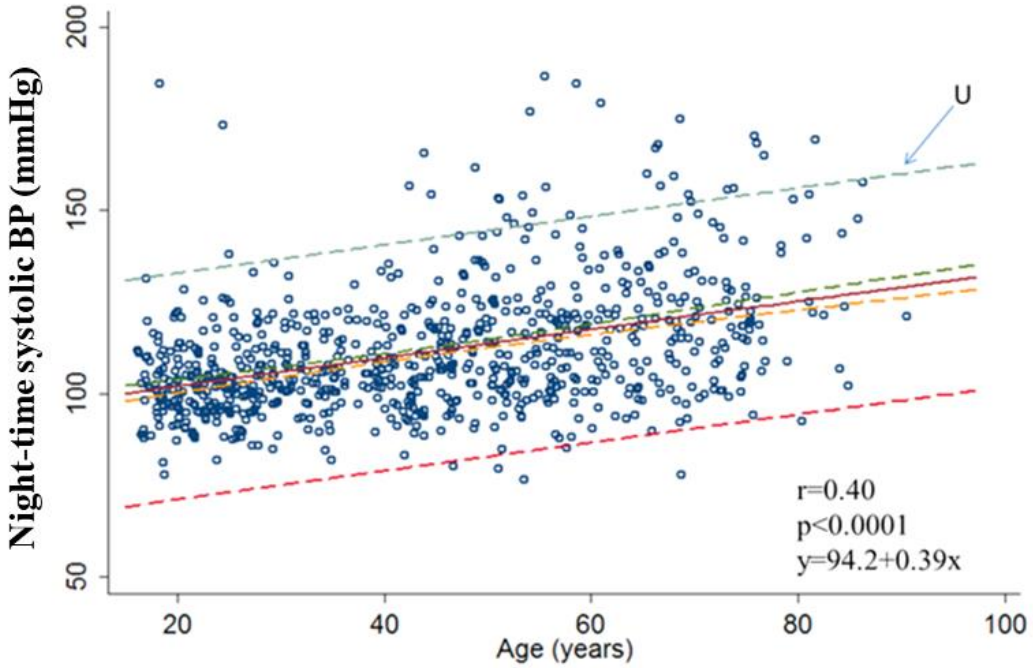


Figure 3.7: Night-time systolic blood pressure vs age 530 healthy participants with complete ambulatory blood pressure measurements. This figure shows the regression line, the 95% confidence interval and the 95% prediction bands for mean and individual values of the night-time systolic blood pressures. The portion labelled **U** represents the movement from normal to elevated values.

3.5.4.6. Diagnostic threshold for night-time diastolic BP

The procedure described in section 3.3.4 yielded a threshold of 80 mmHg for night-time diastolic blood pressure. (Figure 3.8).

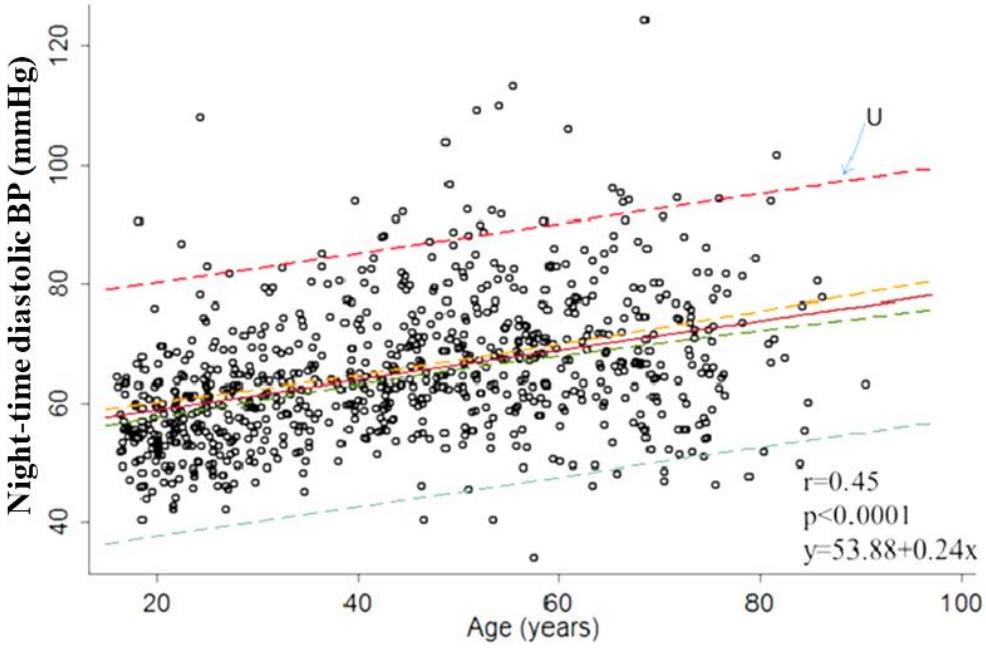


Figure 3.8: Night-time diastolic blood pressure vs age in 530 healthy participants with complete ambulatory blood pressure measurements. This figure shows the regression line, the 95% confidence interval and the 95% prediction bands for mean and individual values of the night-time systolic blood pressures. The portion labelled **U** represents the movement from normal to elevated values.

3.5.4.7. Diagnostic threshold for ambulatory blood pressure by age group.

We determined the thresholds for ambulatory blood pressure for different age groups per decade increase in age by using the 95% prediction band for ABP values at the mean age for each age group. This showed an increase in ABPM threshold values and confidence intervals with increasing age, with the lowest values in the 18-28 age group and the highest in the 73-85 age group (Table 3.3). The average increase in ABP values was 1.5 mmHg with each decade increase in age. The sharpest increase in ABPM threshold was seen in night-time systolic BP which had an average increase in ABPM threshold of 3 mmHg per decade increase in age.

Table 3.3 Ambulatory blood pressure thresholds in different age groups.

Age (years)	18-28	29-39	40-50	51-61	62-72	73-85
N	169	86	80	67	35	20
ABPM reference values (mmHg) with 95% CI						
24 Hr SBP	133 (131.32-134.67)	134 (131.81-136.18)	135 (131.85-138.15)	136 (131.66-140.32)	138 (132.38-143.62)	140 (131.90-148.09)
24 Hr DBP	82 (80.86-83.14)	83 (81.31-84.68)	84 (81.84-86.15	85 (82.11-87.89)	87 (83.16-90.84)	89 (84.47-93.53)
Daytime SBP	135 (135.34-138.66)	138 (135.32-140.47)	139 (135.84-142.16)	140 (135.79-144.21)	141 (135.57-146.43)	142 (133.66-150.33)
Daytime DBP	89 (87.80-90.19)	90 (87.97-92.02)	91 (88.72-93.28)	91(88.11-93.89)	92 (88.11-95.89)	92 (87.48-96.52)
Night-time SBP	124 (122.06-125.93)	128 (125.79-130.20)	132 (128.49-135.50)	137 (132.02-141.98)	138 (131.30-144.69)	139 (129.77-148.23)
Night-time DBP	77 (75.60-78.39)	79 (77.13-80.87)	79 (76.64-81.36)	81 (77.89-84.11)	82 (77.49-86.51)	83 (77.28-88.72)

ABPM= Ambulatory blood pressure monitoring; SBP= Systolic blood pressure; DBP= Diastolic blood pressure; CI= Confidence interval.

3.6. Discussion

In this study, thresholds for ambulatory blood pressure in Africans living in Africa were determined. The results suggest that at 38 years old, ambulatory BP may be abnormally increased if the following thresholds are exceeded: 135 mmHg 24-hour, 140 mmHg daytime and 130 mmHg night-time for systolic BP and 85 mmHg 24-hour, 90 mmHg daytime and 80 mmHg night-time for diastolic BP. We also showed that these thresholds increase with age by an average of 1.5 mmHg with each decade increase in age. To our knowledge, this is the first time that reference values for ambulatory blood pressure have been determined in people of Africans ancestry living in Africa.

The average ambulatory BP values shown in this study are similar to other studies conducted in different races and in different geographical locations (O'Brien *et al.,* 1991; Kikuya *et al.,* 2007). These findings contradict previous studies which have shown that subjects of African ancestry have higher BP values as compared to those of European ancestry (Mancia *et al.,* 1983b; Pickering *et al.,* 2006). In a study by Wang and colleagues, the investigators showed that ambulatory BP values were higher in blacks compared to whites and these ethnic differences were more pronounced at night (Wang *et al.,* 2006a). The night time differences were because blacks have reduced nocturnal BP dipping compared to whites. Similarly, a meta-analysis involving 2852 participants showed that blacks had significantly higher ambulatory BP values and reduced nocturnal dipping compared to whites (Profant *et al.,* 1999).

Consistent with other studies, our data show that office BP is higher than either daytime ambulatory BP or 24-hour BP (Mancia *et al.,* 1995; Staessen *et al.,* 1993a; Omvik *et al.,* 2003; Staessen *et al.,* 1996). The reason for this difference has not been firmly established but is assumed to be due to a sympathetic response elicited in an office environment (Staessen *et al.,* 1996). The reason for the difference in 24-hour BP and office BP is that office BP does not account for nocturnal decreases in BP. Our results suggest that night-time systolic BP might be on average 4 mmHg (24-hour), 5 mmHg (daytime) and 3 mmHg (night-time) higher in men than in women, this is similar to the results obtained by Thayer *et al* (2016).

The uniqueness of our findings may be due to our population sample. Our study sample consists of people of African descent living in Africa while most studies were conducted in African American populations (Urbina *et al.,* 2008; Chobanian *et al.,* 2003). The socio-

economic, geographic and cultural differences between black South Africans and African Americans may account for the observed BP differences between these groups. Our findings highlight an important principle; studies conducted in African Americans cannot necessarily be representative of African populations living in Africa.

It should be highlighted that our recommended thresholds are higher than those of the European Society of Hypertension (ESH) which recommend thresholds of 130/80 mmHg for 24-hour BP, 135/85 mmHg for daytime BP and 120/70 mmHg for night-time BP (O'Brien *et al.,* 2013). This might be because of different population sizes and because data derived from an ABPM study from a cohort like ours representing an African population living in Africa was not included in the determination of the ESH recommended thresholds. Pending outcome-based studies to determine the actual target organ, morbidity and mortality effects of the thresholds determined in this study, our recommendations should be considered preliminary. The thresholds determined herein can thereafter be very important in helping to properly diagnose hypertension, especially identification of white coat hypertension and exposure of masked hypertension.

Though our sample size is the largest conducted in this population, it is still small in epidemiological terms. Notwithstanding, our findings might be readily extrapolated, because our study was based on a general population. Indeed, our population demographics are representative of South Africans of African ancestry because they are comparable to those previously reported (Shiburi *et al.,* 2006). Moreover, we applied a validated protocol and maintained strict quality control (O'Brien *et al.,* 1991). For ambulatory BP monitoring, we adhered to the recommendations of the European Society of Hypertension (O'Brien *et al.,* 2003).

3.7. Conclusions

In conclusion, pending authentication in outcome-based studies, 135/85 mmHg for 24 hour systolic/diastolic BP, 140/90 mmHg for daytime BP, 130/80 mmHg for night-time BP might be considered as thresholds to identify abnormal ambulatory BP in young adults of African descent. Outcome based studies should also be conducted in this population so that these ambulatory BP values can be used to accurately predict morbidity and mortality.

CHAPTER 4

Nocturnal Blood Pressure Dipping and Urinary Sodium Excretion in People of African Ancestry: The Role of Aldosterone.

4.1 Introduction

Over 24-hours, nocturnal BP values are normally 15% lower than day time BP values (Pickering *et al.,* 2001). A non-dipping blood pressure (BP) profile is defined as failure of the night-time BP values to drop at least 10% of the daytime values. This is equivalent to a night-to-day BP ratio greater than 0.9 (Head *et al.,* 2010). This attenuated decline in nocturnal BP is an independent predictor of stroke, left ventricular hypertrophy, congestive heart failure, carotid intima-media thickening and microalbuminuria (Birkenhager *et al.,* 2007; O'Brien *et al.,* 1988; Verdecchia *et al.,* 1990). Mechanisms responsible for the development of the non-dipping BP profile remain unclear but there are suggestions that autonomic dysfunction, abnormal renal sodium handling, poor sleep quality, nocturnal decrease in cardiac output and hormonal mechanisms are responsible (Redon *et al.,* 2008; Zacharieva *et al.,* 2004).

Some studies have reported an ethnic variation in the prevalence of non-dipping with black populations having a higher prevalence of nocturnal BP non-dipping (Murphy *et al.,* 1991). Since abnormal renal sodium handling has been suggested as one of the mechanisms responsible for non-dipping, it is possible that increased salt retention due to salt-sensitivity in Africans may be responsible for the attenuated decline in nocturnal BP (Sowers *et al.,* 1988). Even though the relationship between urinary sodium excretion and BP has been established (Mente *et al.,* 2014), more evidence is required on the impact of urinary excretion rate on nocturnal BP dipping in salt sensitive African populations. This is further compounded by the fact that most of the evidence that shows a higher prevalence of non-dipping in blacks was not established in black people living in Africa (Murphy *et al.,* 1991; Muntner *et al.,* 2017; Sherwood *et al.,* 2011). Studies conducted in Africans have either focused on hypertensive (Mvunzi *et al.,* 2017) or on HIV positive patients (Borkum *et al.,* 2017). Hence to date, the prevalence of nocturnal BP non-dipping in people of African descent living in Africa has not been adequately described.

4.2. Aim

The aim of this study was to determine the prevalence of nocturnal BP non-dipping and to investigate the role of urinary sodium excretion rate and possible hormonal input in the attenuation of nocturnal BP dipping in people of African ancestry living in Africa.

4.3. Objectives

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

4.1.2.1. To determine the prevalence of nocturnal BP non-dipping in an African population using ambulatory blood pressure monitoring.

4.1.2.2. To investigate the role of 24-hour urinary sodium excretion in the failure of normal nocturnal decline in BP.

4.1.2.3. To investigate the role of plasma Aldosterone in the failure of normal nocturnal decline in BP.

4.4. Methods

4.4.1. Study Population

Details about the study population are given in chapter 2 of this thesis. Of the 1,219 participants that enrolled in the study, only 796 had complete ambulatory BP monitoring reports. Participants were classified as dippers if their nocturnal drop in systolic BP was >10% and as non-dippers if they had a nocturnal drop in systolic BP of <10%.

4.4.2. Procedures

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

4.4.3. Statistical analysis

All data were analyzed using STATA (StataCorp LLC Texas USA) data analysis and statistical software version 13.0. The five readings obtained from the conventional BP measurements were averaged to obtain a single systolic and diastolic BP reading. Ambulatory BP data were expressed as 24-hour, daytime and night-time average systolic and diastolic pressures. Comparisons between dipper and non-dipper groups as well as gender groups were done using independent Student's t-test. Multivariate regression analysis was used to determine association between 24-hour urinary sodium excretion and blood pressure parameters. Multivariate regression analysis was also used to determine association between dipping and some general demographic parameters. Data were expressed as means ± standard deviation (SD) for continuous variables. Categorical variables are expressed as absolute or relative frequencies or as percentages. A P-value <0.05 was considered as statistically significant.

4.5. Results

4.5.1. General characteristics of the participants

The study population consisted of 796 participants. The mean age of the study population was 44±18 years with 36% being male and 64% being female. Of the total population, 383 (48%) were classified as being dippers and 413 (52%) as non-dippers based on their 24-hour BP pattern. Non-dippers were older than dippers (48±18 vs 40±17 years). Non-dippers were also more likely to be hypertensives when compared with dippers (66% vs 34%) but had a lower recorded alcohol usage (7% vs 16%). Demographic and clinical data of the total population are represented in Table 4.1.

Table 4.1. General and clinical characteristics of the participants

	All subjects	Dippers	Non-dippers	P
Number	796	383	413	
Age (years)	44.20 ± 18.13	40.41 ± 17.55	47.69±18	<0.0001
Weight (kg)	76.3±16.9	75.0±17.1	77.6±17.7	0.1081
Height (m)	164.50±8.60	165.4±7.1	163.6±6.6	0.1162
BMI ($kg.m^{-2}$)	28.91±7.62	27.88 ± 7.11	29.84±7.96	0.0003
Smokers (%)	15	18	12	
Alcohol use (%)	21	22	19	
Diabetics (%)	8.50	33.82	66.18	
Hypertensives (%)	24.20	33.67	66.33	
Systolic BP (mmHg)	128.30±20.99	124.89±17.63	131.45±23.27	<0.0001
Diastolic BP (mmHg)	83.70±11.80	82.48±10.98	84.84±12.41	0.0047

BMI, body mass index, pulse wave velocity. Data is presented as mean ± SD or percentage.

4.5.2. Hemodynamic parameters

Compared with dippers, non-dippers had higher 24 hour systolic (121.72±10.80 vs 114.94±11.70, p<0.0001) and diastolic BP (74.90±11.30 vs 70.46±8.50, p<0.0001), night time systolic (119.30±17.70 vs 103.48±10.97, p<0.0001) and diastolic BP (70.22±12.00 vs 59.84±8.67, p<0.0001) as well as higher conventional systolic BP (131.50±23.00 vs 124.90±18.00, p<0.0001). Percentage dip in systolic (3.00±0.16 vs 15.70±3.05, p<0.0001) and diastolic (10.97±1.56 vs 23.17±2.07, p<0.0001) BP were also significantly lower in non-dippers when compared with dippers. There were no other significant differences observed in the remaining demographic and clinical parameters (Table 4.2).

Table 4.2 Hemodynamic parameters of the participants

	All participants	Dippers	Non-Dippers	P
N (%)	796	383 (48)	413 (52)	
Conventional SBP (mmHg)	128.30±21.00	124.90±18.00	131.50±23.00	<0.0001*
Conventional DBP (mmHg)	83.70±11.80	82.50±11.00	84.80±12.00	0.0823
24Hr SBP (mmHg)	118.30±10.70	114.94±11.70	121.72±10.80	<0.0001*
24Hr DBP (mmHg)	72.63±10.20	70.46±8.50	74.90±11.30	<0.0001*
Daytime SBP (mmHg)	122.45±14.66	121.84±12.30	123.11±16.94	0.2243
Daytime DBP (mmHg)	77.62±10.40	77.18±9.13	78.12±11.62	0.2032
Night-time SBP (mmHg)	111.33±17.19	103.48±10.97	119.30±17.70	<0.0001*
Night-time DBP (mmHg)	64.92±9.20	59.84±8.67	70.22±.12.00	<0.0001*
% SBP Dip	9.08±2.08	15.70±3.05	3.00±0.16	<0.0001*
% DBP Dip	16.34±4.10	23.17±2.07	10.97±1.56	<0.0001*

SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure. Dip=dipping. Data is presented as mean ± SD or absolute values and %. A P value ≤ 0.05 depicts significant difference between dippers and non-dippers.

4.5.3. Electrolyte and hormonal profiles of participants

Dippers had higher levels of 24-hour urinary sodium excretion (UNa^+) (111.0 ±80.8 vs 100±76.1 mmol/day, p=0.0402) when compared to non-dippers. Aldosterone levels were higher in non-dippers when compared with dippers (207.7±173.6 vs 188.4±149.6 ng/dL p=0.005) however there was no significant difference in renin levels between the two groups. Aldosterone-to-renin ratio was significantly higher in non-dippers compared to the dippers (31.9±60.1 vs 22.8±39.4, p=0.0124) (Table 4.3).

Table 4.3 Electrolyte and hormonal profiles of dippers and non-dippers.

	All participants	Dippers	Non-Dippers	P
N (%)	796	383 (48)	413 (52)	
Urine K^+ (mmol/day)	28.40±21.80	30.50±22.10	26.60±21.40	0.0124*
Urine Na^+ (mmol/day)	105.60±78.40	111.00±80.8	100.00±76.10	0.0402*
Na^+/K^+	4.37±3.19	4.10±2.00	4.60±3.90	0.0203*
Aldosterone (pg/ml)	198.50±162.70	188.40±149.60	207.70±173.60	0.0105*
Renin (pg/ml)	36.50±72.50	37.50±76.50	35.70±68.70	0.0700
Ald/Ren	27.4±51.20	22.80±39.40	31.90±60.10	0.0124*

K^+ = Potassium, Na^+ = Sodium, Ald/Ren = Aldosterone to Renin ratio. Data is presented as mean ± SD or absolute values and %. A P value ≤ 0.05 depicts significant difference between dippers and non-dippers.

4.5.4. Prevalence of the non-dipping BP pattern in the general population

The overall prevalence of the non-dipping BP pattern in the study participants was 52%. The prevalence was slightly higher in females when compared to males (54% vs 49%) but this was not statistically significant (Figure 4.1).

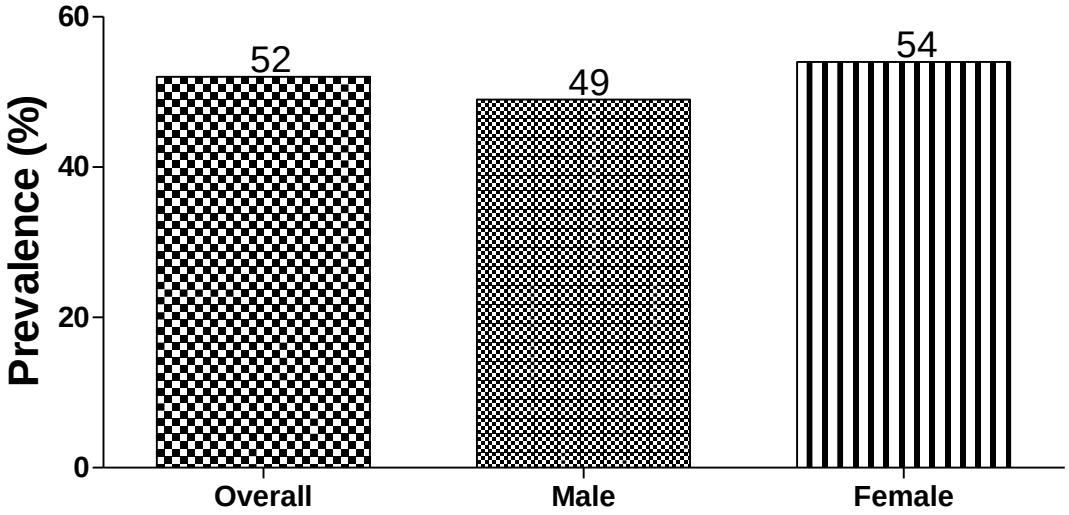


Figure 4.1. Prevalence of the non-dipping blood pressure pattern in the general population.

4.5.5. Prevalence of the non-dipping BP pattern by age group and gender.

Participants were further stratified into different age ranges by gender. The lowest age range was between 18 and 30 years and the highest was from 60 years and above. The prevalence of NDP increased steadily with age in the female participants however, in the male participants, it increased from the 18-30 years age range to the 30-40 age range and flattens out only to increase again after the 50-60 years age range. The prevalence of NDP was consistently higher in females in all age ranges ($p<0.001$) (Figure 4.2).

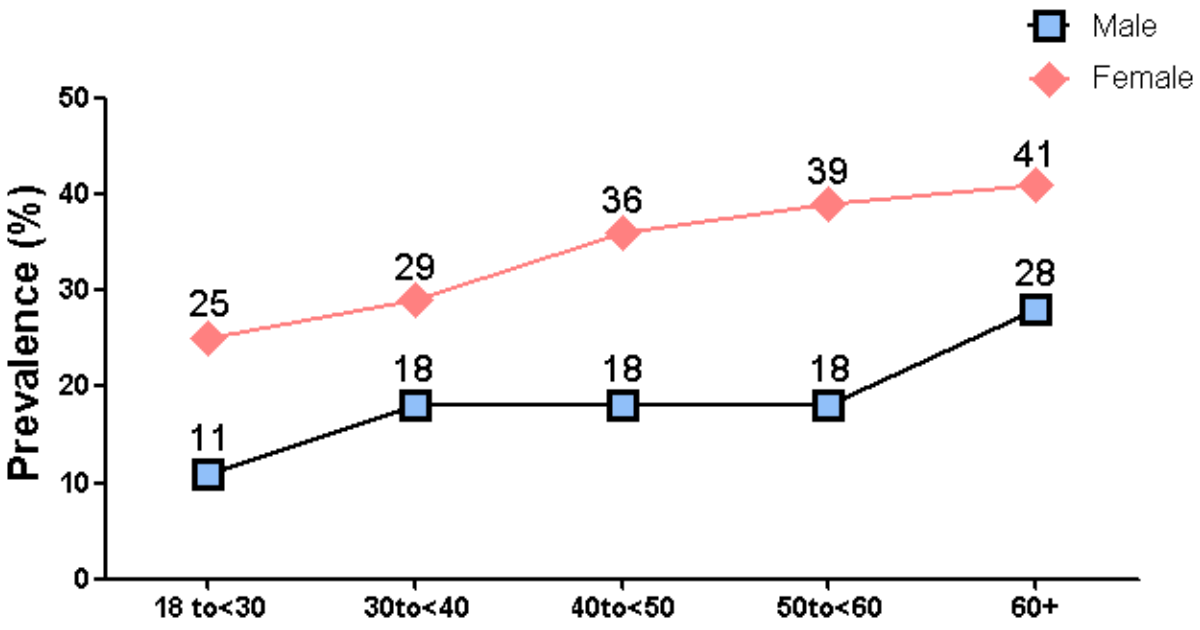


Figure 4.2. Prevalence of the non-dipping blood pressure pattern by age group and gender

4.5.6. Relationship between 24-hour urinary sodium excretion and blood pressure parameters in dippers and non-dippers

Following multiple regression analyses controlling for age, gender, and hypertension, we observed a significant relationship between 24-hour urinary sodium excretion and conventional, 24 hour and day time systolic and diastolic BP, night time systolic BP but not night time diastolic BP in non-dippers. Urinary sodium excretion was also significantly associated with both systolic and diastolic dipping in non-dippers. There was however no correlation between 24-hour urinary sodium excretion and BP parameters in dippers (Table 4.4).

Table 4.4 Results of multiple linear regression analysis for the relationship between 24-hour sodium excretion and blood pressure parameters in dippers and non-dippers.

	Dippers			Non-dippers		
	r^2	CI	P	r^2	CI	P
24 Hour SBP (mmHg)	0.03	-0.10 to 0.16	0.6660	0.18	0.07 to 0.29	0.0022*
24 Hour DBP (mmHg)	-0.02	-0.15 to 0.11	0.7540	-0.17	-0.28 to -0.05	0.0044*
Night time SBP (mmHg)	0.04	-0.09 to 0.17	0.5420	0.13	0.01 to 0.24	0.0385*
Night time DBP (mmHg)	-0.11	-0.23 to 0.02	0.0930	-0.10	-0.21 to 0.02	0.1022
Daytime SBP (mmHg)	0.03	-0.09 to 0.16	0.6112	0.18	0.07 to 0.29	0.0023*
Daytime DBP (mmHg)	-0.001	-0.13 to 0.13	0 .9942	-0.18	-0.29 to 0.06	0.0027*
Conventional SBP (mmHg)	0.02	-0.11 to 0.15	0.7932	0.12	0.002 to 0.23	0.0466*
Conventional DBP (mmHg)	-0.05	-0.18 to 0.08	0.4320	-0.13	-0.24 to -0.01	0.0342*
Systolic BP dipping (mmHg)	0.04	-0.10 to 0.16	0.5083	-0.13	-0.25 to 0.01	0.0406*
Diastolic BP dipping (mmHg)	0.08	-0.10 to 0.25	0.4106	-0.14	-0.24 to -0.04	0.0057*

SBP=Systolic blood pressure, DBP=Diastolic blood pressure, CI= Confidence interval. Age, gender, BMI, hypertension and diabetes were corrected for in each multivariate analysis. *Statistical significance.

4.5.7. Relationship between dipping and general and clinical parameters

Following multivariate regression analysis, neither BMI nor gender were associated with dipping, only age and hypertension status were sssociated with dipping.

Table 4.5 Results of multiple linear regression analysis for the relationship between dipping and some general and clinical parameters.

	β	CI	P
BMI	0.003	-0.002 to 0.008	0.270
Gender	0.040	-0.037 to 0.118	0.310
Age	0.004	0.002 to 0.006	0.001*
Hypertension	0.096	0.006 to 0.185	0.036*

CI = Confidence interval, BMI = Body Mass Index. * Statistical significance

4.6. Discussion

The main finding of the present cross-sectional study is a 52% prevalence of nocturnal non-dipping in people of African ancestry living in Africa which may be due to sodium retention, mediated by abnormally high aldosterone concentration. The present study also showed an association between urinary sodium excretion and 24-hour BP, daytime BP, night-time BP and blood pressure dipping status in non-dippers but not in dippers. To our knowledge this is the first study to determine the prevalence of nocturnal non-dipping in the target population and to also describe a mechanism that might be responsible for it.

The prevalence of nocturnal non-dipping BP as determined in the present study is relatively high compared to other population groups. A study in a Pakistan population showed a prevalence of 31.7% (Almas *et al.,* 2016). Even though African Americans have been shown to have a high prevalence of nocturnal BP non-dipping when compared to Caucasian Americans (46.2% vs 21.9% $p<0.01$) (Sherwood et al 2011), it is still considerably lower than that of our study population. We also observed slight gender differences in the prevalence. The prevalence was slightly higher, in females (54%) compared to males (49%). This is similar to the findings by Muntner *et al.,* (2017), which showed a slightly higher prevalence of non-dipping in black females compared to black males (40.9 vs 37.5 %) and similarly, a higher prevalence of non-dipping in white females when compared with white males (21.2% vs 19.8%). The gender differences though present but not significant, may be insufficient to play a role in dipping. This was confirmed by our results which showed no significant relationship between gender and dipping.

Our results also showed a higher prevalence of non-dipping amongst hypertensive individuals when compared with normotensives. This is consistent with a study by Friedman and colleagues which showed a non-dipping BP pattern prevalence of 25% amongst normotensives and 42.3% in treated hypertensives (Friedman *et al.,* 2009). After adjusting for age, gender and BMI, multivariate regression analysis also showed significant relationship between hypertension status and dipping. Our finding is consistent with that of Irvin and colleages where they showed a significant relationship between hypertension and non-dipping status in 540 patients with treated hypertension after adjusting for age, gender, obesity and other confounders (Irvin *et al*., 2018). Our results indicate that hypertension might be a determinant of blood pressure dipping status in this population.

Similar to other studies, daytime BP was similar in dippers and non-dippers in this population in spite of different night-time BP values. Ragot and colleagues compared autonomic nervous system activity in dipper and non-dipper hypertensives. Similar to our findings, their results showed no significant difference in the daytime BP values between dippers and non-dippers (Ragot *et al.,* 1999). Similarly, there was no significant difference in the daytime and nigh-time heart rates between dippers and non-dippers in both studies. The implication of our findings is that the physiological mechanisms that drive the high prevalence of non-dipping in this population target night-time BP exclusively.

In a multivariate regression analysis, neither BMI nor gender was associated with dipping; only age and urinary sodium excretion were associated with dipping. The relationship between age and dipping has been well established and is not unique to our study. Staessen *et al.,* (1997) showed an increase in the incidence of non-dipping with increasing age. However, urinary sodium handling may be different in this population when compared to other populations. An earlier study conducted by our research group (Bochud *et al.,* 2009) showed that black South Africans reabsorb more of the filtered sodium in the proximal tubule and less in the distal tubule when compared to Europeans. These results prove that there is an increase in salt retention in our population. This highlights the possibility that the increased incidence of non-dipping in this population is likely related to renal sodium handling. This further confirms the important role of urinary sodium excretion in the attenuation of nocturnal BP dipping in this population. Previous studies that have investigated the relationship between salt and dipping have not shown the difference in the impact of salt between dippers and non-dippers (Centonza *et al.,* 2000; Staessen *et al.,* 1993b; Staessen *et al.,* 1991).

The mechanisms responsible for the role of sodium excretion in the attenuation of dipping in non-dippers need further explanation. The most plausible explanation is the increase in nocturnal BP while daytime BP remains unchanged in non-dippers. This could be due to differences in night-time renal salt handling between dippers and non-dippers. Our results show that non-dippers had a significantly higher aldosterone concentration which was not related to an increase in renin since plasma renin concentrations remain similar between the two groups. As a result, the non-dippers had a greater aldosterone-to-renin ratio. We therefore hypothesised that the abnormally high aldosterone concentration in the non-dippers resulted in an increased sodium retention as indicated by the lower urinary sodium excretion in this group. The subsequent increase in plasma sodium results in increased pressure natriuresis, a process characterised by increased BP with the aim of

increasing sodium excretion. However, a few studies (Centonza *et al.,* 2000; Staessen *et al.,* 1993b; Staessen *et al.,* 1991) have shown that even though pressure natriuresis is active over 24-hours, it reaches its peak at night. Consequently, non-dippers have a higher night-time BP resulting in reduced percentage nocturnal BP dipping. A reduction in salt intake in this population may help reverse the non-dipping blood pressure status and its consequent target organ effects as 24-hour salt excretion has been shown to be a major factor in non-dipping in this population.

Our study is a cross sectional one and precludes conclusions concerning causal relationships.
Also, modifying the urine collection protocol from 24-hour urine collection to 'day time urine' and 'night-time urine' could have shed more light on the differences between daytime and night-time urinary sodium excretion rate.

4.7. Conclusions

In this cohort of black people living in Africa, there is a high prevalence of nocturnal BP non-dipping. This high prevalence may be linked to dietary sodium intake. The high prevalence could also be mediated by high plasma aldosterone. Future studies should be conducted in this population to determine the impact of non-dipping on cardiovascular target organ damage.

CHAPTER 5

Determinants of Arterial Stiffness in a Black African Population: Role of the Nocturnal Non-Dipping Blood Pressure Profile.

5.1 Introduction

Increased arterial stiffness as measured by carotid femoral pulse wave velocity (CFPWV) has been shown to be an independent predictor of cardiovascular morbidity and mortality (Laurent *et al.*, 2001; Meaume *et al.,* 2001). Measurement of arterial stiffness using CFPWV is the gold standard for assessment of central arterial stiffness because it is easy to use, replicable and non-invasive (Laurent *et al.*, 2006). Although, it has been shown that ageing and high blood pressure are the main determinants of arterial stiffness (Avolio *et al.,* 1983; Benetos *et al.,* 2002; Lebrun *et al.,* 2002), other factors like obesity, diabetes, dietary salt intake, high plasma aldosterone concentration have been shown to be associated with arterial stiffness (Lacolley et al., 2002; Blacher et al., 1997; Gates et al., 2004; Bagrov et al., 2004; Alecu *et al.,* 2006; Ketel *et al.,* 2010)

A non-dipping blood pressure profile is described as failure of the nocturnal blood pressure values to fall by at least 10% of the daytime values or a less than 10% dip in nocturnal BP relative to diurnal BP (Head *et al.,* 2010). The non-dipping BP profile is associated with target organ damage and has a strong predictive index of cardiovascular morbidity and mortality (Cuspidi *et al.,* 2004; Hansen *et al.,* 2006). Amongst other target organ effects of non-dipping, arterial stiffness is one of them.

It is however unknown if the nocturnal non-dipping blood pressure pattern can modify the relationship between earlier mentioned risk factors and arterial stiffness as measured by carotid femoral pulse wave velocity. We believe this possibility should be investigated as we have reported a high prevalence of the non-dipping blood pressure pattern in this population in the previous chapter of the present thesis and furthermore because the non-dipping blood pressure pattern is associated with worse cardiovascular outcomes (Liu *et al.,* 2003; Rahman *et al.,* 2005). Additionally, since the nocturnal non-dipping blood pressure pattern and arterial stiffness are both strong predictors of adverse cardiovascular events, there might be some interaction between the two.

5.2 Aim

To investigate the possible determinants of arterial stiffness in non-dippers of African ancestry.

5.3 Objectives

5.3.3 To investigate if conventional cardiovascular risk factors are determinants of arterial stiffness as measured by carotid femoral pulse wave velocity in a community of African ancestry.

5.5.4 To establish if the nocturnal non-dipping blood pressure profile interacts with the relationship between conventional cardiovascular risk factors and arterial stiffness as measured by carotid femoral pulse wave velocity.

5.4 Methods

5.4.1. Study Population

Details about the study population are given in chapter 2 of this thesis. Of the 1,219 participants that enrolled in the study, only 796 had complete ambulatory BP monitoring reports. Participants were classified as dippers if their nocturnal drop in systolic BP was >10% and as non-dippers if they had a nocturnal drop in systolic BP of <10%.

5.4.2. Procedures

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

5.4.3 Statistical analysis

All data were analyzed using STATA (StataCorp LLC Texas USA) data analysis and statistical software version 13.0. The five readings obtained from the conventional BP measurements were averaged to obtain a single systolic and diastolic BP reading. Ambulatory BP data were expressed as 24-hour, daytime and night-time average systolic and diastolic pressures. Comparisons between dipper and non-dipper groups were done using independent Student's t-test. Data are expressed as means ± standard deviation (SD) for continuous variables. Categorical variables are expressed as absolute or relative frequencies or as percentages. Age, hypertension, diabetes, obesity and waist circumference were significantly correlated with arterial stiffness in the univariate regression analysis. These variables were then included in the multivariate regression model to see if they were independent correlates of arterial stiffness. Because renin and aldosterone concentrations and aldosterone-to-renin ratios were not normally distributed, in data analysis they are expressed as log renin and square root aldosterone. A P-value <0.05 was considered as statistically significant.

5.5 Results

5.5.1. Demographic and clinical characteristics

The study population consisted of 796 participants. The mean age of the study population was 44±18 years with 36% being male and 64% being female. Of the total population, 383 (48%) were classified as being dippers and 413 (52%) as non-dippers based on their diurnal BP pattern. Non-dippers were older than dippers (48±18 vs 40±17 years). Non-dippers had higher pulse wave velocity (PWV) when compared with dippers (6.96±2.98 vs 5.89±2.34). Non-dippers had higher conventional systolic BP (131.45±23.27 vs 124.89±17.63, $p<0.0001$) Non-dippers were also more likely to be hypertensive when compared with dippers (66% vs 34%) but had a lower recorded alcohol usage (7% vs 16%). Demographic and clinical data of the total population are represented in Table 5.1.

Table 5.1 Demographic and clinical characteristics according to blood pressure dipping status.

	All subjects	Dippers	Non-dippers	P
Number	796	383	413	
Age (years)	44.20 ± 18.13	40.41 ± 17.55	47.69±18	<0.0001
Weight (kg)	76.3±16.9	75.0±17.1	77.6±17.7	0.1081
Height (m)	164.50±8.60	165.4±7.1	163.6±6.6	0.1162
Waist circumference	89.72±16.13	87.14±14.87	92.10±16.88	0.017
BMI (kg/m^2)	28.91±7.62	27.88 ± 7.11	29.84±7.96	0.0003
Smokers (%)	15.00	18.00	12.00	
Alcohol use (%)	21.00	22.00	19.00	
Diabetics (%)	8.50	33.82	66.18	
Hypertensive (%)	24.20	33.67	66.33	
Systolic BP (mmHg)	128.30±20.99	124.89±17.63	131.45±23.27	<0.0001
Diastolic BP (mmHg)	83.70±11.80	82.48±10.98	84.84±12.41	0.0823
PWV (m/s)	6.45±2.74	5.89±2.33	6.96±2.98	<0.0001

BMI = body mass index, PWV = pulse wave velocity. Data is presented as mean ± SD or percentage.

5.5.2. Relationship between pulse wave velocity and cardiovascular risk factors

Following univariate analysis, there was a positive significant relationship between pulse wave velocity and Age, hypertension, waist circumference and obesity (all Ps <0.0001). There was however a significant negative correlation between pulse wave velocity and diabetes (P <0.0001). History of smoking, alcohol consumption and gender were not associated with pulse wave velocity. The univariate analysis is presented in table 5.2.

Table 5.2 Univariate relationships between pulse wave velocity and cardiovascular risk factors.

	β	CI	P
Age	0.099	0.091 to 0.107	<0.0001*
Hypertension	2.910	2.485 to 3.340	<0.0001*
Diabetes	-3.081	-3.780 to -2.383	<0.0001*
Obesity	0.879	0.460 to 1.298	<0.0001*
Waist circ	0.062	0.049 to 0.074	<0.0001*
Smoking	0.321	-0.230 to 0.871	0.254
Alcohol	-0.153	-0.651 to 0.343	0.544
Gender	0.285	-0.134 to 0.705	0.182

CI = confidence interval; R^2 = adjusted R^2. Waist circ. = waist circumference. *Depicts significant relationship.

5.5.4 Dipping status and the relationship between pulse wave velocity and age

Independent of common confounders, a significant interaction between PWV and nocturnal BP dipping status was associated with age ($P<0.0001$). This translated into a stronger positive relationship between PWV and age in participants with nocturnal non-dipping blood pressure pattern ($R^2 = 0.486$) when compared with dippers ($R^2 = 0.355$).

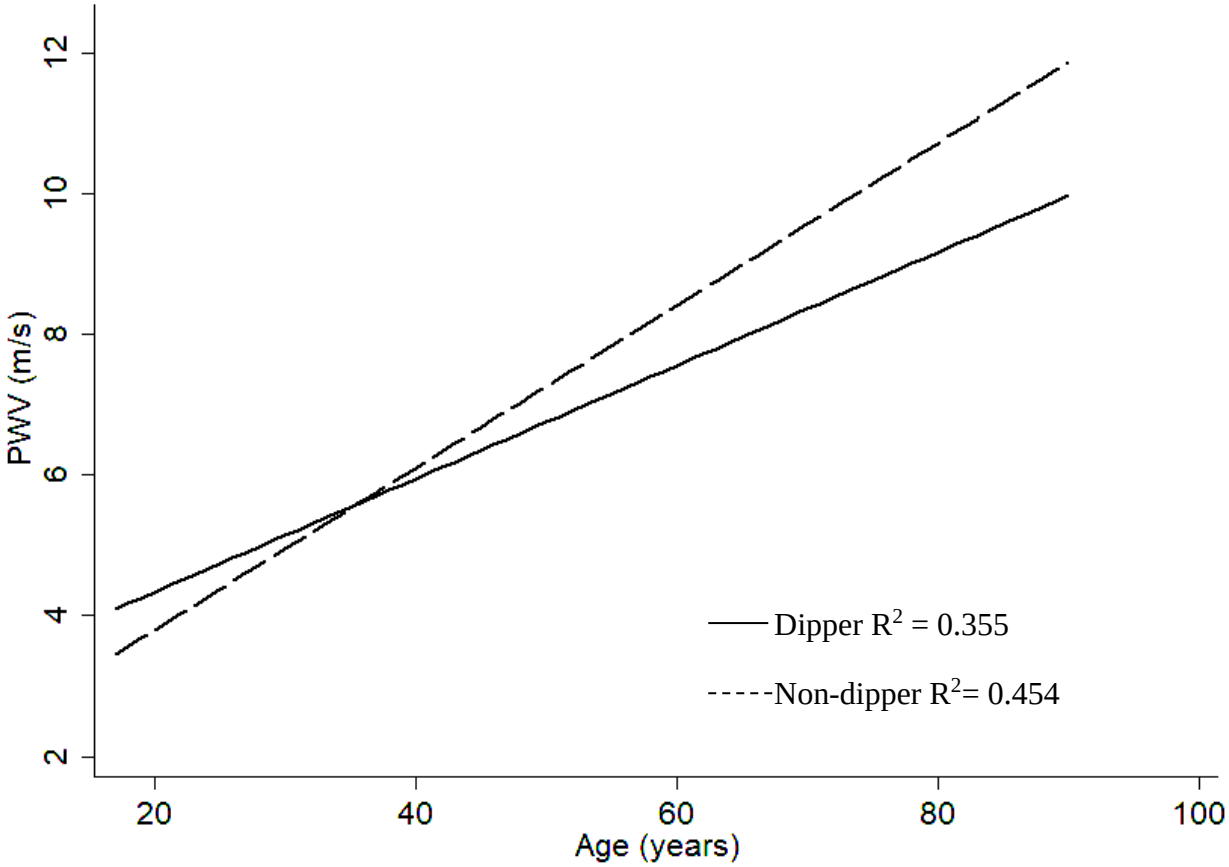


Figure 5.1. Effect of dipping status on the relationship between pulse wave velocity and age. PWV = Pulse Wave Velocity. R^2 = Adjusted R^2

5.5.5. Dipping status and the relationship between PWV and Gender

Independent of common confounders, a significant interaction between PWV and nocturnal BP dipping status was associated with gender ($P<0.0001$). This translated into a higher PWV in non-dipper males than in non-dipper females ($p<0.0001$).

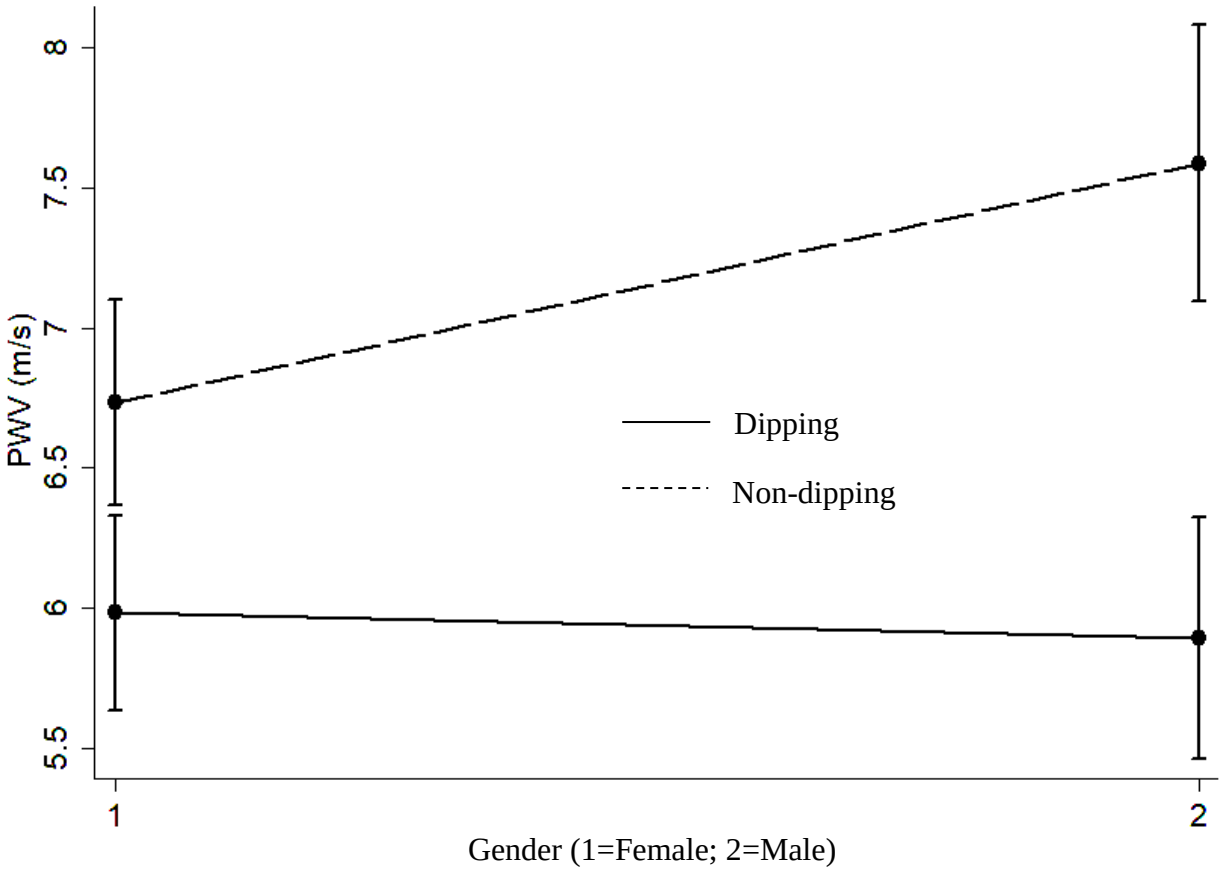


Figure 5.2. Interaction of non-dipping with the relationship between PWV and gender

5.5.3. Multivariate relationships between pulse wave velocity and cardiovascular risk factors.

Multiple regression analysis between PWV and the cardiovascular risk factors associated with PWV in the univariate regression analysis showed that, in the total study population, age, hypertension and diabetes were independent determinants of PWV irrespective if nocturnal blood pressure dipping status (Table 5.3). The relationship between PWV and age was however stronger in the non-dipping group when compared to the dipping group. On the other hand, the relationship between PWV and diabetes was weaker in the non-dipper group when compared to the dipper group. Waist circumference was an independent correlate of PWV only in the dipper group. The remainder of the parameters were not significantly associated with PWV in the general study population and in the dipping sub-groups. In each multivariate analysis, confounding variables including age, hypertension, diabetes, obesity, smoking, alcohol consumption and gender were adjusted for as appropriate.

Table 5.3 Multivariate relationships between pulse wave velocity and significant cardiovascular risk factors from the univariate analysis.

	Total sample population		Dippers		Non-dippers	
	β (CI)	P	β (CI)	P	β (CI)	P
Age	0.080 (0.069 to 0.091)	<0.0001*	0.060 (0.045 to 0.073)	<0.0001*	0.100 (0.082 to 0.118)	<0.0001*#
Hypertension	1.130 (0.696 to 1.528)	<0.0001*	0.93 (0.371 to 1.483)	0.001*	1.034 (0.457 to 1.706)	0.001*
Diabetes	-1.425 (-1.958 to -0.817)	<0.0001*	-1.760 (-2.498 to -0.867)	<0.0001*	-1.146 (-1.928 to -0.312)	0.007*#
Obesity	-0.429 (-0.972 to -0.087)	0.073	-0.568 (-1.179 to -0.055)	0.06	-0.092 (-1.050 to 0.338)	0.811
Waistc	0.009 (-0.003 to 0.027)	0.222	0.03 (0.010 to 0.049)	0.005*	-0.009 (-0.030 to 0.017)	0.426

Waistc= Waist circumference. CI=confidence interval. Data is presented as mean ± SD. * depicts significant relationship between PWV and different parameters. #depicts significant difference in β between dipper groups.

5.5.7. Relationship between carotid femoral pulse wave velocity, blood pressure parameters and RAAS parameters according to nocturnal blood pressure dipping status.

Following multiple regression analysis adjusting for age, hypertension, diabetes, obesity, smoking, alcohol consumption and gender, there was a significant negative correlation between 24-hour urinary sodium-to-potassium ratio and pulse wave velocity in the total study population ($p = 0.02$) but this relationship was lost when participants were stratified according to blood pressure dipping status. This suggests that 24-hour urinary sodium-to-potassium ratio is an independent determinant of PWV in the general population but not in nocturnal BP dipping sub-groups. There was a significant positive correlation between PWV and aldosterone-renin-ratio in the general study population but this relationship was lost when participants were stratified according to blood pressure dipping status. There was however no correlation between PWV and other RAAS parameters following multivariate regression analysis.

Table 5.4 Multivariate relationship between carotid femoral pulse wave velocity and some RAAS parameters according to blood pressure dipping status.

	Total sample population		Dippers		Non-dippers	
	β (CI)	P	β (CI)	P	β (CI)	P
Urine Na^+ (mmol/day)	-0.002 (-0.004 to 0.0003)	0.089	-0.003 (-0.005 to 0.0008)	0.068	-0.002 (-0.005 to 0.003)	0.499
Urine K^+	0.0001 (-0.009 to 0.009)	0.981	-0.006 (-0.017 to 0.004)	0.261	0.008 (-007 to 0.025)	0.292
Na^+/K^+	-0.070 (-0.129 to -0.009)	0.020*	-0.102 (-0.230 to 0.024)	0.113	-0.055 (-0.128 to 0.016)	0.131
Sqrt aldosterone (pg/ml)	0.009 (-0.014 to 0.032)	0.449	0.013 (-0.017 to 0.044)	0.392	0.005 (-0.029 to 0.040)	0.766
Log renin (pg/ml)	0.300 (-0.434 to 0.134)	0.300	-0.211 (-0.572 to 0.149)	0.250	-0.094 (-0.547 to 0.360)	0.685
ARR	0.004 (0.001 to 0.007)	0.009*	-0.0003 (-0.010 to 0.010)	0.995	0.001 (-0.009 to 0.007)	0.120

Na^+ = 24-hour sodium excretion, Na^+/K^+ = 24-hour sodium-potassium ratio, Log renin = Log plasma renin concentration, Sqrt aldosterone (Square root serum aldosterone concentration. ARR = Aldosterone to Renin ratio, CI=confidence interval. Data is presented as mean ± SD%. * depicts significant relationship between PWV and different parameters. #depicts significant difference between dippers and non-dippers.

5.6. Discussion

The present study investigated the determinants of arterial stiffness and the possible influence of the nocturnal non-dipping blood pressure profile (NDP) in modifying the relationship between these determinants and arterial stiffness in a community of black Africans living in Africa. In this cross-sectional study, age, hypertension and diabetes were independent determinants of arterial stiffness as measured by carotid femoral pulse wave velocity (CFPWV). These relationships can however be modified by the non-dipping blood pressure pattern. Sodium-to-potassium ratio and aldosterone-renin-ratio were also independent determinants of arterial stiffness if the nocturnal blood pressure dipping status was not considered. To our knowledge, this is the first time that role of NDP in modifying the relationship between the above-mentioned cardiovascular risk factors and arterial stiffness has been investigated.

In multivariate regression analysis, hypertension was an independent determinant of arterial stiffness. This relationship was still maintained when participants were further stratified according to blood pressure dipping status, although this relationship was not significantly stronger in the non-dipping sub-group. It has been documented that hypertension is associated with arterial stiffness (Mitchell *et al.,* 2012; Okada *et al.,* 2012). In a large population study with 1,769 participants (974 women), an elevated arterial stiffness as measured by CFPWV was strongly correlated with a high risk for incident hypertension (Kaess *et al.,* 2012). Another relatively smaller study showed similar results in 187 hypertensive participants and 296 normotensive participants (Benetos *et al.,* 2002). In this study, arterial stiffness was assessed using CFPWV. Other studies have also shown similar results (Lebrun *et al.,* 2002; Mackey *et al.,* 2002). The main mechanism by which hypertension leads to arterial stiffness is by causing hypertrophy of the medial layer of the blood vessel wall (Benetos *et al.,* 1993; Safar *et al.,* 1994). It has also been shown that hypertension can cause excessive collagen production (Xu *et al.,* 2000) which is a major feature of arterial stiffness (Lakatta *et al.,* 2003). Whether arterial stiffness causes hypertension or the other way round is still a topic of debate. It has been shown that elevated blood pressure increases pulsatile aortic wall stress which accelerates elastin degradation (O'Rourke *et al.,* 1999; O'Rourke *et al.,* 2002; McEniery *et al.,* 2010) hence hypertension is viewed as a form of vascular aging that can lead to arterial stiffness. Conversely, it has been

shown that high arterial stiffness in normotensive individuals are associated with hypertension progression and an increased risk of incident high blood pressure during follow up (Dernellis *et al.,* 2005; Kaess *et al.,* 2012; Najjar *et al.,* 2008). The present study also shows that age is one of the most important determinants of arterial stiffness in this population. This relationship is however stronger in non-dippers. This is in line with other studies investigating the determinants of arterial stiffness in different populations although the non-dipping component was not included (Laogun *et al.,* 1982; Asmar *et al.,* 1995; Avolio *et al.,* 1983). The wall of large arteries like the aorta loses distensibility and becomes thicker as age advances (Folkow *et al.,* 1993) leading to an increase in pulse wave velocity (PWV). This increase in arterial stiffness leads to a reduction in the reservoir function of the conduit arteries closer to the heart leading to an increase in systolic BP and pulse pressure (Greenwald *et al.,* 2007). Following multivariate regression analysis, we showed that diabetes is an independent determinant of arterial stiffness irrespective of nocturnal blood pressure dipping status. This relationship was however blunted in the non-dipping group. Alecu and colleagues showed similar results (Alecu *et al.,* 2006), although the non-dipping component was not considered. They showed in 207 participants a strong correlation between diabetes and arterial stiffness and also a stronger relationship between pulse wave velocity and age in diabetic individuals when compared with non-diabetic individuals. A relatively larger study with 1,415 participants showed higher prevalence of arterial stiffness amongst diabetic participants. They also showed a higher risk of increased arterial stiffness amongst diabetic individuals in a 5 year follow-up study (de Oliviera Alvim *et al.,* 2013).

In this salt sensitive African population, salt intake as measured by 24-hour urinary sodium excretion, was not a determinant of arterial stiffness as measured by CFPWV. This is in line with a study conducted in the same population (Redelinghuys *et al.,* 2010). This study consisted of 653 randomly recruited participants of African ancestry and investigated the relationship between urinary salt excretion and cardiovascular hemodynamic parameters. They found no association between 24-hour urinary sodium excretion and pulse wave velocity. Our findings are however in sharp contrast to the study by Sonoda and colleagues (Sonoda *et al.,* 2012) where it was shown that salt intake was independently associated with pulse wave velocity. This study however used brachia-ankle pulse wave velocity to measure arterial stiffness and may have accounted for varying observations. Other studies have shown similar contrasting results (He *et al*., 2009; Jablonski *et al*., 2013). A high salt diet has been shown to cause an increase in vascular smooth muscle tone and hypertrophy coupled with

thickening of the vascular medial layer and excessive production of collagen (Gu *et al.,* 1998; Safar *et al.,* 2000; Partovian *et al.,* 1998), all components of arterial stiffness. The relationship between urinary sodium excretion and arterial stiffness may have been masked by an unknown mechanism. We believe this might be the case as it has been shown in the same population that obesity masks the relationship between urinary sodium excretion and blood pressure (Maseko *et al*., 2018). This however does not undermine the role of sodium in other cardiovascular diseases (Strazullo *et al*., 2009; Umesawa *et al.,* 2008). Our observation showing no relationship between aldosterone and pulse wave velocity is in contrast with several studies (Lacolley *et al.,* 2002; Blacher *et al.,* 1997) despite it being reported that aldosterone increases the production of endothelin-1 which is a known vasoconstrictor, and which also has fibrotic effects on the vascular wall (Park *et al.,* 2001). Just like urinary salt intake, the relationship between aldosterone and arterial stiffness may have been masked by a currently unknown mechanism. Twenty-four-hour urinary sodium-to-potassium ratio was independently associated with pulse wave velocity. This is in line with the findings of Zhang and colleagues (Zhang *et al.,* 2018) where they showed an independent association between urinary sodium to potassium ratio and arterial stiffness using CFPVW in 309 individuals. It is however not surprising because it has been shown that urinary sodium to potassium ratio is a stronger predictor of cardiovascular hemodynamics than either sodium or potassium alone (Mente *et al.,* 2014). Urinary sodium to potassium ratio was however not independently associated when participants were stratified according to nocturnal blood pressure dipping pattern suggesting that it may only be important if nocturnal blood pressure dipping pattern was not considered. We show that ARR is an independent determinant of PWV in our population. This is similar to the findings of Mahmud and colleagues (Mahmud *et al.,* 2005). This is however not surprising as it has been shown that Aldosterone-to-renin ratio (ARR) is more reproducible than just measuring aldosterone concentrations only (Lim *et al.,* 2002). As with urinary sodium to potassium ratio, ARR was not associated with PWV when participants were stratified according to nocturnal blood pressure dipping status. The lack of association between 24-hour urinary sodium excretion and PWV probably translated into the observations with the RAAS components as it has been shown that salt intake is strongly associated with the RAAS (Campese *et al.,* 1982).

The present study showed that NDP is associated with higher pulse wave velocity. This is similar to the findings of Castelpoggi and colleagues where they observed a positive association between NDP and arterial stiffness in 600 resistant hypertensive patients

(Castelpoggi *et al.,* 2009). Another study that investigated the relationship between NDP and arterial stiffness in 56 hypertensive patients showed similar results (Cicek *et al.,* 2013). The relationship between NDP and arterial stiffness has also been studied in patients with different conditions and in general populations where they also found significant associations (Celik *et al.,* 2015; Jerrard-Dune *et al.,* 2007). The exact mechanism via which NDP causes arterial stiffness has not been fully elucidated. However, NDP is associated with nocturnal sympathovagal imbalance ((Kario *et al.,* 2002) which has also been associated with arterial stiffness (Subha *et al.,* 2014).

The non-dipping pattern was associated with a significantly higher pulse wave velocity in males when compared with females. This is the first time that this has been demonstrated. Although studies have shown a higher PWV amongst non-dippers when compared with dippers (Castelpoggi *et al.,* 2009; Jerrard-Dune *et al.,* 2007), gender differences were not considered. There was no gender difference in PWV within the dipper group. It has been shown that differences do not exist in arterial stiffness between males and females (Waddell *et al.,* 2001) without consideration for dipper status, although there is still some controversy about which gender has a steeper increase in age related arterial stiffness (Alghathrif *et al.,* 2013; Wen *et al.,* 2015; Nethononda *et al.,* 2015; Tomiyama *et al.,* 2003). We hypothesize that non-dipping induced changes in vascular wall properties are more severe in males when compared with females.

Finally, as mentioned earlier, the present study showed a steeper increase in age related arterial stiffness as measured by CFPWV with non-dipping. To our knowledge, this is the first time this has been investigated. This suggests that the presence of NDP in old age might lead to a worse case of arterial stiffness and hence overall adverse cardiovascular outcome. This should not be surprising as the damaging effects of age on the arterial walls coupled with non-dipping induced arterial stiffness might summate and lead to an overall worse prognosis. This is very likely as it is known that high PWV and NDP becomes more prevalent with age (Nichols *et al.,* 2005; O'Brien *et al.,* 1988; Marinakis *et al.,* 2003). The above observations suggest that NDP is an important criterion to consider in age related arterial stiffness and interventions geared towards reversing the non-dipping BP pattern might be beneficial in improving overall prognosis.

Our results showed no interactions of non-dipping in the relationship between other cardiovascular risk factors and PWV.

This study has its limitations. First, this is a cross-sectional study and cause-effect relationships cannot be established, secondly, sleep apnoea which usually associated with NDP was not investigated in this study and may have been a confounder in the multivariate relationships.

5.7. Conclusions

In a community of black Africans living in Africa, age, hypertension and diabetes are independent determinants of arterial stiffness. The relationships between these cardiovascular risk factors and arterial stiffness can however be altered in different ways by the non-dipping blood pressure pattern.

CHAPTER 6

Gender Differences in Non-dipping Related Arterial Stiffness in Individuals of African Ancestry.

6.1 Introduction

Arterial stiffness is a known marker of arteriosclerosis and subclinical atherosclerosis and is an independent predictor of future adverse cardiovascular events (Blacher *et al.,* 1999). Increase in arterial stiffness has been shown to be an independent predictor of all cause cardiovascular morbidity and mortality in patients with end-stage renal disease, hypertension and diabetes (Blacher *et al.,* 1999; Laurent *et al.,* 2001; Cruickshank *et al.,* 2002). It is known that arterial walls stiffen with advancing age and the main changes include luminal enlargement and depletion of the elastic properties mainly in the large elastic arteries leading to atherosclerosis (Izzo *et al.,* 2001). A non-dipping blood pressure profile (NDP) is associated with increased arterial stiffness and other cardiovascular target organ damage and is also a stronger predictive index of cardiovascular morbidity and mortality (Ohkubo *et al.,* 2002; Cuspidi *et al.,* 2004; Hansen *et al.,* 2006). We show in chapter 4 that there is a high prevalence of NDP in our target population. Additionally, in chapter 5 we show that NDP is associated with a steeper age-related increase in arterial stiffess and that NDP is associated with a higher PWV in males than in females. Although gender differences in artrial stiffness and age-related arterial stiffness have been reported (Alghathrif *et al.,* 2013; Wen *et al.,* 2015), the potential involvement of NDP has not been described. Serum lipid profile has accounted for gender differences in different pathologies (Kolovou *et al.,* 2009; Schianca *et al.,* 2012) including arterial stiffness (Łoboz-Rudnicka *et al.,* 2018). In this chapter, I investigate the role of serum lipid profile in gender differences in NDP related arterial stiffness.

6.2. Aim

We aimed to investigate gender differences in non-dipping related increase in arterial stiffness in a population of African ancestry.

6.3. Objectives

5.1.2.1 To investigate gender differences in non-dipping related arterial stiffness as measured by carotid femoral pulse wave velocity (CFPWV) in a population of black Africans.

5.1.2.2 To identify a possible role of serum lipid profiles in non-dipping related arterial stiffness in a population of black Africans.

6.4 Methods

6.4.1. Study Population

Details about the study population are given in chapter 2 of this thesis. Of the 1,219 participants that enrolled in the study, only 796 had complete ambulatory BP monitoring reports. Participants were classified as dippers if their nocturnal drop in systolic BP was >10% and as non-dippers if they had a nocturnal drop in systolic BP of <10%.

6.4.2. Procedures

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

6.4.3 Statistical analysis

All data were analyzed using STATA (StataCorp LLC Texas USA) data analysis and statistical software version 13.0. Conventional blood pressure was measured 5 consecutive times and the values were averaged to obtain single systolic and diastolic BP readings. Comparisons between dipper and non-dipper groups as well as gender groups were done using independent Student's t-test. Data were expressed as means ± standard deviation (SD) for continuous variables. Categorical variables are expressed as absolute or relative frequencies or as percentages. A P-value <0.05 was considered as statistically significant.

6.5. Results

6.5.1. Demographic and clinical characteristics

The study population consisted of 796 participants. The study population was classified according to gender and BP dipping status. There was a total of 288 males and 508 females, of the 288 males, 148 were classified as dippers while 140 were classified as non-dippers. Of the 508 females 235 were classified as dippers while 273 were classified as being non-dippers. In both gender groups, non-dippers were older than dippers; 49.79±18.84 vs 39.06±17.88, p<0.0001 in the male group and 46.61±17.45 vs 41.25±17.33, p=0.0003 in the female group. Body mass index was similar in all groups irrespective of dipper status and gender although non-dipper females had higher BMI than non-dipper males (32.00±8.22 vs 25.63±5.30 p<0.05). Waist circumference was higher in the male non-dipper group when compared with the male dipper group (88.23±15.22 vs 83.42±13.31, p=0.02) and also higher in the female non-dipper group when compared with the female dipper group (94.13±17.38 vs 89.50±15.35, p=0.0008). Non-dipper females had a higher waist circumference when compared with non-dipper males (94.13±17.38 vs 88.23±15.22, p<0.05) There were more hypertensive participants in the non-dipper groups when compared with the dipper groups irrespective of gender. There were also more diabetic participants in the male non-dipper group when compared to the male dipper group. Serum triglycerides concentration did not

differ between dippers and non-dippers but non-dipper females had lower triglycerides concentration when compared with non-dipper males ($p<0.05$). Serum HDLc concentrations were lover in non-dipper groups but did not reach statistical significance however, it was significantly lower in the non-dipper male group when compared to the non-dipper female group ($p<0.005$). Serum LDLc levels did not differ between dipper and non-dipper groups however, LDLc levels were higher in the female non-dipper group when compared with the male non-dipper group ($p<0.05$). There were no differences in other clinical and demographic characteristics. These results are presented in table 5.1.

Table 6.1 Demographic and clinical characteristics of participants according to gender and blood pressure dipping status.

	Males				Females			
	All	Dippers	Non-Dippers	P	All	Dippers	Non-dippers	P
N (%)	288 (36)	148(51.40)	140(48.61)		508 (64)	235(46.25)	273(53.74)	
Age (years)	44.2±19.09	39.06±17.88	49.79±18.84	<0.0001*	44.14±17.58	41.25±17.33	46.61±17.45	0.0003*#
BMI (kgm^{-2})	24.96±5.14	24.32±4.92	25.63±5.30	0.35	31.13±7.89	30.12±7.38	32.00±8.22	0.30#
Waist circ (cm)	85.76±14.45	83.42±13.31	88.23±15.22	0.002*	91.98±16.62	89.50±15.35	94.13±17.38	0.0008*#
Smokers (%)	36%	36%	31%		5%	6%	3%	
Alcohol use (%)	38%	40%	36%		11%	11%	10%	
Hypertensive (%)	38%	25%	50%		40%	33%	46%	
Diabetics (%)	10%	5%	14%		8%	6%	5%	
TCHOL	4.47	4.45±1.05	4.48±0.99	0.401	4.67	4.59±1.03	4.57±1.31	0.425
TRGL	1.46	1.32±0.98	1.61±2.05	0.06	1.13	1.06±0.58	1.19±1.14	0.06#
HDLc	1.35	1.40±0.53	1.30±0.43	0.04*	1.44	1.47±0.44	1.44±0.38	0.205#
LDLc	2.48	2.45±0.87	2.50±0.87	0.3131	2.72	2.63±0.94	2.79±1.06	0.091#

BMI = body mass index; Hip circ, hip circumference; Waist circ, waist circumference; TCHOL = Total cholesterol; TRGL = Triglycerides; HDLc = High density lipoprotein cholesterol; LDLc = Low density lipoprotein cholesterol. Data were presented as mean ± SD or number (%). *A P value ≤ 0.05 depicts significant difference between dippers and non-dippers of same gender. # depicts significant difference between non-dipper males and non-dipper females.

6.5.2. Carotid femoral pulse wave velocity (CFPWV) by gender and dipping status

In both male and female groups, non-dippers had higher CFPWV when compared with dippers; 7.53±3.60 (n=130) vs 5.74±2.47 (n=134), $p<0.0001$ and 6.64±2.52 (n=232) vs 5.98±2.23 (n=203), $p=0.0021$ respectively. Non-dipper males however had significantly higher CFPWV when compared with non-dipper females (7.53±3.60 vs 6.64±2.52, $p=0.0031$). These results are presented in Figure 5.2.

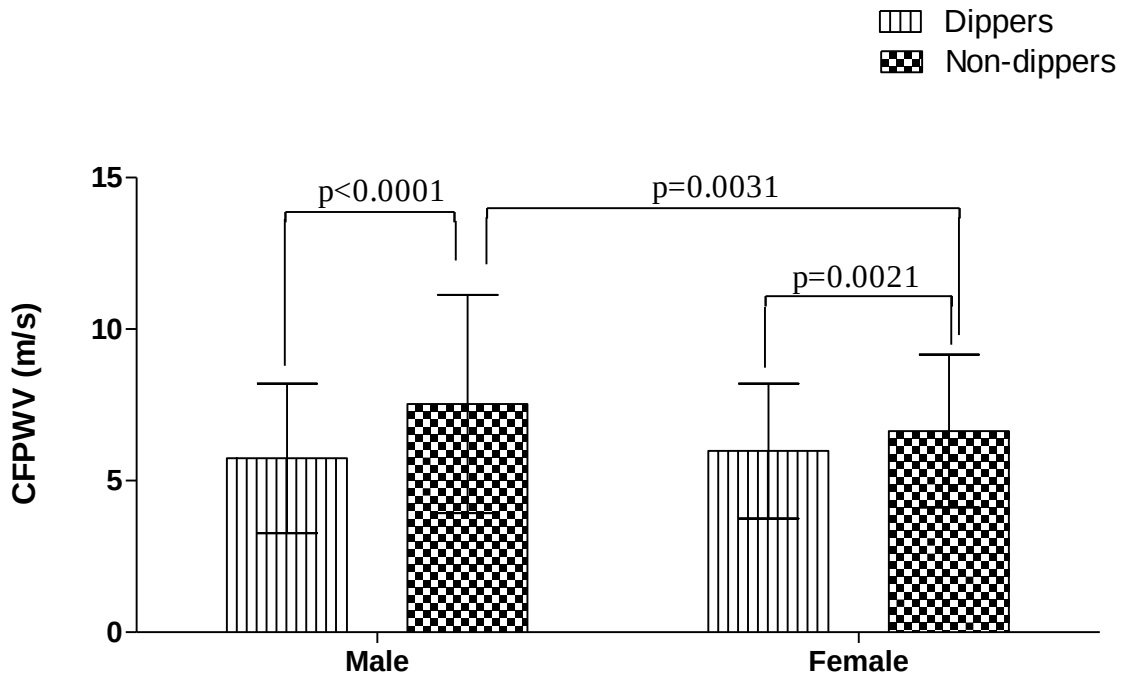


Figure 6.1. Carotid femoral pulse wave velocity (CFPWV) of participants according to blood pressure dipping status and gender. Results are presented as mean±SD.

6.5.3. Relationship between age and carotid femoral pulse wave velocity (CFPWV)

Carotid Femoral Pulse Wave Velocity increased with age amongst males and females irrespective of nocturnal non-dipping blood pressure dipping status. The age-related increase in CFPWV was however steeper in males when compared to females ($p<0.0001$) in the general population. The age-related increase in CFPWV was steeper in both non-dipper males and females when compared to the general population ($p<0.0001$). This relationship was however still steeper amongst non-dipper males when compared to non-dipper females ($p<0.0001$) although females exhibited a greater change in age related arterial stiffness when the general population was compared to non-dippers ($p<0.0001$).

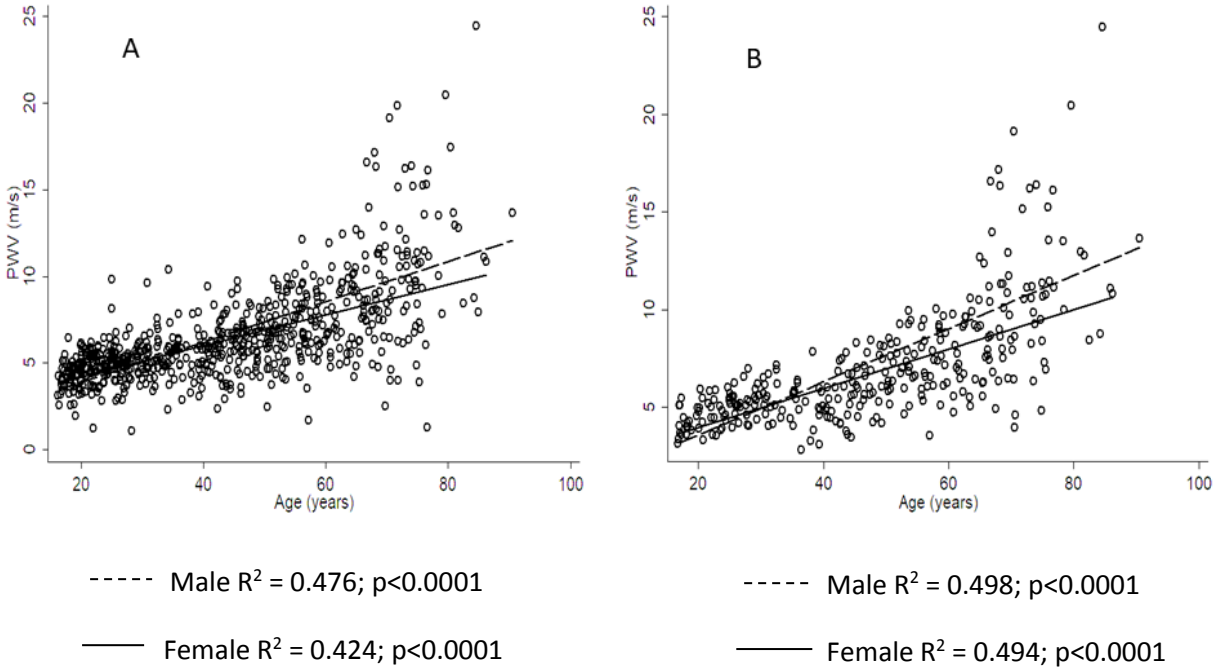


Figure 6.2. Age related increase in pulse wave velocity. Panel A represents the general population while panel B represents non-dippers. PWV = Pulse Wave Velocity.

6.6. Discussion

In this study, we investigated the gender differences in non-dipping related increase in arterial stiffness and the effect of non-dipping in gender differences in age related arterial stiffness as measured by CFPWV in a population of black Africans. The main findings of this study are that male non-dippers of African ancestry have greater arterial stiffness and greater age related increase in arterial stiffnes when compared with females and that this difference may have been mediated by significantly lower levels of high-density lipoprotein cholesterol (HDLc) coupled with higher levels of triglycerides in non-dipper males. To our knowledge, this is the first time that age and gender components have been studied in the relationship between the non-dipping BP pattern and arterial stiffness.

The present study shows an increase in CFPWV with age irrespective of gender, with males having a steeper increase in age related arterial stiffness when compared to females irrespective of dipper status. These observations are consistent with those of Alghathrif and colleagues where they showed in a population of 777 individuals with 354 men and 423 women that men had a steeper longitudinal increase in CFPWV with advancing age when compared with women, which makes the males to have higher CPFPWV values after their fifth decade. Our results are also in line with another study by Wen *et al.,* (2015) in which arterial stiffness was assessed by cardio-ankle vascular index (CAVI) in 18, 336 subjects and it was observed that aging led to a higher arterial stiffness in men when compared with women. Magalhães and colleagues also showed that males above 50 years old had a greater pulse wave velocity when compared to age matched females (Magalhães *et al*., 2013). In contrast to our findings however are the observations by Nethononda and colleagues where it was observed that females showed a steeper increase in aortic stiffness with age when compared with males (Nethononda *et al.*, 2015). This study consisted of 777 individuals with 408 females and 369 males. Pulse wave velocity was however assessed using cardiovascular magnetic resonance. Similarly, the study by Tomiyama and colleagues reported that age induced arterial stiffness as measured by brachial ankle pulse wave velocity was higher in females when compared to males. This study consisted of 7,881 subjects with 4,488 males and 3,393 females (Tomiyama *et al*., 2003).

The differences in observations between the latter mentioned studies are not quite clear, but despite their large sample sizes, they employed different methods to assess arterial stiffness. It is however known that CFPWV is currently the gold standard for the assessment of arterial

stiffness (Laurent *et al.,* 2006) hence we believe that our observations might hold more validity. All the studies mentioned above have been conducted in general populations and the nocturnal BP dipping statuses of the participants were not taken into consideration. The reports that studied the non-dipping BP pattern alongside arterial stiffness did not consider age and gender differences (Jerrard-Dunne *et al.,* 2007; Chen *et al.,* 2018). Our results suggest that NDP induced arterial stiffness is more severe in males when compared to females causing them to have a higher non-dipping related arterial stiffness and a steeper increase in age related arterial stiffness when compared with females.

The present study shows a significantly lower serum HDLc in non-dipper males when compared with non-dipper females. This is consistent with other findings (Habib *et al*., 2005; Kolovou *et al.,* 2009) although the non-dipping component was not included. The role of HDLc in arterial stiffness has been studied and it has been highlighted that low levels of HDLc is a risk factor for cardiovascular and cerebrovascular disease (Assmann *et al.,* 2004). Similarly, different studies have shown an inverse relationship between HDLc and pulse wave velocity, suggesting that higher levels of HDLc might be associated with an improved arterial distensibility (Zhao *et al.,* 2013; Kozakova *et al.,* 2015). Wang and colleagues in a cross-sectional study showed that in 15, 302 participants, HDLc had an inverse relationship with CFPWV (Wang *et al.,* 2013). Because of the large population of this study and similarity in technique used in the assessment of aterial stifnes, we are confident about the validity of our results. Of interest also is the study by Shen and colleagues where it was shown that high serum levels of HDLc are associated with a lower risk of arterial stiffness specifically in women older than 50 years of age but not in age matched men and not in individuals younger than 50 years old (Shen *et al*., 2017). This might have accounted for the slower age-related increase in arterial stiffnes in females. The exact mechanism behind the relationship between HDLc and CFPWV is not quite clear but it has been postulated that high HDLc levels are inversely proportional to advanced glycation end products levels (AGEs) (Chang *et al.,* 2011) which stimulate inflammatory pathways and stress signaling and can damage elastin molecules in the vascular wall leading to arterial stiffness (Zieman *et al.,* 2005). It has also been shown that HDLc has beneficial effects on the vascular wall via its anti-apoptotic, anti-inflammatory and antithrombotic properties (Campbell *et al*., 2013).

The males in our cohort had greater levels of triglycerides irrespective of dipping status. This is consistent with the findings of Kolovou and colleagues (Kolovou *et al.,* 2009; Habib *et al*., 2005). The role of triglycerides in arterial stiffness has also been investigated. Wang and colleagues in a community-based study, showed in 1, 447 participants that lower levels of

triglycerides were significantly associated with decreases in CFPVW (Wang *et al.*, 2016) even after adjusting for confounding variables. Zhao and colleagues also showed similar results (Zhao *et al.,* 2014). They showed that serum triglycerides had a positive relationship with brachial ankle pulse wave velocity (baPWV) in 1133 participants aged 50 to 90. Other studies are also in line with the above findings (Chen *et al*., 2018; Urbina *et al.,* 2013). Elevated triglyceride concentrations have been shown to impair the beneficial functions of HDLc thereby promoting atherosclerosis (Greene *et al.,* 2001). Elevated triglycerides levels have also been shown to increase the release of endothelin-1 which significantly facilitates endothelial dysfunction, which is an important contributor to arterial striffness (Maggi *et al*., 2004; Jagla *et al.,* 2001). The levels of triglycerides may have contributed towards observed differences in arterial stiffness

We also show in this study that male non-dippers had higher LDLc concentrations when compared with non-dipper females. This is similar to previously reported findings (Farid *et al.*, 2017; Spinneker *et al.,* 2012). High serum levels of LDLc have been attributed to higher degrees of arterial stiffness (Bjornstad *et al.,* 2015). This study was however conducted in 267 adolescents with type 1 diabetes mellitus. Anotheer study conducted in 1,133 middle aged to elderly Chinese participants also showed that LDLc had a positive correlation to arterial stiffness as measured by baPWV (Zhao *et al.,* 2014). Female non-dippers however showed higher serum levels of LDLc and a lower level of arterial stiffness when compared with non-dipper males, meaning that LDLc concentrations might not explain the gender differences in arterial stiffness and age related arterial stiffnes in this population based on earlier cited studies. This suggests that LDLc might be less important at determining arterial stifnesss when compared with HDLc and triglycrides in this population. This assumption may be supported by trials that have shown significant residual cardiovascular risk even when LDLc levels have reached treatment target levels. This residual cardiovascular risk has been attributed to high triglycerides levels despite normal LDLc levels (Sampson *et al.,* 2012). It has also ben shown that levels of HDLc have better prognostic capabilities when compared to levels of LDLc (Nilsson *et al.,* 2009).

The significantly higher pulse wave velocity in non-dipper males could have also been accounted for by the higher prevalence of diabetes in this group. Alecu and colleagues showed in 207 participants a strong correlation between diabetes and arterial stiffness and also a stronger relationship between pulse wave velocity and age in diabetic individuals when compared with non-diabetic individuals (Alecu *et al.,* 2006), although the non-dipping component was not considered. A relatively larger study with 1,415 participants showed

higher prevalence of arterial stiffness amongst diabetic participants. They also showed a higher risk of increased arterial stiffness amongst diabetic individuals in a 5 year follow-up study (de Oliviera Alvim *et al.*, 2013).

Our study population was modest in size and consisted entirely of black Africans living in Africa, this might limit the generalizability of our findings. Also, our study was a cross-sectional one, therefore, cause-effect relationships can not be established.

6.7. Conclusions

The nocturnal non-dipping blood pressure profile is associated with higher arterial stiffnes and a steeper age-related increase in arterial stiffness. Serum HDLc and serum triglycerides levels might be important targets for therapy in improving arterial compliance with or without the presence of non-dipping.

CHAPTER 7

Contextual Narrative and Conclusions

7.1 Introduction

In the chapters 3 to 6 of the present thesis, I provide comprehensive discussion of the findings of my research. In this chapter, I will however give a contextual narrative for the earlier chapters 3 to 6 and then discuss the possible clinical significance of my research as well as its limitations.

Ambulatory blood pressure monitoring (ABPM) has over time given us better understanding of the circadian behaviour of blood pressure and is said to be a better predictor of cardiovascular target organ damage when compared with conventional blood pressure measurement techniques (Kuznetsova *et al.,* 2000). The information provided by ABPM is also more reproducible than that derived from conventional blood pressure measurement (Krakoff *et al.,* 2013). Ambulatory blood pressure monitoring has immense advantages including correct diagnosis of white coat hypertension and exposing masked hypertension (Pickering *et al.,* 1988, Franklin *et al.,* 2013, Peacock *et al.,* 2014). Hence its use could prevent unnecessary use of chronic medication and avoid underestimation of cardiovascular risk due to hypertension respectively (Lovibond *et al.,* 2011, Myers *et al.,* 2005). Ultimately, the use of ABPM in the clinical diagnosis of hypertension could significantly reduce the financial burden that hypertension places on the healthcare system especially in a country like South Africa where the prevalence of hypertension is about 41% (Ntuli *et al.,* 2015).

The nocturnal non-dipping blood pressure pattern (NDP) is an abnormality of circadian blood pressure behaviour in which night time BP values do not fall by at least 10% of daytime values (Peacock *et al.,* 2014) and it is said that individuals with this type of blood pressure pattern are more likely to develop adverse cardiovascular complications like left ventricular hypertrophy, congestive heart failure, stroke, carotid intima-media thickening and microalbuminuria (Fan *et al.,* 2010, Hoshide *et al.,* 2003). Several mechanisms have been proposed to be responsible for NDP including autonomic dysfunction (Kario *et al.,* 2002), abnormal renal sodium handling (Uzu *et al.,* 1996), poor sleep quality (O'Shea *et al.,* 2000) and hormonal mechanisms (Hoshide *et al.,* 2003, Zelinka *et al.,* 2004). It is said that black people have higher ambulatory BP values when compared with Caucasians (Urbina *et al.,* 2008) but ABPM reference values for normal ambulatory blood pressure have not been determined in people of African ancestry. It has also been shown that NDP is more prevalent among black individuals but the actual prevalence of NDP and the factors responsible for it in an African community is currently unknown. In this present thesis, I have been able to address

both issues. One of the pathologies associated with NDP is arterial stiffening. Arterial stiffnes has different determinants and different populations. In this thesis, I have been able to describe the determinants of arterial stiffness in a population of black Africans living in Africa. I have also been able to describe the influence of NDP in the relationship between these determinants and arterial stiffness. Additionally, I was able to show a gender interaction in the relationship between NDP and arterial stiffness. There is still some controversy as to which gender has a steeper age-related arterial stiffness. In the present thesis, I investigated gender differences in NDP related arterial stiffness and the effect of NDP on age related arterial stiffness in males and females in people of African ancestry.

Subsequently, I will be discussing how findings of the present thesis have contributed to our knowledge concerning ABPM and the non-dipping blood pressure pattern.

7.2 Reference Values for Ambulatory Blood Pressure Monitoring in People of African Ancestry

The findings in the present thesis suggest that people of African ancestry have similar ambulatory blood pressure values when compared to other ethnic groups. This is in contradiction to the premise that black individuals have higher ambulatory blood pressure values when compared to other ethnic groups. The difference in observations might be due to socio-economic, geographic and cultural differences between South African blacks and other black people who live in developed countries. This highlights an important principle that studies conducted in African Americans or in black individuals who live in other developed countries of the world might not necessarily be considered as representative of African populations living in Africa. Pending validation by prospective outcome-based studies, data from the present thesis suggests that the following ambulatory blood pressure values should be considered as thresholds for the diagnosis of abnormal ambulatory BP in young adults of African descent: 135/85 mmHg for 24-hour BP, 140/90 mmHg for daytime BP and 130/80 for night time BP. The thresholds for normal ambulatory blood pressure values as determined in this present thesis have very important diagnostic value as it will lay the foundation for future outcome-based studies that will eventually herald the inclusion of ABPM in the clinical diagnosis of hypertension in South Africa. This will ensure early exposure of masked

hypertension and help identify white coat hypertension; hence unnecessary chronic drug usage as well as underestimation of cardiovascular risk will be avoided.

7.3 Nocturnal Blood Pressure Non-Dipping and Urinary Sodium Excretion in People of African Ancestry: The Role of Aldosterone.

Although it is thought that NDP is more prevalent amongst black people, the actual prevalence of NDP in people of African ancestry is unknown. Different factors have also been proposed to be responsible for NDP as discussed in the introduction section of this chapter. Data from this present thesis points strongly towards abnormally high plasma aldosterone levels as well as low 24-hour urinary sodium excretion secondary to retention. I show in this present thesis that there is a 52% prevalence of NDP in the study population. This is quite high and should be of great concern especially because there is already a high burden of hypertension in South Africa. A combination of a high prevalence of NDP coupled with a high prevalence of hypertension may predispose the population to an even higher risk of developing adverse cardiovascular complications. Therefore, efforts geared towards reducing the prevalence of NDP in this population will go a long way to minimize this risk. I recommend adequate dietary potassium consumption which has been shown to increase natriuresis and hence lower nocturnal BP especially in salt sensitive individuals (Wilson *et al.,* 1999). Potassium is also known to induce the release of high amounts of kallikrein which has been shown to antagonize the activities of the renin angiotensin aldosterone system (Campbell *et al.,* 2003) and consequently reduce blood pressure. An equally important finding in this present thesis is that there is a significant correlation between 24-hour urinary sodium excretion and dipping in non-dippers but not in dippers. This suggests that 24-hour urinary sodium excretion is an independent predictor of the non-dipping blood pressure pattern in this population.

7.4 Determinants of Arterial Stiffness in a Black African Population: Role of the Nocturnal Non-Dipping Blood Pressure Profile.

Increased arterial stiffness as measured by carotid femoral pulse wave velocity has been shown to be an independent predictor of cardiovascular morbidity and mortality (Laurent *et al.,* 2001; Meaume *et al.,* 2001). It is one of the pathologies associated with NDP. In this

thesis I define the determinants of arterial stiffness in a black African population. I demonstrate that age, hypertension and diabetes are independent predictors of arterial stiffness. I also demonstrate that NDP strengthened the relationship between arterial stiffness and age but blunted the relationship between diabetes and arterial stiffness. This is the first time that the role of NDP in modifying the relationship between arterial stiffness and its determinants has been studied. Since age is the main determinant of arterial stiffness, the exacerbation of age-related arterial stiffness by NDP should be considered a serious clinical issue. An equally important finding is that NDP leads to significantly higher levels of arterial stiffness in males when compared to females. This suggests the presence of gender differences in NDP related arterial stiffness. Therefore, males might be at a significantly higher risk of increased arterial stiffness if they are non-dippers when compared to female dippers or non-dippers. It is recommended that at old age, routine ambulatory blood pressure monitoring, and assessment of pulse wave velocity be conducted to determine dipping status and level of arterial stiffness respectively. Since arterial stiffening is inevitable at old age, efforts should be made to reverse the nocturnal non-dipping blood pressure pattern (as described in the previous section) in those who have it. This could possibly improve overall cardiovascular prognosis that would have otherwise been worsened by NDP. This might be particularly important in this population as we report a high prevalence of NDP in the previous chapter.

7.5 Gender Differences in Non-dipping Related Arterial Stiffness in Individuals of African Ancestry.

As seen in the previous chapter, I report gender differences in NDP induced arterial stiffness, with males more prone to a higher NDP related arterial stiffness. I also report a steeper increase in age related arterial stiffness in male non-dippers when compared with female non-dippers. I investigate the involvement of lipid profile in the determination of this gender difference. I demonstrate that high density lipoprotein cholesterol (HDLc) and triglycerides might explain these gender differences. I show that high levels of HDLc and low levels of triglycerides in female non-dippers have a vascular protective effect and might explain why they have lower arterial stiffness when compared with their male counterparts. These findings highlight a possible role of NDP in modifying the serum concentrations of the above-mentioned lipids and thereby induce arterial stiffness. This is the first time that the role of

serum lipids in gender differences in non-dipping related arterial stiffness has been studied. The above results suggest that interventions that can increase serum HDLc levels and reduce triglycerides levels can be of immense benefit in reversing non-dipping related arterial stiffness. These interventions may however be more beneficial in old age, with more consideration for males.

7.6 Limitations

During this thesis, I have highlighted the limitations of each study conducted. I will like to emphasize a couple of the limitations in this section. In the determination of reference values for ambulatory blood pressure monitoring, I believe that the sample size is relatively small in epidemiological terms although it is the largest population size for this type of study in the target population. Additionally, outcome-based studies were not conducted to specifically identify adverse cardiovascular outcomes associated with ambulatory blood pressure reference values above the recommended values presented in this current thesis. In the determination of the possible role of aldosterone and urinary sodium excretion in nocturnal non-dipping blood pressure pattern, I acknowledge that the study was a cross-sectional one and it precludes conclusions about causal relationships. In addition, the urine collection protocol did not include ‘day-time’ and ‘night- time’ urine; this could have shed more light on the diurnal and nocturnal pattern of urinary electrolyte excretion and its relationship with ambulatory blood pressure parameters. In investigating the determinants of arterial stiffness, sleep apnoea which usually associated with NDP was not investigated and may have been a confounder in the multivariate relationships. In the determination of gender differences in non-dipping related arterial stiffness, I also recognize that the study was a cross-sectional one and hence, cause-effect relationships cannot be established. Finally, all studies presented in the present thesis were conducted in black people of African ancestry, hence, generalization of our findings to other populations might be limited.

7.7 Conclusions

In conclusion, the findings presented in the present thesis have filled the gap in knowledge about reference values for normal ambulatory blood pressure in a community of African ancestry. This carries a lot of diagnostic importance when it comes to exposure of masked hypertension and identification of white coat hypertension. Second, data from the present thesis has filled the gap in knowledge about the possible mechanism behind NDP and its prevalence in people of African ancestry. I show that the prevalence of the nocturnal non-dipping BP pattern in this population is 52%. I also show that the nocturnal non-dipping BP profile is mediated by high plasma aldosterone levels and sodium retention. Third, observations from the present thesis have established the determinants of arterial stiffness and showed that non-dipping can modify the relationship between arterial stiffness and its determinants in people of African ancestry. Interventions that can reverse NDP may reverse NDP induced target organ damage. Finally, results from this thesis have added to the knowledge regarding gender differences in arterial stiffness. I show that males have higher non-dipping related arterial stiffness when compared with females. Serum concentrations of HDLc and triglycerides might explain the gender difference. Interventions that can increase serum HDLc levels and reduce triglycerides levels can be of immense benefit in reversing non-dipping related arterial stiffness.

APPENDICES

Appendix A

Ethical clearance certificate

R14/49 Mr Abdulraheem Bawa-Allah et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170213

NAME: (Principal Investigator)	Mr Abdulraheem Bawa-Allah et al
DEPARTMENT:	Physiology Hypertension Clinic, Cardiovascular and Genomic Research Unit
PROJECT TITLE:	Nocturnal Non-Dipping Blood Pressure Profiles and Cardiovascular Target Organ Damage
DATE CONSIDERED:	24/02/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Muzi Maseko
APPROVED BY:	Professor P. Cleaton-Jones Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/08/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical)

Principal Investigator Signature

Date 3rd August 2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B

“Turn-it-in” Plagiarism report

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ORIGINALITY REPORT

15%	3%	14%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Witwatersrand Student Paper	1%
2	academic.oup.com Internet Source	1%
3	Blood Pressure Monitoring in Cardiovascular Medicine and Therapeutics, 2016. Publication	1%
4	"Sex-Specific Analysis of Cardiovascular Function", Springer Nature America, Inc, 2018 Publication	<1%
5	G NORTON. "Reference Values for SphygmoCor Measurements in South Africans of African Ancestry", American Journal of Hypertension, 01/2006 Publication	<1%
6	"Pediatric Hypertension", Springer Nature, 2018 Publication	<1%
7	"Sunday 28 August 2016", European Heart Journal, 2016 Publication	<1%

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