The effects of reverse dipping on cardiovascular target organ damage in a black South African population

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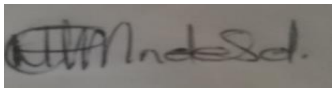
A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, for the Degree of **Master of Science in Medicine**.

Johannesburg 2021

I, Nolwazi Farewell Mndebele 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.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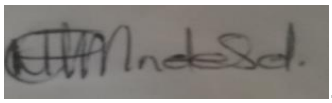
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Prof. Muzi J. Maseko (Supervisor) Dr Nokuthula Nkosi-Mafutha (co-supervisor)

Date: 09 September 2021 Date: 09 September 2021

Dedications

This dissertation is dedicated to; my late Father, Elvis Mfishane Mndebele; my mother, Anita Ntombikhona Mthethwa; my sisters, Buyile, Phethile, Queen, Xolile and Nomcebo Mndebele, and my two Best friends Nomcebo Magagula and Nokukhanya Mkhomazi. I would like to extend my utmost gratitude and appreciation, your payers, support, encouragement, and words of reassurance during the course of the research did not go unnoticed.

My contributions to data collection and data analysis

I declare that I collected Most of the data myself. I collected the Blood pressure, anthropometric, sphygmocor, intima media thickness and 24-hour urinary excretion data. I conducted the questionnaires with the help of my colleagues, Caroline Mokwena and Kgothatso Nkoana. Echocardiography data was collected with the help of a qualified nurse. Urine samples were sent to an accredited laboratory for analysis. I analysed the data with the assistance of my main supervisor, Professor Muzi J. Maseko.

Abstract

Reverse dipping has been associated with target organ damage and cardiovascular risk, with hypertension being its most common risk factor. It has been clearly attributed to be a predictor of cardiovascular mortality and to present with a poor clinical prognosis. However, its clear effect and etiology on cardiovascular (CV) target organ damage has not been sufficiently investigated, especially in the black community. In the present study we measured pulse wave velocity (PWV), conventional blood pressure (BP), ambulatory BP, anthropometric measurements, intima media thickness, and 24-hour urinary excretion in 796 black South Africans. Echocardiographic assessment was also performed, and blood samples were collected to assess plasma hormone concentrations. The prevalence of dipping, non-dipping and reverse dipping was 48.0%, 39.0%, and 13.0% respectively. Dippers had the lowest average 24-hour systolic and diastolic BP of 114.8/70.3 mm Hg, followed by non-dippers at 119.3/73.7 mm Hg, with the reverse dippers having the highest 24-hour systolic and diastolic BP of 128.2/78.7. mm Hg. All the groups (dippers, non-dippers, and reverse dippers) had normal 24-hour and daytime BP. The nighttime blood pressures of the dippers and non-dippers were within normal range even though these pressures were significantly different between the two groups. On the other hand, the nighttime BP of the reverse dippers (132.2/77.7 mm Hg) was above the threshold of 120.0/70.0 mm Hg indicating that they were on the nighttime hypertensive range.

The plasma renin concentrations of the three groups were not significantly different. However, the plasma aldosterone concentrations of the reverse dippers were higher than those of the dippers and non-dippers. Urine electrolyte excretion of sodium and potassium was not significantly different between the three groups. However, the sodium-to-potassium ratio of the reverse dippers was significantly different than that of the dippers. Similarly, the urinary magnesium of the reverse dippers was significantly different than that of the dippers.

Investigations of the cardiovascular target organs show that the pulse wave velocity of the reverse dippers was significantly higher than that of the dippers and non-dippers. The left ventricular mass index (LVMI) of the dippers and non-dippers was not significantly different while the LVMI index of reverse dippers was significantly increased when compared to both the dippers and non-dippers.

In conclusion, our results show that despite the normal daytime BP of the reverse dippers, their nighttime BP is high, and this translates to preclinical cardiovascular pathology. The mechanisms that drive the increased nighttime BP may be mediated by the high plasma aldosterone and aldosterone-to-renin ratio in this group.

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First, I would like to extend my utmost gratitude to my main supervisor, Professor Muzi J. Maseko, for your support and patience throughout the course of the research project. It has been a great privilege and an honour to be guided and be under your supervision. Your strength of character, assistance and sound judgement are what made this degree a success. I would also like to thank my co-supervisor Dr Nokuthula Nkosi-Mafutha for her financial support and Mrs Nkele Maseko for sharing your skills and knowledge with us regarding data collection and for always being available to help.

Great appreciation and thanks is extended to the participants who volunteered to participate in the study, your participation made this research project a success. Additionally, I would like to thank my colleagues for always being willing to help, special thanks go to Bongubuhle Mlambo, your warmth and welcoming nature made me feel at home away from home which allowed me to perform at my fullest potential. Above all, I want to thank the Almighty God who made it all possible.

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List of abbreviations

ABP	Ambulatory blood pressure
ABPM	Ambulatory blood pressure monitoring
ACC/AHA	American College of cardiology / American heart association
AGT	Angiotensinogen
AI	Augmentation index
BMI	Body mass index
BP	Blood pressure
CIMT	Carotid intima media thickness
CV	Cardiovascular
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DBPC	Conventional diastolic blood pressure
DBPD	Day-time diastolic blood pressure
DBPN	Night-time diastolic blood pressure
DP	Dippers
E/A	Early to late diastolic filling
HNRL	Human nutrition research laboratory
IQR	Interquartile range

LVED	Left ventricular end diastolic diameter
LVES	Left ventricular end systolic diameter
LVH	Left ventricular hypertrophy
LVMI	Left ventricular mass index

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CHAPTER ONE

INTRODUCTION

1. Hypertension and target organ damage.

It has been proven over years now, that hypertension is one of the leading causes for cardiovascular diseases and deaths worldwide (Nadar *et al*., 2006). Hypertension is a common illness, and it has been considered a significant public health problem (Addo *et al*., 2007), with the rapidly increasing contributing factors such as obesity, physical inactivity, an unhealthy nutrition (WHO, 2003), the consumption of alcohol and an aging population (Peer *et al*., 2013). In 2018, 80.0% of hypertension cases across different countries were seen in low- and middle-income countries (Kearney *et al*., 2005; Kishore *et al*., 2016). The global burden of hypertension is further complicated by the fact that hypertension is underdiagnosed (Addo *et al*., 2007; Monakali *et al*., 2018). Furthermore, its low level of detection, treatment, and control, following its asymptomatic nature is further complicating its management (Addo *et al*., 2007; Monakali *et al*., 2018). Hypertension in urban regions is thought to be as high as in developed countries with the change of lifestyle due to the transition resulting from urbanisation and westernisation (WHO, 2003).

The increasing poor detection and inadequate management of hypertension increases the chances for complications with potential damage to primary target organs such as the heart, brain, kidneys, and arterial blood vessels, with a worsened prognosis (Addo *et al*., 2007). Hypertension, in most cases coexists with other cardiovascular risk factors such as diabetes, obesity, and hyperlipidaemia, resulting in a global increase in morbidity and mortality (WHO, 2003). In high-income countries, the prevalence of hypertension has decreased over the years (Peer *et al*., 2013). In Africa however, it has remained unchanged or has increased in some countries (Peer *et al*., 2013). Peer *et al* (2013) observed that the prevalence of hypertension is high and rising in the urban black population of Cape Town.

Studies have shown that people of African descent are at high risk for target organ damage compared to their white counterparts for a given BP (BP) (Addo *et al*., 2009). Target organ damage has been associated with a higher systolic and diastolic BP, independently or together (Addo *et al*., 2009). Many mechanisms are involved in the pathogenesis of hypertensive target organ damage and these include changes in the

renin angiotensin aldosterone system (RAAS), endothelial activation, increased thrombogenesis, platelet activation, and collagen turn over (Addo *et al*., 2009). The summary is shown in the figure below.

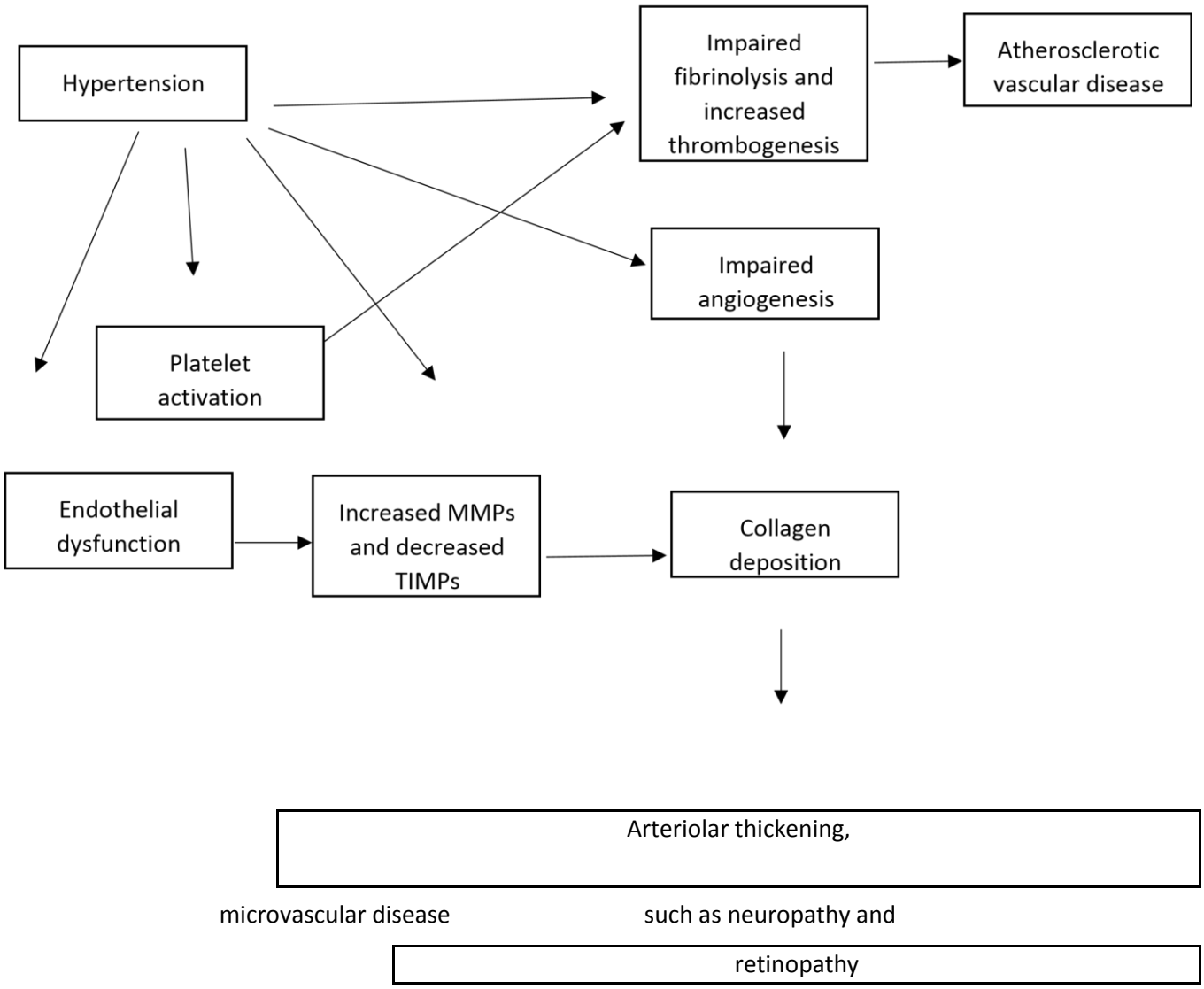

Figure 1.1: Interrelationships between the different processes in the pathogenesis of target organ damage in hypertension (adapted from Nadar *et al*., 2006).

MMP; matrix metalloproteinases, TIMP; tissue inhibitors of MMP.

2. Diagnosis of hypertension

The diagnosis of hypertension is the first step at alleviating the global burden of hypertension. However, this is dependent on the accurate measurement of BP to accurately diagnose and manage hypertension (Muntner *et al*., 2019). Systolic (SBP) and diastolic (DBP) BP are the most commonly used BP measurements since they are readily established cardiovascular (CV) risk factors and can be estimated directly (Muntner *et al*., 2019). When considered individually, SBP and DBP are associated with an increased risk of CV disease (Muntner *et al*., 2019). Some studies however, did not find any association between DBP and CV risk (Muntner *et al*., 2019). Systolic BP risk for CV disease is independent of DBP (Muntner *et al*., 2019). Several other BP measures can be derived from SBP and DBP, these include mean-arterial pressure (1/3 SBP+ 2/3 DBP or DBP + 1/3), for individuals with a normal heart rate, estimates the overall arterial BP during a complete cardiac cycle; Mid BP (Averaged SBP and DBP values); and pulse pressure (SBP DBP), a marker for arterial stiffness (Muntner *et al*., 2019). Two methods may be used in the diagnosis of hypertension, conventional BP and/or ambulatory BP monitoring (ABPM).

2.1. Limitations of conventional BP

Conventional BP is the most widely used technique for the diagnosis and management of hypertension. Individuals with a conventional BP ≥140/90 mm Hg are diagnosed as hypertensive. However, recently updated guidelines (American College of Cardiology/American Heart Association (ACC/AHA) 2017 guidelines) define hypertension as a sustained office BP greater than 130 mm Hg and 80 mm Hg for SBP and DBP respectively (Dadlani *et al*., 2019).

It is crucial to consider the fact that BP varies throughout the day and night as explained by the normal circadian rhythms of BP, and to also acknowledge the event of the morning surge, a phenomenon that conventional BP measurements does not account for (Dadlani *et al*., 2019). This then justifies the reason why ambulatory BP monitoring

is preferred, considered and of interest in many clinical studies (including the present study) over conventional BP.

2.2. Superiority of ambulatory blood pressure monitoring.

Ambulatory BP monitoring (ABPM) refers to the measurements of BP over a 24-hour period to identify variations in BP patterns (Dadlani *et al*., 2019). It is used when there is a large difference between sequential conventional BP readings or when there is a large difference between home and office BP readings (Kaczorowski and Dawes, 2012). This technique is gradually replacing conventional BP, with its numerous advantages over conventional BP (O'Brien *et al*., 2013). Ambulatory BP monitoring, as indicated by a number of longitudinal and cross-sectional studies is mostly used to exclude or confirm the possibility of white coat hypertension and/or masked hypertension, and to identify differing 24-hour BP profile (O'Brien *et al*., 2013). A number of studies have shown that 24-hour ABPM had better predicted CV outcome than do conventional BP levels (O'Brien *et al*., 2013).

Ambulatory BP monitoring allows for the detection of circadian changes of BP, including nighttime dipping and the morning surge (Dadlani *et al*., 2019). Moreover, ABPM provides a large number of readings and provides evidence for the effectiveness of antihypertensive drugs (Dadlani *et al*., 2019).

3. Nighttime blood pressure dipping.

3.1. Non-dipping.

BP varies over 24 hours; it is high during the day and low during the night (Routledge *et al*., 2007). Normally, BP decreases by 10% to 20% during the night (Routledge *et al*., 2007). Individuals that lack this decline in nighttime BP, that is, their BP declines less than 10% during the night, are classified as non-dippers (Gradman *et al*., 2011). A large

body of evidence exists around the strong association between a non-dipping profile and target organ damage at a vascular, cardiac, and cerebrovascular level (Routledge *et al*., 2007). A prospective study reported that CV events in non-dippers were 3 times as many compared to dippers (Individuals who had a nighttime dip in BP of 10-20%) (Gradman *et al*., 2011). Other studies reported an increased risk of chronic renal disease, myocardial infarction, heart failure and stroke (Gradman *et al*., 2011). To add on, a high prevalence of left ventricular hypertrophy and diastolic dysfunction was reported among non-dippers as well as microalbuminuria and endothelial dysfunction (Gradman *et al*., 2011).

3.2. Reverse dipping.

Twenty-four-hour ambulatory BP monitoring has unearthed another variant phenomenon that is opposite to the non-dipping phenomenon that was discussed above. It is called reverse dipping. Individuals who present with this condition have an abnormally high night-time BP that rises above the daytime BP values. (Yan *et al*., 2015). The mechanisms responsible for the abnormal dip in BP during the night are not well understood (Routledge *et al*., 2007). However, literature suggests that this may be as result of an increased sympathetic nervous system activity throughout the night (Routledge *et al*., 2007). Reverse dipping has been commonly reported in patients with type 2 diabetes mellitus, progressive renal dysfunction, and obstructive sleep apnoea (Cuspidi *et al*., 2017).

Several studies reported a strong association between reverse dipping and target organ damage. Yan *et al*. (2015) observed that reverse dipping may increase the risk for carotid atherosclerosis and the early formation of carotid plaque, especially in hypertensive individuals. Kim *et al*. (2013) observed in their study that reverse dipping may be an independent predictor of CV death and a worsened prognosis (Fagard, 2009).

Not much research has been done on reverse dipping, more focus has been put on non-dipping. This calls for the need for more research to be done around reverse dipping especially in the black population that has been declared highly salt sensitive compared to their white counterpart and are at high risk for most of the risk factors

associated with reverse dipping (Grillo *et al*., 2019). These risk factors include but not limited to: high dietary salt and fat, sedentary lifestyle, higher sensitivity to alcohol, genetic traits, socioeconomic status, stress, and excessive adiposity (Fuchs, 2011). The prevalence of these risk factors were reported to be higher in black Americans compared to white Americans (Fuchs, 2011)

4. Prevalence of reverse dipping.

The prevalence of reverse dipping has been reported mostly in population-based studies as well as in a considerable number of clinical settings which constituted those with hypertension, sleep apnoea syndrome, diabetes mellitus, and chronic kidney disease (Cuspidi *et al*., 2017). The common trend in these studies is that a greater percentage of the population exhibit a dipping pattern of BP, with reverse dippers being the least (Abdalla *et al*., 2017). However, reverse dippers have a poor prognosis compared to dippers and non-dippers (Abdalla *et al*., 2017). In comparison to the other groups, reverse dipping is mostly common in older patients, males and smokers (current or previous) (Cuspidi *et al*., 2017). In a cross-sectional study of black people with treated and untreated hypertension, a small difference in the prevalence of nondipping and reverse dipping was observed (Mvunzi *et al*., 2017). However, the prevalence of non-dipping was higher in the group with untreated hypertension, with a smaller percentage showing a reverse dipping pattern (Mvunzi *et al*., 2017). In another population-based study of black people, the prevalence of reverse dipping was associated with a higher prevalence of LVH and Left ventricular mass index (LVMI) (Abdalla *et al*., 2017). Recently, it was discovered that reverse dippers were in a higher risk for lacunar infarction (small infarcts in the deep cerebral white matter, basal ganglia, or pons, presumed to result from the occlusion of a single small perforating artery supplying the subcortical areas of the brain), which supported the hypothesis that reverse dipping is highly associated with atherosclerosis (Yan *et al*., 2015).

5. Determinants of reverse dipping.

5.1. Dietary salt intake.

It has been well supported that BP responds to dietary salt intake in individuals who are salt sensitive, a phenomenon characterized by BP that varies with changes in salt intake (Sachdeva and Weder *et al*., 2006; Grillo *et al*., 2019). A higher prevalence of salt sensitivity has been found in hypertensives, blacks and elderly subjects compared to normotensives, whites, and younger subjects, respectively. Physiologically, salt balance is maintained by pressure-natriuresis and as previously mentioned, BP is normally lowest at night and so is salt excretion (Sachdeva and Weder *et al*., 2006). However, if salt is retained, as seen in most hypertensives, during the day, BP increases during the night to compensate for the pressure needed to excrete it, and that is how non-dipping and progressively reverse dipping result (Sachdeva and Weder *et al*., 2006).

De la Sierra *et al*. (1995) confirmed this by showing in their study that salt sensitive hypertensives retained a non-dipper pattern during both low and high salt intake (Sachdeva and Weder *et al*., 2006). Additionally, they also showed that salt resistant hypertensives (Individuals whose BP is not affected by dietary salt intake) presented with a blunted BP dip during the night after switching from a low to a high salt diet (Sachdeva and Weder *et al*., 2006). Salt sensitive subjects are prone to retain salt during the day, and thus have a higher night/day salt excretion ratio, an event that has been associated with a higher night-time BP (as seen in non-dippers and reverse dippers) (Sachdeva and Weder *et al*., 2006). Kimura *et al*. (2010) also demonstrated a diminished BP dip and a higher mean arterial pressure in highly salt sensitive patients, this was irrespective of the mechanism that caused the salt sensitivity (ultrafiltration capability and enhanced tubular sodium reabsorption). Finally, it was shown that both circadian rhythms of salt and BP were restored by salt restriction or rather a low salt diet (Kimura *et al*., 2010).

There is gap in literature with regards to dietary salt intake and reverse dipping, available literature focuses on non-dipping and salt sensitivity. Therefore, there is a huge gap to investigate the relationship and association of dietary salt intake and reverse dipping in the future.

5.2. Obesity.

Several studies have found a link between obesity induced hypertension and impaired dipping patterns of BP (Kang, 2010; Hall *et al*., 2010; Fabbian *et al*., 2013). The mechanisms that may be involved in this association include altered kidney function, obesity induced hormone elevation, sympathetic nervous system activation, vascular structural changes, and endothelial dysfunction (Dubielski *et al*., 2016). Moreover, obesity is a risk factor for obstructive sleep apnoea, a common cause of reverse dipping and non-dipping patterns of BP (Dubielski *et al*., 2016). Moczulska *et al*. (2020) concluded that patients with morbid obesity frequently present with hypertension of the reverse dipping pattern.

There are significant similarities between the mechanisms of the causes of nondipping, reverse dipping, and obesity induced hypertension; renal sodium reabsorption is increased in obese people, thus resulting in salt retention (Dubielski *et al*., 2016). The excess weight in obese people may result with changes in the histology of the renal medulla, consequently compressing the renal tubules, reducing their cross section thus prolonging the time required for salt reabsorption (Dubielski *et al*., 2016). There is a strong association between plasma levels of renin, angiotensinogen, angiotensin II, aldosterone and obesity, independently of salt retention (Dubielski *et al*., 2016). This association was explained with the fat enveloping the kidneys that creates pressure that induces the secretion of renin (Dubielski *et al*., 2016). Other factors include the antidiuresis effect of insulin in insulin resistant obese people (Dubielski *et al*., 2016) and leptin which is associated with insulin resistance, type 2 diabetes mellitus, masked hypertension and body fat (Thomopoulos *et al*., 2009). Moreover, leptin acts directly on the paraventricular nucleus to increase sympathetic nerve activity (Zhigang *et al*., 2020).

The cluster of these metabolic factors increases the risk for many cardiovascular diseases (Wolk *et al*., 2003). Additionally, both visceral fat and central adiposity in obese people increase the risk for obstructive sleep apnoea (Wolk *et al*., 2003). Epidemiologic data found a link between obesity, obstructive sleep apnoea and hypertension, all of which are risk factors for reverse dipping (Wolk *et al*., 2003). Obstructive sleep apnoea induces sustained increases in BP during the night through other mechanisms that include but not limited to increased arterial stiffness and low grade systemic chronic inflammation (Giacomo *et al*., 2014). A large body of evidence is centred on obesity and reverse dipping increasing cardiovascular risk, there is a scarcity in the investigation of the clear association and mechanisms of reverse dipping and obesity. Available analysis focuses non-dipping.

6. Reverse dipping and target organ damage.

Many studies have demonstrated a strong association between high BP and target organ damage, both structurally and functionally (Ramachandran *et al*., 2019).

6.1. Arterial stiffness.

Arterial stiffness is mainly determined by aging and BP (Castelpoggi *et al*., 2009). It depends on the material and geometric nature of the arterial walls and the pressure at which the arteries distend (Castelpoggi *et al*., 2009; Park *et al*., 2019). Moreover, the reduced compliance of large arteries could have a compromise on the sensitivity of arterial baroreceptors, thus resulting in abnormal variations in BP (Castelpoggi *et al*., 2009). In addition, collagen deposited in the intimal layer of the arterial walls by the cells of the smooth muscles progressively reduce arterial compliance (Drucaroff *et al*., 2014). Drucaroff *et al*. (2014) observed that, arterial stiffness was increased in nighttime hypertensive men of all ages and in women older than 50 years. Moreover, arterial stiffness has been strongly linked to CV disease such as heart failure, myocardial infarction, stroke, dementia, renal disease and overall mortality
(Ramachandran *et al*., 2019). Zieman *et al*. (2005) observed that arterial stiffening is most common in patients with chronic renal disease, diabetes and metabolic syndrome.

The relationship between a non-dipping status and arterial stiffness has been conflicting among studies. Some studies have observed a relationship; with PWV (Pulse wave

velocity) being highest among non-dippers and reverse dippers compared to dippers (Asmar *et al*.,1996 ; Lekakis *et al*., 2005). Conversely, some studies did not find a relationship at all (Grandi *et al*.,2002). This was further explained by the fact that these studies used different classification methods, with the latter defining a non-dipper status based on a less than 10% drop in BP over fixed time intervals which proved poor reproducibility (Asmar *et al*.,1996 ; Lekakis *et al*., 2005; Grandi *et al*.,2002). JerrardDunne *et al*. (2007) also suggested the use of the historic definition of a BP drop less than 10% for non- dippers and alternatively using the ratio of the average nighttime to daytime BP, which is equal to or greater than 1 for reverse dippers. Conformably, this proved to improve reproducibility (Jerrard-Dunne *et al*.,2007). Additionally, JerradDunne *et al*. (2007) discovered that the association between a reverse dipping pattern and PWV remained significant even after it was adjusted for gender, BMI, age, mean arterial pressure, and smoking, reverse dippers had a higher PWV compared to nondippers and dippers. This was also true for Castelpoggi *et al*. (2009).

6.2. Left ventricular hypertrophy.

Like arterial stiffness, Left Ventricular hypertrophy (LVH) is a high risk for CV disease and can be defined by an increase in the mass of the left ventricle and an increase in the accumulation of collagen within the myocardium (Khaznadar *et al*., 2018). Left ventricular hypertrophy is influenced by cardiac load and it varies with age, sex, race, body size, BP and diabetes status (Khaznadar *et al*., 2018). A lot of controversies exist between the association of gender and LVH, Studies show that LVH is more prevalent in females; others observed the opposite while others found no association between gender and LVH (Fesler, P *et al*., 2003; Gardin *et al*., 1995; Lozano *et al*., 2006). Moreover, LVH in hypertensive people is a risk factor for insulin resistance and high levels of insulin (Khaznadar *et al*., 2018). And evidence shows that it causes heart arrhythmias (Khaznadar *et al*., 2018).

In a population-based study of black people, an association between reverse dipping and the prevalence of LVH and LVMI was observed (Abdalla *et al*., 2017). Interestingly, the association was found only in patients who were on antihypertensive medication, suggesting that reverse dipping may be benign in patients not taking antihypertensive

drugs (Abdalla *et al*., 2017). On the contrary, no interaction was found between antihypertensive drugs and dipping patterns of BP on LVH (Abdalla *et al*., 2017).

6.3. Diastolic dysfunction.

Diastolic dysfunction can be defined by the event whereby the heart is unable to relax fast enough as a result of the increased resistance in the filling of either one or both ventricles (Satpathy and Mishra, 2006). Moreover, diastolic dysfunction has been found to be a consequence of LVH (Khaznadar *et al*., 2018). McCullough *et al*. (2002) and Ahmed *et al*. (2003) observed that 40 % of patients with heart failure were able to preserve systolic function with an abnormal diastolic function. In addition, McCullough *et al*. (2002) and Ahmed *et al*., (2003) observed that diastolic dysfunction increases with age and is more prevalent among older women. Common risk factors of diastolic dysfunction include but not limited to, increased dietary salt intake, hypertension, aging, obesity, and infections (Satpathy and Mishra, 2006).

Several studies found an association between ventricular diastolic dysfunction (of both the right and the left ventricle) and reverse dipping; ventricular systolic function was preserved completely in both reverse dippers and non-dippers (ivanovic *et al*., 2013). Diastolic function of both the left and right ventricles deteriorated progressively from extreme dippers to reverse dippers (ivanovic *et al*., 2013). The possible reasons for these findings were suggested to be the result of the great sympathetic activation in both reverse and non-dippers, which causes a blunted dip in BP or even an increased night-time BP in reverse dippers (ivanovic *et al*., 2013). Other possibilities included insulin resistance and the renin-angiotensin-sympathetic interaction, which result in cardiac remodelling and alteration in the state of BP dipping (ivanovic *et al*., 2013).

6.4. Problem statement and justification.

Reverse dipping is a harmful physiological phenomenon frequently documented in patients with type 2 diabetes mellitus, hypertension, obstructive sleep disorders, and chronic kidney disease. Furthermore, reverse dipping is strongly associated with cardiovascular morbidity and mortality and has been shown to present with a poor clinical prognosis. Despite the well-documented studies on the association of reverse dipping and cardiovascular target organ damage globally, the prevalence of reverse

dipping is unknown in South Africa. Furthermore, its association with cardiovascular target organ damage has never been established in South Africans.

7. Objectives.

The objectives of the present study were therefore to:

1. Determine the prevalence of reverse dipping in a population of African ancestry.
2. Investigate the association between reverse dipping and arterial stiffness by measuring pulse wave velocity.
3. Determine the impact of reverse dipping on left ventricular geometry by determining the left ventricular mass index using echocardiography.
4. Determine the impact of reverse dipping on kidney function by determining urinary electrolyte concentration.
5. Investigate the role of dietary salt intake on the pathophysiology of reverse dipping by determining the association between 24-hour urinary salt excretion and reverse dipping.

CHAPTER TWO

METHODOLOGY

2.1. Study group

A total number of 796 South Africans of black ancestry were randomly recruited from areas around Johannesburg, South Africa. The study took place in the Human Nutrition Research Laboratory (HNRL) in the university of the Witwatersrand, Medical school campus, School of Physiology, 7th floor. The minimum age of participation was 18 years and there was no upper age limit. Prior to commencement of the examinations and measurements, the details of the study were thoroughly explained to the participants and if they agreed to participate to the study, they were requested to sign an informed consent form. A standard self-administered questionnaire (adapted with permission from the initial study by Maseko *et al*., 2006) was then presented to and completed by the consenting participants. The questionnaire requested specific answers to date of birth, sex, medical history, hypertension status, diabetes mellitus status and kidney disease, previous and current medication (an lgesic use included), prior and current occupation, education level, smoking, daily alcohol consumption, caffeine consumption, physical activity and history of family ailment for hypertension and cardiovascular events. For females, menstrual history, pregnancy history and the use of oral contraceptive. Most of the questions simply required a “Yes”- “No” answer, but participant’s understanding was evaluated by asking some short answers (Refer to appendix 3). Assistance was only given when it was requested. A majority of the participants were reasonably proficient in English.

Figure 2.1: A representation of Johannesburg where the study took place.

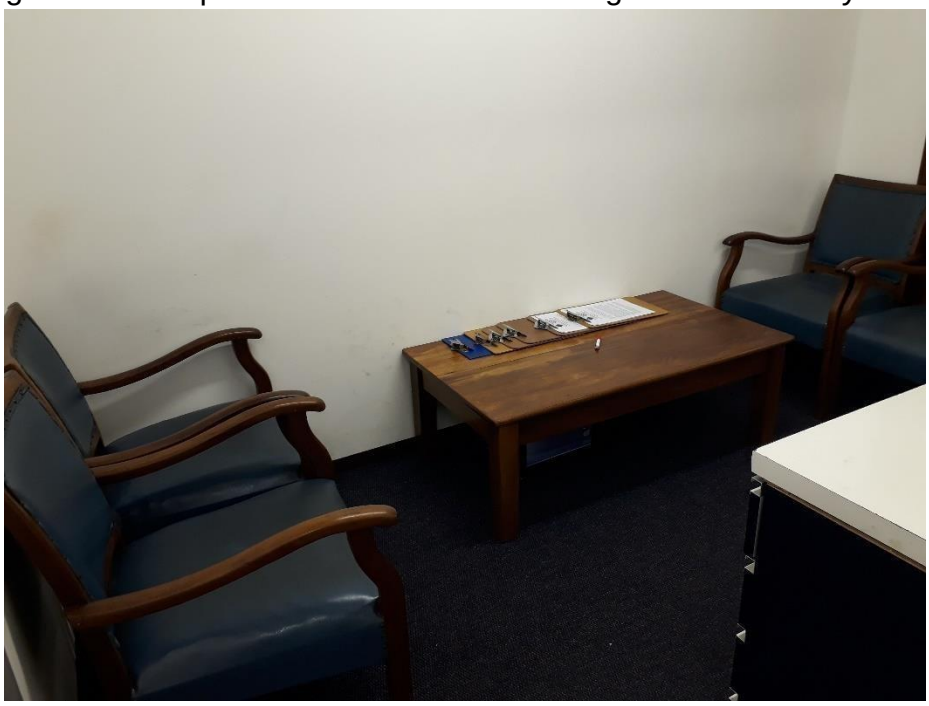

Figure 2.2: Reception area in the NHRL clinic in the School of Physiology, Faculty of Health Sciences, University of the Witwatersrand.

2.2. Anthropometric measurements

Participants were requested to remove their clothing and shoes, and a medical gown was given to them to wear. This was done to allow access to measurement areas with no interference.

Anthropometric measurements were taken according to the international standards for anthropometric assessments (Marfell-Jones *et al*., 2012). Weight measurements were recorded using an electronic scale (Healthometer Professional, McCook, IL, USA) to the nearest 0.1 kg. Height was measured using a stadiometer (Seca portable stadiometer, Hamburg, Deutschland, Germany) to the nearest 0.1 cm. When the measurement was taken the head of the participant was placed in the Frankfort plane with the body standing upright and a sliding headboard was lowered to the vertex of the head. Using body weight and height measurements, the participants' body mass indices (BMI) were determined as weight in kilograms divided by height in meters squared (kg/m^2). These findings were used to categories the participants as underweight, normal, overweight, or obese using the cut-off values adapted from WHO, 2000; $<18.5kg/m^2$, $18.5\text{-}24.9kg/m^2$, $25.0\text{-}29.9kg/m^2$, and $\geq 30kg/m^2$ for underweight, normal weight, overweight, and obesity, respectively.

Waist circumference was determined using a tape measure with participants wearing minimal clothing on the abdominal region. Waist circumference was determined at the narrowest width midway between the iliac crest and the lowest rib to the nearest 0.1 cm at the end of a gentle expiration. This was done to determine central adiposity as guided by the cut-off values adapted from Heyward and Wagner, 2004.

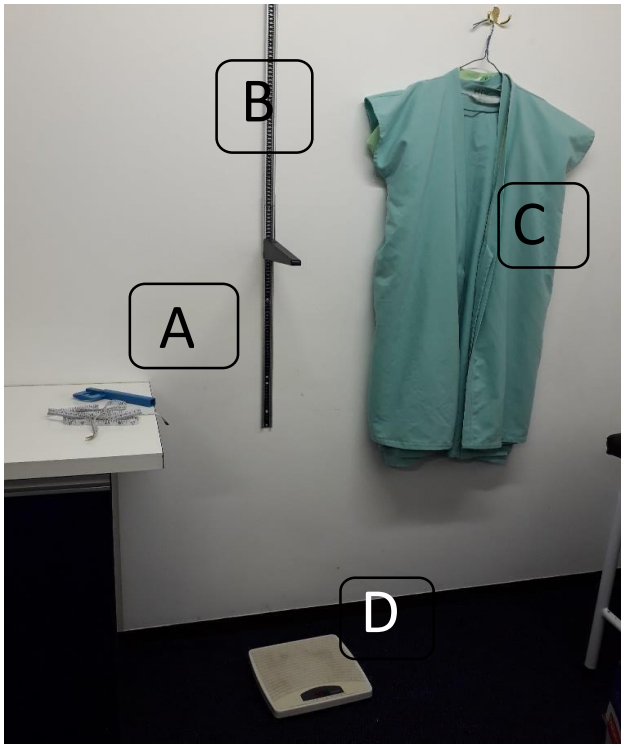

Figure 2.3: general measuring room in the HNRL clinic showing, A: measuring tape and Harpenden skin-fold caliper used to take waist and hip circumference and skin fold measurements respectively; B: stadiometer (Seca portable stadiometer, Hamburg, Deutschland, Germany) used to take height measurements; C: hospital gowns worn by participants to minimize excessive clothing; D: electronic scale (Healthometer Professional, McCook, IL, USA) used to take weight measurements.

2.3. Conventional blood pressure

Participants were allowed to relax for 5 to 10 minutes before BP measurements were taken. Participants assumed a relaxed sitting position (upright with their upper arm positioned so it is level with their heart and feet flat on the floor). An Omron BP monitor was used. Standard cuffs (22 × 12cm) with inflatable bladder were used in participants with a mid-upper arm circumference (MUAC) <31 cm and larger cuffs (31 × 15 cm) were used in participants with a MUAC ≥31 cm. The BP cuff was positioned so that the artery marker pointed to the brachial artery. Measurements were taken five times consecutively on the less dominant arm with at least one minute setting each measurement apart. The five readings were averaged to one systolic and diastolic reading. Conventional BP was taken to record clinical/ in office BP.

Figure 2.4: Omron BP monitor used to take BP measurements.

2.4. Pulse wave velocity

Arterial stiffness was determined by recording carotid and femoral pulse wave velocity (PWV) using augmentation indexes (AI) including central Al (Alc) and peripheral Al (Alp). The method has been previously described by Shiburi *et al*., 2006. At the radial (dominant arm), pulse was recorded by applanation tonometry during an 8-second period using a high-fidelity SPC-301 micro manometer (Millar Instrument, Inc., Houston, Texas), interfaced with a SphygmoCor software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia); version 9.0. This was done to determine pressure components, after participants were allowed to rest for 15 minutes in the supine position. Data with consecutive waveforms that exceeded 5% due to variability in systolic or diastolic BP or the pulse wave signal with amplitude of less than 80mV were discarded.

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

The difference between central systolic BP and the inflection point at the end of the first systolic shoulder was defined as the magnitude of the augmented pressure wave (AP). The difference between central systolic BP and central diastolic BP was used to calculate central PP (PPc), and the formula [central diastolic BP + 1/3(central PP)] was used to calculate mean arterial pressure (MAP).

Aortic PWV was measured from sequential waveform measurements at carotid and femoral sites as previously described (Shiburi *et al*. 2006). The aortic pulse wave was calculated using a validated generalized transfer function. The radial AIp was defined as the ratio of the second to the first peak of the pressure wave expressed in percentage. Distances from the suprasternal notch to the carotid sampling site (labelled as distance A), and from the suprasternal notch to the femoral artery

(Distance B) were measured. The pulse transmit time was determined from the average of 10 consecutive beats (the mean time difference between sites A and B).

Lastly, aortic pulse wave velocity was determined as the ratio of the distance in meters to the transit time in seconds.

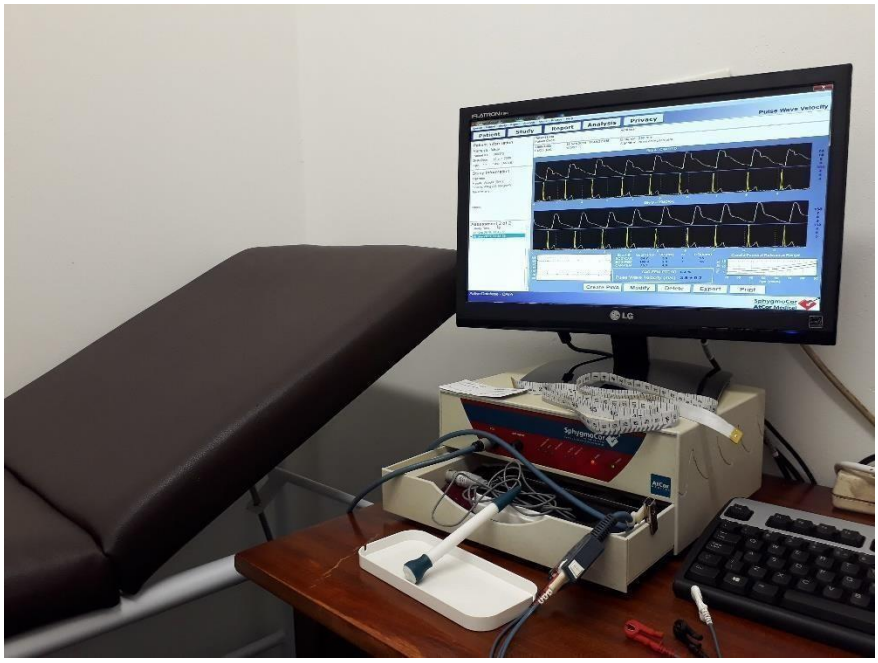

Figure 2.5: SphygmoCor device coupled to an applanation tonometer used to determine central (aortic) haemodynamics and aortic PWV.

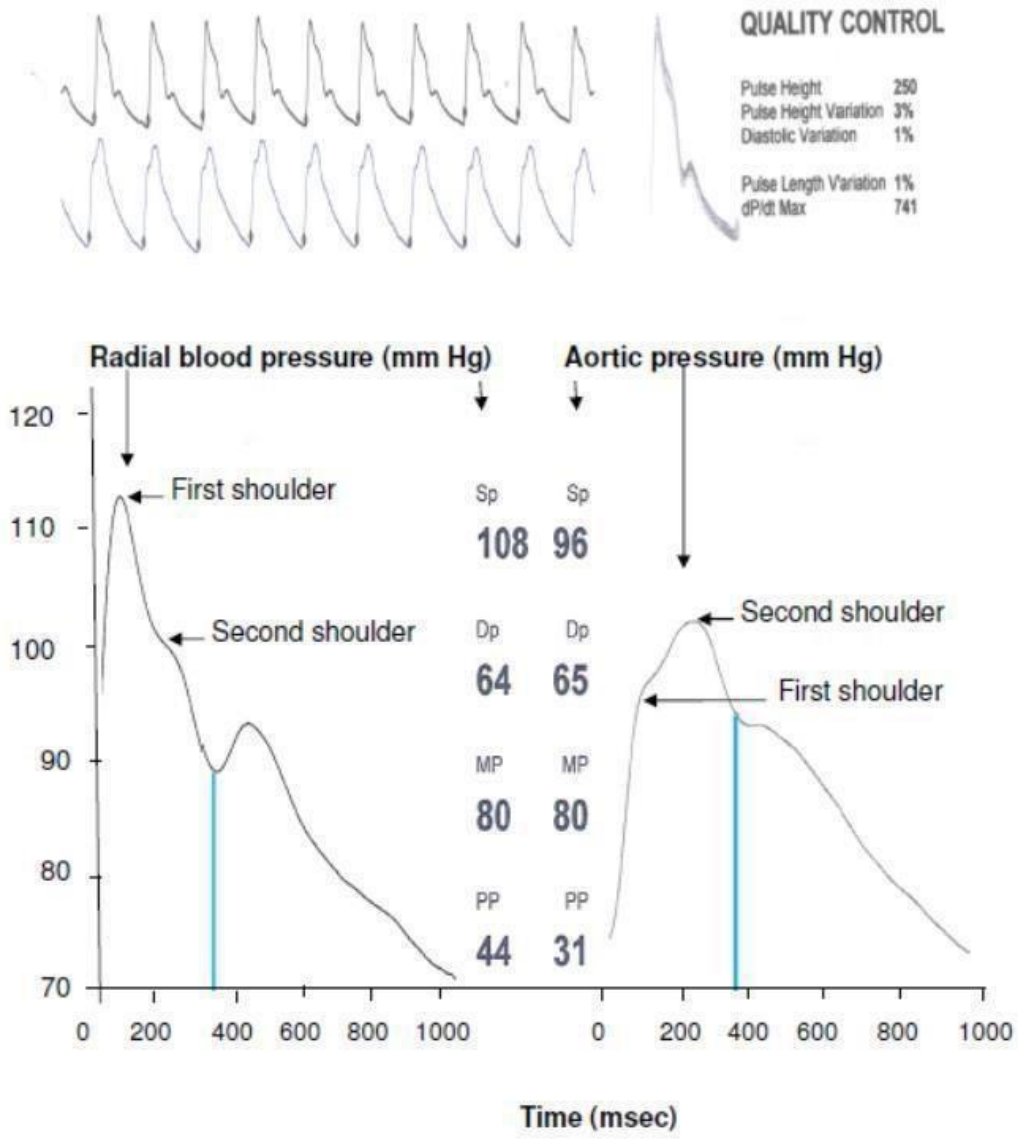

Figure 2.6: Example of a pulse wave recording obtained to determine central hemodynamics. The figure shows the radial artery pulse wave obtained from applanation tonometry (lower left panel) and the aortic pulse wave derived from a population-based transfer function built into the software (lower right panel). The first and second systolic shoulders are identified. Quality control assessments are shown in the top panel. Sp, systolic BP (BP); Dp, diastolic BP; MP, mean arterial pressure; PP, pulse pressure (Shiburi *et al*., 2006).

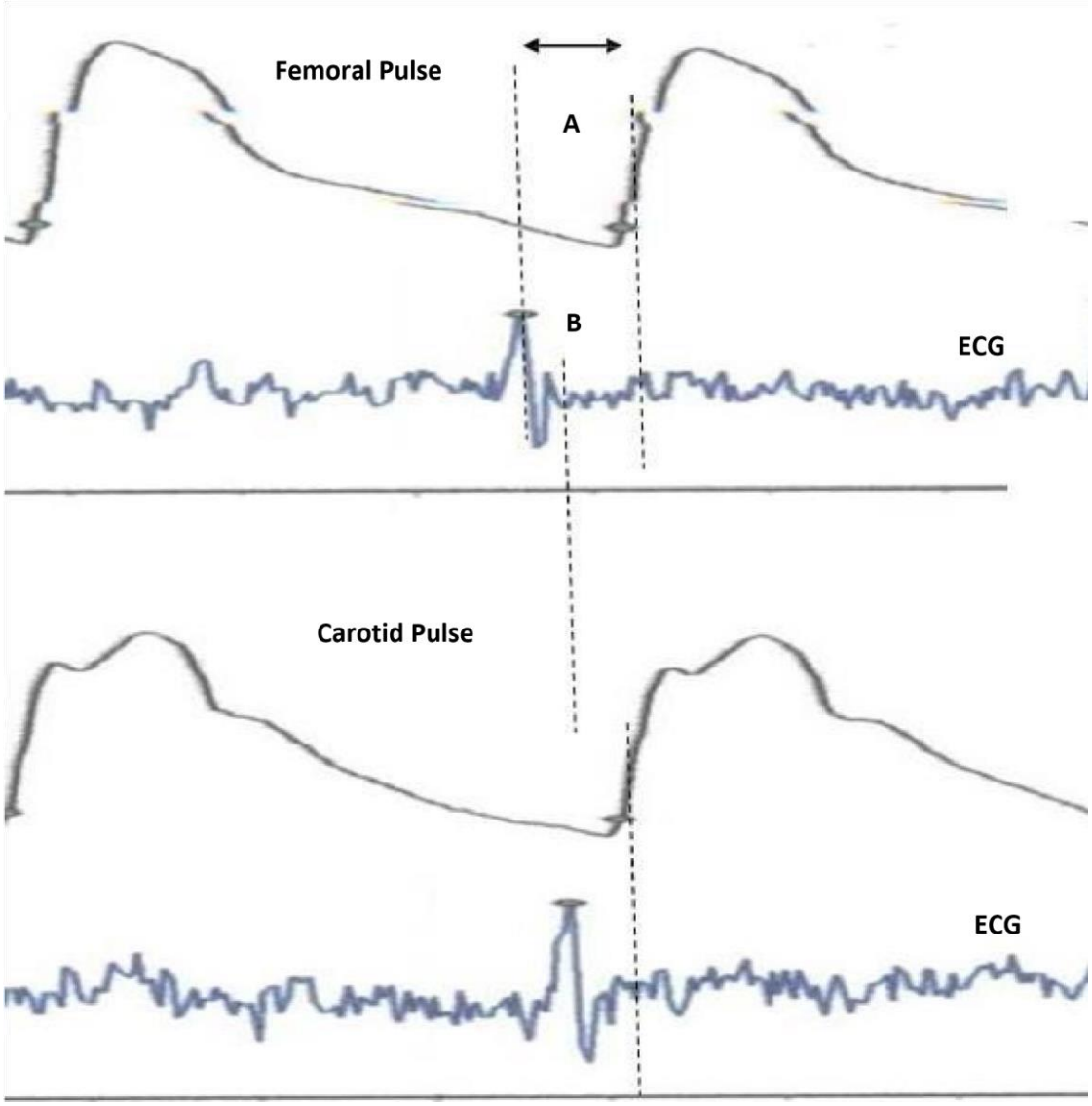

Figure 2.7: Examples of femoral and carotid artery pulse waves obtained using applanation tonometry from the same participants. Together with simultaneous electrocardiographic (ECG) recordings aortic PWV (PWV) is calculated. The arrows indicate the time between electrical events and the arterial pressure changes in the carotid and femoral arteries used to calculate PWV (Shiburi *et al*., 2006).

2.5. Echocardiographic assessment

Echocardiographic measurements were used to assess the size of the left ventricle to detect left ventricular hypertrophy, which is an underlying risk factor for coronary heart disease. The measurements were performed according to the method described by Skudicky *et al*. (2002) and analyzed according to the American Society of

Echocardiographic convention (Sahn *et al*., 1978). A trained technician conducted the measurements. A pulse color Doppler Hewlett Packard model 5500 recorder coupled to a 2.5 MHz transducer was used to conduct a two-dimensional guided M-mode echocardiography. The transducer was placed on the chest perpendicular to the chest wall or pointed inferiorly and laterally at the end of the long axis (Sahn *et al*., 1978). Data obtained in the short-axis view allowed for measurements of the septal wall thickness, posterior wall thickness, and the left ventricle diameter during late diastole and systole. Left ventricular mass was derived from an anatomically validated formula and indexed to height 2.7 (Devereux *et al*., 1986). Left ventricular wall thickness, left ventricular mean wall thickness, left ventricular hypertrophy, and left ventricular concentricity was defined as described by Nunez *et al*., (2005).

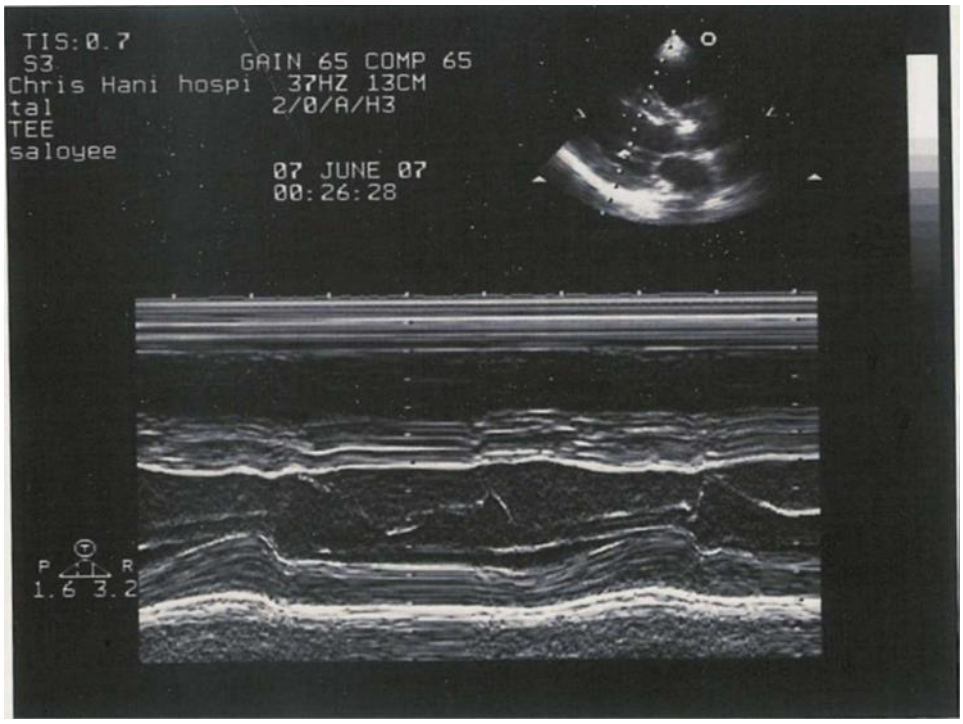

Figure 2.8: Echocardiogram used to assess cardiac structure and function. The picture shows internal dimensions and posterior wall thickness of the left ventricle (Majane, 2009). A- Left ventricular end diastolic (LVED) diameter; B- Left ventricular end systolic (LVES) diameter; C-Left ventricular end systolic posterior wall thickness; D-Left ventricular end diastolic posterior wall thickness; E-Left ventricular end diastolic septal wall thickness; F-Left ventricular end systolic septal wall thickness (Nunez *et al*., 2005).

2.6. Blood sampling

Participants were asked to fast overnight prior to clinical visit. Following an overnight fast, venous blood of about 18 ml was drawn from the median cubital vein of the participants' arm with the help of a professional nurse. The samples were taken to Central Laboratory Services (CLS) for analysis which included Renin, aldosterone, insulin, leptin, Aldo/renin ratio, and angiotensinogen (AGT).

2.7. Ambulatory blood pressure

After all the assessments were done, participants were given a monitor to assess 24hour ambulatory BP. The non-dominant arm was used for measurements. The monitors were calibrated against a mercury manometer. Cuff sizes were the same dimension as explained in conventional BP measurements. Monitors were set at fifteenminute intervals from 06:00–22:00 and one-hour intervals from 22:00–06:00.
Participants kept a diary card to record times for going to bed in the evening and getting up in the morning. Ambulatory BP was expressed as 24-hour, daytime and nighttime systolic and diastolic BP. Time intervals between 06:00 AM and 22:00 PM were considered as daytime, and time intervals between 22:00 PM and 06:00 AM were considered as nighttime. The ABP monitor, which was connected to a cuff around the participant's upper arm, was attached to a belt around the participant's waist. The ABP monitor was small enough that the participant could go about their normal daily life and even sleep with it on.

Figure 2.9: Ambulatory blood pressure monitor.

2.8. Twenty-four-hour urine assessments

Twenty-four-hour urine samples were collected for the analysis of Ca^{2+}, K^{+}, Na^{+}, Mg^{2+} and NaK (The ratio of sodium to potassium) to assess kidney function. Each participant was issued two urine collection bottles, for both daytime and nighttime urine. These urine bottles were labelled as daytime and night-time sample to separate and differentiate between the two collected samples. This was done to allow separate determination of the electrolyte excretion rates and ratios between daytime and nighttime. Participants were given careful instruction on how to differentiate between the times for the sample collection.

Twenty-four-hour urine bottles were collected from each participant after 24 hours, together with the ABP monitors. Twenty-four-hour urine samples with a volume ≥400 ml/day were considered as an acceptable volume from the participants. The urine samples were then taken to CLS for determination of 24-hour, daytime and night-time urinary excretion rates of Ca^{2+}, K^{+}, Na^{+}, Mg^{2+} and NaK.

Figure 2.10: 24-hour urine bottles given to participants to collect urine.

2.9. Data analysis

Data management and statistical analysis was performed using SAS software, version 9.4 (The SAS Institute Inc., Cary, North Carolina, USA). Normally distributed data was expressed as means ± SD or as frequencies (percentage). The means and standard deviations were determined using the general linear model (GLM). An independent t-test was used to determine the association of the variables (PWV, LVMI, urinary electrolytes and 24h urinary excretion) between the groups (dippers vs non-dippers, dippers vs reverse dippers, and non-dippers vs reverse dippers). Corrections for covariates (age, gender, BMI, hypertension and diabetes status, alcohol intake and smoking) were made using the general linear model. A P-value of less than 0.05 was considered statistically significant.

CHAPTER THREE

RESULTS

Table 3.1 describes the demographic, anthropometric and general clinical characteristics of the participants in the present study. These characteristics include BMI, age, gender, smoking, alcohol intake, hypertension, and diabetes status. Of the total population (796.0), a dipping pattern of night-time BP was observed in 48.0% (383.0) of the participants. Thirteen percent (13.0%) of the participants displayed a reverse dipping pattern while 39.0% (308.0) displayed a non-dipping pattern. The mean age of the total population was 44.2±18.3 years with dippers being the youngest at 40.6±17.6 compared to reverse dippers at 56.0±17.2 ($p < 0.0001$). The mean age of the non-dippers was almost the same as that of the total population at 44.9±17.4. The p-values are shown in table 3.1.1. The mean BMI of the total population was 28.9±7.6, with 15.0% of the participants being smokers, 21.0% consumed alcohol, 39.0 % being hypertensive and 9.0% being diabetic. The mean BMI of dippers was 27.4±7.1, with 18.0% of the participants being smokers, 22.0% consuming alcohol, 31.0% being hypertensive and 17.0% being diabetic. The mean BMI of reverse dippers was 31.4±8.6, with 10.0% of the participants being smokers, 13.0% consuming alcohol, 65.0% being hypertensive and 17.0% being diabetic. The mean BMI of non-dippers was 29.3±7.7 with 17.0% of the participants being smokers, 26.0% consuming alcohol, 47.0% being hypertensive and 15.0% being diabetic. The p-values are shown in table 3.1.1.

Table 3.1: General and clinical characteristics of the study population

Variables	TP	DP	ND	RD
Sample number	796.0	383.0 (48.1%)	308.0 (38.7%)	105.0 (13.2%)
Age (years)	44.2±18.3	40.6±17.6	44.9±17.4[a]	56.0±17.2[ab]
%Female	63.0	61.4	66.6	64.8
BMI (kg/m^2)	28.9±7.6	27.4±7.1	29.3±7.7[a]	31.4±8.57.0[ab]
%Smokers	15.0	18.0	17.0	10.0
%Alcohol intake	21.0	22.0	26.0	13.0
%Hypertensive	39.0	31.0	47.0	65.0
%Diabetic	9.0	7.0	15.0	17.0

TP, Total population; DP, dippers; ND, non-dippers; RD, reverse dippers; BMI, body mass index; Age and BMI data expressed as mean ± standard deviation; $p < 0.05$ considered significant; [a], *p*-value significantly different from dippers; [b], *p*-value significantly different from non-dippers.

Table 3.1.1 P-values of age and BMI when compared between dippers, non-dippers and reverse dippers.

	Age	**BMI**
DP vs ND	0.0413	0.0449
DP vs RD	<0.0001	0.0373
ND vs RD	0.0198	0.0459

DP, dippers; ND, non-dippers; RD, reverse dippers; BMI, body mass index; $p < 0.05$ considered significant.

Table 3.2 displays the haemodynamic characteristics of the study population, which includes conventional and ambulatory BP (ABP) values of the participants. Twenty-four hour and night-time systolic and diastolic BP was significantly different between the three groups (dippers, non-dippers, and reverse dippers). However daytime BP was not significantly different between the three groups (p=0.9011, 0.7930, and 0.9127 for DP vs ND, DP vs RD, and ND vs RD respectively). This was true for both systolic and diastolic BP. The night-to-daytime BP ratio was also significantly different between the three groups for both systolic and diastolic BP. Reverse dippers had a significantly higher systolic night-time BP compared to dippers and non-dippers ($p< 0.0001$ for both dippers and non-dippers), and a normal diastolic BP. Conventional systolic BP was significantly different between the three groups. On the other hand, the conventional diastolic BP of reverse dippers was significantly different for both dippers and non-dippers ($p= 0.0317$ and 0.0400 respectively) while there was no significant difference in the conventional diastolic BP of dippers and non-dippers ($p=0.3403$).

Table 3.3 shows the mean and standard deviation values of plasma hormone concentrations of the total population and the three groups (dippers, non-dippers and reverse dippers). These include renin, aldosterone, insulin, leptin, aldosterone-to-renin ratio, and angiotensinogen. The plasma renin, insulin and angiotensinogen

concentrations of the three groups were not significantly different (Table 3.3.1). The plasma aldosterone concentration of the reverse dippers was significantly different from that of the dippers and non-dippers (p= 0.0155, and 0.0207 respectively) while there was no significant difference in the aldosterone plasma concentrations between dippers and non-dippers (p= 0.3610). Similarly, the plasma concentrations of leptin and the aldosterone-to-renin ratio of the reverse dippers was significantly higher than that of the dippers and non-dippers (p= 0.0343 and 0.0259 for leptin; and 0.0013 and 0.0298 for aldo/renin respectively) while there was no significant difference between the dippers and non-dippers (p= 0.9051 for leptin and 0.6419 for aldo/renin).

Table 3.4 shows the 24-hour urinary excretion rate of four electrolytes (potassium, calcium, magnesium and sodium). Sodium and potassium rates were used to calculate the sodium-to-potassium ratio. No significances were observed in the potassium, calcium, and sodium values for all 3 groups (Table 3.4.1). However, the magnesium 24hour urinary excretion rate of the reverse dippers was significantly higher than that of the dippers and non-dippers (p=0.0286, and 0.0591 respectively). Similarly, the sodium-to-potassium ratio of the reverse dippers was significantly higher than that of the dippers and non-dippers (p= 0.0329, and 0.1097 respectively).

Table 3.2: Haemodynamic characteristics of the study population

Variables	TP	DP	ND	RD
SBP24 (mm Hg)	118.3±15.0	114.8±11.5	119.3±15.5[a]	128.2±19.0[ab]
DBP24 (mm Hg)	72.6±18.2	70.3±8.5	73.7±10.4[a]	78.7±12.4[ab]
SBPN (mm Hg)	111.3±17.2	102.8±10.6	114.3±15.1[a]	132.2±20.3[ab]
DBPN (mm Hg)	64.9±11.7	59.3±8.5	67.5±10.6[a]	77.7±12.7[ab]
SBPD (mm Hg)	122.5±14.7	121.9±12.1	122.2±15.8	125.1±18.8
DBPD (mm Hg)	77.6±10.4	77.2±9.1	77.6±10.9	79.1±12.8
RSBP	0.9±0.1	0.9±0.05	0.9±0.0[a]	1.1±0.1[ab]
RDBP	0.8±0.1	0.7±0.07	0.9±0.1[a]	1.0±0.1[ab]
SBPC (mm Hg)	128.3±21.0	124.9±17.6	129.5±22.9[a]	137.4±23.4[ab]
DBPC (mm Hg)	83.7±11.8	82.5±11.0	83.9±12.1	87.4±13.1[ab]

TP, Total population; DP, dippers; ND, non-dippers; RD, reverse dippers; SBP24, 24-hour systolic BP; DBP24, 24-hour diastolic BP; SBPN, night-time Systolic BP; DBPN, night-time diastolic BP; SBPD, day-time systolic BP; DBPD, day-time diastolic BP; RSBP, night-to-daytime systolic BP ratio; RDBP, night-to-daytime diastolic BP ratio; SBPC, conventional systolic BP; DBPC, conventional diastolic BP; [a], *p*-value significantly different from dippers; [b], *p*-value significantly different from non-dippers.

Table 3.2.1: P- values of the Hemodynamic characteristics of the study population

	SBP24	DBP24	SBPN	DBPN	SBPD	DBPD	SBPC	DBPC
DP vs ND	0.3342	0.0401	0.0171	0.0500	0.8162	0.9011	0.0303	0.3403
DP vs RD	<0.0001	0.0081	<0.0001	<0.0001	0.6611	0.7930	0.0004	0.0317
ND vs RD	<0.001	0.0371	<.0292	0.0292	0.7984	0.9127	0.0194	0.0400

DP, dippers; ND, non-dippers; RD, reverse dippers; SBP24, 24-hour systolic BP; DBP24, 24-hour diastolic BP; SBPN, night-time Systolic BP; DBPN, night-time diastolic BP; SBPD, day-time systolic BP; DBPD, day-time diastolic BP; SBPC, conventional systolic BP; DBPC, conventional diastolic BP; $p < 0.05$ considered significant.

Table 3.3: plasma hormone concentration

Variables	TP	DP	ND	RD
Renin (μIU/mL)	36.6±72.5	37.5±76.5	37.9±72.3	29.5±56.7
Aldosterone (pmol/L)	198.5±162.7	188.5±149.6	192.3±156.9	253.1±209.9[ab]
Insulin (IU/mL)	14.1±16.4	14.6±16.9	12.8±14.9	15.4±18.7
Leptin (ng/mL)	20.2±24.3	23.5±16.9	23.1±21.6	29.4±25.3[ab]
Aldo/renin (pmol/L per μIU/mL)	27.4±51.2	22.7±39.4	24.7±36.5	50.8±95.4[ab]
AGT (pg/mL)	29.3±12.3	29.3±13.0	29.3±12.4	29.6±88.9

TP, Total population; DP, dippers; ND, non-dippers; RD, reverse dippers; AGT, angiotensinogen; [a], *p*-value significantly different from dippers; [b], *p*-value significantly different from non-dippers.

Table 3.3.1: P-values of the plasma hormone concentrations when compared among the 3 groups

	Renin	Aldosterone	Insulin	Leptin	Aldo/renin	AGT
DP vs ND	0.8901	0.1463	0.3610	0.9051	0.6419	0.7315
DP vs RD	0.0714	0.0155	0.5001	0.0343	0.0013	0.5002
ND vs RD	0.7219	0.0207	0.2112	0.0259	0.0298	0.8109

DP, dippers; ND, non-dippers; RD, reverse dippers; AGT, angiotensinogen; $p < 0.05$ considered significant

Table 3.4: Urine electrolyte values

Variables	TP	DP	ND	RD
K^+(mmol/day)	28.4±21.8	30.5±22.1	27.2±21.1	24.8±22.6
Ca^{2+} (mmol/day)	1.6±1.9	1.6±1.7	1.7±2.1	1.2±1.7
Mg^{2+}(mmol/day)	2.7±1.9	2.5±2.1	2.2±1.8	1.7±1.4[a]
Na^+ (mmol/day)	105.6±78.4	111.0±80.8	101.8±73.8	97.9±83.4

NaK	4.4±3.2	4.1±2.0	4.4±2.5	5.4±4.4[a]

TP, Total population; DP, dippers; ND, non-dippers; RD, reverse dippers; NaK, sodium-potassium ratio; [a], *p*-value significantly different from dippers; [b], *p*-value significantly different from non-dippers.

Table 3.4.1: P-values of the urine electrolytes when compared among the three groups.

	K^+	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**NaK**
DP vs ND	0.4131	0.3714	0.0601	0.1938	0.0733
DP vs RD	0.0803	0.1173	0.0286	0.0701	0.0329
ND vs RD	0.1239	0.1839	0.0591	0.2392	0.1097

DP, dippers; ND, non-dippers; RD, reverse dippers; NaK, sodium-potassium ratio; $p < 0.05$ considered significant

Figure 3.1 illustrates the average mean ±standard deviation values of PWV for the three different groups (dippers, non-dippers, and reverse dippers). It illustrates how pulse wave velocity varies among the 3 groups. Pulse wave velocity was significantly different between the three groups (p=0.0159 between dippers and non-dippers, p=0.0028 between dippers and reverse dippers and p=0.0271 between non-dippers and reverse dippers).

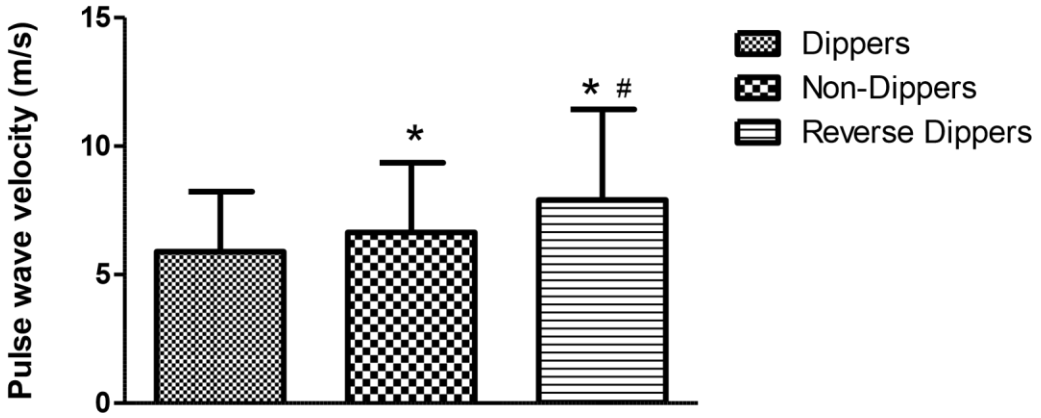

Figure 3.1: Pulse wave velocity for dippers, non-dippers, and reverse dippers. Means corrected for age, sex, BMI, hypertension, diabetes, smoking and alcohol intake. * P value significantly different from dippers; # P value significantly different from non-dippers.

Table 3.1.2; P-values of PWV and E/A when compared among the three groups

	PWV	**E/A**
DP vs ND	0.0159	0.0332
DP vs RD	0.0028	0.0072
ND vs RD	0.0271	0.0149

DP, dippers; ND, non-dippers; RD, reverse dippers; PWV, pulse wave velocity; E/A, early to late ventricular filling

Figure 3.2 illustrates the average mean ± standard deviation of LVMI of the three groups (dippers, non-dippers, and reverse dippers). There was no significant difference in the LVMI between dippers and non-dippers while the LVMI of the reverse dippers was significantly higher than that of the dippers and non-dippers.

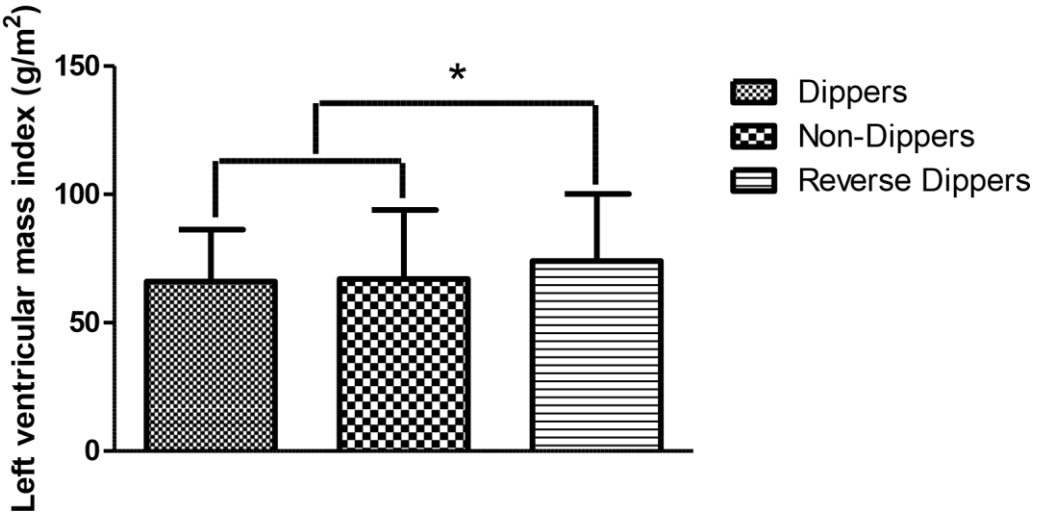

Figure 3.2: Left ventricular mass index for dippers, non-dippers, and reverse dippers. Means corrected for age, sex, BMI, hypertension, diabetes, smoking and alcohol intake. * P value < 0.05.

Figure 3.3 illustrates the average mean ± standard deviation of early-to-late diastolic filling ratio of the three groups (dippers, non-dippers and reverse dippers). The dippers had the highest early-to-late diastolic filling, followed by the non-dippers, and the reverse dippers had the lowest early-to-late diastolic filling. The ratio of the three groups was significantly different.

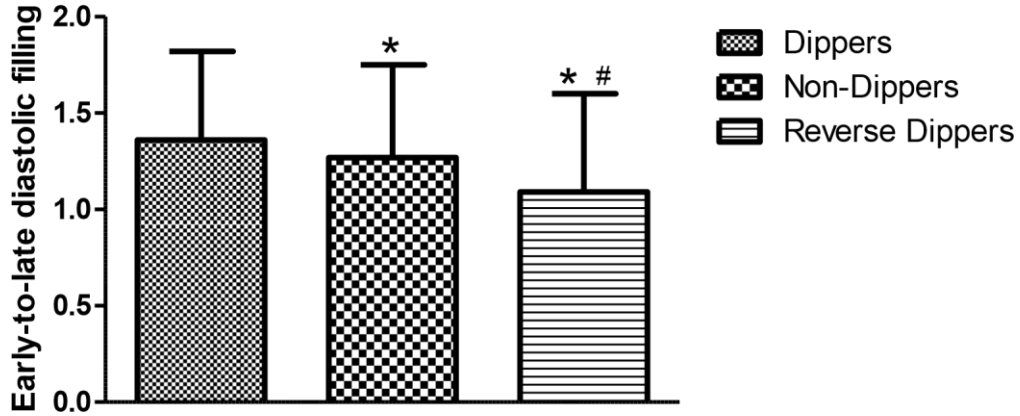

Figure 3.3: Early to late diastolic filling in dippers, non-dippers and reverse dippers. Means adjusted for age, gender, BMI, diabetes, hypertension, smoking and alcohol intake. * P value significantly different from dippers; # P value significantly different from non-dippers.

Figure 3.4 shows the percentage of normotensives among non-dippers and reverse dippers. Among non-dippers, 97.0% of the young adults, 68.0% of the middle-aged group, and 33.0% of the older adults were normotensive. Among reverse dippers, 94.0% of the young adults, 64.0% of the middle-aged group, and 35.0% of the older adults, were normotensive.

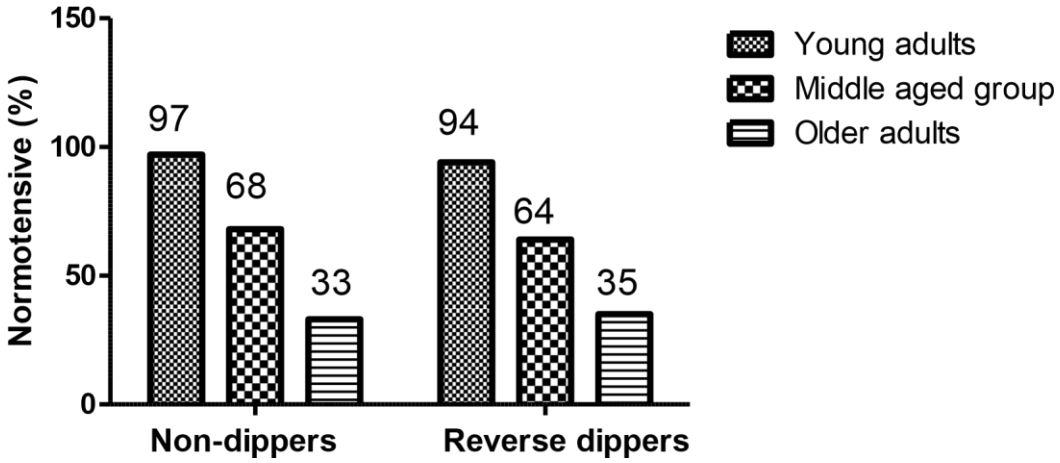

Figure 3.4: Percentage of normotensive individuals classified by age and dipping status.

CHAPTER FOUR

DISCUSSION

4.1. Demographic parameters

Participants were categorized into 3 groups based on the differences between mean systolic BP during the day and mean systolic BP during the night. Participants who had a 10 to 20% fall in night-time BP were categorized as dippers, while participants who had a night-time BP fall of less than 10% were categorized as non-dippers. Participants who had a higher night-time BP compared to daytime BP were categorized as reverse dippers. The prevalence of dipping, non-dipping and reverse dipping was 48.0%, 39.0%, and 13.0% respectively. In a similar population-based study of a black population (Abdalla *et al.*, 2017), the prevalence of dipping, nondipping and reverse dipping was 34.0%, 48.0% and 18.0% respectively. The proportion of nondippers was higher compared to dippers and reverse dippers. In the present study, the highest proportion observed was that of dippers. Factors that could have accounted to the observed differences between the two studies may be that; the participants in the Abdalla and colleagues' study were African American and the participants in the present study were Africans from South Africa. A review study by Zibermit *et al.* (2019) showed genetic and demographical differences between African Americans and others of African descents living in Africa. This is a clear indication that results from studies conducted in African Americans cannot be used as a surrogate for people of African ancestry living in Africa. Besides the difference in geographic location, another difference is that their intervals of measuring BP on their ambulatory BP monitor were different from the present study, their daytime and nighttime periods were defined differently from the present study; their BP was measured in 20 minutes intervals, both during daytime and night-time (which was 20 minutes and 1 hour for day and nighttime respectively in the present study). They defined their daytime as the time between 10 AM and 8 PM (between 6 AM and 22 PM in the present study), and night-time as the time between 12 AM and 6AM (between 22 PM and 6 AM in the present study). Their study population was older than the population in the current study. The age range (21 years and older) was different from the one of the present study (18 years and older). Their average age of non-dippers and reverse dippers was 58.9±11.2 and 62.4±9.7 respectively, whereas in the present study it was 44.86±17.4 and 56.0±17.2 for non-dipping and reverse dipping, respectively. The differences in

the population demographics between the two populations influenced the results of the two populations. The results were different even though both populations can be classified as black. This clearly suggests that studies designed to determine the prevalence of reverse dipping must be population specific because determinants of dipping like daytime and night-time hours influence BP dipping. Hence the importance of the current study which was conducted in people of African ancestry living in Africa.

The prevalence of non-dipping and reverse dipping was high in females compared to males in the present study. These findings were similar to the findings of Abdalla *et al.* (2017). The physiology behind the gender associated differences in BP also agree with the findings of Babatsikou and Zavitsanou (2010); Reckelhoff, (2001); Abdalla *et al.* (2017). However, these findings can be bias as the majority of the study population were females compared to males in these studies. Blood pressure is higher in men until the age of 55 years (Babatsikou and Zavitsanou, 2010), where it is slightly elevated in postmenopausal women (Babatsikou and Zavitsanou, 2010). The mechanism is not clear, however there is evidence that androgens such as testosterone may play a role in gender associated differences in BP regulation (Reckelhoff, 2001). Studies show that after the onset of puberty, when androgen levels are increasing, boys have a higher BP compared to girls of similar age (Reckelhoff, 2001). Additionally, their results also show that nighttime BP does not dip as low in boys as it does in girls post pubescent. Castration studies have shown that castration at a younger age lowered the development of hypertension in male rats (Reckelhoff, 2001). Other studies (Reckelhoff, 2001; Maranon and Reckelhoff, 2013) suggest that the lack of oestrogen at menopause may be the cause for elevated BP in women. Evidence from Chignalia *et al*., an animal model study which observed the effect of testosterone in normotensive and hypertensive male rats, showed that testosterone regulate vascular smooth muscle cells by regulating non receptor tyrosine kinase phosphorylation and *c-src* which mediate vascular contraction and hypertrophy, and regulate events that contribute to increased vascular resistance in hypertension. This may be a contribution to the blunted dip or increase in night-time BP in hypertensive individuals. Female sex hormones may not be the only factor that contribute to the elevation of BP, the simultaneous presence of obesity and type 2 diabetes may also complicate the regulation of BP (Reckelhoff, 2001). The importance of these if findings

is that any intervention strategies that seek to address reverse dipping should consider gender differences.

Diabetes may also play a role in the dipping status of this population as our results show that both non-dippers and reverse dippers had the highest number of diabetics compared to dippers. Chronic increased blood sugar is shown to expose individuals to hypertension, and it has been observed that diabetic individuals tend to have a higher night-time BP (Taghi *et al*., 2018), partly because of autonomic dysfunction (Rossen *et al*., 2014). Type 2 diabetes has been suggested to influence BP through sympathetic activation by hyperinsulinemia and the activation of intrarenal RAAS by hyperglycaemia, hence the higher nigh-time BP in reverse dippers compared to nondippers and dippers (Yan *et al*., 2016). Moreover, Yan *et al*. (2016) showed in their study that diabetes and reverse dipping are interrelated risk factors for each other. Therefore, the findings of our study seem to suggest that control of plasma blood sugar may be paramount to reducing the incidence of reverse dipping in this population.

Our results also indicate that age may play a significant role in the dipping status of this community. Dipping status progresses from dipping to non-dipping to reverse dipping with increasing age. This clearly shows that the attenuation of nighttime BP dipping is strongly influenced by age. When we looked at the proportion of normotensives among non-dippers and reverse dippers by age (figure 3.4), among non-dippers, 97.0% of the young adults, 68.0% of the middle-aged group, and 33.0% of the older adults were normotensive. Among reverse dippers, 94% of the young adults, 64.0% of the middle-aged group, and 35.0% of the older adults, were normotensive. Our results suggested that non-dipping and reverse dipping is mostly common among the black, older, and hypertensive generation and is less common among the younger generation. Our findings matched with several other similar studies (Cuspidi *et al*, 2017; Yan *et al*., 2015).

Adiposity may also play a role in the pathophysiology of reverse dipping as our results also showed that both non-dippers and reverse dippers had a significantly higher BMI compared to dippers. This association may be directly responsible for the high incidence of non-dipping and reverse dipping in overweight/obese individuals observed in our study and similar studies. A study by Dubielski *et al*. (2016). confirms

our findings. Their study showed an association between a blunted night-time BP drop and obesity. The underlying mechanisms may be due to obesity related hormonal changes, increased sympathetic nervous system activation, altered kidney function, vascular structural changes, and endothelial dysfunction (Dubielski *et al*., 2016). This was also relatable with several other studies (Mvunzi *et al*., 2017; Deng *et al*., 2017; Abdalla *et al*., 2017). Additionally, obesity is associated with abnormal sleeping patterns such as obstructive sleep apnoea (OSA) (Pucci *et al*., 2014). Obstructive sleep apnoea has been observed to affect approximately one-fifth of the obese adult population, especially in the United states and its prevalence is estimated to increase because of the rising incidences of obesity (Pucci *et al*., 2014). Pucci *et al*. (2014) suggested that OSA exerts its effect on the CV system through several haemodynamic and chemo-mediated mechanisms. Repeated apnoea and hypoxia episodes seen in obese people who present with OSA result in sleep interruption and consequently the arousal responses of the autonomous nervous system are multiplied (Pucci *et al*., 2014) thus preventing the appropriate dipping of BP at night and/or resulting in an increase in night-time BP (Pandey, 2014). Moreover, night-time PB may increase by 20mm Hg within a few minutes in obese individuals who present with OSA, and indefinitely leading to long-lasting activation of the sympathetic nervous system (Pucci *et al*., 2014). Obstructive sleep apnoea may also induce sustained reverse dipping effects through several other mechanisms which include, but not limited to arterial stiffness, interrupted baroreflex sensitivity, salt sensitivity, and nighttime volume overload (Pucci *et al*., 2014). This was also confirmed by Steinhorst *et al*. (2014) and Genta-Pereira *et al*. (2018). A previous study conducted in our study population indicate that 65% of the population is overweight or obese. Therefore, pharmaceutical interventions alone may not adequately address problems related to reverse dipping, but lifestyle changes that target weight reduction like calorie reduction and exercise should form part of the intervention strategy.

4.2. Reverse dipping and Blood pressure

Our results showed that the 24-hour BP and night-time BP of dippers and non-dippers fall within normal ranges. However, significant differences were observed between the 24-hour and night-time BP of these two groups. Twenty-four-hour and night-time BP of reverse dippers was significantly different from those of dippers and non-dippers. Expectedly, the night-time BP of reverse dippers was significantly higher from those of dippers and non-dippers even after correction for diabetes and hypertension. No significant differences were observed in the day-time BP between all three groups. Most importantly all the daytime BPs, including conventional BP, were within the normal range. This confirms that non-dipping and reverse dipping are BP anomalies that occur only at night. These night-time anomalies that present only at night have serious implications because BP is usually measured in a clinic during the day. Hence these conditions cannot be easily diagnosed as the patients will always present with normal BP during the day. However, a careful look at our results show that the conventional BP of the reverse dippers falls within the pre-hypertensive range according to the ESH guidelines (Williams *et al*., 2018) and stage 1 hypertension according to the more stringent AHA guidelines (Unger *et al*., 2020). On the other hand, the non-dippers fall within the normal range according to the less stringent ESH but when the more stringent AHA guidelines are used, they fall within the prehypertension range. This means these guidelines can be used as an indication of a possible dipping abnormality. Therefore, any person whose BP falls within the prehypertension range should be given an ambulatory BP monitor to investigate their dipping status.

4.3 Possible mechanisms for reverse dipping

Our results further indicate that the night-time systolic BP of the reverse dippers (132 mm Hg) is significantly higher than the daytime systolic BP (125 mm Hg). This significant increase of 7 mm Hg may be explained by the significantly high levels of plasma aldosterone levels and aldosterone-to-renin ratio in reverse dippers (Table 3.3). The mechanism that may be accountable for the high BP with increased aldosterone levels in reverse dippers may be mediated by the

reninangiotensinaldosterone system where aldosterone promotes water and sodium retention while promoting potassium excretion from the body, thus increasing BP (Xanthakis and Vasan, 2013). However, our results indicate that there is no significant difference in the plasma renin and the urinary sodium excretion between the reverse dippers and the other two groups. It is well known that aldosterone not only exerts its effect on the kidneys via the RAAS, it also has extra renal functions that influence BP (Xanthakis and Vasan, 2013). These effects are independent of the renin and sodium status in the blood (Baudrand *et al*., 2017). Aldosterone evokes a fibrotic response in the heart muscles, and causes hypertrophy and inflammation in the vascular system, where it causes the reduction in the bioactivity of nitric oxide, thus causing endothelial dysfunction (Xanthakis and Vasan, 2013). Aldosterone also causes endothelial cells to swell and stiffen (Xanthakis and Vasan, 2013), affecting endothelial-dependant relaxation and contraction of the vascular system, thus increasing BP (Higashi *et al*., 2012

Our results also showed a statistically significant difference between the leptin levels of reverse dippers compared to non-dippers and dippers (Table 3.3). To further explain this, reverse dippers in the present study were characterized by a higher BMI compared to non-dippers and dippers. The reverse dippers were in the obese range while the other two groups were in the overweight range. That could account for the significantly higher levels of plasma leptin in this group compared to the other two groups. Leptin is synthesized by adipose tissue hence its concentrations are higher in the obese group and the leptin directly acts on the paraventricular nucleus to activate the sympathetic nervous system, hence the relatively higher-night-time BP in reverse dippers in the present study. However, this raises another question, why does BP only increase at night while remaining normal during the day? Our results show that even though there is no significant difference in the daytime BP of the three groups (DP, ND and RD), the reverse dippers had a significantly higher night-time BP compared to the other two groups. We speculated that the increased SNS activity at night might not only be due to leptin levels but also due to sleep apnoea. As stated earlier, the reverse dippers are in the obese category. Even though we did not assess sleep apnoea in this study, the association between obesity and sleep apnoea is well established (Pucci *et al*., 2014).), and sleep apnoea is associated with increased SNS activity. In a study by Sherwood *et al*., (2002) they were able to show a positive association

between SNS activity and dipping status. They demonstrated that an increase in SNS activity results in reduced nighttime BP dipping. Therefore, a possibility exists that the obese reverse dippers in our population sample have increased night-time SNS activity due to sleep apnoea. This means in this group the leptin and sleep apnoea effects on SNS activation results in the reverse dipping pattern observed in these individuals.

We also observed that reverse dippers demonstrated significantly low levels of plasma magnesium compared to non-dippers and dippers. A number of studies have shown a relationship between magnesium and BP. The mechanism involved may be centred on the ability of fact that magnesium influences BP by altering vascular tone (Cunha *et al*., 2011). Low levels of magnesium induce the release of nitric oxide, modifying smooth muscle tone in the arteries, thus the concentration of calcium is affected, consequently resulting in vasoconstriction. (Cunha *et al*., 2011). The effects of magnesium could be contributing to the night-time vasoconstriction effects of sleep apnoea leading increased night-time BP resulting in the observed reverse dipping pattern.

4.3. Reverse dipping and cardiovascular target organ damage

4.3.1 Arterial stiffness

We assessed arterial stiffness by measuring PWV in the three groups (DP, ND and RD). Our results showed a significant difference between the pulse wave velocity (PWV) of the three groups. Moreover, our results also suggested that PWV increases as night-time BP increases, as demonstrated by the histogram in figure 3.1. These findings can be explained by the differences in night-time BP, aldosterone, aldosterone-to-renin ratio, BMI, and leptin levels among the 3 groups.

Reverse dippers had a significantly higher night-time systolic BP compared to nondippers and dippers. A higher night-time systolic BP may impact arterial structure and function, and thus increasing PWV. Moreover, changes in vascular structure reduces the sensitivity of baroreceptor reflexes of the arteries, resulting in abnormal BP variations (Park et al., 2019). These can be linked to night-time autonomic imbalance favouring the overstimulation of the sympathetic nervous system and/or

low-grade chronic system inflammation (Castelpoggi *et al*., 2009). These results were also consistent with several other studies (Cicek *et al*., 2012; Kim *et al*., 2014; Lekakis *et al*., 2005; Asar *et al*., 1996).

Reverse dippers had a significantly higher plasma aldosterone and aldosterone-to renin ratio compared to non-dippers and dippers. The mechanisms of aldosterone are usually due to its effect on renal sodium handling. Aldosterone causes sodium retention and potassium excretion. However, as stated earlier, this does not seem to apply in this population as urinary sodium excretion is similar in the three groups. So, the effects of aldosterone on arterial stiffness are independent of the effects of salt on BP. The high aldosterone-to-renin ratio in the reverse dippers indicates that the high aldosterone is not dependent on renin secretion. Hence the relationship between aldosterone and arterial stiffness is mostly due hyperaldosteronism induced arterial wall fibrosis. Hyperaldosteronism has been observed to increase the content of collagen and fibrosis not only on arterial walls but also on the ventricles (Mahmud *et al*., 2005). Evidence on the association between aldosterone and arterial stiffness is seen on an experiment conducted on salt-fed rats where aldosterone led into increased pulse pressure and arterial stiffness through changes in the density of collagen and elastin which is prevented by the aldosterone antagonist, eplerenone (Mahmud *et al*., 2005). Furthermore, Aldosterone was also observed to decrease systemic compliance (Mahmud *et al*., 2005) and increases arterial pressure in patients with chronic hypertension, thus increasing arterial stiffness (El-Gharbawy *et al*., 2000).

Reverse dippers also had significantly higher levels of plasma leptin compared to nondippers and dippers. Adipose tissue in obese individuals, as seen with this group, produce leptin, a hormone independently associated with PWV (Wang *et al*., 2013). Leptin induces the proliferation and migration of aortic smooth muscle cells, promote the growth of endothelial cells and angiogenesis, and affects the production of nitric oxide in endothelial cells, all of which have been associated with arterial stiffness (Wang *et al*., 2013). Our results were consistent with the findings of D'Elia *et al*. (2020) and Wang *et al*. (2013).

4.4. Left ventricular mass index.

Our results showed that there were no significances between the average LVMI of dippers and non-dippers, while the LVMI in reverse dippers was significantly higher compared to dippers and non-dippers. Ivanovic *et al*., demonstrated in their study that left ventricular structure and function was more impaired in reverse dippers and nondippers compared to dippers. They also showed in their study that ventricular function and structure progressively deteriorated with abnormal BP dipping patterns, from extreme dippers to reverse dippers (Ivanovic *et al*., 2013). Though our results are similar to the findings of similar studies (Ivanovic *et al*., 2013; Abdalla *et al*., 2017), the uniqueness of our population is that non-dippers are similar to dippers in that they do not show preclinical cardiac pathology. Only the reverse dippers had a significantly higher LVMI than the dippers. This difference remained even after correcting covariates like age, gender, BMI, hypertension and diabetes status, alcohol intake and smoking. This highlight that the effects of the high aldosterone and high night-time BP influence LVMI independent of other covariates.

Aldosterone's effect within the myocardium is through the receptors of the mineralocorticoid, which enhances the deposition of both collagen and extracellular matrix, as well as fibrosis, which eventually leads to left ventricular hypertrophy (ElGharbawy *et al*., 2000). Night-time BP among reverse dippers in the present study was also associated with increased LVMI. The mechanism on how a raised night-time BP causes an increased LMVI is not clear, as some studies did not find any association between night-time BP and LVMI but rather day-time BP (Cuspidi *et al*., 2010). However, there is a link between obesity and LVMI even after correction for age and BP (Cuspidi *et al*., 2014), as seen in the present study among reverse dippers. Visceral fat, which has been shown to be the more metabolically active component of fat deposit may influence increased LVMI by secreting bioactive molecules such as angiotensin II and inflammatory cytokines (Cuspidi *et al*.,2014). Also, the high blood volume and growth-stimulating effects of high plasma insulin and insulin resistance which are highly associated with central adiposity, may result in left ventricular hypertrophy (Cuspidi *et al*., 2014). Moreover, the confounding effects of obesity and abnormal sleeping patterns may be in part responsible for the increased night-time BP in reverse

dippers. This is also true as Cunha *et al*., mentioned in their study that left ventricular structure and function abnormalities are as a result of the heart's adaptive response to pressure overload, as seen with the relatively higher night-time BP in reverse dippers in the present study.

4.5. Early to late ventricular diastolic filling

Our results showed that dippers had a significantly higher early-to-late diastolic filling ratio (E/A), followed by non-dippers. Reverse dippers significantly had the lowest E/A. The ratio of the three groups was significantly different. These findings matched with the findings of Ivanovic *et al* and Aydin *et al.* (2005), where they demonstrated that E/A deteriorated progressively from extreme dippers to reverse dippers, although our study only compared dippers with non-dippers and reverse dippers. Other studies only compared dippers and non-dippers (Aydin *et al.*, 2005). However, the common findings in these studies were that diastolic function was reduced with a reduced nighttime BP fall, and was further reduced in the extremities, from extreme dippers to reverse dippers. This was true for both right and left ventricular diastolic function (Ivanovic *et al*., 2013).

In the present study, reverse dippers were characterised by a higher night-time and conventional systolic BP, rather than diastolic BP. Agreeably, Mohammed *et al*., observed in their study that diastolic dysfunction was strongly associated with systolic BP rather that diastolic BP, hence the reduced E/A in non-dippers and reverse dippers in our study. The mechanisms that may be accountable for these changes may include reduced left ventricular relaxation and compliance (Lalande and Johnson, 2008). In individuals with a higher-than-normal BP, as a compensatory mechanism to restore ventricular wall stress, the left ventricular wall is thickened, leading to left ventricular concentric hypertrophy (Lalande and Johnson, 2008). Thus, left ventricular compliance and relaxation is reduced and E/A is reduced (Lalande and Johnson, 2008).

4.6. Conclusion

In conclusion, in the present study, the prevalence of reverse dipping was low (13.2%) compared to dippers (48.1%) and non-dippers (38,7%). However, reverse dipping was shown to be in correlation with the highest incidence of CV events both structurally and functionally, compared with non-dippers and dippers. Our results also showed that despite the normal daytime BP of the reverse dippers, their nighttime BP is high, and this translates to preclinical cardiovascular pathology. The mechanisms that drive the increased nighttime BP may be mediated by the high plasma aldosterone and aldosterone-to-renin ratio in this group. Furthermore, in reverse dippers, the effects of aldosterone on BP were independent of the levels of plasma renin and urine sodium, and this led to the observed higher night-time BP in this group. Of particular concern, reverse dipping studies on the black population is very scanty, considering the facts that cardiovascular risk factors vary with genetics and demographics. Therefore, the approach on interventions may also need to be adjusted.

4.7. Limitations

In the present study several limitations were observed. There was no balance in the number of males and females; this was because more women participated in the study. As a result, this could produce results that may appear as bias because of the imbalance in males and females. As, generally, men have a negative outlook on human medical research while women view it as free medical screening. This was seen in several other studies of similar nature.

CHAPTER FIVE

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Appendix 1: Ethical clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Ms NF Mndebele
School of Physiology
Medical School
University

E-mail: 814027@students.wits.ac.za

CC: Supervisor: Dr MJ Maseko, et al <Muzi.Maseko@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2019/10/01

REF: *R14/49*

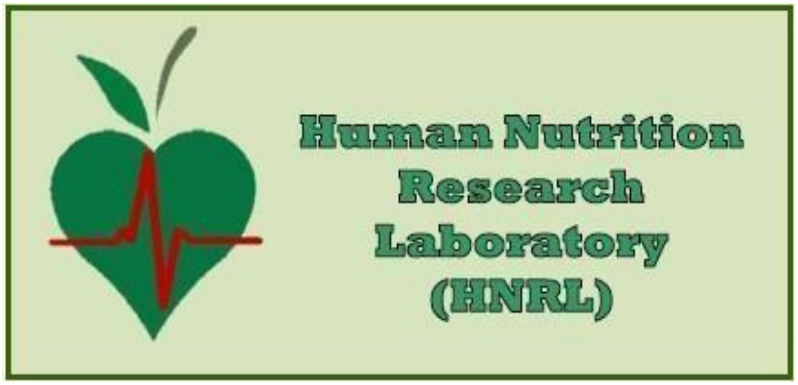
PROTOCOL NO: **M190662** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Reverse dipping and target organ changes in a South African population*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

Appendix 2: Consent form



Human Nutrition

Research Laboratory

South African Hypertension and Diet Study (SAHDS)

Written consent form

The aim and procedures of this study have been explained to me by the investigators. I have read and understood the information sheet provided. I have also had the opportunity to ask questions and have considered the answers given to me. I understand that participation in this study is voluntary, that I may withdraw my consent at any time; and that if I choose to do so my decision will not have any negative impact on me in any way.

I hereby freely give my informed consent to participate in this study.

Name of participant : _____

Participant code : _____ **Signature :** _____

Date : _____

To be filled by the investigators

I confirm that I have fully explained the nature of the study and the procedures to be performed to the above-named participant.

Name : _____

Signature : **Date**

_____

:

_____

Appendix 3: Questionnaire

1.	**Personal information**								
	Participant No.		Gender		Male		Date of Birth		Ethnicity
1.					Female		Age		
2.	**Medical History**								
2.1	**History of non-communicable diseases?**		YES		NO		**Examples, Hypertension, Chronic Kidney disease, Diabetes**		
	If YES, specify								
2.2	**Are you using hypertension treatment or Medicinal plants?**						YES		NO
2.2.1	**If YES, how long (in years)?**								
2.2.2	**Describe the regimen**								
2.3	**Please, specify whether some of your relatives currently suffer (or have suffered) from high blood pressure (hypertension)**								
2.3.1	**Your Father**						Yes		No
	Your grandfather (Father's side)						Yes		No
	Your grandmother (Father's side)						Yes		No
	Your mother						Yes		No
	Your grandfather (mother's side)						Yes		No
	Your grandmother (Mother's side)						Yes		No
	1 or more of your own children						Yes		No
	1 or more of your own grandchildren						Yes		No
	1 or more brothers or sisters of your father						Yes		No

Unknown					
Unknown					
Unknown					
Unknown					
Unknown					
Unknown					
Unknown					
Unknown					
Unknown					
Unknown					

	1 or more brothers or sisters of your mother	Yes		No		Unknown	
	1 or more of your own brothers or sisters	Yes		No		Unknown	

2.4	**Marital status**	Single		Married		Widowed					
2.5	**Are you still attending school?**	Yes		No							
2.6	**Which is the highest level of education which you successfully completed?**										
	Do you currently exert any professional or occupational activity?	**Yes**		**No**							
2.7	If yes: What is your present occupation? Please, provide a detailed description of your current job below.										
2.8	**If you currently do not exert any professional activity, which was the last job you had?**										
2.8.1		**Years**									
2.8.2	**Please, provide a detailed description of your activities at your previous work.**										
3.	**Please, specify whether you suffer from diseases affecting your:**										
3.1.1	**Heart**	Yes		No		Unknown					
3.1.2	**Kidneys**	Yes		No		Unknown					

4.	**Were you ever told by a doctor or health professional that you have an elevated blood pressure?**	Yes		No		Unknown		
5.	**Are you currently in good health?**	Yes		No		Unknown		
6	**Did you ever take or are you taking now drugs which eliminate salt and make you pass urine more frequently or in larger amounts? If yes, explain below**	Yes		No		Unknown		
7.	**Did you ever take pain-killers on a regular basis, for instance against headaches, tooth pain, painful periods, etc... ?** **If yes, explain below**			Yes		No		

8.	**Did you take medicines during the past 2 weeks? if yes, explain below**	Yes		No	
9.1	**Do you currently smoke? If yes, explain the frequency, type and amount per day**	Yes		No	
9.2	**Did you smoke in the past? If yes, explain below**				
10.1	**Do you currently consume alcoholic beverages? If yes, explain the frequency, type and amount per day below**	Yes		No	

10.2	**Did you consume alcohol beverages in the past? If yes, explain**	Yes		No	

11	**Do you drink coffee or caffeine-containing beverages (cola) on a regular base? If yes, give details below**	Yes		No	
12.	**Do you practice any sports activities on a regular basis? If yes, explain the frequency, type and intensity below**	Yes		No	
Please note that this section is for females only					
13.1	**Did you already have your periods?**	Yes		No	
13.2	**Have you ever taken "The Pill"? If yes, explain the regimen below**	Yes		No	
13.3	**Have you ever used other contraceptive methods? If Yes, explain the regimen below**	Yes		No	
14.1	**Are you pregnant at present?**	Yes		No	Unknown
14.2	**Have you been pregnant before?**	Yes		No	

15.	**This section is for women 30 years and older**				
15.1	**Do you still have your periods? If No, explain below**	Yes		No	
15.2	**At present are your periods suppressed by taking "the pill"?**	Yes		No	
15.3	**At present are your periods suppressed by any other contraceptive method? If Yes, explain the regimen w belo**	Yes		No	
END					

DECLARATION

Please, provide your signature to give your approval or disapproval to use this information in our study.

Signature: _____ Date: _____

Appendix 4: Plagiarism report

814027:The_effects_of_reverse_dipping_on_cardiovascular_targ.

ORIGINALITY REPORT

10%	3%	9%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Poster Abstracts", Journal of Clinical Hypertension, 04/2009 Publication	1%
2	Submitted to University of Witwatersrand Student Paper	1%
3	Harry Harianto, Michael Valente, Soenarno Hoetomo, Mahesan Anpalahan. "The Clinical Utility of Ambulatory Blood Pressure Monitoring (ABPM): A Review", Current Hypertension Reviews, 2015 Publication	<1%
4	eprints.soton.ac.uk Internet Source	<1%
5	watermark.silverchair.com Internet Source	<1%
6	P. Mendes-Ferreira, C. Maia-Rocha, R. Adao, A. P. Lourenco et al. "Monday, 27 August 2012", European Heart Journal, 2012 Publication	<1%

7	"Selected Abstracts From XXXIV National Congress of the Italian Society of Hypertension (SIIA), Milan, 5–7 October 2017", High Blood Pressure & Cardiovascular Prevention, 2017 Publication	<1%
8	Evrim B. Turkbey, Robyn L. McClelland, Richard A. Kronmal, Gregory L. Burke et al. "The Impact of Obesity on the Left Ventricle", JACC: Cardiovascular Imaging, 2010 Publication	<1%
9	worldwidescience.org Internet Source	<1%
10	Jeyaraj D. Pandian, Andrew A. Wong, Douglas J. Lincoln, James P. Davis et al. "Circadian blood pressure variation after acute stroke", Journal of Clinical Neuroscience, 2006 Publication	<1%
11	"Abstracts: Vascular Biology, XIIth International Vascular Biology Meeting, 12-16 May, 2002, Karuizawa, Japan", Endothelium, 2009 Publication	<1%
12	"Minutes of The 43rd General Assembly of The European Association for The Study of Diabetes", Diabetologia, 2008 Publication	<1%
13	Juliet Addo, Liam Smeeth, David A. Leon.	

	"Hypertensive Target Organ Damage in Ghanaian Civil Servants with Hypertension", PLoS ONE, 2009 Publication	<1%
14	Robert H Fagard. "Dipping pattern of nocturnal blood pressure in patients with hypertension", Expert Review of Cardiovascular Therapy, 2014 Publication	<1%
15	Florence Gunawan, Hui Yi Ng, Christopher Gilfillan, Mahesan Anpalahan. "Ambulatory Blood Pressure Monitoring in Type 2 Diabetes Mellitus: A Cross-sectional Study", Current Hypertension Reviews, 2019 Publication	<1%
16	Łukasz J. Krzych, Andrzej Bochenek. "Blood pressure variability: Epidemiological and clinical issues", Cardiology Journal, 2013 Publication	<1%
17	"Sleep Disorders Medicine", Springer Science and Business Media LLC, 2017 Publication	<1%
18	Debbie L. Cohen, Yonghong Huan, Raymond R. Townsend. "Ambulatory Blood Pressure in Chronic Kidney Disease", Current Hypertension Reports, 2013 Publication	<1%
	Dogan Erdogan, Hakan Gullu, Mustafa	

19	Caliskan, Ibrahim Yildirim, Taner Ulus, Muhammet Bilgi, Haldun Muderrisoglu. "Coronary flow reserve in dipper and non-dipper hypertensive patients", Blood Pressure, 2009 Publication	<1%
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