AN ATTENUATED NOCTURNAL BLOOD PRESSURE DIPPING IS ASSOCIATED WITH PUMP DYSFUNCTION IN A POPULATION OF AFRICAN ANCESTRY

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

Johannesburg, 2021.

DECLARATION

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

Mandisa Vinolia Ngema .....on this…….....day of…..... 2021

I certify that the studies contained in this dissertation have the approval of the Committee for Research in Human Participants of the University of the Witwatersrand, Johannesburg. The ethics approval number is **M18-05-20.**

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Muzi J. Maseko (Supervisor)	Franswell T. Nyundu (Co-supervisor)
Date……..	Date……...

DEDICATIONS

This dissertation is dedicated to my mother (Nomusa Sylvia Ngema), aunt (Dudu Carol Ngema) and grandmother (Esnet Thulile Ndlovu).

In loving memory of my late father

Sboniso Wiseman Ndlovu

1974-2007

Thank you for your prayers and relentless support during the course of this degree. I am grateful.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS DISSERTATION

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 Pressure Monitoring*, *24*(3), pp.103-109.

ABSTRACT

Background: Non-dipper pattern, defined as an absence of nocturnal decline in blood pressure, is associated with an increase in cardiovascular events. Salt-sensitivity has been reported as a key contributor to a non-dipping profile. In numerous occasions, non-dipping and salt-sensitivity has been investigated in populations with health conditions such as hypertension and renal dysfunction. In this study, we investigated the impact of non-dipping on the cardiovascular system by measuring carotid-femoral pulse wave velocity (PWV) as an index of arterial stiffness, and the ejection fraction (EF) in a salt-sensitive black population of African ancestry, with no health complications.

Methods: Nine hundred and forty-eight participants were recruited from the metropolitan areas around Johannesburg. Pulse wave velocity and echocardiography measurements were measured. After 24-hour ambulatory BP measurements, 796 participants were selected because they had complete 24-ambulatory measurements. Data was categorized into normal and overweight/obese body mass index (BMI).

Results: Of the 283 participants with normal BMI, 151 were non-dippers and 132 were dippers in the study. A stepwise multiple linear regression model was fitted with the normal BMI SBP dipping percentage as the dependent variable, and PWV and EF as independent variables. After adjusting for covariates, PWV showed a positive association (r^2 = 0.13; P = 0.04) and a strong negative association was demonstrated between SBP dipping percentage and EF (r^2 = -0.23; P = 0.001).

Conclusion: An attenuated nocturnal fall in BP results to an increased in arterial stiffness and consequently left ventricular pump dysfunction in black normotensive non-dippers with normal BMI.

ACKNOWLEDGEMENTS

I would like to thank my main supervisor, Prof. Muzi Maseko and my co-supervisor Dr. Thami Nyundu for the relentless support throughout this journey. Being under your supervision has been truly an honour. I am thankful for the guidance and skills I learnt throughout this project. I am grateful for the knowledge and wisdom that you have contributed. It would not have been possible without you.

Also, I would like to thank my colleagues in the Human Nutritional Research Laboratory for the skills, support and contribution in assisting me. The volunteers who participated in this research study, many thanks to you, God bless you. To the Head of School of Physiology, Prof. Willie Daniels, I am grateful for the financial support, the constant check-ups and for challenging me professionally and personally. I would like to extend my gratitude to Dr. Hamzah Bello and Dr. Yusuf Suleiman for guidance and endless support. I would like to thank my friends Brian Nkosi, Sluleko Mkhize, Boitumello Mokwena, Cebile Xulu and Dumisani Mthembu for inspiring me to do better and for motivating me in difficult times during this degree.

MY CONTRIBUTION TO DATA COLLECTION AND ANALYSIS

I declare that I collected the 24-hour urinary and 24-hour ambulatory blood pressure data. The anthropometry, pulse wave velocity and blood data were collected by a qualified nurse, and echocardiography data was collected by an experienced observer. Blood and urine samples were taken to an accredited laboratory, National Health Laboratory Services (NHLS) for analysis. Data analysis was performed by my main supervisor and I.

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ABBREVIATIONS

2-D	two-dimension
ABPM	Ambulatory Blood Pressure Monitor
AIDS	Acquired Immune Deficiency Virus
AHA	American Heart Association
Aldo:Renin	Aldosterone-to-Renin ratio
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
BHS	British Hypertension Society
BMI	Body Mass Index
BP	Blood Pressure
β	Beta
CI	Confidence Interval
CIMT	Carotid Intima Media Thickness
CVDs	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
N:D	Night-time to Day-time Ratio
DP	Dippers
°C	Degrees Celsius
EF	Ejection Fraction
ESH	European Society of Hypertension
ECG	Electrocardiograph
HR	Heart Rate

HIV	Human Immunodeficiency Virus
IVS	Interventricular Septal Wall Thickness
LV	Left Ventricle
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
LVMI	Left Ventricular Mass Index
M-mode	Monodimensional
MMPs	Matrix Metalloproteinases
NHLS	National Health Laboratory Services
NCDs	Non-Communicable Diseases
NDP	Non-Dippers
NEFAs	Non-Esterified Fatty Acids
OSA	Obstructive Sleep Apnea
PNS	Parasympathetic Nervous System
PP	Pulse Pressure
PWV	Pulse Wave Velocity
PWT	Posterior Wall Thickness
RAS	Renin-Angiotensin System
RAAS	Renin-Angiotensin-Aldosterone System
RSBP	Night-time to Day-time Systolic Blood Pressure Dipping Ratio
RDBP	Nigh-time to Day-time Average Diastolic Blood Pressure Dipping Ratio

SD	Standard Deviation
SBP	Systolic Blood Pressure
SAS	Statistical Analysis Software
SNS	Sympathetic Nervous System
SA	South Africa
SAs	South Africans
SAHDS	South African Hypertension Diet Study
TGF-β1	Transforming Growth Factor beta 1
TOD	Target Organ Damage
USA	United States of America
WHO	World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Cardiovascular diseases

The epidemiologic transition describes changing patterns of population distributions in relation to changes of fertility, mortality, leading causes of death, and life expectancy,(McKeown, 2009) and is divided into five phases proposed by Omran (2005). In the past, infectious diseases such as tuberculosis and human immunodeficiency virus (HIV) were a leading cause of mortality (Mathers and Loncar, 2006). However, the epidemiologic transition showed a shift from infectious diseases being the leading cause of mortality and morbidity to the predominance of non-communicable diseases (NCDs) such as cancer, diabetes mellitus, chronic respiratory diseases as well as cardiovascular diseases (CVDs) having substantial increase in morbidity and mortality rate (Yusuf *et al*., 2001; Mathers and Loncar, 2006).

Every country or region in the world is at a different phase of the epidemiologic transition (McKeown, 2009). According to a report by the 2014 World Health Organization (WHO) on NCDs (chapter 1, page 11 Figure. 1.4), black people from Africa have the second highest probability of dying from one of the main NCDs. This report places Africa in the first and second stage of the epidemiologic transition whereby a long-term shift occurs in mortality and disease patterns involving pandemics of infection being gradually displaced by degenerative diseases as the main form of primary cause of death and morbidity. The report also demonstrated that South Africans (SAs) have a 25% probability of dying from NCDs (chapter 1, page 11 figure 1.5a), indicating an increase in the prevalence of CVDs such as coronary and congenital heart disease, cerebrovascular disease, peripheral arterial disease.

It has been estimated by the WHO's Global Heart Initiative (2016) that CVDs are the leading cause of deaths and disabilities worldwide, accounting for 17.9 million deaths per year which makes up 31% of total global deaths. In South Africa (SA), CVDs are the leading cause of death after acquired immune deficiency syndrome (AIDS), and more people die from CVD than all cancers combined (The Heart and Stroke Foundation of South Africa, 2016). Due to urbanization, individuals with risk factors such as hypertension, dyslipidemia, obesity, and diabetes have been reported to be at a higher risk of CVDs (Yusuf *et al*., 2001; Steyn *et al*., 1991). Other risk factors that are linked to CVDs include an unhealthy diet, tobacco smoking and lack of physical activity (WHO, 2014). According to Yusuf et al. (2001), aspects linked to CVD can be classified into two categories: risk factors and risk markers. Risk factors are those that have been proven to be casual. Risk markers are those that show association with CVDs but for

whom a cause and effect association is yet to be proven (Yusuf *et al*., 2001). Risk markers that show association with CVDs include psychological factors (depression, stress), low socioeconomic status, and breakdown in social structures such as loss of cohesion and social support (Yusuf *et al*., 2001). Risk factors that are causally linked to CVD include elevated low-density lipoprotein, tobacco consumption, low high-density lipoproteins, elevated blood glucose, obesity, physical inactivity, diet and high blood pressure (BP) (Yusuf *et al*., 2001). Amongst these risk factors, hypertension has a substantial impact on the burden of CVD worldwide. Hypertension has been estimated to cause about 12.8% total deaths worldwide which equates to 7.5 million deaths (WHO, 2014).

1.2 Overview of hypertension

Blood pressure is defined as lateral pressure exerted by the blood on the wall of the blood vessels while flowing through them (Khurana, 2008). Normal systolic blood pressure (SBP) is 120 mm Hg (range: 110-130 mm Hg) and it is the maximum BP during the ventricular systole. Diastolic blood pressure (DBP) is 80 mm Hg (range: 70-90 mm Hg) and it is the minimum pressure during ventricular diastole. According to the European Society of Hypertension (ESH), individuals with a BP ≥140/90 mm Hg are regarded as hypertensive (McComack *et al*., 2019). Hypertension can be classified as either primary or secondary. According to the Centre for Disease Control and Prevention report (2020), 90% of all cases are primary hypertension, with no known cause. The other 10% of cases are secondary hypertension, and this type of hypertension results from other known factors such as obesity, high salt intake.

Even though the exact cause of primary hypertension is not known, there are various risk factors that have been correlated with the condition. These risk factors are also correlated with other CVDs (Boutayeb, 2006) and can be categorized into non-modifiable and modifiable risk factors. The non-modifiable risk factors are characteristics in the individual that cannot be adjusted or changed (British Heart Foundation, 2020). These risk factors include race, family history, sex, age genetic composition (WHO, 2014). On the other hand, modifiable risk factors of primary hypertension are those lifestyle patterns that can be adjusted or changed to avert the development of the disease. These modifiable risk factors include lack of exercise, obesity, high fat diet, excessive salt intake, alcohol consumption and tobacco use (Butta *et al*., 2005). According to WHO (2014), Africa has the highest prevalence of hypertension and approximately over 46% of

African adults have hypertension. To add to this problem, more than 50% of people with hypertension are unaware of their condition (Steyn *et al*., 2001).

Non-pharmacological approaches such as consumption of a healthy diet and increased physical activity to the reduction of BP are generally recommended as the preliminary approach for the treatment of hypertension (Vamvakis *et al*., 2017). Patients who do not show any reduction in BP after four to six months are directed to drug therapy (Brown and Bussell, 2011). Of the people diagnosed with hypertension, only a third is on treatment, and of those, only a third has adequate control of their BP (Steyn *et al*., 2001). This makes it apparent that there is poor management of hypertension. In addition to poor management of hypertension, there is still controversy on which BP measuring technique/device is reliable for the diagnosis of hypertension (O'Brien *et al*., 2003). There are three BP measuring techniques stated by the ESH i.e. auscultatory, automated and ambulatory BP monitoring/measurement (O'Brien *et al.*, 2003). Of the three, the auscultatory technique of measuring BP is considered the conventional way to measure BP. However, due to the controversy of the accuracy of auscultatory technique, efforts were made to improve the technique with automated devices. Simultaneously, awareness of the white-coat hypertension, defined by the elevation of BP caused by the presence of a medical doctor, led to development of methods that provide profiles of BP behaviour outside clinical settings i.e. ambulatory technique (Franklin *et al.*, 2013).

1.3 Diagnosis of hypertension

1.3.1 Conventional BP measuring technique

Manual BP measuring technique entails the use of a mercury sphygmomanometer and a stethoscope for auscultation. For decades, the use of these devices has been regarded as the conventional BP measuring technique and it remains as such (Ogedegbe and Pickering, 2010). However, this technique has three important limitations. Firstly, BP is not static but distinguished by a significant degree of variability over a 24-hour period, as a result of both diurnal fluctuations and behaviour-induced changes (Mancia and Grassi, 2000). At best, only three measurements are taken at frequent intervals and this cannot recognize an individuals' 24-hour BP variation (O'Brien *et al*., 2013). Secondly, conventional BP is associated with white-coat hypertension (Pickering *et al*., 2006 and Mancia *et al*., 1987). The elevation of BP in the presence of a physician leads to wrong diagnosis and consequently needless management which

could be detrimental to the individual's health. Thirdly, the conventional technique cannot be used to diagnose masked hypertension i.e. the patient's clinic or office BP is normal, but home BP measurements are elevated (≥140/90 mm Hg) (Mancia *et al*., 2009; Papadopoulos and Makris, 2007). As a result of these limitations, the value of conventional BP in predicting CVD in an individual is poor and it may not reflect individuals true BP's. This led to the development of automated alternatives to the mercury sphygmomanometer.

1.3.2 Automated BP measuring technique

Automated techniques employ oscillometric measurements and electronic calculations rather than auscultation. Compared with conventional BP measurement, these newer techniques are more duplicable and not subject to observer bias or digit preference (Staessen *et al*., 2000). For this reason, automated techniques are slowly replacing conventional BP measurement. However, the introduction of automated BP devices in clinical practices has come with new predicaments. Questions such as: how many measurements are needed to approximate a patient's normal BP, what are normal values for the automated BP devices, how do anti-hypertensive treatment affect automated BP devices, and to what extent can automated BP devices predict prognosis? (Verbek *et al*., 2005). In addition, concerns have been raised about the accuracy of oscillometric devices. When the device is calibrated, it is accepted that a Grade A British Hypertension Society (BHS) validated oscillometric device may have an error of >15 mm Hg for 5% of the time (Cao *et al.*, 2015). Putting this into a clinical setting, an error of 10 mm Hg could possibly lead to the misclassification of 100,000 adults in terms of their BP status (Medicines and Healthcare Products Regulatory Agency, 2003).

One of the major limitations of using the oscillatory method occurs when a patient has a cardiac arrhythmia (improper beating of the heart) and therefore a large variation in BP from beat to beat (Ogedegbe and Pickering, 2010). In atrial fibrillation, defined as rapid heart rate (HR), for example, stroke volume increases and therefore BP varies, depending on the previous pulse interval, and the oscillatory method is not able to notice this variation (Beevers *et al*., 2001). It is for this reason that the Medicines and Healthcare Products Regulatory Agency recommendations state that where use of oscillometric devices is not clinically appropriate, other methods should be used to measure the BP. Therefore, the ambulatory technique was developed as an alternative to amend the limitations and questions arising from the use of mercury sphygmomanometer and automated BP techniques.

1.3.3 Ambulatory BP measuring technique

Ambulatory BP monitoring is a technique used to measure BP over a 24-hour period, whilst living a normal daily life. The technique uses an automated monitor and a cuff. The monitor is programmed to inflate after specific time intervals. The device records and stores all BP measurements and computer software is used to access those (O'Brien *et al.*, 2013). Ambulatory blood pressure monitoring provides a full 24-hour profile on BP eg. summary statistics for the overall 24-hour period, day-time and night-time BP. Other parameters include HR, BP variability, average BP, pulse pressure (PP), load index, BP load, reduction/dipping percentage of BP, the distribution pattern of BP, and trough/peak ratio.

1.4 Benefits of using ambulatory blood pressure monitor

1.4.1 Diagnosis of hypertension

Although conventional BP is common in the diagnosis of hypertension, it may not mirror the true BP levels. Ambulatory BP monitoring overcame the limitations of conventional BP, allowing intermittent BP measurement during an individual's normal daily activities. The use of ambulatory BP monitor (ABPM) also eliminates the white coat effect, can identify masked hypertension, early morning hypertension, and excessive variability, which correlate with target-organ damage (TOD) and CVDs outcomes (O'Brien, 2011). In addition, clinical trials have shown that ABPM values correlate more closely than conventional BP with hypertension-associated TOD (Ohkubo *et al.*, 2000 and Staessen *et al.*, 1999). Thus, the use of ABPM is expanding in clinical trials and practice, as it provides a closer association to prognosis than conventional BP measurements (O'Brien *et al.*, 2013). In addition, ABPMs are deemed to be a leading method to quantify or assess BP levels and the diagnosis of hypertension. For these reasons', clinicians are steadily moving towards the use of ABPMs (O'Brien, 2003; Redon and Lurbe, 2014).

1.4.2 Prediction of CVD events using ABPM

In a study conducted by Dolan et al. (2005), it was shown that ABPM better predicted a five-year cardiovascular risk by using night-time SBP, night-time DBP, and day-time SBP. In addition,

PP, which represents the force that the heart generates each time it contracts; has been associated with CVDs (Buda *et al.*, 2018). Other studies have reported that average 24-hour PP is an independent predictor of cardiovascular mortality and morbidity in seemingly healthy subjects with essential hypertension (Verdecchia *et al*., 2001). Nakano et al. (2005) further advocated the association of 24-hour PP with cardiovascular events in type 2 diabetic patients.

Another aspect of the importance of ABPM is that it provides a prognostic value in cerebrovascular events (Eguchi *et al*., 2012). Of the various ABPM parameters, the night-time BP has been found to be a substantial predictor of silent cerebrovascular events (Eguchi *et al*., 2009). Kario et al. (2003) showed that elderly subjects with morning surge of BP had an increased occurrence of multiple cerebral infarcts. Apart from these benefits, the overriding significance of ABPM comes with its ability to give the circadian change of BP (Hermida, 2007). This has enabled clinicians to give the correct diagnosis on the type of hypertension the patient has and consequently, the right and appropriate treatment.

1.4.3 Circadian BP rhythm: diurnal and nocturnal BP measurements using ABPM

Blood pressure follows a biological clock called the circadian rhythm. The BP circadian rhythm is influenced by various physiological factors (hormones, autonomic nervous system, and temperature) over a 24-hour period. These physiological factors affect BP differently during the night (nocturnal) and during the day (diurnal) leading to BP variability which can only be recorded by the ABPM (Morris *et al*., 2012).

Blood pressure fluctuates over a 24-hour sleep-wake cycle, with values falling after midnight and rising in during the day. Blood pressure normally decreases at night-time by at least ≥ 10% of SBP compared to that of day-time, this phenomenon is known as nocturnal BP dipping and this change can only be observed through the use of an ABPM (Sherwood *et al*., 2019). The importance of nocturnal BP dipping is that it reduces hemodynamic load placed on the heart, arteries, and kidneys for prolonged periods (Brian *et al*., 2017).

Investigation in the area of hypertension control has focused progressively on the clinical relevance of nocturnal BP dipping in predicting TOD and improving the control of BP

(Routledge *et al*., 2007; Verdecchia *et al*., 2001). The prognostic value of nocturnal BP dipping was documented in a novel cohort study that had 10 populations by Fan et al. (2010).

1.5 Clinical significance of nocturnal BP dipping

For decades there has been a lot of controversy in the definition of nocturnal BP dipping. Some authors considered night-time mean arterial pressure (MAP) (Friedman and Logan, 2009), some considered night-time SBP (Myers *et al*., 1999) while others used night-time and day-time SBP/DBP (Mohamed *et al*., 2003) overall as a measure of dipping at night between 00:00-02:00am. However, the American Heart Association (AHA) recommends the use of average night-time and day-time SBP in defining nocturnal BP dipping (Kairo, 2019). In addition, for an individual to be considered a dipper, the average night-time SBP must be 10%-20% lower than day-time SBP (Myers *et al*., 1999; O'Brien *et al*., 2003; Bloomfield and Park, 2015).

The nocturnal SBP dipping percentage is calculated as (1- average night-time SBP/average day-time SBP) x 100 and based on this percentage, nocturnal BP dipping is classified into four categories (Hermida *et al*., 2013; Kairo, 2019). Non-dippers are those individuals who show a decrease of SBP within the range of 0-9.9%. Dippers are individuals with a decrease in SBP between 10-20% and extreme dippers are individuals who show a SBP decrease of >20% (Hermida *et al*., 2013). There are individuals who exhibit a decrease of < 0% in SBP and are called reverse dippers. Although reverse dipping has adverse complications compared to other categories, its prevalence is relatively low compared to non-dipping (Cuspidi *et al*., 2017; Yan *et al*., 2015). As a consequence, non-dipping has been, at most, the main area of focus in research because of its high prevalence and association with CVD and TOD.

Over the years, the decrease of BP at night has been investigated across different populations with or without health complications concerning CVD. Numerous studies reported a correlation between the absence of nocturnal BP dipping (non-dipping) and cardiovascular risk (O'Brien *et al*., 2013; Boggia *et al*., 2007; Sega *et al*., 2005).

1.6 Non-dipping BP

Non-dipping of BP is detrimental as it is a risk factor for TOD and CVD. The mechanism/s by which non-dipping BP occurs remains unclear. However, a number of factors have been identified as possible mechanisms fundamental for the deficits in nocturnal BP dipping. According to studies, non-dipping occurs to be impacted by psychological factors, quality of sleep, obesity and autonomic nervous system (ANS) dysfunction (Fallo *et al*., 2002; Tun *et al*., 1999; Portaluppi *et al*., 1990).

1.6.1 Potential physiological mechanisms that lead to non-dipping

1.6.1.1 Psychological factors and poor sleep quality as determinants of non-dipping

Non-dipping is relatively more prevalent among individuals with various disease states (Loredo *et al*., 2004). However, it may also occur among healthy individuals, and stress along with poor sleep quality has been implicated as a cause (Fallo *et al*., 2002). According to Mezick et al. (2010), non-dipping is associated with high hostility and low life purpose. Similarly, Fallo et al. (2002), found a significant relationship between stressful life circumstances such as divorce and non-dipping. These factors i.e. stress and high hostility, are considered as psychological illnesses. In response to these psychological illnesses, stress hormones are released (e.g. cortisol, epinephrine and norepinephrine) which affect sleep quality. Cortisol is an adrenocorticotropic hormone that causes vasoconstriction and increases BP to enhance the delivery of oxygenated blood (Nussey and Whitehead, 2013). In addition, excitation of the sympathetic nervous system (SNS) releases epinephrine and norepinephrine that results in the elevation of BP (Nussey and Whitehead, 2013). The synergy of a prolonged physiological response exerted by these hormones ultimately results in hypertension. When these events occur during the night-time, nocturnal hypertension occurs and consequently non-dipping ensues.

Numerous studies have documented the association between nocturnal BP dipping and quality of sleep (Sachdev and Weder, 2006; Fabiana *et al*., 2012; Loredo *et al*., 2004; Pickering and Kairo, 2001). It has been shown that sleep is associated with changes in body temperature, SNS and cerebral activity as well as hormonal secretions (Baker *et al*., 2001; Morris *et al*., 2012). Good quality of sleep has been shown to associate with a decline in BP at night i.e. dipping (Tun *et al*., 1999). Physiological mechanism that can explain this, is that good quality of sleep favours the parasympathetic nervous system (PNS) thereby decreasing cardiac output and peripheral resistance (Sherwood *et al*., 2011). In addition, suppression of the SNS decreases the level of catecholamines (epinephrine and norepinephrine), decreasing nocturnal BP thus favouring dipping. However, with poor quality of sleep, the SNS activity is increased leading to high levels of catecholamines, which increase HR and nocturnal BP thus favouring non-dipping. In obese patients with obstructive sleep apnoea (OSA) syndrome, a relatively high sympathetic tone or an increased concentration of circulating catecholamines are likely operatives in the non-dipping pattern observed with these conditions (Birkenhäger and Meiracker, 2007).

1.6.1.2 Obesity and non-dipping

Obesity is a well-recognized risk factor for the development of hypertension, especially visceral obesity (Jian *et al*., 2016; Davy and Orr, 2009). There is a significant similarity between the mechanism/s of obesity-induced hypertension and non-dipping considered in different studies (Fabbian *et al*., 2013; Kang *et al*., 2013; Kanbay *et al*., 2008). Pathological factors of increased BP in obese individuals that may play role in impaired nocturnal BP fall include: OSA and SNS activation (Tun *et al*., 1999; Suzuki *et al*., 1996).

Obesity is considered as a major risk factor for the development of OSA (Fritscher *et al*., 2007; Peppard *et al*., 2000; Wolk *et al*., 2006). This has been documented by previous studies (Morrison *et al*., 2007; Rudnick *et al*., 2007). It has been shown that due to obesity OSA may be aggravated because of fat deposition in specific sites (Schwartz *et al*., 2008). In obese individuals, there is fat deposition in tissues surrounding the upper respiratory airway resulting in a small lumen and increase in collapsibility of the upper airway, predisposing to apnea (Schwab *et al*., 2003). In addition, fat deposits around the thorax reduce chest compliance and functional residual capacity and may increase oxygen demand (Maholtra and White, 2002). The physiological feedback following increase in oxygen demand is stimulation of the SNS activity leading to increase HR and BP (Dubielski *et al*., 2016).

Evidence has shown increased SNS activation in obese patients (Grassi *et al*., 1995). Potential factors that have been proposed to contribute to an increase in SNS activity in obesity include an increased RAAS activity (da Silva *et al*., 2009; Wang and Sherer, 2008). It has been documented that adipose tissue contains components of the renin-angiotensin system (RAS) (Cassis, 2000). In obese individuals, the production of RAS components is exacerbated, thus contributing to RAAS and its consequences such as an increase in SNS activity through angiotensin II. In addition, activation of RAAS seems to play a crucial role in the cause of obesity related hypertension by stimulation of aldosterone and direct effects on the kidney to promote increase Na^+ retention (de Paula *et al*., 2004; Hall *et al*., 2003).

1.6.1.3 Salt-sensitivity and non-dipping

Salt-sensitivity is defined as a change in BP in response to a change in NaCl intake (Dong, 2018). An increase of 5-10% BP or > 4mm Hg in mean BP are two commonly used cut-off values in response to a change in NaCl intake (Felder *et al*., 2013; Sanada *et al*., 2011). A study conducted in hypertensives that were maintained on a high or low NaCl diet for one week, found that in the salt-sensitive group that was on either a high and low NaCl diet, the dipping pattern was diminished compared to the salt-resistant group (Uzu *et al*., 1996). These findings were consistent with Higashi et al. (1997), where they confirmed that the change in BP at night was lower in the salt-sensitive compared to salt-resistant patients during high NaCl intake. Although mechanisms of diminished nocturnal BP dipping in salt-sensitive individuals remain unclear, studies have suggested that impaired renal excretory capacity is responsible for this occurrence (Kimura, 2001; Kimura and Brenner, 1997). Salt-sensitivity is controlled by the kidneys and BP characterized by high salt-sensitivity indicates impaired renal function (SA Majid *et al*., 2015). When NaCl intake is excessive in patients with salt-sensitive hypertension, the defect of Na^+ excretory capability becomes evident, resulting in elevated BP during night- time (Kimura *et al*., 2010). This nocturnal hypertension compensates for impaired natriuresis during day-time and enhances pressure natriuresis during night-time thus leading to the manifestation of a non-dipper BP pattern. Apart from salt-sensitivity, other studies have suggested that non-dipping is associated with autonomic dysfunction (Kohara *et al*., 1995; Nakano *et al*., 2001; Portaluppi *et al*., 1990).

1.6.1.4 Autonomic dysfunction is associated with non-dipping

A study reported that the dysfunction in the ANS is associated with non-dipping is (Portaluppi *et al*., 1990). The generally accepted mechanism is related to the imbalance between the SNS and parasympathetic nervous system (PNS) tone. Specifically, failure of the ANS to shift from sympathetic to parasympathetic during night-time. In patients with essential hypertension, it has been shown that a decrease in parasympathetic and an increase in SNS may contribute to the occurrence of the non-dipping phenomenon (Kohara *et al*., 1995; Nakano *et al*., 2001). It has been shown that an abnormal sympathetic versus vagal balance plays an important role in the development of a decline in nocturnal BP dipping in hypertensives (Hojo *et al*., 1997). A study reported that non-dippers have a relatively low sympathetic nervous tone and decreased PNS activity during day-time (Ragot *et al*., 1999). As a consequence, the

abnormality in the ANS could impact vascular tone and HR thus resulting in blunted night-time BP decline.

Evidently, as hypertension is associated with CVD, non-dipping has also been found to result in cardiovascular TOD such as increased arterial stiffness and, left ventricular dysfunction (Saeed *et al*,. 2014; Cicek *et al.*, 2013; Ajayi *et al*., 201; Schutte *et al*., 2011).

1.7 Non-dipping is associated with cardiovascular target organ damage

1.7.1 Non-dipping results in arterial structural changes

Many studies have, and continue, to investigate the association between carotid intima-media thickness (CIMT) and non-dipping because CIMT has been validated to be a predictor of atherosclerosis and CVD events (Lorenz *et al*., 2018). In the optimizing participant experience and response to topical analgesic (OPERA) study, non-dippers had the highest CIMT compared to dippers after correcting for confounding variables (Vasunta, 2012). Consistent with the OPERA study, Atabek et al. (2014) found an inverse correlation between CIMT and dipping. They concluded that increased CIMT and impaired SBP dipping may indirectly predict the degree of atherosclerosis. In a study by Lee et al. (2011) these findings were further substantiated where an increased in CIMT was observed in non-dippers with type I diabetes. In addition, similar findings were noted in newly diagnosed hypertensives (Chatzistamatiou *et al*., 2012). Different to these studies, Saeed et al. (2014) investigated young and middle-aged stroke survivors (15-60years). This study found an increased in pulse wave velocity (PWV) in the individuals; indicating an early aortic stiffening that was nearly three-fold more common in non-dippers. In a cross-sectional study with untreated hypertensive patients, Cicek et al. (2013) found an independent association between diminished nocturnal BP decline and PWV. In a follow-up study by Gallineo et al. (2018) in hypertensive patients undergoing chronic hemodialysis with a non-dipping pattern, PWV was independently associated with cardiovascular mortality. Similar findings were observed in a study of patients with Behcet's disease (Celik *et al*., 2015). From these studies, it is evident that non-dipping is associated with arterial TOD in individuals with pre-existing health conditions as the ones mentioned. To the best of my knowledge, no study has investigated the effects of non-dipping on PWV in a generally healthy population.

1.7.2 Non-dipping results in left ventricular remodelling and dysfunction

In previous studies, echocardiographic investigations for research and clinical purposes in patients with hypertension have reported a variety of cardiac alterations such as left ventricular hypertrophy (LVH) and systolic dysfunction. Evidence suggests that hemodynamic stress plays a major role in the development of LVH (Cuspidi *et al*., 2010). A large body of evidence indicates two pathological processes that changes the myocardial structure in hypertensive patients leading to LVH (Cuspidi *et al*., 2006; Rossi, 1998). These processes are referred as myocyte hypertrophy and interstitial fibrosis. In hypertensive LVH, unbalanced increase in fibrous tissue and myocyte volume components are responsible for the myocardial texture and function (impaired systolic shortening) (Díez, 2009). Long-term pressure load plays a key role in the overexpression of fatal isoforms of contractile proteins such as β-troponin, β-myosin heavy chain and α-chain, which is indicative of myocyte hypertrophy (Cuspidi *et al*., 2010).

Studies have investigated non-dipping, 24-hour BP variability with LVH in different populations with different health conditions. Of particular interest, in black population these parameters have also been investigated. However, due to limitations such as small sample size (Mezue *et al.*, 2016), participants with medical condition (Ajayi *et al.*, 2011; Woodiwiss *et al.*, 2018), use of measuring techniques that are not golden standard (Schutte *et al.*, 2011) observed in the studies, there is still a need for more research in profiling BP dipping status in order to ascertain cardiovascular outcomes in individuals with non-dipping status. A study conducted in hypertensive black Nigerians found an association between LVH and non-dipping, indicating cardiac dysfunction (Ajayi *et al*., 2011). This finding is consistent with other studies that have been conducted in individuals with known, untreated and controlled hypertension (Sorof *et al*., 2002; Abdel-Rheim *et al*., 2016; Woodiwiss *et al*., 2018).

Considering the amount of extensive research on hypertension related CVDs, cardiac remodelling is more prevalent in hypertensives compared to normotensives, thus the presence LVH is expected in hypertensives. Interestingly, few studies have shown LVH that exists in normotensive non-dippers (Mezue *et al*., 2016; Schutte *et al*., 2011). In a black Jamaican population of 23 normotensives, Mezue et al. (2016) showed an increase in concentric remodelling, concentric and eccentric hypertrophy in the left ventricular geometry of non-dippers. However, the sample size of this study was relatively small to be considered a representative sample. This calls for larger studies to confirm these findings. In close relation to

the current population of interest i.e. black South Africans (SAs), a study conducted in . dorsalis pedis artery segment was measured. Lastly, echocardiography is highly recommended and is the golden technique in examining cardiac geometry (Lang *et al*., 2015), however they used electrocardiography (ECG) to measure LVM.

1.7.3 Rationale

Evidence suggests that salt-sensitivity is more prevalent in black individuals compared to whites (Lindhorst *et al*., 2007; Richardson *et al*., 2013; Wright *et al*., 2003). Of importance to this study, Maseko et al. (2006) showed that black South Africans that we investigated in the current study possess the salt-sensitivity trait. To the best of my knowledge no study has investigated the non-dipping pattern and its implications on cardiovascular TOD in a black population of African ancestry.

1.8 Objectives

In a salt-sensitive black population of African ancestry we:

1. determined the impact of obesity on nocturnal BP dipping
2. investigate the relationship between 24-hour urinary electrolytes excretion rate and nocturnal BP dipping
3. determined the relationship between nocturnal BP dipping and PWV as a marker of arterial stiffness
4. determined the impact of an attenuated decline in nocturnal BP on EF as a marker of left ventricular function

CHAPTER TWO: METHODS

2.1 Study participants

The present study was conducted in accordance with the guidelines outlined in the Helsinki Declaration. The study was approved by The University of the Witwatersrand Committee for Research on Human Subjects (Medical) (approval number M18-05-20). The study forms part of ongoing study of the South African Hypertension Diet Study (SAHDS). The study design has been described previously (Maseko *et al*., 2006). I randomly recruited 948 participants from the metropolitan areas of Johannesburg (Soweto, Daveyton and Vosloorus). Participants gave informed, written consent to participate in the study. South Africans of black African ancestry from different tribes, Zulu, Venda, Sotho, Pedi, Swati, Tswana, Xhosa, Tsonga and Ndebele with SA ancestral background tracing back from grandparents were randomly recruited in Johannesburg and the surrounding areas and townships. The minimum age for participating was 18 and there was no upper age limit. No participants of mixed, Asian, or European ancestry were recruited, and no Khoi-San participants were recruited. Any participant that suffered or had been previously diagnosed with cardiovascular, pulmonary or renal problems, chronic physical or psychological disorders, diabetes or any recurring illnesses, taking any chronic medication, pregnant or on any supplements was excluded from the study. Of the 948 participants, 796 (399 males, 457 females) were selected because they had complete a 24-hour ABPM and 24-hour urine samples collected.

2.2 Site where measurements were conducted

The measurements were carried out at the SAHDS facility at the School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg, South Africa. Participants were transported to the SAHDS Laboratory on Monday, Wednesday and Fridays, which are the designated clinic days between 08:00-15:00, during which time all the required measurements were carried out.

2.3 Clinical, demographic and anthropometric measurements

A standardized questionnaire was administered, to obtain demographic and clinical data. The questionnaire was made available in English, but trained study assistants (Mercy Mashao, Edgar Phukubje) familiar with the home (first) languages assisted with the completion of each

questionnaire. Only same-sex assistants were used to assist each participant with the completion of the questionnaire. Assistance was only provided when requested. Nonetheless, the majority of participants were reasonably proficient in the English language (primary and secondary education is largely conducted in the English language). The questionnaire sought specific answers to date of birth, gender (designated as either male or female), past medical history, including the presence or absence of hypertension, diabetes mellitus, renal disease, prior cardiovascular events, such as myocardial infarction, stroke, and angina pectoris. Past use of medications (analgesics), antihypertensive use (pills used to lower BP) and pills used to lower blood sugar were also evaluated. Cigarette smoking history was assessed as either smoked in the past or smoking presently. Previous and present history of the consumption of alcoholic beverages (beer, traditional beer, or any form of alcohol and daily quantity) was assessed. Caffeine consumption (number of cups of coffee), frequency of physical activity and family history of hypertension, diabetes mellitus, or heart disease was also recorded. For female participants, the histories of monthly menstrual flow, previous or present use of contraceptive pills, and previous history of past pregnancy, were also recorded. Most of these questions required a simple response of either –YES‖ or –NO‖ as answers. If there was any uncertainty as to the answers given to the questions asked at the initial visit, then this information was requested during the next visit (collection of ABPM and 24-hour urine samples).

2.4 Anthropometry

Body weight was measured using an adult weighing scale and approximated to the nearest 0.5 kg with participants wearing indoor clothes with no shoes. Height was measured using a Stadiometre and expressed in metres. Body mass index (BMI) was then derived from weight and height as weight in kg, divided by height squared (kg/m^2), and this was employed as a marker of adiposity. Obesity was defined as a BMI of ≥ 30 kg/m^2 and overweight as a BMI between $\geq 25 < 30$ kg/m^2.

2.5 Blood sampling analysis

After an overnight fasting period of 12 hours in all participants, a trained nurse-technician obtained blood samples from antecubital vein from the participants lying in a supine position.

Samples were collected in potassium ethylenediaminetetraacetic acid tubes and processed at room temperature until centrifugation, carried out for 15 minutes. After cell removal, plasma was rapidly frozen and kept at -20℃. After collecting a substantial number of samples (~100), samples were taken to an accredited National Health Laboratory Service (NHLS) to measure plasma aldosterone (ng/dl), renin (mU/l) and leptin (ng/ml) concentrations. Aldosterone-to-renin ratio was calculated with aldosterone as a numerator expressed in ng/dl and renin as a denominator expressed as mU/l (Lonati *et al*., 2014). The hormone concentrations were naturally log transformed, because none appeared to be normally distributed (Hussain *et al*., 2014). Diabetes mellitus or an abnormal blood glucose control was defined as use of insulin or a glycated haemoglobin value > 6.1% (Bennett *et al*., 2007).

2.6 BP measurements

BP and HR were measured by a trained nurse-technician using OMRON HBP-1300 Professional BP Monitor (OMRON Corp., hiokoji Horikawa, Kyoto Japan) that is calibrated to meet the standards of the mercury sphygmomanometer. Five consecutive BP readings were obtained, at least 30 seconds apart in a sitting position after 10 minutes of rest using an appropriately sized cuff for each of the participants, and the average of the five readings was subsequently employed for statistical analysis. Standard cuffs were used with an inflatable bladder with a length of 22 cm and a width of 12 cm, except when arm circumference exceeded 31 cm larger cuffs with a 31 x 15 cm bladder were employed. Participants were considered to be hypertensive if they had a systolic BP of ≥ 140 mm Hg and/or a diastolic BP of ≥ 90 mm Hg.

2.7 Ambulatory measurements

Twenty-four-hour ambulatory BP monitoring was performed using oscillometric monitors (Spacelabs,model 90207 A). The most commonly used (ABPM) is the SpaceLabs . Its accuracy has been validated by the official BHS and the United States Association for the Advancement of Medical Instrumentation protocols for validating the accuracy of automated BP devices. Ambulatory monitors were calibrated monthly against a mercury manometer. The non- dominant arm was selected for BP monitoring. Standard cuffs with an inflatable bladder suitable

for the participant's arm circumference were used. Participants were shown how to fit, remove and switch on the monitor should they need to take a bath. The monitors were programmed to measure BP at 20-minute intervals from 06:00 to 22:00 and at 30-minute intervals from 22:00 to 06:00. Participants kept a diary card for the duration of the recordings to note the time of going to bed in the evening and time of waking-up in the morning. These times were used to determine the wake and sleeping periods. Participants also recorded the time when they took medication, smoked, drank caffeine or alcohol, depending on which was applicable to each participant. Participants were asked to pursue their normal daily activities but not to participate in any activity that involved intense physical activity during the 24-hour period, and to keep the cuff arm steady during measurements. On completion of the recordings, data were transferred to a computer for analysis using Spacelabs.Ink software. Participants enrolled in the study whose ambulatory BP measurements did not meet the pre-specified quality criteria (if there were less than 10 readings for day-time and night-time each) were excluded from the analysis. These approaches are standard approaches and have been published by AHA (Muntner, 2019). The equation documented by Kairo et al. (2019) was used to determine dipping % i.e. dipping % = (1-average night-time SBP/average day-time SBP) x 100. However, for statistical analysis only the average night-time SBP and average day-time SBP ratio was used to classify dippers and non-dippers. Dippers were defined as participants whose night-time to day-time dipping ratio of SBP was equal to and between ≥ 0.8 and ≤ 0.9. Non-dippers were defined as participants whose night-time to day-time dipping ratio of SBP was > 0.9 and ≤ 1. When these ratios are inserted in the above equation and converted to a dipping %, the classifications documented by Hermida et al. (2013) are evident and applicable.

2.8 Urinary electrolytes excretion rates

Timed urine samples were obtained over a 24-hour period. Two 3 litre containers were issued to the participants to collect urine over a 24-hour period. An accredited NHLS laboratory measured the concentrations and calculated the excretion rates of twenty-four hour Na^+, K^+, Mg^{2+} and Ca^{2+}. Excretion rates were 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. Twenty-four hour urine creatinine and urine volume results were not reported in this study. Samples with urine volumes < 300 mL/day were also assumed to be incomplete urine

collections. These approaches were previously used (Maseko *et al.*, 2006; Muzi *et al.*, 2018).

2.9 Aortic hemodynamics

After participants had rested for 15 minutes in the supine position, the waveform at the radial (dominant arm) pulse was recorded by applanation tonometry during an 8-seconds period using high-fidelity SPC-301 micromanometer (Miller Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, Version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) (see Figures 2.1). The pulse wave was calibrated by manual measurement (auscultation) of the brachial BP, taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphymoCor software (see Figures 2.1). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal or were less than 80 Mv were discarded. All measurements were made by a single experienced technician, unaware of the clinical history of the participants and with a low degree of intra-observer variability and a high degree of reproducibility.

Aortic PWV was measured from the sequential waveform measurements as described previously (Shiburi *et al*., 2006). Pulse wave transit time i.e. the duration taken for the wave to travel from the carotid to the femoral site, was determined as the difference between the time taken to generate the femoral and the carotid pulse waveforms, as shown in Figure 2.2. To assess differences in time of the generation of the carotid and femoral pulse wave form, a 3-lead ECG was performed simultaneously with pulse waveform sampling. The time delay in the pulse waves between the carotid and femoral locations was determined using the R wave of the ECG as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which pulse wave travels was determined as difference between distance from the femoral sampling site to the suprasternal notch (site B), and the distance from the carotid to the suprasternal notch is termed (site A). Pulse wave velocity was calculated as a distance in meters divided by the transit time in seconds. Values of PWV >10 m/sec were considered as an elevated aortic PWV (Van Bortel *et al*., 2012).

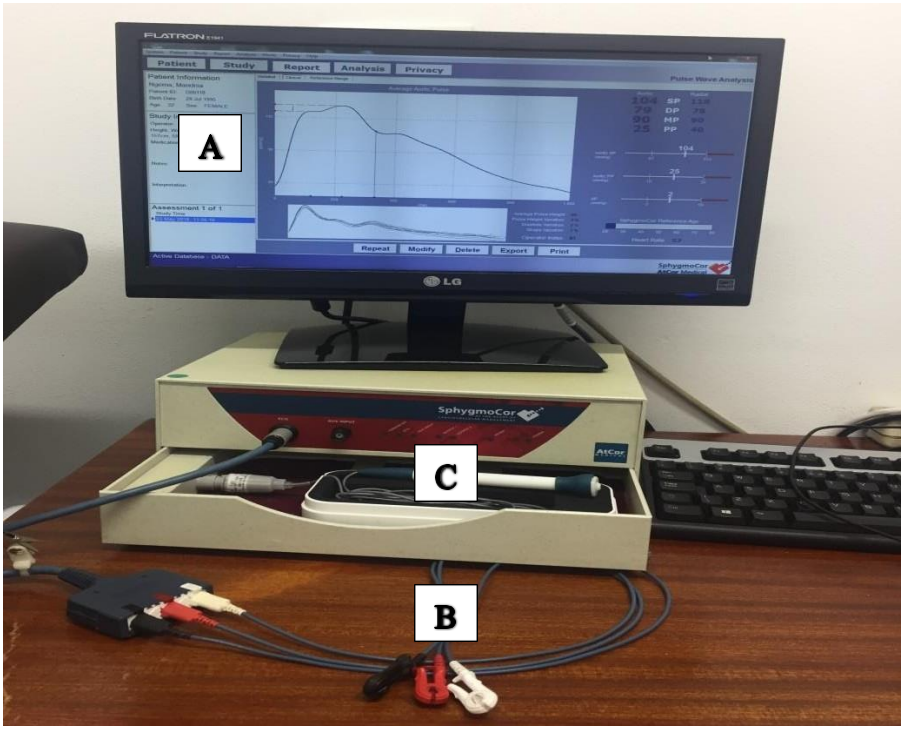

Figure 2.1: SphygmoCor device employed to determine aortic hemodynamics. A is the SphygmoCor machine, B are electrocardiogram leads, and C is the pulse pen.

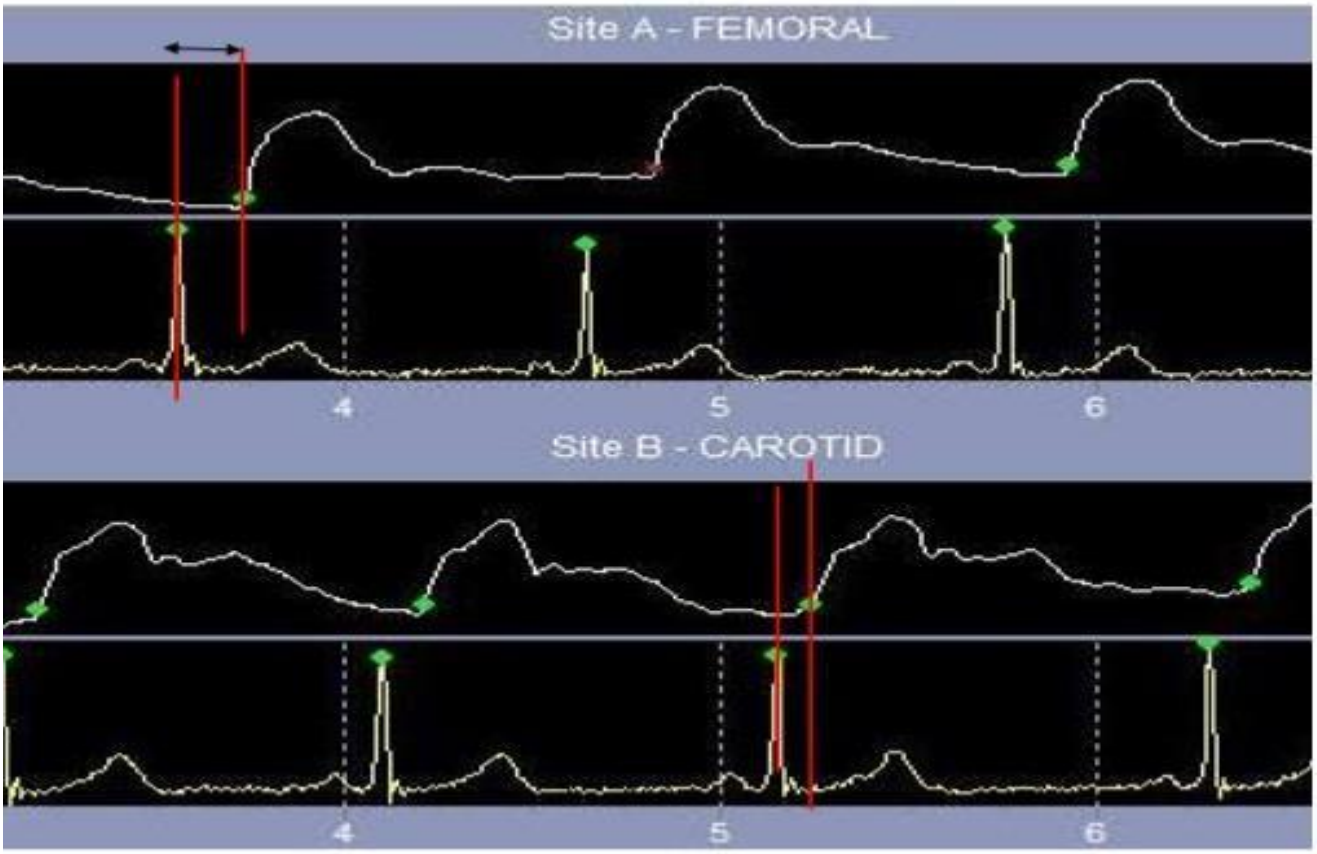

Figure 2.2: Assessment of aortic PWV. Femoral and carotid artery pulse waves are obtained using applanation tonometry. Together with simultaneous electrocardiographic recordings aortic PWV is calculated. The red lines indicate the time between electrical events and the arterial pressure changes in the carotid and femoral arteries used to calculate PWV. See text for a further description.

2.8 Echocardiography

Echocardiographic measurements were performed by an experienced observer who was unaware of the clinical data of the participants. All measurements were recorded and stored offline using echocardiography (Acuson SC2000 Diagnostic ultrasound system; Siemens Medical Solutions, USA, Inc.). Echocardiography was performed with the participants placed in the partial left decubitus position, with the head of the bed elevated to the 30° position. Participants were first assessed for mitral and aortic valve abnormalities using two-dimensional (2-D) guided colour Doppler imaging. To determine left ventricular mass (LVM), the transducer probe (2.5 MHz)

was placed over the left intercostal space at the mid-clavicular line, with the probe marker pointing to the right shoulder in order to obtain a long axis parasternal view window. Left ventricular (LV) dimensions were obtained from 2-D guided (long axis parasternal view) M-mode echocardiography in the short axis of the LV, according to guidelines (Sahn *et al*., 1978). To obtain M-Mode images in the short axis of the LV, a sample bar was placed at right angles to the LV posterior wall, and as close to the mitral valve as possible without the images of the mitral valve appearing in the M-Mode recording. The interventricular septal wall thickness (IVS) at end diastole and end systole, the posterior wall thickness (PWT) at end diastole and end systole and the end diastolic and end systolic internal dimensions of the LV were measured only when appropriate visualization of both the right and the left septal surfaces occurred and where the endocardial surfaces of both the septal and posterior wall were clearly visible (as depicted in Figure 2.3). Left ventricular mass was calculated using an anatomically validated standard formula (Devereux *et al*., 1986), and indexed to height$^{1.7}$ (LVMI). Left ventricular hypertrophy (LVH) was defined as an LVMI-height$^{1.7}$ >80 g/m$^{1.7}$ for men, and 60 g/m$^{1.7}$ for females (Chirinos *et al*., 2010). Left ventricular function i.e. EF was calculated by dividing the volume of blood pumped from the left ventricle per beat (stroke volume) by the volume of blood collected in the left ventricle at the end of diastolic filling (end-diastolic volume) and multiplied by 100. Left ventricular dysfunction was defined as EF < 40% (Paulus *et al*. 2007). From the parameters obtained from the echocardiography, only EF was reported in this study.

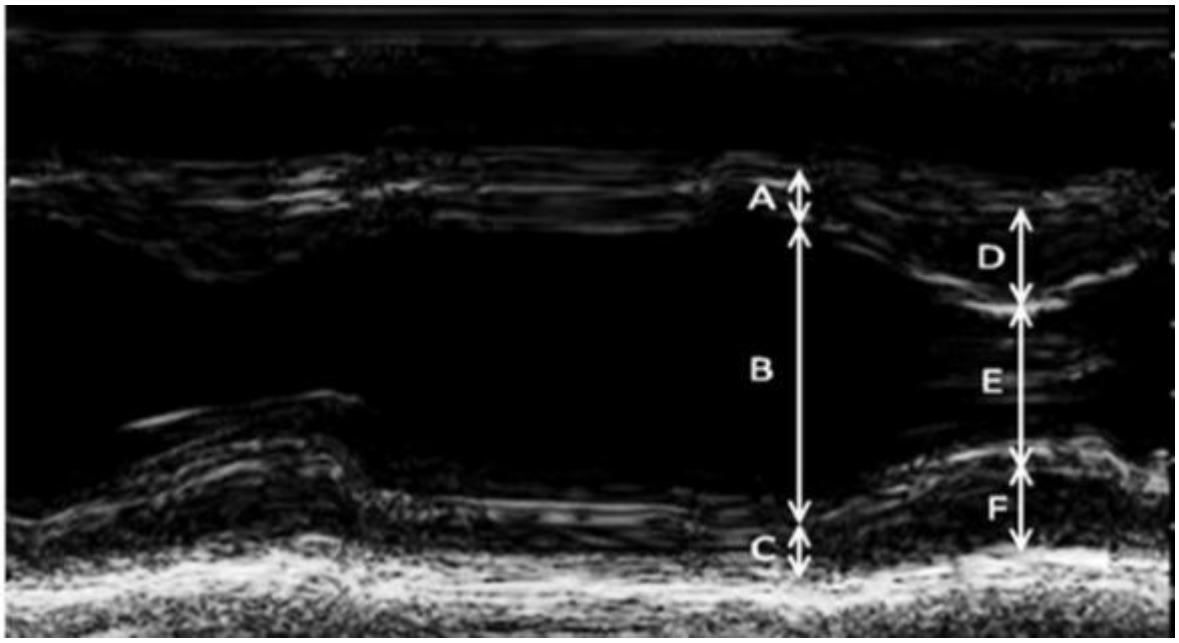

Figure 2.3: Two-Dimensional M-mode guided echocardiographic image to assess LV dimensions. A is interventricular septal wall thickness in diastole, B is left ventricular end diastolic diameter, C is posterior wall thickness in diastole, D is interventricular septal wall in systole, E is left ventricular internal diameter in systole, and F is posterior wall thickness in systole.

2.9 Data analysis

Database management and statistical analyses were performed using SAS software version 9. (SAS Institute Inc., Cary, North Carolina, USA). For all parameters, data was categorized into normal BMI (< 25 kg/m^2) and overweight/obese (≥ 25 kg/m^2). Each category was further divided into two sub-groups i.e. dippers and non-dippers. Data from individual participants were averaged and expressed as mean ± SD or proportions. The level of significance was set at $P < 0.05$, any P value greater than 0.05 was considered not significant. Comparisons of hemodynamic parameters and urinary electrolytes excretion rates between dippers and non-dippers for both categories were made using two-way analysis of variance (ANOVA) after adjusting for covariates i.e. age, sex, alcohol, smoking, diabetes and hypertension. In addition, an ANOVA was used to compare PWV between non-dippers and dippers of normal and overweight/obese. Lastly, a stepwise multiple linear regression for the normal BMI category was

used to determine the association between day-time to night-time SBP ratio and 24-hour Na^+, PWV and EF.

CHAPTER THREE: RESULTS

3.1 General and clinical characteristics

General and clinical characteristics of the sub-groups are listed in Table 3.1 as mean ± SD. Data was categorized into two groups i.e. normal BMI and overweight/obese. In this population overall, the percentage of normal BMI individuals was 35.55% and for overweight/obese was 64.44%. Each group was further divided into two sub-groups i.e. dippers and non-dippers. For the overweight/obese group, there were more females compared to males in sub-groups, 73% for dippers and 72% for non-dippers. In addition, there was an overall higher prevalence of hypertension and diabetes in individuals who had overweight/obese compared to normal BMI. However, overall smoking and alcohol use occurred to be higher in the normal BMI compared to overweight/obese group.

Table 3.1 General and clinical characteristics

	All patients (n=796)	Normal[a]		Overweight[b]/Obese[c]	
		NDP (n=151)	DP (n=132)	NDP (n=281)	DP (n=232)
Age (years)	41.55±16.53	38.10±19.20	30.10±14.70	52.20±15.10	45.80±17.10
Female (%)	67	50	43	72	73
BMI (kg/m^2)	27.22±4.18	21.60±2.30	21.30±2.30	33.70±6.60*	32.20±5.50#
Smokers (%)	15	23	28	7	12
Alcohol use (%)	21	27	30	16	17
Diabetic (%)	9	5	3	14	8
Hypertensive (%)	29	20	7	42	32

BMI, body mass index; NDP, non-dippers; DP, dippers; n, number of participants. [a]Normal BMI is defined as < 25 kg/m^2, [b]overweight defined as ≥ 25 < 30 kg/m^2 and obese defined as ≥ 30 kg/m^2. Data is presented as mean ± SD or percentage. *Significantly different ($p<0.05$) from the normal weight non-dippers. # Significantly different ($p<0.05$) from the overweight/obese non-dippers.

3.2 Hemodynamic parameters

Following results of ABPM in Table 3.2, 24-hour, night-time and day-time SBP was compared between dippers and non-dippers of across both groups after correcting for confounding variables. In a normal BMI group, 24-hour SBP between dippers and non-dippers showed a significant difference ($P = 0.01$). Night-time SBP also showed a significant difference ($P < 0.0001$), however, day-time showed no significant difference ($P = 0.23$). Similar findings were observed in the overweight/obese group, whereby a significant difference was found in the 24-hour ($P < 0.0001$) and night-time ($P < 0.0001$) SBP between dippers and non-dippers, except for day-time ($P = 0.53$). The ratio for dippers was 0.85±0.00 and for non-dippers was 0.96±0.01 for the normal BMI individuals. In the overweight/obese individuals, the ratio for dippers was 0.84±0.01 and for non-dippers was 0.97±0.01. A ration between 0.8 and 0.9 indicated a normal dipping % i.e. 10-20%. Conventional SBP was significantly higher in overweight/obese non-dippers than dippers ($P < 0.001$). Similarly, a significantly high conventional SBP was detected in normal BMI non-dippers versus dippers ($P = 0.02$).

Table 3.2 Hemodynamic parameters

	[a]Normal			[b]Overweight/[c]Obese		
	NDP (n=151)	DP (n=132)	P	NDP (n=281)	DP (n=232)	P
Ambulatory SBP (mm Hg)						
24-hour	116.90±15.40	112.60±9.80	0.01	123.80±17.20	116.20±12.30	<0.0001
Night-time	114.30±16.70	101.00±8.70	<0.0001	121.60±18.60	103.90±11.40	<0.0001
Day-time	115.50±15.10	119.80±10.70	0.23	124.90±17.10	123.40±12.80	0.53
Ambulatory DBP (mm Hg)						
24-hour	72.20±10.40	69.10±8.1	0.01	75.90±11.30	71.10±8.60	<0.0001
Night-time	67.30±11.60	58.30±8.40	<0.0001	76.40±11.90	60.03±8.50	<0.0001
Day-time	74.50±10.40	76.20±8.90	0.26	79.20±11.80	77.90±9.20	0.30
N:D ratio						
RSBP	0.96±0.01	0.85±0.00	<0.0001	0.97±0.01	0.84±0.01	<0.0001
RDBP	0.89±0.09	0.077±0.07	<0.0001	0.90±0.08	0.77±0.07	<0.0001
Conventional BP (mm Hg)						
SBP	123.60±21.70	118.60±15.10	0.02	135.10±23.10	128.90±17.90	<0.001
DBP	81.60±11.70	79.40±10.10	0.08	86.40±12.50	84.50±11.10	0.05

BMI, body mass index; NDP, non-dippers; DP, dippers; n, number of participants; %, percentage; SBP, systolic BP; DBP, diastolic BP; N:D, night-time to day-time ratio; RSBP, night-time to day-time systolic BP dipping ratio; RDBP, night-time to day-time diastolic BP dipping ratio. [a]Normal BMI is defined as < 25 kg/m^2, [b]overweight defined as $\geq 25 < 30$ kg/m^2 and obese defined as ≥ 30 kg/m^2. Corrected for age, sex, smoking, alcohol use, hypertension and diabetes. Data is presented as mean ± SD or percentage. $P < 0.05$ was considered significant.

3.3 Blood hormonal parameters and twenty-four hour urinary electrolytes excretion rates

Log transformed plasma hormones are shown in Table 3.3 expressed as mean ± SD. No statistical comparison was made. However, a clinically high aldosterone levels in normal BMI non-dippers (2.05 ng/dl) compared to overweight/obese non-dippers (1.97 ng/dl). Consistent with other studies (Maseko *et al*., 2018; Adamska-Patruno *et al*., 2018), leptin was elevated in overweight/obese individuals for both non-dippers (1.31 ng/ml) and dippers (1.32 ng/ml). Ejection fraction was normal for all the subgroups. Following 24-hour urine electrolytes results in Table 3.4, we compared all the electrolytes excretion rates between dippers and non-dippers on across both groups after correcting for confounding variables. For normal BMI, 24-hour Na^{+} excretion rate was significantly higher in dippers (122.93 mmol/day) compared to non-dippers (95.20 mmol/day). However, 24-hour Na^{+} excretion rate in overweight/obese individuals was relatively the same between dippers (103.90 mmol/day) and non-dippers (103.70 mmol/day), hence no difference was detected (P = 0.78). Twenty-four hour potassium (P = 0.04) and 24-hour Mg^{+2} excretion rate (P = 0.01) both showed a significant difference between dippers and non-dippers of normal BMI group. In contrast, only 24-hour K^{+} excretion rate (P = 0.04) showed a significant difference between non-dippers and dippers in the overweight/obese group.

Table 3.3 Blood hormonal parameters and ejection fraction

	[a]Normal		[b]Overweight/[c]Obese	
	NDP (n=151)	DP (n=132)	NDP (n=281)	DP (n=232)
Aldosterone(nmol/L)	0.07±1.05	0.07±1.20	0.07±1.16	0.06±1.28
Renin (mU/l)	1.19±0.61	1.23±0.47	1.09±0.61	1.19±0.57
Aldo:Renin	2.97±1.75	2.99±1.88	2.71±2.34	2.93±1.98
Leptin (ng/mL)	0.67±0.71	0.61±0.74	1.31±0.51	1.32±0.48
EF (%)	65.68±8.43	67.86±8.16	66.55±9.82	66.99±8.77

BMI, body mass index; n, number of participants; NDP, non-dippers; DP, dippers; Aldo:Ren, aldosterone to renin ratio; EF, ejection fraction. [a]Normal BMI is defined as < 25 kg/m^2, [b]overweight defined as ≥ 25 < 30 kg/m^2 and obese defined as ≥ 30 kg/m^2 . Data is presented as mean ± SD.

Table 3.4 Twenty-four hour urinary electrolytes excretion rates.

	[a]Normal			[b]Overweight/[c]Obese		
	NDP (n=151)	DP (n=132)	P	NDP (n=281)	DP (n=232)	P
Na^+ (mmol/day)	95.20±72.00	122.93±10.50	0.02	103.70±78.01	103.90±62.70	0.78
K^+ (mmol/day)	24.50±13.90	30.60±24.20	0.04	27.70±22.60	30.40±20.80	0.04
Mg^{+2} (mmol/day)	2.09±1.88	2.81±2.29	0.01	2.05±1.69	2.30±1.93	0.16
Ca^{+2} (mmol/day)	1.55±2.27	1.94±1.89	0.15	1.54±1.87	1.36±1.51	0.36
Na^+/K^+	4.86±5.69	4.59±2.50	0.65	4.47±2.66	3.84±1.58	0.02

BMI, body mass index; n, number of participants; NDP, non-dippers; DP, dippers; Na^+, sodium; K^+, potassium; Mg^{+2}, magnesium; Ca^{+2}, calcium, Na^+/K^+, sodium-to-potassium ratio. [a]Normal BMI is defined as < 25 kg/m^2, [b]overweight defined as ≥ 25 < 30 kg/m^2 and obese defined as ≥ 30 kg/m^2 .Two-way ANOVA was used. Corrected for age, sex, smoking, alcohol use, hypertension and diabetes. Data is presented as mean ± SD. P < 0.05 was considered significant.

3.4 Pulse wave velocity and association results

Results of PWV are represented graphically in Figure 3.1. Comparison of PWV between dippers and non-dippers on both groups was conducted. Analysis on PWV showed a significant difference between the normal BMI non-dippers and dippers (P = 0.01) and between the overweight/obese non-dippers and dippers (P = 0.01). A stepwise multiple linear regression model was fitted with the normal BMI day-time to night-time SBP ratio as the dependent variable and 24-hour Na^+, PWV and EF as independent variables (Table 3.5). After adjusting for covariates, 24-hour Na^+ was negatively associated with day-time to night-time SBP ratio (r^2 = -0.15; P = 0.04) while PWV showed a positive association (r^2 = 0.13; P = 0.04). Furthermore, a statistically highly significant negative correlation association was demonstrated between day-time to night-time SBP ratio and EF albeit a weak correlation (r^2 = -0.23; P = 0.001).

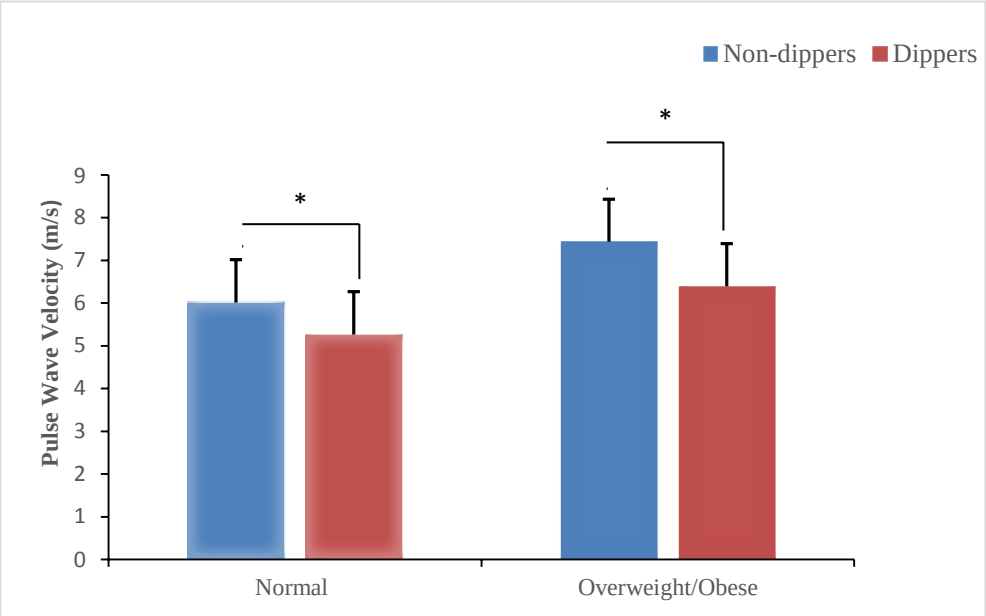

Figure 3.1 Graph showing PWV between dippers and non-dippers with normal or overweight/obese. BMI; body mass index. Normal BMI is defined as < 25 kg/m^2, overweight defined as ≥ 25 < 30 kg/m^2 and obese defined as ≥ 30 kg/m^2 . Corrected for age, sex, smoking, alcohol use, hypertension and diabetes. Data presented as mean ± SD. * depicts P < 0.05.

Table 3.5 An association between non-dipping and 24-hour Na^+, PWV and EF in normal BMI

	RSBP			RDBP		
	r^2	CI	P	r^2	CI	P
24-hour Na^+(mmol/day)	-0.15	-0.29 to -0.004	0.04	-0.20	-0.33 to -0.66	0.01
PWV ($m.s^{-1}$)	0.13	0.03 to 0.25	0.04	0.10	0.04 to 0.20	0.19
EF (%)	-0.23	-0.37 to -0.09	0.001	-0.17	-0.31 to -0.25	0.02

RSBP, night-time to day-time systolic BP dipping ratio; RDBP; night-time to day-time diastolic BP dipping ratio; CI, confidence interval; Na^+, sodium; PWV, pulse wave velocity; EF, ejection fraction. Adjusted for age, sex, smoking, alcohol, diabetes and hypertension. $P < 0.05$ depicts significant difference.

CHAPTER FOUR: DISCUSSION

4.1 Overview of the general characteristics

The results of the current study indicate that there is a high prevalence of overweight/obesity in our community sample, especially in females (Table 1.1). These findings are confirmed by other studies conducted in South Africa (Malhotra *et al*., 2008; Cois and Day, 2015; Micklesfield *et al*., 2013), which showed that obesity is more common in black women. Malhotra et al (2008) reported the prevalence of obesity of 53.4% and 18.7% among women and men, respectively. Cois and Day (2015) studied obesity trends in a longitudinal study and found a high overall rate change in BMI per decade among women (+1.82 kg/m^2) compared to men (+1.03 kg/m^2).

The etiological factors that could possibly explain the observed gender difference in obesity are cultural-bound beliefs, urbanization, socio-economic status and menopause. In previous studies, it was reported that a larger body size was preferred over a lean body size among SA women (Mvo, 1999; Puoane *et al.,* 2002). This preference stems from a widely-held belief among black SA women, that large people are happy and healthy, whereas those who are slender are perceived to experience personal problems and that they may have diseases such as HIV/AIDS (Matoti-Mvalo and Puoane, 2011). The study was conducted in an urban township, Khayelitsha, Cape Town in black women between the ages of 18-65 years. Since the population of the current study was dominantly from an urban township, i.e Soweto and consisted of black women between the ages of 18-65 years, I therefore speculate that the increased prevalence of obesity in women in this study could be explained by the results presented by Matoti-Mvalo and Puoane (2011).

In addition, our population can be described as middle class in terms of socio-economic status, as they reside in an urban township. A study by Maholtra et al. (2008) that was conducted in an urban township Khayelitsha confirmed that an urban township largely constitutes of middle to high socio-economic status residents and have access to water, electricity and are in close proximity to shopping centres. This contributes to physical inactivity and consequently weight gain. In addition, more and more people in this region are being exposed to, and are adopting the urban lifestyle that has obesogenic habits (Ghose, 2017). This transition contributes to weight gain through consumption of Western diet (Yaya and Ghose, 2019).

With the understanding of the physiology of females and taking into consideration the average age (49 years) of the population under study, one cannot ignore that most females in this study are in their menopausal years. Females generally have a greater percentage of body fat compared

to males, although the causes for excess body fat are not clearly elucidated (Lovejoy, 2003). However, it has been shown that reproductive hormones influence body fat distribution in women, in particular oestrogens and androgens. Oestrogens promote the accumulation of fat in femoral and gluteal regions and androgens in the abdominal (Kozakowski *et al*., 2017) and mammary regions (Rebuffe-Scrive *et al*., 1986). At menopause, there is a shift in the ratio of androgens to oestrogens whereby ovarian oestrogen production ceases whilst androgen production continues (Lovejoy, 2003). This may lead to increased abdominal fat distribution contributing to obesity in women. This could account for the high percentage of overweight/obese women in this study population.

Besides the differences in body fat composition between the genders, data showed that alcohol use and smoking was higher in individuals with normal BMI compared to the overweight/obese for both sub-groups in both dippers and non-dippers. There was a two-fold increase in alcohol consumption and smoking in normal BMI non-dippers compared to overweight/obese non-dippers. These findings are confirmed by a study conducted in Soweto (Muzi *et al*., 2018) which showed an increased alcohol intake and smoking in individuals with normal BMI. In the study Muzi et al. (2018), alcohol intake was 33.1% and 18% for normal and overweight/obese, respectively; for smoking, 26.4% and 9.1% for normal and overweight/obese, respectively. The reason for the differences between the two groups could be that overweight/obese individuals may have adopted a healthy lifestyle after being conscious of their BMI status. Paradoxically, the positive lifestyle change could be the reason for the overweight/obese. Although smoking cessation has notable health benefits, it has been shown to be associated with weight gain (Peltzer, 2008). This could account for the increase in BMI in individuals with low prevalence of smoking in this study population.

4.2 Ambulatory BPs in normal and overweight/obese groups

In Table 3.2, findings of the current study show that the 24-hour SBP was < 130 mm Hg for both dippers and non-dippers with normal or overweight/obese. Based on the AHA guidelines for High BP Research (Ben-Dov *et al*., 2006), this BP can be classified as normal. These findings were reported by a study conducted earlier on this population (Muzi *et al*., 2018), which showed normal ambulatory 24-hour SBP in both normal and overweight/obese status individuals.

When participants were stratified according to BMI status, the ambulatory 24-hour and night-time SBPs, as we all conventional BPs of the non-dippers were significantly higher than those of the dippers in each group. Contrary, there was no significant difference in the day-time BPs between the dippers and non-dippers of each BMI group. Further analysis revealed that there was a greater difference in the 24-hour SBP between non-dippers and dippers within the overweight/obese group (123.80 mm Hg - 116.20 mm Hg = 7.60 mm Hg) compared to normal BMI group (116.90 mm Hg - 112.60 mm Hg = 4.20 mm Hg). A similar trend was observed for where a big difference was observed in night-time SBP between non-dippers and dippers of the overweight/obese group (121.60 mm Hg - 103.90 mm Hg = 17.70 mm Hg) compared to the normal BMI group (114.3 mm Hg – 101 = 13.3 mm Hg). The diastolic BPs followed the same trend as the systolic BPs.

The daytime SBPs and DBPs in the DP group are relatively similar to the conventional BPs (119.80±10.70 mm Hg vs 118.60±15.10 mm Hg, respectively in normal BMIs), yet for the NDP group the conventional BPs are much higher (115.50±15.10 mm Hg vs 123.60±21.70 mm Hg, respectively for normal BMIs). In the overweight/obese BMI group, the conventional BPs are higher than daytime BPs in both the DP and NDP We speculate that for DP, the similarity between conventional and daytime SBP/DBP was because the DP, although normotensive, their BP was in the lower range of the normal BP. We speculate that the high conventional BP in NDP of normal BMI and overweight/obese DP and NDP is because of the white-coat effect.

These findings indicate that BMI is an independent predictor of BP in this population. The effect of BMI on BP is more pronounced on nocturnal BP of overweight/obese non-dippers. This is evidenced by the fact that even though 24-hour, daytime and conventional BPs in this group are within normal range, nocturnal BP is above the threshold of 120/70 mm Hg (Ben-Dov *et al.*, 2006). This clearly indicates that as BMI increases, only nocturnal BP increases while daytime BP remains unchanged. This results in a reduced difference between daytime and night- time BP. These findings confirm findings of earlier studies which have shown a higher incidence of non-dipping in overweight/obese individuals (Kotsis *et al*., 2005; de la Sierra *et al*., 2009). Possible mechanisms responsible for these findings have been documented. In a review by Hossain et al. (2007), it was stated that the growing prevalence of overweight and obesity propels the upsurge of diabetes and hypertension, and studies have shown that diabetes and hypertension are associated with non-dipping in overweight/obese individuals (Perk *et al*., 2002; Holl *et al*., 1999). However, as stated earlier, in the current study I corrected for diabetes, hypertension,

alcohol intake, smoking, age and gender when the average BPs were calculated for each group. This means that the effects of BMI on dipping observed in this population are independent of these covariates. The most plausible explanation for the attenuated decline in nocturnal BP in this population could be the differences in plasma aldosterone concentrations between the dippers and non-dippers. Plasma aldosterone concentration is higher in non-dippers compared to dippers for both the normal BMI and the overweight/obese groups.

4.3 Mechanisms of non-dipping in the normal BMI group

In the current study population, out of the 283 participants with normal BMI status, 53% of the participants were non-dippers. To the best of my knowledge, this is the first study to have found a noticeable non-dipping prevalence in individuals with normal BMI as one would expect a relatively small percentage of non-dippers with normal BMI status since it is interpreted as an ideal, healthy range. Understandably, this finding called for a further search at other possible factors that could explain our results. The possible explanation for normal BMI in non-dippers of this population group is that black individuals are known to be salt-sensitive (Fray and Douglas, 2013; Calhoun and Oparil, 1995), and it has been shown that the non-dipping profile is frequently associated with salt-sensitivity whether obese or not (Polónia *et al*., 2014). Indeed, Muzi et al. (2018) demonstrated that the population of the current study is salt-sensitive. In their study, Muzi et al. (2018) used 24-hour Na^+ excretion as an index of dietary salt intake in normotensive normal BMI individuals. The incidence that is accompanied by salt-sensitivity is that; black individuals understudy have poor Na^+ excretory capability that contributes to impaired pressure natriuresis (Bankir *et al*., 2008). Studies conducted in black and white populations have reported a low 24-hour Na^+ excretion in blacks compared to whites (Charlton *et al*., 2005; Bankir *et al*., 2007). Expectedly, due to impaired pressure natriuresis, during the day due to Na^+ retention, increase of Na^+ reabsorption occurs at night thereby increasing BP. In such cases, nocturnal hypertension is inevitable.

In order to substantiate the impaired natriuresis in understudy population, I assessed the plasma hormones that regulate BP and urinary Na^+ excretion rate. Data showed that plasma aldosterone was higher in non-dippers compared to dippers in the normal BMI group (Table 3.3). In order to support this finding, I evaluated the 24-hour Na^+ urinary excretion rate. Interestingly, non-dippers with normal BMI had a significantly low 24-hour Na^+ excretion rate compared to the dippers. To verify the role of aldosterone on BP in the understudy population, Tu et al (2014) investigated racial differences in sensitivity of BP to aldosterone between black and white individuals. BP was positively associated with aldosterone in blacks compared to whites ($P = 0.004$) and there was a significant increase in night-time SBP in blacks versus whites ($P = 0.006$) (Tu *et al*., 2014). Although this study did not measure Na^+ excretion rate, it was concluded that aldosterone is a significant determinant of BP in blacks.

The findings of the current study can be collectively explained by the renin-angiotensin-aldosterone-system mechanism. Explained by Yee et al. (2010), renin converts blood circulating

angiotensinogen into angiotensin I. Angiotensin I is then converted to angiotensin II by the angiotensin-converting-enzyme found in the vascular endothelial cells of the lungs. Angiotensin II exerts two effects: causes blood vessels to constrict thereby increasing BP and stimulates the release of hormone aldosterone. Aldosterone leads to Na^+ reabsorption in the kidneys thereby elevating BP. For this reason, the significant decrease in Na^+ excretion in normal BMI non-dippers can be explained by the elevated aldosterone levels. It is therefore apparent that aldosterone hormone is responsible for the non-dipping status in the normal BMI individuals in our population.

4.4 Mechanisms of non-dipping in the overweight/obese group

Plasma aldosterone concentration was higher in non-dippers compared to dippers in the overweight/obese group (Table 3.3). It has been observed that pathological factors of increased BP in overweight/obese individuals that may play a role in impaired nocturnal BP fall include impaired pressure natriuresis (Hall, 2000) and obesity-driven hormones (Tun *et al*., 1996). Adipose tissue excretes aldosterone-releasing factors, thereby stimulating aldosterone secretion independently of the systemic RAS (Kwarazaki and Fujita, 2016). Prolonged secretion of aldosterone leads to sustained renal Na^+ reabsorption and K^+ excretion. In this way overweight/obese individuals require a high BP to maintain Na^+ balance indicating impaired renal natriuresis which consequently plays a role in the development of hypertension in obesity (Kwarazaki and Fujita, 2016).

In the overweight/obese group, plasma aldosterone was higher in non-dippers (1.97 ng/dL) compared to dippers (1.87 ng/dL). However, 24-hour Na^+ excretion showed no significant difference between non-dippers and dippers. In contrast, 24-hour K^+ excretion showed a significant difference whereby non- dippers had low 24-hour K^+ compared to dippers. Since 24-hour Na^+ excretion is the same between non-dippers and dippers in overweight/obese group, it is worth mentioning that the main contributor on dipping is the 24-hour K^+ excretion. On account of elevated 24-hour K^+ excretion (indicating high dietary K^+ intake) in dippers, this causes a decrease in BP in this group hence the presence of a dipping pattern. Effects of K^+ on lowering BP have been demonstrated in previous studies (He *et al.*, 2005; Macgregor *et al.*, 1982). One of the proposed mechanisms is that K^+ has anti-hypertensive effects (Staruschenko, 2018). Some suggest that K^+ could act as a diuretic agent and resulting in the reduction of extracellular fluid volume, which in turn result in decreased BP (Brandis *et al.*, 1972). An alternative mechanism

of action is that K^+ may alter the activity of the RAS and reduce angiotensin influences on vascular, adrenal, or renal receptors (Vander, 1970). However, if dietary K^+ intake is low, BP increases. This observation was evident in the non-dippers hence the non-dipping pattern.

In overweight/obese non-dippers, the significantly low 24-hour K^+ excretion contributed to the significantly high 24-hour Na^+/K^+ excretion ratio. It has been well documented that high Na^+ intake can trigger Na^+ retention, which can result in increased blood volume and subsequently an increase in BP (Martin and Fischer, 2012). In addition, excess dietary Na^+ intake has negative effects on arteries independent of high BP. It has been reported that small changes in dietary Na^+ intake can have a large impact on the endothelial function (Oberleithner *et al.*, 2007). The mechanism at which this occurs is that, high dietary Na^+ intake mechanically stiffens endothelial cells by decreasing nitric oxide release thereby increasing vascular smooth muscle tone (Kusche-Vihrog and Oberleithner, 2012). When the Na^+/K^+ ration is elevated, this result in the dominance of adverse effects of Na^+ and the K^+ BP lowering effects are cancelled. As a result, BP increases, and this has been previously reported (Perez and Chang, 2014). Since the 24-hour Na^+/K^+ excretion ratio was significantly high in non-dippers, it causes an increase in BP. When BP fails to dip at night a non- dipping pattern manifests in non-dippers.

Although plasma aldosterone was clinically higher in non-dippers compared to dippers, 24-hour Na^+ excretion rate did not show any significant difference, rather it was relatively the same for non- dippers and dippers. This implies that, in our population not only aldosterone is associated with the 24-hour Na^+ excretion but other deeply involved factors should be considered such as the hormone leptin. Leptin was clinically elevated in individuals with overweight/obese with non-dippers and dippers (Table 3.3). As previously reported by Muzi et al. (2018), the current study found high leptin levels of 43.0 ng/mL (equivalent to 1.63 ng/mL) in overweight/obese versus normal 9.9 ng/mL (equivalent to 0.99 ng/mL) individuals. Leptin has been reported to be a mediator of SNS activation, especially renal SNS activity in animal (Haynes *et al*., 1997) and human studies (Grøntved *et al*., 2011; Straznicky *et al*., 2008) resulting in leptin-induced BP elevation. This could be considered as a mechanism responsible for impaired natriuresis. I can speculate that in the population understudy, the overweight/obese non-dippers can be explained by the findings of Grøntved and Straznicky's (2008). That impaired natriuresis, which is evidently supported by low 24-hour Na^+ excretion, is due to leptin in overweight/obese individuals.

Besides electrolytes excretion rates and plasma hormones, another probable cause of the elevated night-time SBP in overweight/obese individuals could be OSA. Although the present study I did not assess on OSA, previous studies have indicated that OSA is associated with increased BMI i.e. overweight/obese (Chen *et al*., 2014; Vorona *et al*., 2005; Watabane *et al*., 2010). It has been shown by numerous studies that OSA, due to overweight/obese, contributes to various health conditions including elevated night-time BP (Phillips and O'Driscoll, 2013; Konecny *et al*., 2014). The mechanism that supports this association is that; in overweight/obese individuals due to fat deposition in the upper respiratory tract the air-passage narrows resulting in a decrease in muscle activity in this region (Dempsey *et al*., 2010 and Jehan *et al*., 2017). This ultimately leads to hypoxic and apneic events resulting in sleep apnea. In simpler terms, the narrowing of blood vessels in the upper respiratory airways leads to decreased oxygen in the blood. This, therefore, results in decreased tissue oxygen available in the blood vessels and body tissues. The body's response towards this event is increased SNS activation leading to increased night-time BP.

4.5 Impact of non-dipping on arterial stiffness and ejection fraction in normal BMI group

To the best of my knowledge, this is the first study to have found a noticeable non-dipping prevalence in individuals with normal BMI as one would expect a relatively small percentage of non-dippers with normal BMI status since it is interpreted as an ideal, healthy range. I therefore investigated arterial stiffness using PWV, and EF in order to assess CVD risk. Although PWV was not >10 m/s in any of the sub-groups, our results showed a significantly higher PWV in non-dippers (6.01 m/s) compared to dippers (5.26 m/s) with normal BMI ($P = 0.01$). To the best of my knowledge, this is the first study to have found increased arterial stiffness in normotensive non-dippers with normal BMI. This finding indicates that the effects of non-dipping on arterial stiffness are independent of BMI status in the population understudy.

The uniqueness of the current study is that the population was classified into normal and overweight/obese. Although some studies have reported increased CVD and TOD risk in normotensive non-dippers (Aranda *et al*., 2010; Hermida *et al*., 2012), these studies had an overall of overweight/obese BMI status. In a study conducted by Aranda et al. (2010), BMI status was reported to be 28.0 kg/m^2 and a study by Hermida et al. (2012) had an average of 30.2 kg/m^2. Therefore, due to this limitation, the mechanism/s responsible for the aforementioned studies cannot be ascribed to our findings.

In order to find the possible mechanism that accounts for findings of the current study, a stepwise multiple linear regression was performed. The relationship dipping with 24-hour Na^+, PWV and EF was assessed. Data demonstrated that a negative relationship exists between non-dipping and 24-hour Na^+ excretion in both RSBP (r^2 = -0.15, P = 0.0439) and RDBP (r^2 = -0.20, P = 0.0066) (Table 3.5). This decrease in 24-hour Na^+ excretion implies a relatively increased renal Na^+ retention which exacerbates a blunted nocturnal fall in BP through pressure natriuresis as previously explained.

However, PWV was only modestly associated with RSBP (r^2 = 0.13, P = 0.0440) but not RDBP (r^2 = 0.10, P = 0.1926). This relationship has been documented by other studies, however, in populations that either had a pathology or with overweight/obese BMI status (Celik *et al*., 2015). For example, Celik et al (2015) found a correlation between non-dipping and 24-hour and night-time PWV in patients with Behcet's disease. Behcet's disease is a rare disorder that causes inflammation in the blood vessels throughout the body (MayoClinic, 2019). For this reason, we cannot explain findings of the present study based on these studies due to the observed differences in these populations versus the population understudy. I, therefore, used the salt-sensitive phenomenon to explain and provide mechanism/s for the findings of the present study. It has been demonstrated that salt-sensitivity predicts a higher rate of cardiovascular events and TOD, independently of BP (Morimoto *et al*., 1997). It has been documented that salt-sensitivity, as a result of impaired natriuresis, is implicated in multiple pathways of endothelial dysfunction (Bragulat *et al*., 2001; Argawal *et al*., 2013). One plausible mechanism is through the overexpression of the active form transforming growth factor beta-1 (TGF-β1) (Ying *et al*., 2008). TGF-β1 is a multifunctional cytokine that regulates proliferation, cell migration, cell apoptosis and differentiation of endothelial cells. However, in its active state, it is most importantly a potent stimulator of collagen synthesis as well as an inhibitor of matrix metalloproteinases (MMPs) (Sanders, 2009). The suppression of MMPs is permissive to collagen deposition in the vasculature and, in turn, causes fibrosis of the tunica intima which further worsens arterial stiffness. Therefore, it is worth mentioning that despite the normal PWV in normotensive non-dippers with normal BMI, impaired natriuresis significantly results in the elevated PWV consequently, arterial stiffening.

Furthermore, EF was negatively associated with both RSBP (r^2 = -0.23, P = 0.0019) and RDBP (r^2 = -0.13, P = 0.0215). Although EF was normal, this finding suggests that a progressive blunted nocturnal fall in BP results in a decreased left ventricular EF. Previous studies in non-

black populations have shown that, non-dipping is associated with TOD (Hoshide *et al.*, 2003; Soylu *et al.*, 2009; Hermida *et al.*, 2013). Hoshide et al. (2003) studied 74 normotensive Japanese and showed that brain natriuretic peptide, atrial natriuretic peptide and LVMI were increased in non-dippers compared to dippers. Although the study had a small sample size and population was overweight, Mezue et al. (2016) found increased relative wall thickness, LVM and LVMI in non-dippers. An increase in these parameters is an established evidence of cardiac remodeling and LVH which signify early cardiac dysfunction. Similar observations were documented in normotensive blacks. The mechanism that explains findings of the current study is that; ventricular ejection generates a primary pressure wave that moves away from the heart at a finite speed i.e PWV, which increases arterial stiffness (London and Guerin, 1999). The primary wave is reflected at any particular point of the arterial tree, generating a reflected wave traveling backward towards the ascending aorta (Nichols and O'Rourke, 1991; Murgo *et al*., 1980). Increase in arterial stiffness exacerbates increase PWV which is likely to result in an early return of the reflected waves. This implies that reflected waves may return during systolic phase rather than diastolic phase, thus augmenting the systolic part of the primary wave and contributing further to increase SBP. Chronically elevated SBP causes cardiac remodeling eg. LVH resulting in pump dysfunction. This mechanism validates the results in Table 3.4. Therefore I conclude that in the subgroup of non-dippers with normal BMI in this population, impaired natriuresis as seen with 24-hour Na^+ excretion rate, results in failure of nocturnal BP to dip. The rise in nocturnal BP then leads to a blunted nocturnal fall in BP. The synergy of these events leads to increased risk of CVD which we observed; increase in arterial stiffness and reduced ejection fraction.

CHAPTER FIVE: CONCLUSIONS

5.1 Conclusion

In summary, findings of the current study demonstrated that overweight/obese has adverse effects on the BP circadian rhythm, especially in night-time BP. I further showed the possible mechanisms that led to the findings of the current study that, salt-sensitivity contributes to non-dipping in normal BMI individuals. Moreover, data revealed that in the overweight/obese group low 24-hour K^+ excretion and elevated 24-hour Na^+/K^+ excretion resulted in non-dipping. In addition, leptin also contributed in non-dipping in the overweight/obese group. In this sub-group, I then concluded that, in the face of overweight/obese status there is a high probability of blunted nocturnal fall in BP. With regards to non-dippers with normal BMI, our study showed that a blunted nocturnal fall in BP is associated with increased arterial stiffness and reduced EF and to the best of my knowledge, this is the first study to have made this observation. Given the results of the stepwise multiple linear regression, the present study suggests that impaired natriuresis results in an attenuated nocturnal fall in BP which leads to increased arterial stiffness and consequently left ventricular pump dysfunction in black normotensive non-dippers with normal BMI status. These findings emphasize the importance of establishing dipping status to guide stratification in an inherently high-risk population for the consequence of hypertension.

5.2 Limitations

This study must be interpreted within the context of the following limitations. Urinary collection was not separated into day and night-time; rather it was over a 24-hour period. Separating urine into day and night-time would have enabled me to know exactly the electrolytes excretion rates during night-time considering that the main focus of this study was night-time BP. In addition, participants were not given an option to report missing urine over the 24-hour period. This would have a possibility of underestimating the true electrolytes excretion. Also, if participants collected urine over a period of >24-hour, excretion rates would have been overestimated. There is a need for more research in profiling BP dipping status in patients with normal BP, taking into consideration the above-mentioned limitations.

5.3 Possible clinical implications and recommendations

Numerous organizations have been established with a goal of reducing the morbidity and mortality rate caused by CVDs. These organizations target mainly individuals with various health complications. However, to this date, CVDs are still the leading cause of mortality and morbidity. From the findings of this study, it is worth mentioning that there is a possibility that lean individuals may manifest with CVDs 10-20 years later full medical screening, including use of ABPM is not conducted. Thus, contributing more if not equally, towards the exponential increase in mortality and morbidity rates caused by CVDs. Healthcare institutions should implement the use ABPM for all patients. In addition, the use of ABPM in medical screening facilities such as medical aid institutions should be compulsory. From the data obtained from the ABPM, an individual would be aware of their risk of CVD and make necessary lifestyle changes if they are non-dippers. This may reduce prevalence of CVDs.

CHAPTER SIX: REFERENCES

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APPENDICES

Appendix 1: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Ms M Ngema et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180520

NAME: (Principal Investigator)	Ms M Ngema et al
DEPARTMENT:	School of Physiology Medical School University
PROJECT TITLE:	The relationship between nocturnal blood pressure dipping and carotid intima media thickness in a salt-sensitive African population
DATE CONSIDERED:	25/05/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr MJ Maseko
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	27/08/2018

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 on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
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 reviewed in **May** and will therefore be due in the month of **May** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2: Change of title

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

26 November 2019
Person No: 732553
TAA

Ms MV Ngema
P. O. Box 3118
Ds House No. 2381 Dube Village-groutville
Stanger
4450
South Africa

Dear Ms Mandisa Ngema

Master of Science in Medicine: Change of title of research

I am pleased to inform you that the following change in the title of your Dissertation for the degree of **Master of Science in Medicine** has been approved:

From:	**The relationship between nocturnal blood pressure dipping and carotid intima media thickness in a salt-sensitive African population**
To:	**An attenuated nocturnal blood pressure dipping is associated with pump dysfunction in a salt-sensitive African population**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix 3: Aldosterone and Aldosterone/Renin table

Table 3.3.1 Blood hormonal parameters that are non-parametric

	Normal BMI[a]		Overweight/Obese[b]	
	NDP(n=151)	DP(n=132)	NDP(n=281)	DP(n=232)
Aldosterone/Renin	3.32(3.07)	3.40(3.54)	3.25(5.51)	3.37(3.93)
Aldosterone	2.29(1.09)	2.20(1.45)	1.03(0.37)	2.25(1.66)

BMI, body mass index; NDP, non-dippers; DP, dippers; n, number of participants. [a]Normal BMI is defined as < 25 kg/m^2, [b]overweight defined as $\geq 25 < 30$ kg/m^2 and obese defined as ≥ 30 kg/m^2. Data is presented as median(IQR).

Appendix 4: Plagiarism report

732553:Mandisa_Masters_for_plagiarism_2nd_attempt.docx

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