# IS 24-HOUR AUGMENTATION INDEX A BETTER INDICATOR OF ARTERIAL STIFFNESS COMPARED TO CLINIC AUGMENTATION INDEX?

**Wantonda Papinah Mukhovha**

**(1834012)**

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

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**Declaration**

I, Wantonda Papinah Mukhovha, declare that this dissertation is my own, unaided work. It is being submitted for the degree Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been previously submitted for any degree to the current or any other institution.

I certify that the assessments contained in this dissertation have the approval of the Committee for Research in Human Subjects (Medical) of the University of the Witwatersrand, Johannesburg. The ethics approval number is **M220737**.

Wantonda Papinah Mukhovha

Signed on the: 24 July 2024

I certify that the above statement is correct:

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|  |  |  |
| Prof. Muzi Maseko | Prof. Nqoba Tsabedze | Edgar Phukubje |
| (Supervisor) | (Co-supervisor) | (Co-supervisor) |
| Date: 24 July 2024 | Date: 24 July 2024 | Date: 19 July 2024 |

## Dedication

This dissertation is dedicated to my mother Makumbelo Magugumela, my siblings, my uncle, and my partner Miranda Magagula. Thank you for your unending support.

**Abstract**

Arterial Stiffness is a major independent risk factor that is strongly associated with an increased risk of developing cardiovascular diseases (CVDs), making it an important marker in the assessment of CVD risk. Therefore, in an effort to reduce the rising incidence of cardiovascular diseases in South Africa, it is crucial to determine the best indicator for arterial stiffness. Currently two indices are used to measure arterial stiffness, pulse wave velocity (PWV) and augmentation index (AI). Since arterial stiffness measurement techniques were developed much later than blood pressure (BP) measurement techniques, important lessons can be learned from BP measurement. Studies have indicated that 24-hour BP measurement is a much better tool of measurement compared to a once off conventional BP measurement. This creates a possibility that 24-hour arterial stiffness assessment is a better tool for measuring arterial stiffness compared to a once off clinic arterial stiffness measurement. Therefore, in this study we compared 24-hour AI to in-clinic AI and also assessed gender differences. Previous studies conducted on 24-hour AI were focused on establishing normal 24-hour AI reference values. To date, no studies have been conducted to compare 24-hour AI to in-clinic AI. Moreover, gender differences in the 24-hour arterial stiffness profile have never been studied. We recruited 125 individuals of black African descent and took anthropometric measurements. We measured both conventional BP and 24-hour BP. Pulse wave analysis was performed to obtain both in-clinic and 24-hour AI. In the total population, 24-hour augmentation index (AI24) was significantly higher than in-clinic augmentation index (AIC) ($p<0.0001$). When participants were stratified according to gender, AI24 was significantly higher than AIC in both men ($p<0.0001$) and women ($p<0.0001$). Night-time augmentation index (AIN) in the total population was significantly higher than daytime augmentation index (AID) ($p=0.0143$). When participants were stratified according to gender, our results show that AIN was only significantly higher in women ($p=0.0291$) and not in men. Our results show that AIC may grossly be underestimating the prevalence of arterial stiffness and its adverse effects on cardiovascular target organs. Secondly, our results also show that there are gender differences in arterial stiffness fluctuations over the 24-hour period. In men, daytime arterial stiffness does not differ from night-time arterial stiffness. However, in women arteries become stiffer during the night-time compared to the daytime. These findings indicate that special attention must be given to night-time arterial stiffness because it may be more closely related to target organ damage than daytime arterial stiffness.

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**List of Abbreviations**

| | |
|---|---|
| ADH | Antidiuretic hormone |
| AI | Augmentation index. |
| AIC | Clinic augmentation index. |
| AID | Daytime augmentation index. |
| AIN | Night-time augmentation index. |
| Ang-II | Angiotensin-II |
| AT1R | Angiotensin-II type 1 receptor. |
| AI24 | 24-hour augmentation index. |
| B-DBP24 | 24-hour brachial diastolic blood pressure |
| B-MAPC | clinic brachial mean arterial pressure |
| B-MAP24 | 24-hour brachial mean arterial pressure |
| BMI | Body mass index |
| BP | Blood pressure |
| B-PPC | Clinic brachial pulse pressure |
| B-PP24 | 24-hour brachial pulse pressure |
| B-SBPDIP | Brachial systolic blood pressure dipping |
| C-DBPC | Clinic central diastolic blood pressure |
| C-DBPD | Daytime central diastolic blood pressure |
| C-DBPN | Night-time central diastolic blood pressure |
| C-DBP24 | 24-hour central diastolic blood pressure |
| C-MAPC | Clinic central mean arterial pressure |
| C-MAP24 | 24-hour central mean arterial pressure |
| C-PPC | Clinic central pulse pressure |
| C-PP24 | 24-hour central pulse pressure |
| CRP | C-Reactive Protein |

| | |
|---|---|
| C-SBPC | Clinic central systolic blood pressure |
| C-SBPD | Daytime central systolic blood pressure |
| C-SBPN | Night-time central systolic blood pressure |
| C-SBP24 | 24-hour central systolic blood pressure |
| CVD | Cardiovascular Diseases |
| DBPC | Clinic diastolic blood pressure |
| DBPD | Daytime diastolic blood pressure |
| DBPN | Night-time diastolic blood pressure |
| eNOS | Endothelial nitric oxide synthase |
| GFR | Glomerular filtration rate |
| HT | Hypertension |
| IL-1 | Interleukin-1 |
| IL-1β | Interleukin-1 beta |
| IL-6 | Interleukin-6 |
| IL-8 | Interleukin-8 |
| IL-10 | Interleukin-10 |
| IR | Insulin resistance |
| JNK | c-Jun N-terminal kinases |
| LDL-chol | Low density lipoprotein cholesterol |
| MMP | Matrix metalloproteinases |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NO | Nitric oxide |
| PI3K | Phosphatidylinositol 3-kinase pathway |
| PWV | Pulse wave velocity |
| RAAS | Renin angiotensin aldosterone system |
| ROS | Reactive oxygen species |

| | |
|---|---|
| SD | Standard deviation |
| SNS | Sympathetic nervous system |
| SOCS | Suppressor of cytokine signalling |
| SOD | Superoxide dismutase. |
| TNF-α | Tumour necrosis factor alpha |
| VSMC | Vascular smooth muscle cells |
| WC | Waist circumference |
| WHO | World health organization |
| WHR | Waist to hip ratio |
| WHtR | Waist to height ratio |

## Preface

Arterial stiffness is characterised by the arteries losing their elastic ability because of thickened and stiffened blood vessel walls. The development of arterial stiffness is associated with various risk factors such as age, hypertension, smoking, alcohol consumption, ethnicity, gender, dyslipidaemia, and obesity. Arterial stiffness is associated with target organ damage by decreasing blood flow to the organs which makes it an important marker for the assessment of cardiovascular disease risk. Therefore, the measurement of arterial stiffness is of paramount importance because it can help prevent adverse cardiovascular outcomes. Arterial stiffness is determined using pulse wave velocity and augmentation index.

Pulse wave velocity is the velocity at which pressure waves propagate down the arterial tree during systole. It is currently regarded as the gold standard method of measuring arterial stiffness, however, it faces a limitation of being recorded as a once off clinical measurement. According to existing evidence, once off measurements have been found to be less accurate than 24-hour measurements which poses the question of: “how accurate are once off clinical measurements of arterial stiffness?”.

The answer to the abovementioned question can currently be obtained by measuring augmentation index, which is another important parameter used to assess arterial stiffness, because the current existing methods of measuring augmentation index can assess arterial stiffness both as a once off measurement and over a 24-hour period. Studies on 24-hour augmentation index have been conducted, however, these studies were focused on establishing normal reference values for 24-hour augmentation index and not focused on determining the difference between a once off measurement of arterial stiffness and a 24-hour measurement of arterial stiffness. Therefore, this study aims to cover the gaps that were not covered by the existing literature.

# CHAPTER 1

(Introduction)

## 1.1 Cardiovascular Diseases

Cardiovascular diseases (CVDs) continue to be the world's leading cause of mortality worldwide (World Health Organization (WHO), 2021). They are responsible for the deaths of approximately 17.9 million people each year, globally and in South Africa, they are responsible for approximately 17.3% of all deaths that occur (Heart and Stroke Foundation South Africa, 2017). These diseases are a group of illnesses that affect the heart and blood vessels that supply vital organs such as the brain, heart, kidneys, and eyes (Gaziano *et al.*, 2006). They include coronary heart disease, cerebrovascular disease, congenital heart disease, rheumatic heart disease, peripheral arterial disease, deep vein thrombosis and pulmonary embolism (World Health Organization (WHO), 2021). Arterial Stiffness is one major independent risk factor that has been found to be strongly associated with increased risk of developing CVDs (Bonarjee, 2018).

## 1.2 Arterial Stiffness

Arterial stiffness is characterized by the arteries losing their elastic ability as a result of thickened and stiffened blood vessel walls that occur as a result of increased proliferation of vascular smooth muscle cells (VSMCs) within the arterial walls (Zieman *et al.*, 2005). This is due to an upregulation in the activity of matrix metalloproteinases (MMPs) that are responsible for the degradation of elastin within the blood vessel wall. Vascular smooth muscle cells migrate from the arteries tunica media into the arteries tunica intima where they differentiate into fibroblasts which in turn increases the secretion of collagen (Bonarjee, 2018; Kocemba *et al.*, 1998; Zieman *et al.*, 2005). These structural changes in the blood vessel walls observed in arterial stiffness will also result in the arteries losing their ability to adequately transport blood to organs causing target organ damage (Tan & Thakur, 2021).

According to existing evidence, various risk factors such as age, hypertension, smoking, alcohol consumption, ethnicity, gender, dyslipidaemia, and obesity can be associated with the development of arterial stiffness (Kim *et al.*, 2014; Lin *et al.*, 2015; Sun, 2015). These risk factors are then classified under two main categories, namely non-modifiable and modifiable risk factors (Dahlöf, 2010). Non-modifiable risk factors are risk factors that cannot be changed such as age, ethnicity, gender, and genetics (Brown *et al.*, 2020). Whereas modifiable risk factors are risk factors than can be changed by intervention, for example hypertension,

smoking, alcohol consumption, unhealthy diet, physical inactivity, and obesity (Brown *et al*., 2020).

### 1.2.1 The impact of non-modifiable risk factors on arterial stiffness.

#### 1.2.1.1 Age.

Studies have shown that arterial stiffness increases with age in both men and women. According to these existing studies, aging is associated with the development of arterial stiffness by increasing the degradation of elastin while promoting the accumulation of stiffer collagen, resulting in a decreased elastin to collagen ratio (Kohn *et al.*, 2015; Sun, 2015; Wen *et al.*, 2015). One of the mechanisms in which an increase in age increases the degradation of elastin and the accumulation of stiffer collagen is by increasing the activity of MMPs, which is thought to contribute to arterial stiffness by inducing the degradation of elastin fibres while causing fibrosis through increased collagen deposition (Kohn *et al*., 2015). This ultimately results in endothelial inflammation which promotes the proliferation of VSMCs (Peeters *et al.*, 2017; Wang *et al.*, 2015).

#### 1.2.1.2 Gender.

Sex hormones such as testosterone and oestrogen also play a role in age related changes in arterial compliance (DuPont *et al*., 2019; Nethononda *et al.*, 2015). Of these sex hormones, oestrogen, a hormone predominantly found in postpubescent females and premenopausal females, is the one that has been found to significantly decrease arterial stiffness. It also has more cardioprotective properties compared to testosterone, a hormone predominantly found in postpubescent males (DuPont et al., 2019; Karas *et al.*, 1994). Studies that have assessed the arterial stiffness lowering effects of oestrogen by comparing arterial stiffness between prepubescent and postpubescent males and females, found that arterial stiffness was higher in prepubescent females compared to prepubescent males, but after puberty, males had stiffer arteries compared to females (DuPont *et al*., 2019; Nethononda *et al.*, 2015). This reaffirms the arterial stiffness lowering effects of oestrogen (DuPont et al., 2019; Nethononda et al., 2015). Oestrogen decreases arterial stiffness by activating nitric oxide synthase found in endothelial cells, which will increase the production of NO (Nevzati *et al.*, 2015; White, 2002). The NO produced will induce vasodilation and maintain the elasticity of the arteries, thus decreasing the risk of developing arterial stiffness (White, 2002). On the other hand, testosterone has been found to be associated with arterial stiffness, however, the mechanism in which testosterone

contributes to the development of arterial stiffness is not well understood (Kelly & Jones, 2013).

#### 1.2.1.3 Ethnicity.

In addition to age and gender, studies have shown that ethnicity also contributes to the development of arterial stiffness (Cruz *et al.*, 2020; Schutte *et al.*, 2020). According to these studies, people of black African descent have a higher risk of developing arterial stiffness compared to any other ethnic group (Cruz *et al.*, 2020; Schutte *et al.*, 2020). The reason behind people of black African descent being at a higher risk of developing arterial stiffness is because their genetic makeup consists of a lysine specific demethylase 1 (LSD1) single nucleotide polymorphism, which makes them sensitive to salt compared to any other ethnic group (Parksook *et al.*, 2022; Sowers *et al.*, 1988). Salt-sensitivity in people of black African descent results in increased sodium reabsorption and decreased sodium excretion in the kidneys, which results in increased plasma sodium concentrations (Grillo *et al.*, 2019). Increased plasma sodium concentrations lead to the stiffening of arteries by increasing the production of reactive oxygen species (ROS) such as superoxide and inhibiting angiotensin-2 (Ang-II). Ang-II is crucial for the activation and expression of superoxide dismutase (SOD), an enzyme that breaks down superoxide. As a result, the reduction in Ang-II and the rise in ROS levels cause oxidative stress in endothelial cells which results in endothelial dysfunction (Younus, 2018). Endothelial dysfunction will stimulate the proliferation of VSMCs which results in vascular hypertrophy (Edwards & Farquhar, 2015; Zieman *et al.*, 2005). In addition to the role salt-sensitivity plays in the development of arterial stiffness independent of BP, it has also been found that salt-sensitivity is the most common cause of hypertension in a population of black African descent (Grillo *et al.*, 2019). Since salt-sensitivity is associated with increased urinary sodium reabsorption, the increased urinary sodium absorption will lead to an increase in plasma sodium concentrations (Balafa & Kalaitzidis, 2021; Elijovich *et al.*, 2016). The increased plasma sodium concentrations will result in the activation of osmoreceptors located in the hypothalamus (Cuzzo *et al.*, 2024). These activated osmoreceptors will result in the secretion of antidiuretic hormone (ADH), which will promote fluid retention in the kidneys causing an increase in BP (Cuzzo *et al.*, 2024).

### 1.2.2 The impact of modifiable risk factors on arterial stiffness.

#### 1.2.2.1 Hypertension.

Hypertension is one of the modifiable risk factors that have been found to be strongly associated with the development of arterial stiffness (Kim *et al.*, 2014; Lin *et al.*, 2015; Sun, 2015). One of the mechanisms in which hypertension contributes to the development of arterial stiffness is by causing damage to the endothelial cells (Brandes, 2014). The mechanisms in which hypertension causes endothelial damage are well understood. One of the mechanisms in which hypertension causes damage to endothelial cells is through the over-activation of renin-angiotensin-aldosterone system (RAAS) as a result of decreased blood flow to the kidneys linked with hypertension (Fountain & Lappin, 2020). The overactivated RAAS will result in the over-production of Angiotensin-II (Ang-II) which binds to the angiotensin-II type 1 receptors (AT1R) located on endothelial cells (Kawai *et al.*, 2017). Once Ang-II is bound to AT1R, it will stimulate the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, within the endothelial cells, which will increase the production of reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals (Schulz *et al.*, 2011). The abovementioned ROS causes damage to endothelial cells by decreasing the activity of nitric oxide (Schulz *et al.*, 2011). The damaged endothelial cells will become more permeable, which allows low-density lipoprotein cholesterol (LDL-Chol) to enter the subendothelial space. Once in the subendothelial space, they will be oxidized resulting in vascular inflammation which in turn allows the circulating monocytes to enter the subendothelial space (Woollard & Geissmann, 2010). Once these monocytes enter the subendothelial space, they differentiate into macrophages that will act as scavengers, and engulf the oxidized LDL-Chol to form foam cells (El Hadri *et al.*, 2021). After the foam cells have been formed, macrophages in the subendothelial space will secrete pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), which will promote the apoptosis of the foam cells resulting in the formation of an atheromatous plaque which also contributes to the stiffening of arteries (El Hadri *et al.*, 2021). The pro-inflammatory cytokines secreted by macrophages will also contribute to the stiffening of arteries by increasing the proliferation of the VSMCs, and by promoting the migration of VSMCs from the tunica media into the tunica intima where they will differentiate into fibroblasts which will increase the production of collagen which contributes to stiffening the arteries (Zieman *et al*., 2005). The mechanism in which hypertension contributes to the formation of atherosclerosis is shown below in figure 1.1.

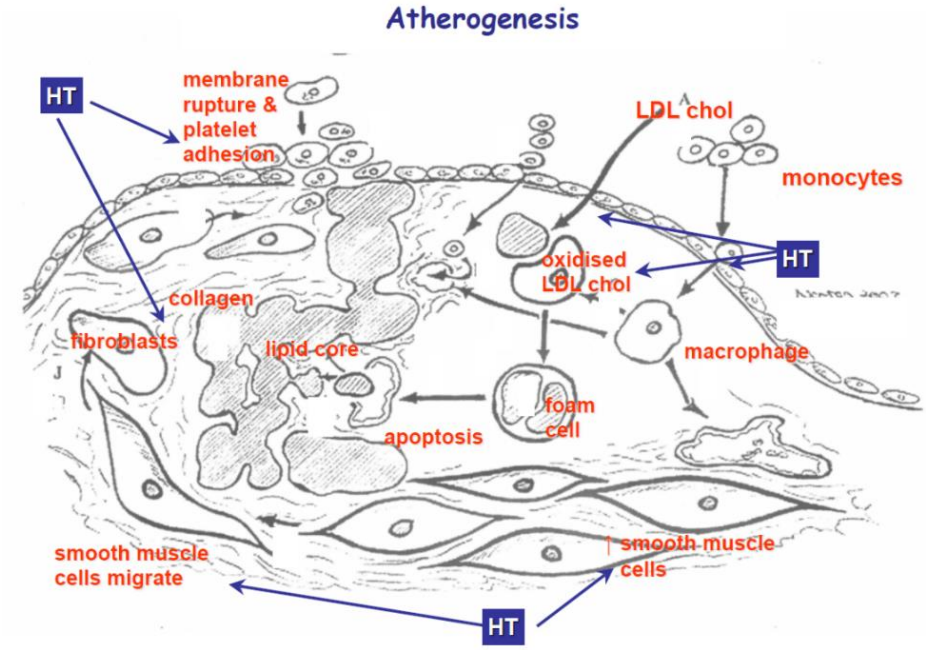



**Figure 1.1:** A visual illustration showing the role hypertension play in the development of atherosclerosis. HT, hypertension; oxidised LDL-chol, oxidised low-density lipoprotein cholesterol (Peterson; 2020).

### 1.2.2.2 Obesity.

Obesity/overweight is another modifiable risk factor that is associated with increased arterial stiffness (Aroor *et al.*, 2018; Para *et al.*, 2021). The mechanisms that link obesity with increased arterial stiffness are well understood. Obesity causes arterial stiffness by increasing the expression and secretion of adipokines, namely tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and leptin (Nakamura *et al.*, 2014; Para *et al.*, 2021). These adipokines will lead to insulin resistance (IR) through the activation of inhibitory molecules such as the suppressor of cytokine signalling (SOCS) and the c-Jun N-terminal kinases (JNK) which suppresses insulin signalling (Kwon & Pessin, 2013). One of the proposed mechanisms in which IR can result in arterial stiffness is by causing endothelial dysfunction (Muniyappa & Sowers, 2013). The endothelial dysfunction in IR is a result of an impaired phosphatidylinositol 3-kinase-dependent (PI3K) signalling pathway as a consequence of a suppressed insulin signalling pathway (Muniyappa & Sowers, 2013). The impaired PI3K pathway will cause endothelial dysfunction: by decreasing the expression and activation of endothelial nitric oxide synthase (eNOS), by decreasing the amount of eNOS substrates and co-factors, by increasing

the degradation of NO to form ROS, and by increasing the secretion of endothelin-1 (Potenza *et al.*, 2009). The effects IR has on endothelial cells is shown in figure 1.2.

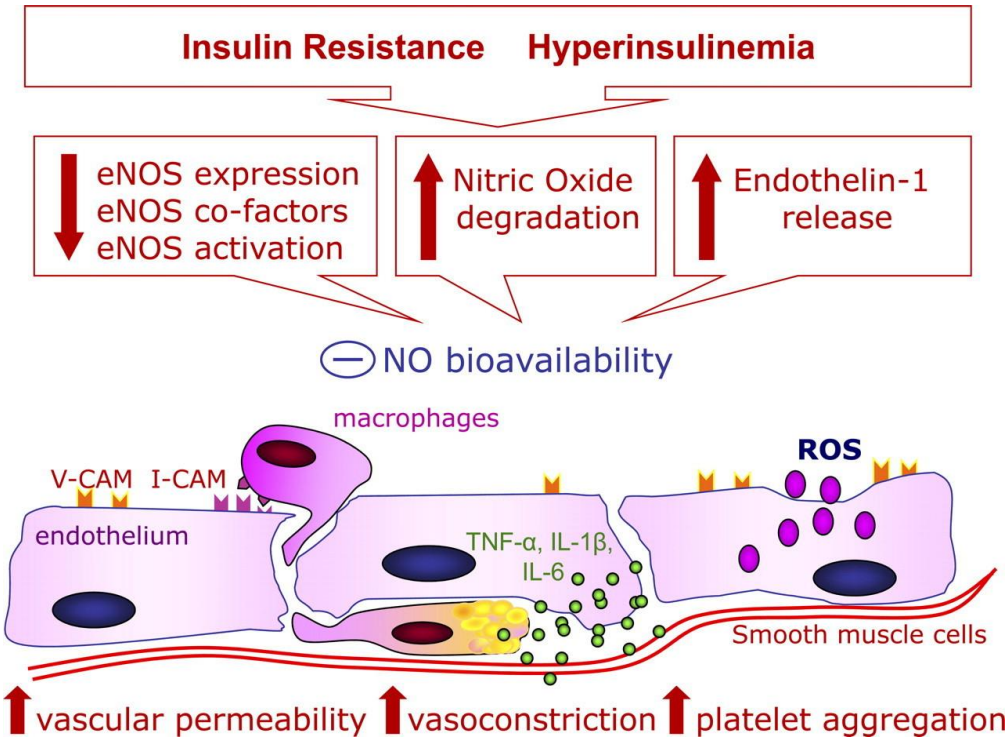



**Figure 1.2:** Endothelial dysfunction under insulin resistance and hyperinsulinemia. Decreased endothelial nitric oxide synthase (eNOS) protein expression or function, increased reactive oxygen species (ROS) formation/NO degradation, and enhanced endothelin-1 (ET-1) release are among mechanisms by which insulin resistance and hyperinsulinemia may contribute to endothelial dysfunction. Endothelial dysfunction increases expression of adhesion molecules, favours macrophage infiltration and release of proinflammatory cytokines (green spheres), induces proliferation of VSMC, and increases platelet aggregation. VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; TNF-α, tumour necrosis factor alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; NO, nitric oxide; ↑, increase (Potenza *et al.*, 2009).

#### 1.2.2.3 Smoking.

Smoking is currently regarded as one of the leading modifiable risk factors that plays a role in the development of arterial stiffness (Doonan *et al.*, 2010; Messner & Bernhard, 2014). Smoking contributes to the development of arterial stiffness in various mechanisms. One of those mechanisms involves smoking causing an inflammatory state by increasing the production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, and decreasing the production of anti-inflammatory cytokines such as IL-10 (Arnson *et al.*, 2010; Doonan *et al.*,

*2010)*. The pro-inflammatory cytokines will stimulate the VSMCs to start producing c-reactive protein (CRP) (S. Park & Lakatta, 2012). The locally produced CRP will promote vascular inflammation which ultimately results in endothelial dysfunction (S. Park & Lakatta, 2012). Endothelial dysfunction will stimulate the migration of VSMC from the tunica media to the tunica intima, where these VSMCs change their phenotype from VSMCs to fibroblasts and start producing collagen which causes the stiffening of arteries (Milutinović *et al*., 2020; Zieman *et al.*, 2005).

Another mechanism in which smoking contributes to the development of arterial stiffness is by causing hypertension (Doonan *et al.*, 2010). Smoking causes hypertension by stimulating the secretion of adrenaline and noradrenaline, as a result of nicotine binding to nicotine acetylcholine receptors located on the peripheral postganglionic sympathetic nerve endings and the adrenal medulla (Haass & Kübler, 1997). The secreted adrenaline and noradrenaline will result in the constriction of blood vessels which in turn will increase BP causing hypertension (Nagao *et al.*, 2021). The increased BP will exert mechanical stress on the arterial walls, which will ultimately lead to the damaging of the arterial walls (Doonan *et al.*, 2010). The damaged arterial walls will contribute to arterial stiffness by promoting vascular hypertrophy and by increasing the deposition of calcium and collagen within the arterial walls (Doonan *et al.*, 2010).

#### 1.2.2.4 Alcohol consumption

Alcohol consumption is associated with development of arterial stiffness (Del Giorno *et al.*, 2022). The mechanisms in which alcohol causes arterial stiffness are well known. One of the mechanisms in which alcohol consumption contributes to arterial stiffness is by causing hypertension (Beilin & Puddey, 2006; Tasnim *et al.*, 2020). According to existing studies, the mechanism in which alcohol consumption causes hypertension is by decreasing the baroreceptor activity which results in the over-activation of the sympathetic nervous system (Husain *et al.*, 2014; Tasnim *et al.*, 2020). The over-activation of the sympathetic nervous system will result in hypertension by causing vasoconstriction (Husain *et al.*, 2014). Furthermore, the vasoconstriction caused by the activation of the sympathetic nervous system will decrease blood flow to the kidneys which will result in a decreased glomerular filtration rate (GFR) and the activation of the renin-angiotensin-aldosterone-system (RAAS). The activation of RAAS will also contribute to the development of hypertension by increasing water retention in the kidneys and by also promoting vasoconstriction (Piano, 2017).

#### 1.2.2.5 Physical activity.

Physical activity/exercise was found to have an inversely proportional relationship with arterial stiffness, where an increase in physical activity/exercise reduces the risk of developing arterial stiffness (W. Park *et al.*, 2017). According to existing evidence, the mechanisms in which physical activity/exercise reduces arterial stiffness is by improving vascular endothelial function (Maeda *et al.*, 2008; W. Park *et al.*, 2017; Saz-Lara *et al.*, 2021). Physical activity/exercise improves vascular endothelial function by increasing the production of NO and reducing the production of endothelin-1 (W. Park *et al.*, 2017). Another mechanism in which physical activity/exercise reduces arterial stiffness is by decreasing the over-activation of the sympathetic nervous system (SNS). The decreased activation of the SNS would result in a decreased heart rate which increases ventricular filling time and also result vasodilation which would increase blood flow. The increased blood flow will promote endothelial health by delivering nutrients to the endothelial cells, increasing the production of NO, reducing inflammation and removal of metabolic waste (Maeda *et al.*, 2008). On the other hand, when the SNS is over-activated, it can contribute to the development of arterial stiffness (Maeda *et al.*, 2008). The over-activation of the SNS contributes to the development of arterial stiffness by increasing the secretion of catecholamines such as adrenaline and noradrenaline. These catecholamines will contribute to the development of arterial stiffness by causing vascular hypertrophy as a result of catecholamines binding to the alpha-1 adrenergic receptors of VSMC which increases the production of collagen and fibronectin expression (Petrák *et al.*, 2010).

### 1.2.3 Assessment of arterial stiffness.

Pulse wave velocity (PWV), the velocity at which pressure waves propagate down the arterial tree during systole, is one of the methods used to assess arterial stiffness (Pereira *et al.*, 2015). Pulse wave velocity is currently regarded as the "gold standard method" for measuring arterial stiffness, where an increased PWV indicates the presence of arterial stiffness (DeLoach & Townsend, 2008; Pereira *et al.*, 2015). Although PWV is currently considered as the gold standard method to measure arterial stiffness, a possibility exists that PWV might not be as accurate as we think, because the current non-invasive methods used to assess PWV are currently limited to once off clinic measurements. Once off clinic measurements are generally known to be less accurate compared to 24-hour measurements, due to their inability to account for circadian fluctuations and their inability to detect the difference between night-time measurements and day-time measurements. For example, studies have found that clinic BP

measurements have a tendency to over-estimate BP, which often results in the misdiagnosis of hypertension (Miladinović *et al.*, 2021; Pappaccogli *et al.*, 2019). These findings showing that clinic BP measurements overestimates BP poses the question of whether clinic measurements of arterial stiffness also overestimates arterial stiffness?

The answer to this question can currently be obtained by using augmentation index to measure arterial stiffness, since the current existing methods of measuring augmentation index can assess arterial stiffness both in a clinical setting and over a 24-hour period. Augmentation index is another important parameter used to assess arterial stiffness which is derived from ascending aortic pressure waveforms (Wilkinson *et al.*, 2000). During systole, the heart generates a pressure wave which travels down the arterial tree to supply blood to the rest of the body, and this pressure wave generated during systole is referred to as the forward wave or the primary wave (Fantin *et al.*, 2007). As the forward wave moves down the arterial tree, some of the forward wave bounces off the smaller arteries and travels back to the ascending aorta (Fantin *et al.*, 2007). The wave that bounces off the smaller arteries and travels back to the ascending aorta is known as the reflected wave. In arterial stiffness, the amplitude and the speed of the reflected wave are increased which causes augmentation index to increase (Izzo, 2007).

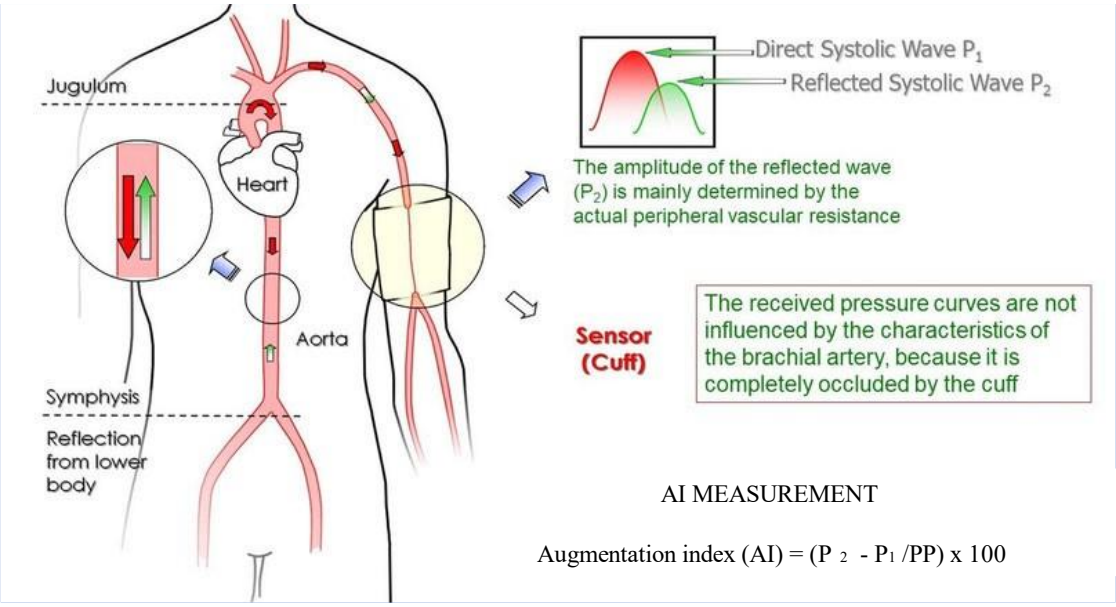



**Figure 1.3:** Diagram illustrating how augmentation index is derived from aortic pressure waveforms. P1, Direct systolic/primary wave; P2, reflected systolic wave; PP, pulse pressure (Systolic blood pressure – diastolic blood pressure).

## 1.3 Justification.

Arterial stiffness has been established as an important marker for the assessment of cardiovascular disease risk (Zieman *et al.,* 2005). Arterial stiffness has been associated with target organ damage by decreasing blood flow to the organs (Tan & Thakur, 2021). Since arterial stiffness is associated with increased risk of cardiovascular disease, its assessment is of paramount importance because it can help prevent adverse cardiovascular outcomes. The emergence of 24-hour SphygmoCor that measures augmentation index over a 24-hour period allows us the opportunity to assess arterial stiffness over a 24-hour period, since existing evidence shows that 24-hour measurements are more accurate than once-off measurements (O'Brien, 2003; O'Brien *et al.,* 2013). Assessing arterial stiffness over a 24-hour period enables us to monitor how arterial stiffness changes throughout the day and night and enables us to monitor how arterial stiffness is influenced by lifestyle choices and behaviour. A study assessing arterial stiffness over a 24-hour period using 24-hour augmentation index has been conducted, however, these studies were conducted to establish normal reference values for 24-hour augmentation index (Kuznetsova *et al.,* 2014) To the best of our knowledge, there is no existing study that has assessed the difference between clinic augmentation index and 24-hour augmentation index. To address this gap, studies that assess the difference between clinic augmentation index and 24-hour augmentation index are needed.

## 1.4 Aim

The aim of the study was to determine whether 24-hour augmentation index is a better indicator of arterial stiffness compared to clinic augmentation index.

## 1.5 Objectives

- To determine 24-hour augmentation index (AI24) and clinic augmentation index (AIC), in a population of African ancestry.
- To compare 24-hour augmentation index and clinic augmentation index between men and women of African ancestry.
- To determine the difference between night-time augmentation index (AIN) and daytime augmentation index, in a population of African ancestry.
- To determine whether arterial stiffness follows the BP circadian pattern by comparing 24-hour ambulatory BP pattern with 24-hour ambulatory augmentation index, in a population of African ancestry.

# CHAPTER 2

(Methods)

## 2. 1 Study group

This study was conducted at the University of the Witwatersrand Faculty of Health Sciences, Johannesburg, South Africa. It was conducted in accordance with the principles outlined in the Helsinki declaration, and it was granted ethical approval by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, approval number **M220737** (see appendix 1). One hundred and twenty-five South Africans of black African ancestry were recruited in and around Johannesburg (Figure 2.1). The minimum age of the participants was 18 years, with no upper age limit.



**Figure 2.1:** An example of an area (Johannesburg, Braamfontein) where some of the participants were recruited.

## 2.2 Clinical and demographic data

Participants were invited to the Human Nutrition Clinic where the details of the study were explained. After giving consent to participate in the study, a standardised questionnaire was administered (Appendix 4). The questionnaire was designed by the Human Nutrition Research Laboratory, in such a way that participants could answer most of the questions with a simple "yes" or "no", with a few sections requiring short answers. The questions that required specific answers were date of birth, gender, previous medical history, family history of hypertension

and cardiovascular events, the presence of non-communicable diseases, prior and current drug therapy, occupation, level of education, smoking status, alcohol and 4.3. For women, the use of contraceptives, history of pregnancies as well as menstrual history were recorded.

### 2.3 Anthropometric measurements

Anthropometric assessments were measured according to the international standards for anthropometric assessments (Stewart *et al.*, 2011). Weight measurements were recorded using an electronic digital scale (Heavy-Duty Digital Floor Scale, Healthometer Professional, USA) to the nearest 0.1 kg. During all the measurements, the participants were asked to take their shoes off, and minimal clothing was worn which allowed access to areas of measurements. Participants were asked to step on the scale and stand still without supporting themselves on any object and weight was recorded when the scale had stopped fluctuating. Height was measured once, using a portable stadiometer (Seca 222 portable stadiometer, Seca Precision for Health, Netherlands) 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. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters ($kg/m^2$). Classification of participant as being underweight, normal, overweight, or obese was done according to table 2.1. Waist circumference was measured 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. Waist circumference cut-off values; normal WC (≤94 cm in men: ≤80 cm in women) vs increased WC (>94cm in men: >80 cm in women) (World Health Organisation (WHO), 2008). Hip circumference was determined using a tape measure at the largest circumference around the buttocks to the nearest 0.1 cm. Waist-to-Hip-Ratio (WHR) was determined by dividing the waist circumference with the hip circumference, where the normal WHR cut-off value for men is ≤0.90 and for women is ≤0.85 (World Health Organisation (WHO), 2008). Waist-to-Height-Ratio (WHtR) was determined by dividing WC with height, where the normal WHtR cut-off value is ≤0.50 in both men and women.

**Table 2.1:** Body mass index (BMI) threshold values.

| BMI ($kg/m^2$) | Weight Classification | Relative Risk of disease |
|---|---|---|
| <18.5 | Underweight | |
| 18.5 – 24.9 | Normal | No risk |
| 25.0 – 29.9 | Overweight | Increased risk |
| 30.0 – 34.9 | Obese (Class I) | High risk |
| 35.0 – 39.9 | Obese (Class II) | Very high risk |
| ≥40.0 | Morbidly Obese | Extremely High risk |

Adapted from World Health Organization, 2000. Obesity: Preventing and managing the global epidemic.

### 2.4 Conventional blood pressure measurements

Brachial blood pressure (BP) was measured using an automated sphygmomanometer (Omron, Kyoto, Japan). The participants were required to rest for 5 to 10 minutes with their legs uncrossed, both feet touching the floor and their back rested against the chair. Participants were required to rest the arm at which the BP was measured on a table that was set beside the chair at an elevation close to the heart level. Caution was taken by using a cuff suitable to the arm circumference of each participant. Five consecutive measurements were taken at one-minute intervals. The five measurements were averaged to determine the participants' systolic and diastolic BP in mm Hg and resting heart rate. Measurements at ≥140 mm Hg systolic and 90 mm Hg diastolic were defined as hypertensive states. Furthermore, participants who received anti-hypertensive treatment were also classified as hypertensives.

### 2.5 Twenty-Four-Hour ambulatory blood pressure measurements

Twenty four-hour BP monitoring was performed using oscillometric monitors (Oscar 2, SunTech Medical Inc., North Carolina, USA) (Figure 2.2). The non-dominant arm was selected for BP monitoring. The size of the cuff was the same as that which was used for conventional BP measurements. The monitors were programmed to measure BP at 15-minute intervals from 06:00 to 22:00 and then 60-minute intervals from 22:00 to 06:00. Participants kept a diary card

for the duration of the monitoring to note the time of going to bed in the evening and getting up in the morning to determine the awake and sleep periods of the day. Participants were also asked to document any oral intake and medication. Participants continued with normal daily activities during the monitoring. Intra-individual means of the ambulatory measurements were weighted by the time-interval between successive recordings (Bawa-Allah *et al*., 2019). Ambulatory BP data were expressed as 24-hour average systolic and diastolic BP, the percentage decrease in BP at night (mean day-mean night/mean day x 100), the difference between day and night BP and the ratio of day-to-night BP.


**Figure 2.2:** Oscar 2, SunTech Medical Inc.

### 2.6 In-clinic augmentation index measurements

Radial artery pulse wave analysis was used to determine in-clinic aortic augmentation index (AI). Subjects remained in a supine position and measurements were taken immediately following the determination of brachial BP. The right radial artery pressure waveforms were recorded using a Millar tonometer (SPC-301; Millar Instruments Houston, Texas, USA) and calibrated to brachial systolic and diastolic pressure. Waveforms were processed using dedicated software (SphygmoCor v7; AtCor, Sydney, Australia). The integral system software was used to calculate an average radial artery waveform and the corresponding central aortic pressure waveforms were generated using a previously validated transfer function. Pressure waveforms not achieving the automatic quality controls specified by the SphygmoCor software

were rejected. The aortic waveforms were subject to further analysis by the SphygmoCor software, to identify the outgoing and reflected pressure waves (augmentation), occurring during systole. Augmentation index was defined as the ratio of augmentation to pulse pressure and were expressed as a percentage (AI = (ΔP/PP) × 100). Time to the foot of the reflected wave (Tr) was also identified. Data from the mean of two central aortic pressure waveforms were taken for each subject.

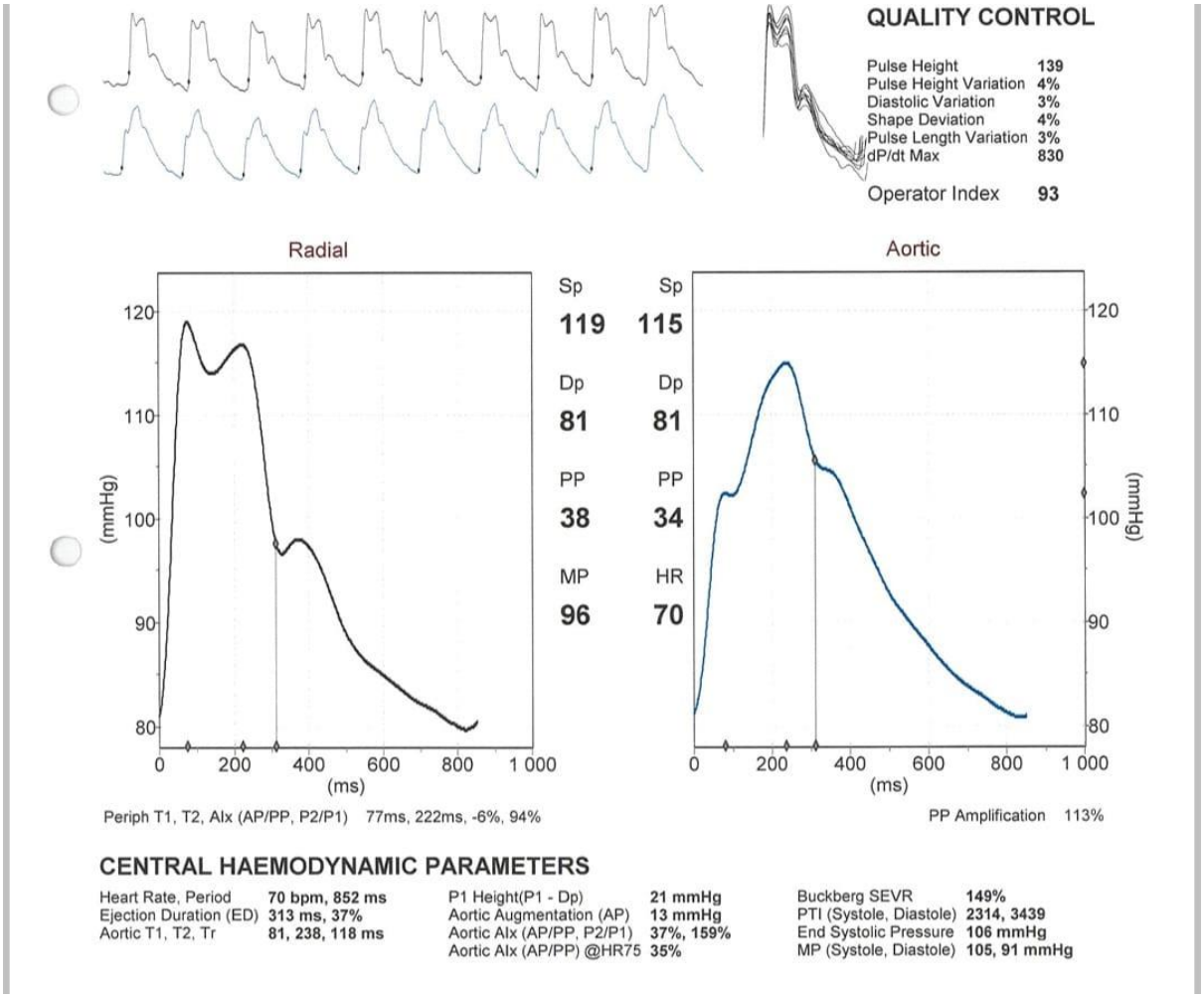



**Figure 2.3:** Example of a pulse wave analysis recording obtained to determine central haemodynamic parameters. The figure shows central hemodynamic parameters, the radial artery pulse wave analysis obtained from applanation tonometry, and the aortic pulse waveforms derived from a population-based transfer function built into the software. Aortic augmentation index corrected for heart rate (AI @HR75) was obtained from the central haemodynamic parameters. Sp, systolic blood pressure (BP); Dp, diastolic BP; MP, mean arterial pressure, PP, pulse pressure; HR, heart rate.

## 2.7 Twenty-Four-Hour augmentation index measurements

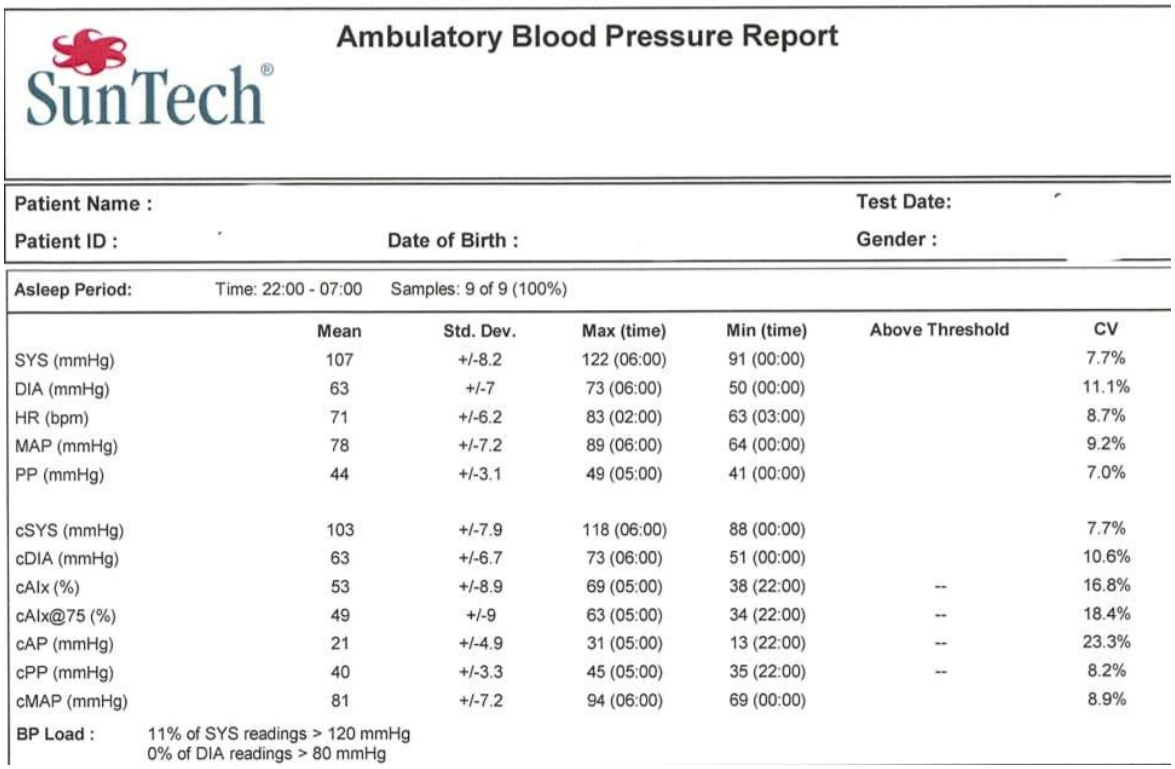
In addition to measuring 24-hour ambulatory BP, the Oscar 2 machine by SunTech Medical also measured 24-hour ambulatory augmentation index using the blood pressure values it has obtained. Augmentation index was measured every 30 minutes from 07h00-22h00 for daytime values (Figure 2.4), and every 60 minutes from 22h00-07h00 for night-time values (Figure 2.5). The device also recorded the 24-hour average augmentation index (Figure 2.6).

**Awake Period:** Time: 07:00 - 22:00 Samples: 34 of 46 (74%)

| | Mean | Std. Dev. | Max (time) | Min (time) | Above Threshold | CV |
|---|---|---|---|---|---|---|
| SYS (mmHg) | 124 | +/-17.6 | 201 (11:00) | 105 (19:32) | | 14.2% |
| DIA (mmHg) | 81 | +/-14.3 | 118 (11:00) | 57 (19:14) | | 17.7% |
| HR (bpm) | 87 | +/-9.9 | 107 (13:58) | 67 (07:00) | | 11.4% |
| MAP (mmHg) | 96 | +/-14.5 | 146 (11:00) | 73 (19:14) | | 15.1% |
| PP (mmHg) | 42 | +/-12.3 | 83 (11:00) | 26 (11:34) | | 29.3% |
| cSYS (mmHg) | 114 | +/-10.1 | 139 (11:34) | 99 (19:14) | | 8.9% |
| cDIA (mmHg) | 81 | +/-13.8 | 118 (11:34) | 58 (19:14) | | 17.0% |
| cAIx (%) | 41 | +/-16.8 | 78 (18:58) | 9 (14:58) | -- | 41.0% |
| cAIx@75 (%) | 47 | +/-15.6 | 84 (16:58) | 26 (14:28) | -- | 33.2% |
| cAP (mmHg) | 14 | +/-8.6 | 36 (18:58) | 2 (14:58) | -- | 61.4% |
| cPP (mmHg) | 32 | +/-8.8 | 54 (08:30) | 20 (11:34) | -- | 27.5% |
| cMAP (mmHg) | 98 | +/-11.8 | 126 (11:34) | 77 (19:14) | | 12.0% |

**BP Load :** 9% of SYS readings > 140 mmHg
15% of DIA readings > 90 mmHg

**Figure 2.4:** Example of daytime blood pressure measurements which included daytime augmentation index that has been corrected for heart rate (cAI@75), and these measurements were obtained using the Oscar 2 machine by SunTech Medical Inc. SYS, systolic blood pressure (BP); DIA, diastolic BP; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; cSYS, central systolic BP; cDIA, central diastolic BP; cAI, central augmentation index; cPP, central pulse pressure; cMAP, central mean arterial pressure.



Ambulatory Blood Pressure Report

SunTech®

Patient Name : Test Date:

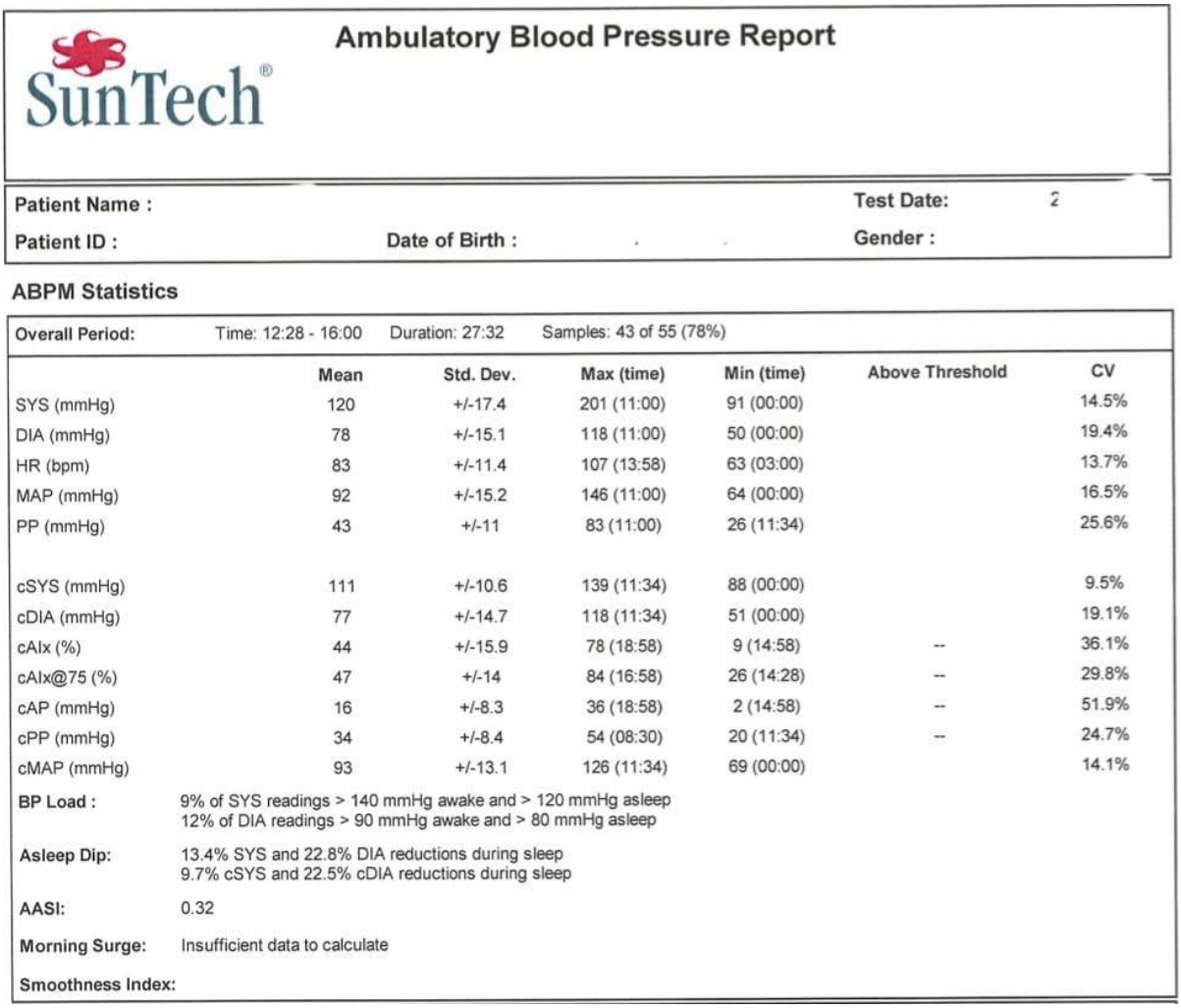
Patient ID : Date of Birth : Gender :

Asleep Period: Time: 22:00 - 07:00 Samples: 9 of 9 (100%)

| | Mean | Std. Dev. | Max (time) | Min (time) | Above Threshold | CV |
|---|---|---|---|---|---|---|
| SYS (mmHg) | 107 | +/-8.2 | 122 (06:00) | 91 (00:00) | | 7.7% |
| DIA (mmHg) | 63 | +/-7 | 73 (06:00) | 50 (00:00) | | 11.1% |
| HR (bpm) | 71 | +/-6.2 | 83 (02:00) | 63 (03:00) | | 8.7% |
| MAP (mmHg) | 78 | +/-7.2 | 89 (06:00) | 64 (00:00) | | 9.2% |
| PP (mmHg) | 44 | +/-3.1 | 49 (05:00) | 41 (00:00) | | 7.0% |
| cSYS (mmHg) | 103 | +/-7.9 | 118 (06:00) | 88 (00:00) | | 7.7% |
| cDIA (mmHg) | 63 | +/-6.7 | 73 (06:00) | 51 (00:00) | | 10.6% |
| cAIx (%) | 53 | +/-8.9 | 69 (05:00) | 38 (22:00) | -- | 16.8% |
| cAIx@75 (%) | 49 | +/-9 | 63 (05:00) | 34 (22:00) | -- | 18.4% |
| cAP (mmHg) | 21 | +/-4.9 | 31 (05:00) | 13 (22:00) | -- | 23.3% |
| cPP (mmHg) | 40 | +/-3.3 | 45 (05:00) | 35 (22:00) | -- | 8.2% |
| cMAP (mmHg) | 81 | +/-7.2 | 94 (06:00) | 69 (00:00) | | 8.9% |

BP Load : 11% of SYS readings > 120 mmHg
0% of DIA readings > 80 mmHg

**Figure 2.5:** Example of night-time blood pressure measurements which included night-time augmentation index that has been corrected for heart rate (cAI@75), obtained using Oscar 2 ABPM machine by SunTech Medical Inc. SYS, systolic blood pressure (BP); DIA, diastolic BP; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; cSYS, central systolic BP; cDIA, central diastolic BP; cAIc, central augmentation index; cPP, central pulse pressure; cMAP, central mean arterial pressure



Ambulatory Blood Pressure Report

SunTech®

Patient Name : Test Date: 2

Patient ID : Date of Birth : Gender :

ABPM Statistics

Overall Period: Time: 12:28 - 16:00 Duration: 27:32 Samples: 43 of 55 (78%)

| | Mean | Std. Dev. | Max (time) | Min (time) | Above Threshold | CV |
|---|---|---|---|---|---|---|
| SYS (mmHg) | 120 | +/-17.4 | 201 (11:00) | 91 (00:00) | | 14.5% |
| DIA (mmHg) | 78 | +/-15.1 | 118 (11:00) | 50 (00:00) | | 19.4% |
| HR (bpm) | 83 | +/-11.4 | 107 (13:58) | 63 (03:00) | | 13.7% |
| MAP (mmHg) | 92 | +/-15.2 | 146 (11:00) | 64 (00:00) | | 16.5% |
| PP (mmHg) | 43 | +/-11 | 83 (11:00) | 26 (11:34) | | 25.6% |
| cSYS (mmHg) | 111 | +/-10.6 | 139 (11:34) | 88 (00:00) | | 9.5% |
| cDIA (mmHg) | 77 | +/-14.7 | 118 (11:34) | 51 (00:00) | | 19.1% |
| cAIx (%) | 44 | +/-15.9 | 78 (18:58) | 9 (14:58) | -- | 36.1% |
| cAIx@75 (%) | 47 | +/-14 | 84 (16:58) | 26 (14:28) | -- | 29.8% |
| cAP (mmHg) | 16 | +/-8.3 | 36 (18:58) | 2 (14:58) | -- | 51.9% |
| cPP (mmHg) | 34 | +/-8.4 | 54 (08:30) | 20 (11:34) | -- | 24.7% |
| cMAP (mmHg) | 93 | +/-13.1 | 126 (11:34) | 69 (00:00) | | 14.1% |

BP Load : 9% of SYS readings > 140 mmHg awake and > 120 mmHg asleep
12% of DIA readings > 90 mmHg awake and > 80 mmHg asleep

Asleep Dip: 13.4% SYS and 22.8% DIA reductions during sleep
9.7% cSYS and 22.5% cDIA reductions during sleep

AASI: 0.32

Morning Surge: Insufficient data to calculate

Smoothness Index:

**Figure 2.6:** Example of 24-hour blood pressure measurements which included 24-hour augmentation index that has been corrected for heart rate (cAI@75), obtained using Oscar 2 ABPM machine by SunTech Medical Inc. SYS, systolic blood pressure (BP); DIA, diastolic BP; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; cSYS, central systolic BP; cDIA, central diastolic BP; cAIc, central augmentation index; cPP, central pulse pressure; cMAP, central mean arterial pressure.

### 2.8 Inclusion criteria

Participants who had all measurements taken and had both clinic and 24-hour augmentation index were included for analysis.

### 2.9 Data Analysis

Database management and statistical analyses were performed using SAS software, version 9.5 (The SAS Institute Inc., Cary, North Carolina, USA). The distribution of data was tested for normality using the Shapiro-Wilk test and descriptive statistics (skewness and kurtosis). Continuous data and characteristics of participants were expressed as mean ±SD or as

frequencies (%). Data was analysed according to gender to determine sex-specific effects. Potential confounders were defined as variables shown in the literature to be predictors of BP, namely: smoking status, alcohol consumption, diabetes, and hypertension. Mann-Whitney U test was used to determine the difference in age, body mass index, waist circumference, waist-to-hip ratio, Waist-to-height ratio, and haemodynamic parameters between men and women. Students unpaired t-test was used to determine the difference in clinic augmentation index, 24-hour augmentation index, daytime augmentation index, and night-time augmentation index between men and women.

# CHAPTER 3
(RESULTS)

The general and clinical characteristics of the study population are shown in Table 3.1. The average age in men (n=53) was 34.9 ±15.8 years and the average age in women (n=72) was 37.5 ±17.1 years. The difference in age between the two groups was not statistically significant (p=0.5302). Based on the BMI reference values, both groups were found to be overweight with an average BMI of 25.7 ±6.1 for men and an average BMI of 29.0 ±15 for women. The difference observed in BMI between the two groups was statistically significant (p=0.0450). Based on the existing gender specific WC reference values, women were at a high risk of developing adverse cardiovascular events with an average WC value of 87.7 ±17.0 cm whilst men were at a lower risk of developing adverse cardiovascular events with an average WC value of 86.7 ±19.2. Additionally, the WHtR in women was above the normal WHtR reference values which also indicated that women are at a high risk of developing adverse cardiovascular events. The prevalence of hypertension was slightly higher in women compared to men. The incidence of smoking and alcohol consumption was slightly higher in men compared to women.

The peripheral haemodynamic parameters of study population are shown in Table 3.2. Men had a significantly higher SBPC (p= 0.0335) and B-SBP24 (p= 0.0027) compared to women. The SBPN observed in men was also significantly higher compared to that of women (p= 0.0090). The participants in the men group also had a significantly higher SBPD (p= 0.0013) compared to the women group. Additionally, the B-MAP24 (p= 0.0281) in men was also found to be significantly higher compared to the B-MAP24 of women. Lastly, the B-PP24 (p= 0.0010) observed in the men group was significantly higher compared to the women group.

The central haemodynamic parameters of the study population are shown in Table 3.3. The C-SBP24 in men was significantly higher compared to that of women (p= 0.0081). Moreover, men had a significantly higher C-SBPN (p= 0.0242) and C-SBPD (p= 0.0031) compared to the C-SBPN and C-SBPD in women. The C-DBPN in men was significantly higher than C-DBPN in women (p=0.0477). Lastly, men also had a significantly higher C-PP24 (p= 0.0045) compared to the C-PP24 in women.

**Table 3.1:** General characteristics of study population

| | Total (n=125) | Men (n=53) | Women (n=72) | p-value |
|---|---|---|---|---|
| Age (years) | 36.4 ±16.5 | 34.9 ±15.8 | 37.5 ±17.1 | 0.5302 |
| BMI ($kg/m^2$) | 27.6 ±9.1 | 25.7 ±6.1 | 29.0 ±15 | 0.0450* |
| WC | 87.3 ±18.3 | 86.7 ±19.2 | 87.7 ±17.0 | |
| WHR | 0.87 ±0.2 | 0.89 ±0.1 | 0.85 ±0.2 | |
| WHtR | 0.52 ±0.1 | 0.49 ±0.1 | 0.54 ±0.1 | |
| Hypertension (%) | 31.2 | 28.3 | 31.9 | |
| Diabetic (%) | 4.0 | 1.8 | 5.5 | |
| Smoker (%) | 8.8 | 9.4 | 8.2 | |
| Alcohol intake (%) | 35.2 | 39.6 | 33.3 | |

Data expressed as mean ± SD or expressed as percentages; BMI, body mass index; Normal BMI ≥ 18.5 and < 25 $kg/m^2$; overweight BMI ≥25 and < 30 $kg/m^2$; Obese BMI ≥30 $kg/m^2$; WC, waist circumference; normal WC (≤94 cm in men; ≤80 cm in women); WHR, waist to hip ratio; normal WHR (≤0.90 in men; ≤0.85 in women); WHtR, waist-to-height ratio; normal WHtR (≤0.50 in both men and women) (*) Represents statistical significance, where $p<0.05$. Mann-Whitney U test was used to compare waist circumference between men and women.

**Table 3.2:** Peripheral haemodynamic parameters of study population

| | Total | Men | Women | p-value |
|---|---|---|---|---|
| SBPC (mmHg) | 122.8 ±16.5 | 126.5 ±16.1 | 120.1 ±16.3 | 0.0335* |
| DBPC (mmHg) | 82.1 ±13.6 | 83.2 ±11.6 | 81.3 ±14.8 | 0.2204 |
| B-SBP24 (mmHg) | 123.6 ±18.6 | 127.6 ±17.5 | 120.4 ±18.9 | 0.0027* |
| B-DBP24 (mmHg) | 74.2 ±11.7 | 76.1 ±12.5 | 72.7 ±10.8 | 0.1057 |
| SBPN (mmHg) | 114.7 ±20.3 | 117.5 ±17.1 | 112.4 ±22.6 | 0.0090* |
| DBPN (mmHg) | 65.6 ±11.4 | 67.4 ±12.2 | 64.1 ±10.7 | 0.0965 |
| SBPD (mmHg) | 126.6 ±19.4 | 131.1 ±18.3 | 122.9 ±19.7 | 0.0013* |
| DBPD (mmHg) | 77.2 ±13.3 | 79.1 ±13.0 | 75.7 ±13.4 | 0.0722 |
| B-MAPC (mmHg) | 96.1 ±12.3 | 97.9 ±13.3 | 94.8 ±11.5 | 0.2425 |
| B-MAP24 (mmHg) | 90.7 ±13.6 | 93.2 ±13.9 | 88.6 ±13.1 | 0.0281* |
| B-PPC (mmHg) | 31.1 ±10.5 | 30.8 ±7.5 | 31.2 ±12.1 | 0.4330 |
| B-PP24 (mmHg) | 49.5 ±9.5 | 51.6 ±7.6 | 47.7 ±10.5 | 0.0010* |
| B-SBPDIP (%) | 8.9 | 10.1 | 7.9 | |

Data expressed as mean ±SD or expressed as percentages; SBPC, clinic systolic blood pressure; DBPC, clinic diastolic blood pressure; B-SBP24, 24-hour brachial systolic blood pressure; B-DBP24, 24-hour brachial diastolic blood pressure; SBPN, night-time systolic blood pressure; DBPN, night-time diastolic blood pressure; SBPD, daytime systolic blood pressure, DBPD, daytime diastolic blood pressure; B-MAPC, clinic brachial mean arterial pressure; B-MAP24, 24-hour brachial mean arterial pressure; B-PPC, clinic brachial pulse pressure; B-PP24, 24-hour brachial pulse pressure; B-SBPDIP, brachial systolic blood pressure dipping; (*) Represents statistical significance, where $p<0.05$. Man-Whitney U test was used to compare haemodynamic parameters between men and women.

**Table 3.3:** Central haemodynamic parameters of study population

| | Total | Men | Women | p-value |
|---|---|---|---|---|
| C-SBPC (mmHg) | 113.5 ±16.7 | 115.0 ±15.8 | 112.4 ±17.4 | 0.3074 |
| C-DBPC (mmHg) | 82.4 ±10.3 | 84.2 ±11.8 | 81.1 ±8.8 | 0.2507 |
| C-SBP24 (mmHg) | 113.7 ±16.7 | 117.3 ±16.1 | 110.8 ±16.8 | 0.0081* |
| C-DBP24 (mmHg) | 75.6 ±10.8 | 77.8 ±11.9 | 73.7 ±9.6 | 0.0558 |
| C-SBPN (mmHg) | 107.5 ±18.9 | 109.7 ±16.0 | 105.7 ±20.9 | 0.0242* |
| C-DBPN (mmHg) | 67.1 ±11.2 | 69.1 ±12.2 | 65.4 ±10.1 | 0.0922 |
| C-SBPD (mmHg) | 115.7 ±17.1 | 119.9 ±16.9 | 112.1 ±16.61 | 0.0031* |
| C-DBPD (mmHg) | 78.6 ±11.3 | 80.9 ±12.3 | 76.6 ±10.1 | 0.0477* |
| C-MAPC (mmHg) | 92.7 ±11.9 | 94.5 ±12.8 | 91.5 ±11.1 | 0.2842 |
| C-MAP24 (mmHg) | 92.7 ±13.1 | 94.7 ±14.0 | 91.1 ±12.3 | 0.1341 |
| C-PPC (mmHg) | 31.1 ±10.4 | 30.8 ±7.6 | 31.3 ±12.1 | 0.4330 |
| C-PP24 (mmHg) | 38.1 ±8.4 | 39.4 ±6.3 | 37.0 ±9.7 | 0.0045* |

Data expressed as mean ±SD; C-SBPC, clinic central systolic blood pressure; C-DBPC, clinic central diastolic blood pressure; C-SBP24, 24-hour central systolic blood pressure; C-DBP24, 24-hour central diastolic blood pressure; C-SBPN, night-time central systolic blood pressure; C-DBPN, night-time central diastolic blood pressure; C-SBPD, daytime central systolic blood pressure; C-DBPD, daytime central diastolic blood pressure; C-MAPC, clinic central mean arterial pressure; C-MAP24, 24-hour central mean arterial pressure; C-PPC, clinic central pulse pressure; C-PP24, 24-hour central pulse pressure; (*) Represents statistical significance, where $p<0.05$. Man-Whitney U test was used to compare haemodynamic parameters between men and women.

Figure 3.1 compares the difference in clinic augmentation index (AIC) between men (n=53) and women (n=72). Our results show that women had a significantly higher AIC compared to men ($p<0.0001$).

Figure 3.2 compares the difference between 24-hour augmentation index (AI24) and clinic augmentation index in the total population. Our results show that AI24 was significantly higher than AIC in the total population ($p<0.0001$).

Figure 3.3 compares the difference between AIC and AI24 in women. Our results show that the augmentation index measured over a 24-hour period was significantly higher than the augmentation index measured in a clinical setting AIC ($p<0.0001$).

Figure 3.4 compares the difference between AIC and AI24 in men. Our results show that the augmentation index measured over a 24-hour period was significantly higher than the augmentation index measured in a clinical setting ($p<0.0001$).

Figure 3.5 compares the difference in AI24 between men and women. Our results show that women had a significantly higher AI24 compared to men ($p<0.0001$).

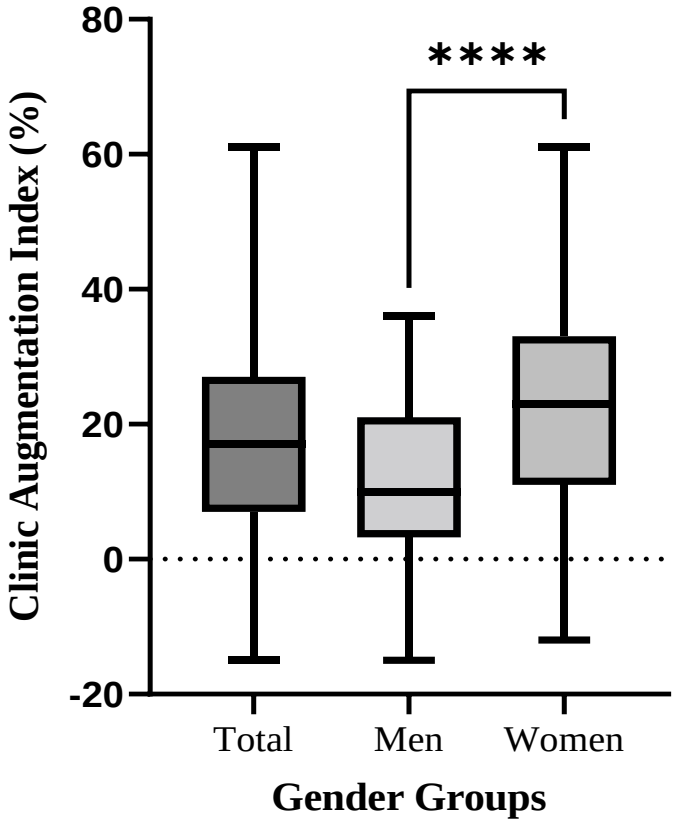



**Figure 3.1:** The comparison of clinic augmentation index between men and women.

The mean and SD for clinic augmentation index (AIC) in the men's group was 11.3 ±12.2 and the mean and SD in the women's group was 22.2 ±14.1 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (****) Represents statistical significance, where $p<0.0001$. Student unpaired t-test was used to determine statistical difference.

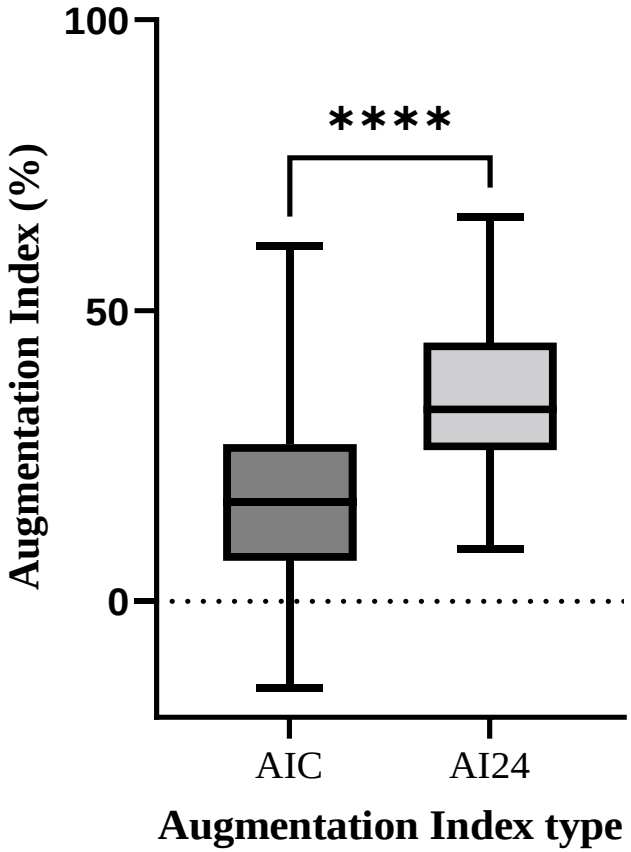



**Figure 3.2:** The difference between clinic augmentation index and 24-hour augmentation index in the total population.

The mean and SD for the clinic augmentation index (AIC) was 17.5 ±14.3 and the mean and SD for the 24-hour augmentation index (AI24) was 34.3 ±12.4 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (****) Represents statistical significance, where $p<0.0001$. Student unpaired t-test was used to determine statistical difference.

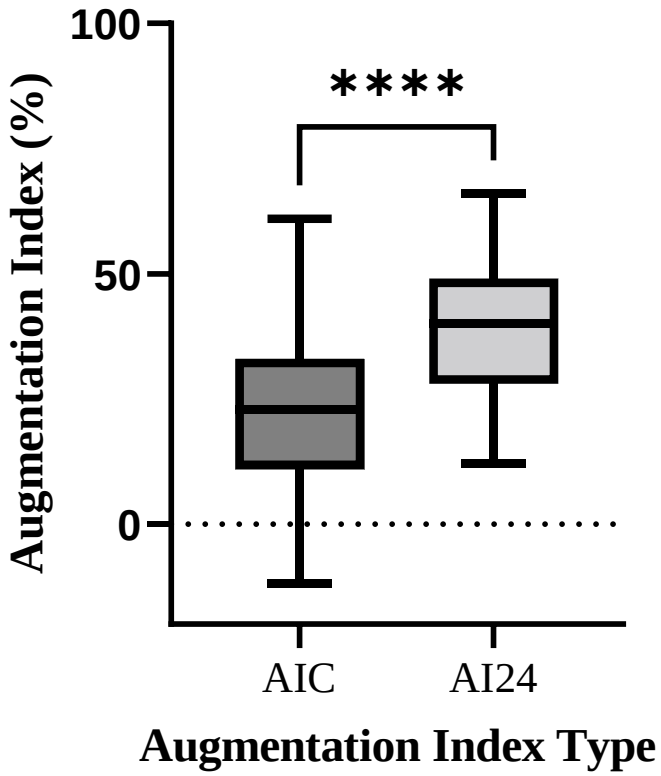



**Figure 3.3:** The difference between clinic augmentation index and 24-hour augmentation index in women.

The mean and SD for the clinic augmentation index (AIC) was 22.2 ±14.1 and the mean and SD for the 24-hour augmentation index was 38.4 ±12.1 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (****) represents statistical significance, where $p<0.0001$. Student unpaired t-test was used to determine statistical difference.

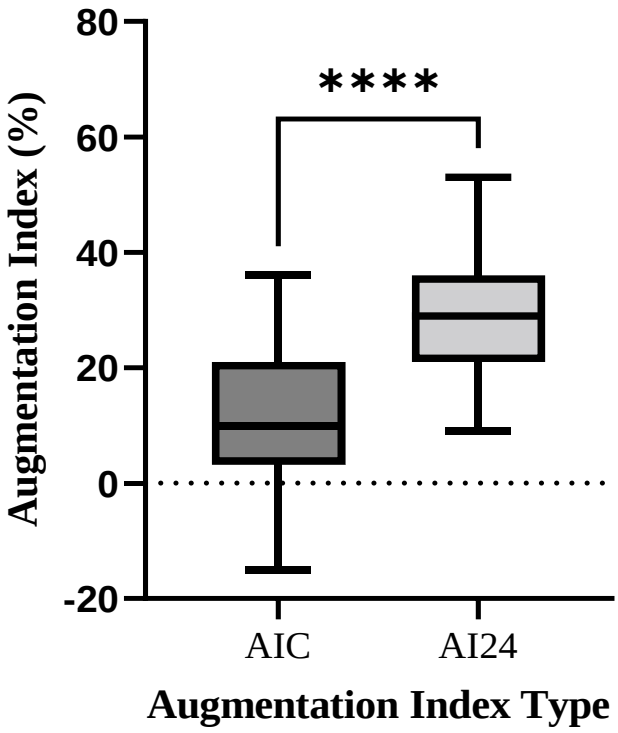



**Figure 3.4:** The difference between clinic augmentation index and 24-hour augmentation index in men.

The mean and SD for the clinic augmentation index (AIC) was 11.3 ±12.2 and the mean and SD for the 24-hour augmentation index was 29.3 ±10.8 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (****) Represents statistical significance, where $p<0.0001$. Student unpaired t-test was used to determine statistical difference.

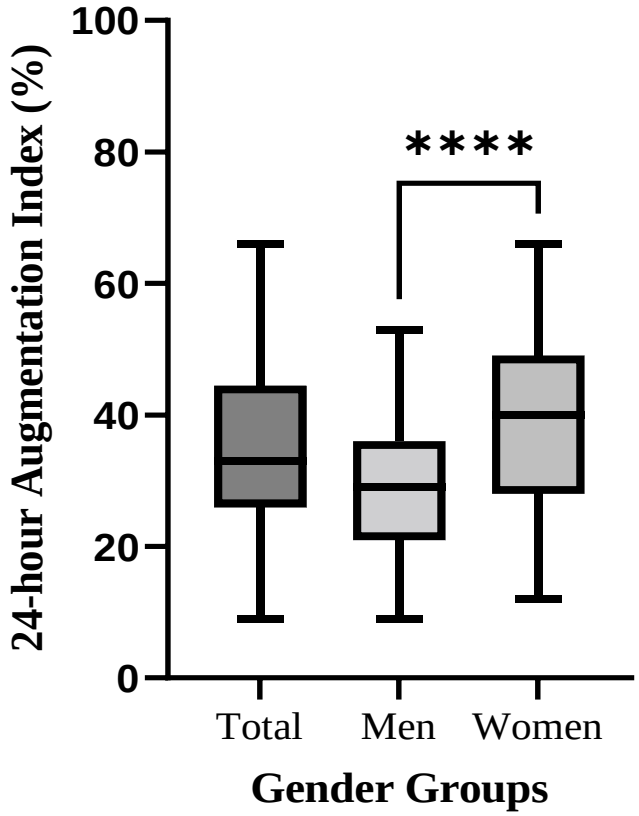



**Figure 3. 5:** The comparison of 24-hour augmentation index between men and women.

The mean and SD for the 24-hour augmentation index (AI24) in the men's group was 29.3 ±10.8 and in the mean and SD in the women's group was 38.4 ±12.1 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (****) Represents statistical significance, where $p<0.0001$. Student unpaired t-test was used to determine statistical difference.

Figure 3.6 compares the difference in AID between men and women. Our results show that during the day, women had a significantly higher augmentation index compared to men ($p=0.0003$).

Figure 3.7 compares the difference in AIN between men and women. Our results show that during the night, women had a significantly higher augmentation index compared to men ($p<0.0001$).

Figure 3.8 compares the difference between AIN and AID in the total population. Our results show that the augmentation index measured during the night-time was significantly higher compared to the augmentation index measured during the daytime ($p=0.0143$).

Figure 3.9 compares the difference between AIN and AID in men. Our results show that there was some slight increase in augmentation index in the night-time, however the increase was not statistically significant ($p=0.1541$).

Figure 3.10 compares the difference between AIN and AID in women. Our results show that the augmentation index in women was significantly higher during the night-time compared to during the daytime ($p=0.0291$).

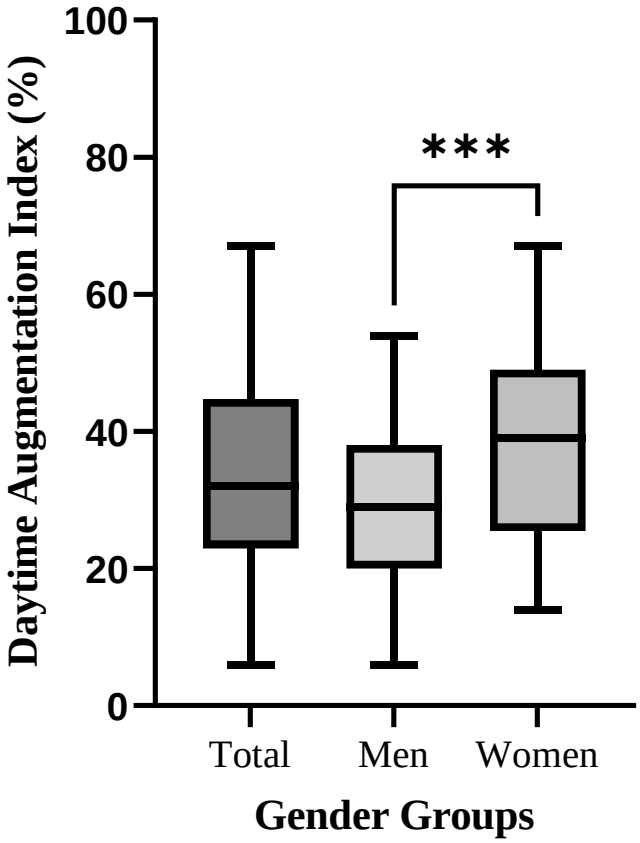



**Figure 3.6:** The comparison of daytime augmentation index between men and women.

The mean and SD for daytime augmentation index (AID) in the men's group was 28.5 ±11.5 and the mean and SD in the women's group was 37.4 ±13.2 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (***) Represents statistical significance, where $p<0.05$. Student unpaired t-test was used to determine statistical difference.

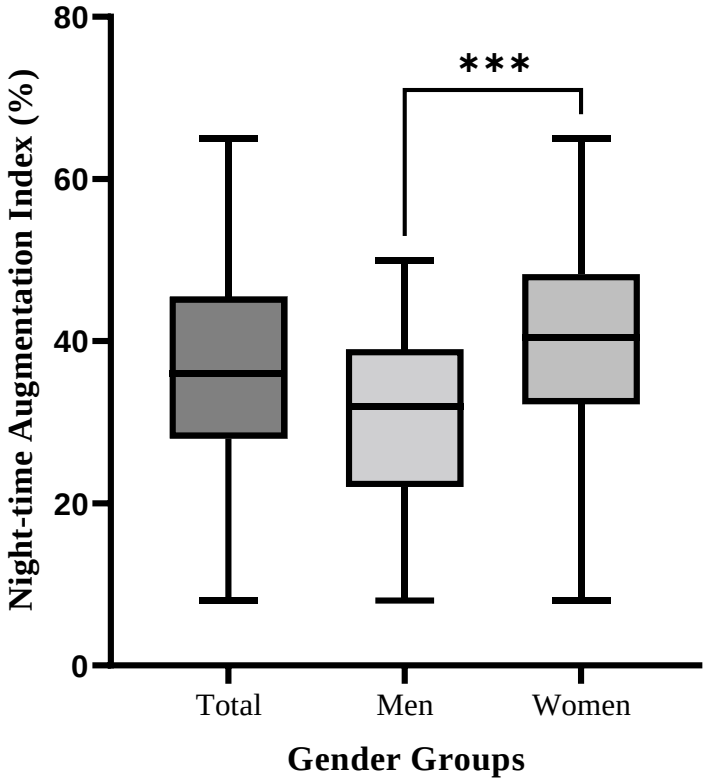



**Figure 3.7:** The comparison of night-time augmentation index between men and women.

The mean and SD for night-time augmentation index (AIN) in the men's group was 31.8 ±10.9 and the mean and SD in the women's group was 42.5 ±12.2 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (***) Represents statistical significance, where $p<0.05$. Student unpaired t-test was used to determine statistical difference.

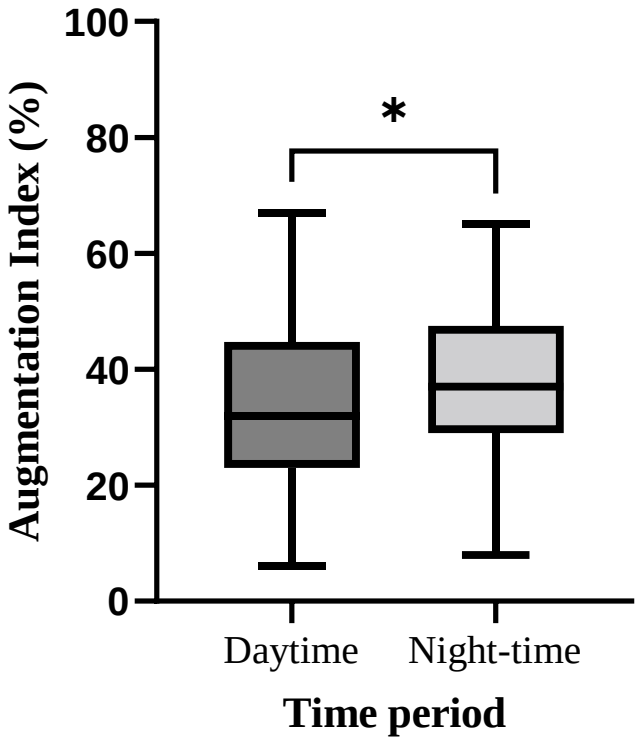



**Figure 3.8:** The comparison between night-time augmentation index and daytime augmentation index in the total population.

The mean and SD for the daytime augmentation index (AID) was 33.4 ±13.2 whilst the mean and SD of the night-time augmentation index (AIN) was 37.7 ±12.8 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (*) Represents statistical significance, where $p<0.05$. Student unpaired t-test was used to determine statistical difference.

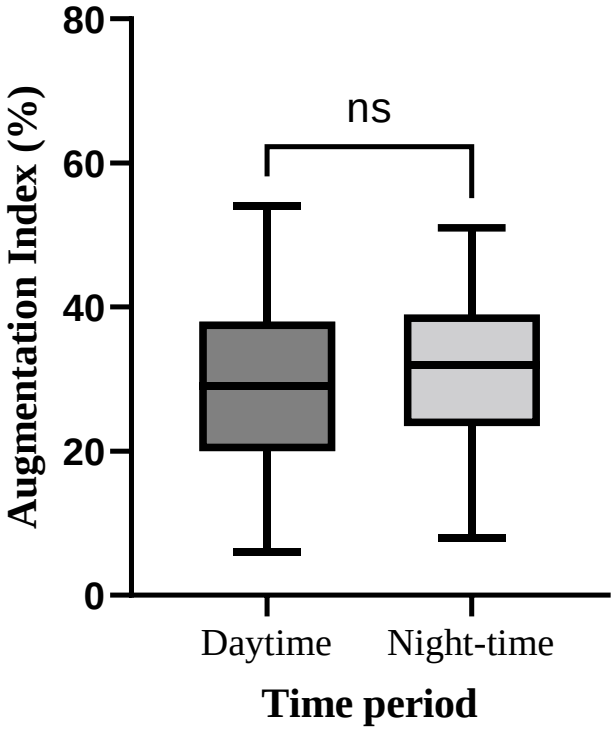



**Figure 3.9:** The comparison between daytime augmentation index and night-time augmentation index in men.

The mean and SD for daytime augmentation index (AID) was 28.5 ±11.5 and the mean and SD for the night-time augmentation index (AIN) was 31.8 ±10.9 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (ns) Represents statistical non-significance, where $p>0.05$. Student unpaired t-test was used to determine statistical difference.

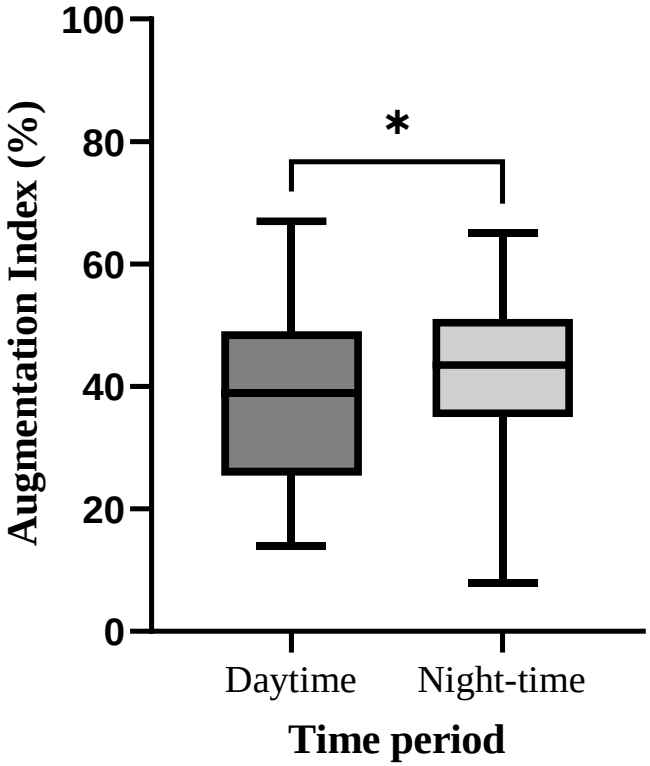



**Figure 3.10:** The comparison between daytime augmentation index and night-time augmentation index in women.

The mean and SD for daytime augmentation index (AID) was 37.4 ±13.2 and the mean and SD for the night-time augmentation index (AIN) was 42.5 ±12.3 corrected for age, hypertension, diabetes, smoking, and alcohol consumption; (*) Represents statistical significance, where $p<0.05$. Student unpaired t-test was used to determine statistical difference.

# CHAPTER 4

(Discussion)

### 4.1 The prevalence of hypertension in a population of black African Descent.

Our results confirm the findings of previous studies which have shown that there is a high prevalence of hypertension in this population (Javed *et al*., 2022; Maseko *et al*., 2018). According to our results, 31.2% of the total population was hypertensive, with women having a slightly higher prevalence of hypertension compared to men (31.9% in women and 28.3% in men). Similarly, a study conducted by Maseko *et al*. (2018) in this population group also found that the prevalence of hypertension was higher in women compared to men (39% in women and 24% in men). Although the women were slightly older than the men, there was no significant difference in age between the two gender groups. Therefore, age could not account for the gender differences in the prevalence of hypertension. Age related hypertension has been well studied, and it was found that an increase in age increases the risk of developing hypertension (Dai *et al.*, 2022; Noon *et al*., 2008; Pinto, 2007). Aging initiates structural and functional changes in the arterial wall, and these changes result in the arteries becoming less compliant and stiff (Sun, 2015). Subsequently, the stiff arteries will contribute to the development of hypertension by increasing the resistance to blood flow and by impairing the relaxation of arteries (Dernellis & Panaretou, 2005; Franklin, 2005; Liao *et al.*, 1999). Therefore, without any significant difference in age, these mechanisms cannot account for the gender differences in the prevalence of hypertension. Another reason why age could not account for the gender differences in the prevalence of hypertension in this study population is that age related changes become more evident above the age of sixty-five (WHO, 2023), whereas the average age of our study population was the mid-thirties for both men and women.

Since our results ruled out the possibility of age being the main reason for the observed gender differences in the prevalence of hypertension, we then investigated whether lifestyle factors such as smoking, alcohol consumption and being overweight might account for the gender differences in the prevalence of hypertension. Our results show that the incidence of smoking and alcohol consumption was almost similar between the two groups. The incidence of smoking was slightly higher in men compared to women (9.4% in men and 8.2% in women). A study conducted by Zhang *et al.* (2019) investigating the prevalence of smoking amongst Chinese adults also found the incidence of smoking to be high in men compared to women. Subsequently, a study conducted by Xie *et al.* (2021) found smoking to be strongly associated with the pathogenesis of hypertension. Smoking contributes to the development of hypertension by stimulating the sympathetic nervous system which is a result of nicotine acting

as an adrenergic agonist (Nagao *et al.*, 2021; Primatesta *et al.*, 2001). Even though we did not look at the relationship between smoking and hypertension in this current study, based on the evidence from other studies, it is possible that smoking contributed to the high incidence of hypertension in this population.

In addition to smoking being associated with the development of hypertension, existing studies have also found alcohol consumption to be strongly associated with the development of hypertension (Piano, 2017; Wildman *et al.*, 2005). According to these existing studies, alcohol contributes to the development of hypertension by decreasing baroreceptor activity thus increasing the activity of the sympathetic nervous system (Piano, 2017; Tasnim *et al*., 2020). Therefore, it is possible that alcohol consumption contributes significantly to the high prevalence of hypertension in this population because of the high incidence of alcohol consumption in both men (35.8%) and women (33.3%). Though alcohol may account for the high incidence of hypertension at a population level, it cannot account for the observed gender differences because, similar to smoking, alcohol consumption does not differ significantly between men and women.

Having ruled out age, alcohol consumption, and smoking as the main contributing factors in the gender difference on the prevalence of hypertension in this population sample, we therefore speculated that overweight/obesity could be the main contributor to the observed differences. Our results, after using four different methods (BMI, WHR, WC and WHtR) of measuring excess bodyweight, show that women were more overweight/obese compared to men. These results confirm the findings of an earlier study conducted by Maseko *et al*. (2018) which showed that the incidence of overweight/obesity is higher in women compared to men in this population. The pathophysiological mechanisms that link overweight/obesity to the development of hypertension are well understood. One of the pathophysiological mechanisms in which overweight/obesity contributes to the development of hypertension involves the increased secretion of insulin (hyperinsulinemia) found in obese/overweight individuals. Hyperinsulinemia is associated with the over-activation of the sympathetic nervous system. The activation of the sympathetic nervous system will increase BP by constricting blood vessels. The constriction of the blood vessels will also reduce blood flow to the kidneys which will result in decreased glomerular filtration resulting in increased sodium reabsorption in the proximal tubules of the kidney and the activation of the renin-angiotensin-aldosterone system

(RAAS), leading to salt and water retention by the kidneys which further increases BP (Jiang *et al.*, 2016; Kotsis *et al.*, 2010; Shariq & McKenzie, 2020).

**4.2 The difference in blood pressure measurements between men and women.**

Despite the prevalence of hypertension being high in women, our results show that the BP of women was lower than that of men. This was true for conventional BP, central and peripheral 24-hour BP, central and peripheral night-time BP, and for central and peripheral daytime BP. Since hypertension is defined as a significant increase in BP, then why do women have a lower BP compared to men despite having a higher prevalence of hypertension? A careful look at our results showed that the average BP for both genders was within the normotensive range. This implies that even though women have a higher incidence of hypertension, their lower BP may be due to adherence to antihypertensive treatment. Though we did not compare differences in the adherence to antihypertensive treatment between men and women in this study, other studies have shown that adherence to antihypertensive treatment is poor in men compared to women (Egan *et al.*, 2010; Hajjar & Kotchen, 2003). Therefore, the high BP of uncontrolled hypertensive males is increasing the average BP of men to be significantly above that of women. Hence, despite men having a lower prevalence of hypertension, their average BP was significantly higher than that of women.

Based on the average age of our participants being the mid-thirties, we speculated that sex hormones such as testosterone and oestradiol might have also contributed to the significant BP variation between the two genders in this study population. Existing studies have found significant evidence that testosterone and oestradiol may have an important influence in BP regulation (Dubey *et al.*, 2002; Maranon & Reckelhoff, 2013; Reckelhoff, 2001). These studies found that testosterone, which is a sex hormone predominantly found in men, was strongly associated with an increase in BP but the physiological mechanism on how testosterone increases BP remains unclear. A study conducted by Wu *et al.* (2000) found that testosterone levels are usually high in men below the age of fifty compared to men above the age of fifty. Based on the previously mentioned findings by Wu *et al.* (2000), our results show that the men in our study are most likely to have high levels of testosterone since their average age was below the age of fifty (34.9 years). This explains why men had significantly higher BP measurements compared to women. On the other hand, oestradiol which is a primary form of oestrogen found in women was found to be strongly associated with a decrease in BP but the physiological mechanism on how oestradiol decreases BP also remains unclear (Dubey *et al.*,

2002; Maranon & Reckelhoff, 2013). Existing studies have found that the concentration of oestradiol is high in premenopausal women compared to menopausal women (Dubey *et al.*, 2002; Maranon & Reckelhoff, 2013). According to WHO, premenopausal women are women below the age of forty-five who can still become pregnant whereas menopausal women are women above the age of forty-five who can no longer become pregnant (WHO, 2021). Additionally, the women in our study can be classified as premenopausal women because their average age is below forty-five. Since premenopausal women have been found to have high levels of oestradiol compared to menopausal women and the effects of oestradiol on BP being known, explains why women in our study had a significantly lower BP measurements compared to men despite having a slightly higher prevalence of hypertension compared to men.

### 4.3 The difference between clinic augmentation index and 24-hour augmentation index.

Despite women having lower BP, our results indicate that women have a higher AIC compared to men, indicating that women have increased arterial stiffness. These findings are consistent with the findings of previous studies (Chester *et al*., 2013; Costa-Hong *et al.*, 2018). However, in our study we further analysed the participants' augmentation index over a 24-hour period to confirm our findings. We speculated that measuring augmentation index over a 24-hour period would be more precise than measuring augmentation index in a clinical setting, since 24-hour measurements are accepted to be more accurate than once off measurements since they account for circadian fluctuations and can detect the differences between daytime and night-time measurements. For example, measuring BP over a 24-hour period has been shown to be more beneficial than measuring BP in a clinical setting, because it can detect white-coat hypertension, night-time hypertension, and BP dipping status (O'Brien, 2003; O'Brien *et al.*, 2013). With existing evidence showing that 24-hour measurements are more accurate and precise than in-clinic measurements, a possibility exists that AI24 may be more accurate than AIC in the assessment of arterial stiffness. Our results show that AIC does indeed underestimate arterial stiffness because we found AI24 to be significantly higher than AIC. To the best of our knowledge, this is the first study to show the superiority of AI24 over AIC in the assessment of arterial stiffness.

When participants were separated according to gender, a similar trend was observed. Twenty-four-hour augmentation index was significantly higher than AIC in both men and women. Most importantly, when men were compared to women, AI24 was significantly higher in women compared to men. This was also true for night-time and daytime augmentations. This confirms

the AIC results which show that women have a higher augmentation index compared to men despite their lower BP. Since BP is known as the strongest determinant of arterial stiffness, what could account for the contradictory findings in this population? One thing that stood out was the differences in the BMI of the two groups. Though both genders were in the overweight BMI category, women had a significantly higher BMI (29.0 kg/m²) compared to men (25.7 kg/m²). The increased adiposity found in overweight/obese individuals resulted in an increased secretion of adipokines such as leptin (Para *et al.*, 2021). Existing studies have found that high plasma levels of leptin (hyperleptinemia) contribute to the pathogenesis of arterial stiffness by inducing endothelial oxidative stress, increasing SNS activation, increasing the secretion of endothelin-1, and by diminishing the bioavailability of nitric oxide which ultimately result in vasoconstriction and decreased vasodilatory effects (Muñoz-Durango *et al.*, 2016). Additionally, a study conducted by Tsai *et al.* (2016) found that hyperleptinemia found in overweight/obese individuals also contributes to arterial stiffening by promoting vascular smooth muscle cell proliferation and migration through the phosphorylation and activation of mitogen protein kinases. The aforementioned mechanisms on how overweight/obesity contributes to the pathogenesis of arterial stiffness affirm our results which show that women had increased arterial stiffness compared to men due to their increased adiposity.

### 4.4 The difference in dipping status between men and women.

Besides increased adiposity, our data shows that women were non-dippers with a BP dipping percentage of 7.9% and men were dippers with a BP dipping percentage of 10.1%. Blood pressure dipping is a phenomenon whereby night-time BP drops by 10-20% from daytime BP, and the inability for night-time BP to drop by 10% or more is known as non-dipping. Existing studies have shown that non-dipping is strongly associated with an increased risk of developing adverse cardiovascular events (Cicek *et al*., 2013; Liakos *et al*., 2019; Verdecchia *et al.*, 1990), which suggests that women in our study are at a higher risk of developing adverse cardiovascular events compared to men. In addition to non-dipping being associated with increased risk of developing adverse cardiovascular events, it has also been associated with premature alterations in the arterial structure and function which mimics the effects of aging and this phenomenon can be referred to as premature vascular aging or arterial stiffening (Cicek *et al.,* 2013). We therefore concluded that the attenuated decline in nocturnal BP of women is one of the contributing factors to their increased arterial stiffness.

To gain a deeper understanding of the impact of the attenuated decline in nocturnal BP of women on arterial stiffness, we looked at the 24-hour augmentation index profile of the population. Our results indicate that the 24-hour ambulatory augmentation index differs from the 24-hour ambulatory BP profile. As explained earlier, normally at night BP drops by 10%, which is a process known as nocturnal BP dipping. However, according to our results, AID is lower than AIN. Indicating that augmentation index increases during sleep. This is a direct opposite of what happens to BP. The physiological mechanisms that lead to a decrease in BP during sleep are well known. One of the physiological mechanisms that leads to a decrease in BP during sleep involves the decreased activity of the sympathetic nervous system as a result of a downward shift in the baroreflex threshold (Sayk *et al.*, 2007). The decreased activity of the sympathetic nervous system is physiologically accompanied by an increased activity of the parasympathetic nervous system. The increased activity of the parasympathetic nervous system will result in a decreased heart rate, cardiac output and total peripheral resistance which results in a decreased BP (Casagrande *et al.*, 2020). The reasons for the increased AIN in the population become apparent when the participants are separated by gender. In men, there is no significant difference between AIN and AID. However, in women, AIN was significantly higher than AID. Since the number of women is much higher than that of men, the high night-time augmentation index is imputed into the whole population resulting in a higher AIN in the total population.

**4.5 The impact obesity has on arterial stiffness.**

The higher augmentation index in women may be due to disruption in renal sodium handling in overweight/obese individuals. The mechanism responsible for the decreased salt excretion in obese/overweight individuals seems to be mediated by the high plasma leptin concentrations usually found in obese/overweight individuals. The chronically high plasma leptin concentrations found in obese/overweight individuals, suppress pressure natriuresis through the upregulation of the sodium/potassium ATPase pumps (Maseko *et al.*, 2018). The upregulation of the sodium/potassium ATPase pumps will result in increased salt retention and decreased urinary salt excretion. Increased salt retention results in hypernatremia which is a condition characterized by high salt levels in the blood.

The increased plasma salt concentrations will cause arterial stiffening by increasing the production of reactive oxygen species (ROS) such as superoxide and suppressing angiotensin-2 (Ang-II) which is responsible for the activation and expression of the enzyme superoxide

dismutase (SOD) (Edwards & Farquhar, 2015). Superoxide dismutase is responsible for breaking down superoxide (Younus, 2018). Therefore, the suppression of Ang-II coupled with the increased production of ROS will cause the endothelial cells to undergo oxidative stress which reduces the bioavailability of NO which results in endothelial dysfunction (Edwards & Farquhar, 2015). In endothelial dysfunction, the endothelial cells will increase the expression of leukocyte adhesion molecules and increase the production of pro-inflammatory cytokines (Taniyama & Griendling, 2003). The increased expression of leukocyte adhesion molecules and pro-inflammatory cytokines will facilitate transmigration of leukocytes into the subendothelial space, once these leukocytes are in the subendothelial space they will produce enzymes that will degrade the blood vessel's elastin which contributes to the stiffening of arteries (Taniyama & Griendling, 2003). The other methods in which endothelial dysfunction causes arterial stiffness is by stimulating the proliferation of vascular smooth muscle cells (VSMC) which will result in vascular hypertrophy, and by stimulating the migration of VSMC from the tunica media into the tunica intima where these VSMC will become fibroblasts and start producing collagen which also contributes to the stiffening of the blood vessels (Zieman *et al.,* 2005).

## 4.6 Conclusion

Our study confirms the high prevalence of hypertension among the population of black African descent, consistent with findings from previous studies. Women in this population exhibit a slightly higher prevalence of hypertension compared to men, which cannot be attributed to age, as there was no significant age difference between genders. Lifestyle factors such as smoking and alcohol consumption, while prevalent, do not account for the gender disparity. Instead, the higher incidence of overweight and obesity among women emerges as a significant contributor, influencing hypertension through mechanisms involving insulin secretion and activation of the sympathetic nervous system. Despite women having lower blood pressure than men, adherence to antihypertensive treatment likely explains this discrepancy. The role of sex hormones, specifically testosterone and oestradiol, further elucidates the blood pressure differences observed.

A key finding in our study is that relying on conventional augmentation measurements in the assessment of arterial stiffness may underestimate the prevalence of arterial stiffness and its adverse effects on cardiovascular target organs. The higher AIC and AI24 observed in women, despite lower BP values compared to men, indicate that increased adiposity determines arterial stiffness above BP in this population. Current intervention strategies that focus solely on the treatment and control of hypertension, while ignoring the high obesity epidemic, may not be beneficial to this population. Even when BP is controlled to target levels, arterial stiffness remains high if adiposity is high. This may explain the rise of cardiovascular disease-related morbidity and mortality in this population despite efforts to treat hypertension. These results highlight the complex interplay of physiological, hormonal, and lifestyle factors in hypertension and arterial stiffness, underscoring the need for targeted interventions to address these disparities in cardiovascular health.

### 4.7 Study limitations and future perspectives.

The cohort of our present study had more women than men. We believe that the reason behind our study having more women than men, despite randomly recruiting participants, may be due to men being reluctant to participate. The low number of men in our study may have created some gender bias. In our study, we did not measure the participants oestradiol and testosterone concentrations to assess the extent of the regulatory effects sex hormones have on blood pressure in a population of African descent. Future studies should measure sex hormones to determine the extent in which they regulate BP in a population of black African descent. Future studies should also measure lipid profiles, leptin, resistin, and adiponectin in order to assess the strength of the relationship these variables have with 24-hour augmentation index. Lastly, future studies should also conduct echocardiography measurements to assess the strength of the relationship between 24-hour augmentation index and left ventricular hypertrophy.

# REFERENCES

Arnson, Y., Shoenfeld, Y., & Amital, H. (2010). Effects of tobacco smoke on immunity, inflammation and autoimmunity. *Journal of Autoimmunity*, **34**, 258-265.

Aroor, A. R., Jia, G., & Sowers, J. R. (2018). Cellular mechanisms underlying obesity-induced arterial stiffness. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, **314**, 387–398.

Balafa, O., & Kalaitzidis, R. G. (2021). Salt sensitivity and hypertension. *Journal of Human Hypertension*, **35**, 184–192.

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

Beilin, L. J., & Puddey, I. B. (2006). Alcohol and hypertension. *Hypertension*, **47**, 1035–1038.

Bonarjee, V. V. S. (2018). Arterial stiffness: a prognostic marker in coronary heart disease. available methods and clinical application. *Frontiers in Cardiovascular Medicine*, **5**, 645-648.

Brandes, R. P. (2014). Endothelial dysfunction and hypertension. *Hypertension*, **64**, 924–928.

Brown, J. C., Gerhardt, T. E., & Kwon, E. (2020). Risk factors for coronary artery disease. In *StatPearls*, **55**, 44-48.

Casagrande, M., Favieri, F., Langher, V., Guarino, A., Di Pace, E., Germanò, G., & Forte, G. (2020). The night side of blood pressure: nocturnal blood pressure dipping and emotional (dys)regulation. *International Journal of Environmental Research and Public Health*, **17**, 8892-8897.

Chester, R. C., Sander, G., Fernandez, C., Chen, W., Berenson, G., & Giles, T. (2013). Women have significantly greater difference between central and peripheral arterial pressure compared to men: the Bogalusa heart study. *Journal of the American Society of Hypertension*, **7**, 379–385.

Cicek, Y., Durakoglugil, M. E., Kocaman, S. A., Cetin, M., Erdogan, T., Dogan, S., Ugurlu, Y., & Canga, A. (2013). Non-dipping pattern in untreated hypertensive patients is related to increased pulse wave velocity independent of raised nocturnal blood pressure. *Blood Pressure*, **22**, 34–38.

Costa-Hong, V. A., Muela, H. C. S., Macedo, T. A., Sales, A. R. K., & Bortolotto, L. A. (2018). Gender differences of aortic wave reflection and influence of menopause on central blood pressure in patients with arterial hypertension. *Biomed Central: Cardiovascular Disorders*, **18**, 123-129.

Cruz, V. P. D., Gonzaga, C. W. O., da Silva, V. B., da Cruz, A. F. P., da Silva, A. B. T., Capingana, D. P., Magalhães, P., Zaniqueli, D. A., Guimarães, A. L. S., Cunha, R. S., Mill, J. G., & Baldo, M. P. (2020). Arterial stiffness in black adults from Angola and Brazil. *The Journal of Clinical Hypertension*, **22**, 1469–1475.

Cuzzo, B., Padala, S. A., & Lappin, S. L. (2024). Physiology, Vasopressin. *In StatPearls*, **6**, 8-13.

Dahlöf, B. (2010). Cardiovascular disease risk factors: epidemiology and risk assessment. *The American Journal of Cardiology*, **105**, 3-9.

Dai, B., Addai-Dansoh, S., Nutakor, J. A., Osei-Kwakye, J., Larnyo, E., Oppong, S., Boahemaa, P. Y., & Arboh, F. (2022). The prevalence of hypertension and its associated risk factors among older adults in Ghana. *Frontiers in Cardiovascular Medicine*, **9**, 616-622.

Del Giorno, R., Maddalena, A., Bassetti, S., & Gabutti, L. (2022). Association between alcohol intake and arterial stiffness in healthy adults: a systematic review. *Nutrients*, **14**, 1207-1224.

DeLoach, S. S., & Townsend, R. R. (2008). Vascular stiffness: its measurement and significance for epidemiologic and outcome studies. *Clinical Journal of the American Society of Nephrology*, **3**, 184–192.

Dernellis, J., & Panaretou, M. (2005). Aortic stiffness is an independent predictor of progression to hypertension in nonhypertensive subjects. *Hypertension*, **45**, 426–431.

Doonan, R. J., Hausvater, A., Scallan, C., Mikhailidis, D. P., Pilote, L., & Daskalopoulou, S. S. (2010). The effect of smoking on arterial stiffness. *Hypertension Research*, **33**, 398-410.

Dubey, R. K., Oparil, S., Imthurn, B., & Jackson, E. K. (2002). Sex hormones and hypertension. *Cardiovascular Research*, **53**, 688–708.

DuPont, J. J., Kenney, R. M., Patel, A. R., & Jaffe, I. Z. (2019). Sex differences in mechanisms of arterial stiffness. *British Journal of Pharmacology*, **176**, 4208–4225.

Edwards, D. G., & Farquhar, W. B. (2015). Vascular effects of dietary salt. *Current Opinion in Nephrology and Hypertension*, **24**, 8–13.

Egan, B. M., Zhao, Y., & Axon, R. N. (2010). US trends in prevalence, awareness, treatment, and control of hypertension. *Journal of the American Medical Association*, **303**, 2043–2050.

El Hadri, K., Smith, R., Duplus, E., & El Amri, C. (2021). Inflammation, oxidative stress, senescence in atherosclerosis: thioredoxine-1 as an emerging therapeutic target. *International Journal of Molecular Sciences*, **23**, 77-100.

Elijovich, F., Weinberger, M. H., Anderson, C. A. M., Appel, L. J., Bursztyn, M., Cook, N. R., Dart, R. A., Newton-Cheh, C. H., Sacks, F. M., & Laffer, C. L. (2016). Salt sensitivity of blood pressure. *Hypertension*, **68**, 7–46.

Fantin, F., Mattocks, A., Bulpitt, C. J., Banya, W., & Rajkumar, C. (2007). Is augmentation index a good measure of vascular stiffness in the elderly? *Age and Ageing*, **36**, 43–48.

Fountain, J. H., & Lappin, S. L. (2020). Physiology, renin angiotensin system. In *StatPearls*, **60**, 78-84.

Franklin, S. S. (2005). Arterial stiffness and hypertension. *Hypertension*, **45**, 349–351.

Gaziano, T., Reddy, K. S., Paccaud, F., Horton, S., & Chaturvedi, V. (2006). Cardiovascular disease: in disease control priorities in developing countries. *The International Bank for Reconstruction and Development*, **33**, 1-51.

Grillo, A., Salvi, L., Coruzzi, P., Salvi, P., & Parati, G. (2019). Sodium intake and hypertension. *Nutrients*, **11**, 1970-1976.

Haass, M., & Kübler, W. (1997). Nicotine and sympathetic neurotransmission. *Cardiovascular Drugs and Therapy*, **10**, 657–665.

Hajjar, I., & Kotchen, T. A. (2003). Trends in prevalence, awareness, treatment, and control of hypertension in the United States. *Journal of the Medical Association*, **290**, 199–206.

Husain, K., Ansari, R. A., & Ferder, L. (2014). Alcohol-induced hypertension: Mechanism and prevention. *World Journal of Cardiology*, *6*(5), 245–252.

Izzo, J. L. (2007). Hemodynamics of hypertension. *Comprehensive Hypertension*, **11**,123–133.

Javed, Z., Haisum Maqsood, M., Yahya, T., Amin, Z., Acquah, I., Valero-Elizondo, J., Andrieni, J., Dubey, P., Jackson, R. K., Daffin, M. A., Cainzos-Achirica, M., Hyder, A. A., & Nasir, K. (2022). Race, racism, and cardiovascular health: applying a social determinants of health

framework to racial/ethnic disparities in cardiovascular disease. *Circulation: Cardiovascular Quality and Outcomes*, **15**, 72-86.

Jiang, S.-Z., Lu, W., Zong, X.-F., Ruan, H.-Y., & Liu, Y. (2016). Obesity and hypertension. *Experimental and Therapeutic Medicine*, **12**, 2395–2399.

Karas, R. H., Patterson, B. L., & Mendelsohn, M. E. (1994). Human vascular smooth muscle cells contain functional estrogen receptor. *Circulation*, **89**, 1943–1950.

Kawai, T., Forrester, S. J., O'Brien, S., Baggett, A., Rizzo, V., & Eguchi, S. (2017). AT1 receptor signaling pathways in the cardiovascular system. *Pharmacological Research*, **125**, 4–13.

Kim, J.-Y., Park, J. B., Kim, D. S., Kim, K. S., Jeong, J. W., Park, J. C., Oh, B. H., & Chung, N. (2014). Gender difference in arterial stiffness in a multicentre cross-sectional study: the Korean arterial aging study (KAAS). *Pulse*, **2**, 11–17.

Kocemba, J., Kawecka-Jaszcz, K., Gryglewska, B., & Grodzicki, T. (1998). Isolated systolic hypertension: pathophysiology, consequences and therapeutic benefits. *Journal of Human Hypertension*, **12**, 621-626.

Kohn, J. C., Lampi, M. C., & Reinhart-King, C. A. (2015). Age-related vascular stiffening: Causes and consequences. *Frontiers in Genetics*, **6**, 112-116.

Kotsis, V., Stabouli, S., Papakatsika, S., Rizos, Z., & Parati, G. (2010). Mechanisms of obesity-induced hypertension. *Hypertension Research*, **33**, 386-393.

Kun Xie, Xiufang Gao, Liwen Bao, Ying Shan, Haiming Shi & Yong Li, K. X. (2021). The different risk factors for isolated diastolic hypertension and isolated systolic hypertension: A national survey. *Biomed Central: Public Health*, **21**, 616-623.

Kuznetsova, T. Y., Korneva, V. A., Bryantseva, E. N., Barkan, V. S., Orlov, A. V., Posokhov, I. N., & Rogoza, A. N. (2014). The 24-hour pulse wave velocity, aortic augmentation index, and central blood pressure in normotensive volunteers. *Vascular Health and Risk Management*, **10**, 247–251.

Kwon, H., & Pessin, J. E. (2013). Adipokines mediate inflammation and insulin resistance. *Frontiers in Endocrinology*, **4**, 71-78.

Liakos, C., Karpanou, E., Grassos, C., Markou, M., Vyssoulis, G., & Tousoulis, D. (2019). Nocturnal blood pressure dipping status and cardiovascular risk in white-coat hypertension individuals. *Journal of Hypertension*, **37**, 10-17.

Liao, D., Arnett, D. K., Tyroler, H. A., Riley, W. A., Chambless, L. E., Szklo, M., & Heiss, G. (1999). Arterial stiffness and the development of hypertension. *Hypertension*, **34**, 201–206.

Lin, L.-Y., Liao, Y.-C., Lin, H.-F., Lee, Y.-S., Lin, R.-T., Hsu, C. Y., & Juo, S.-H. H. (2015). Determinants of arterial stiffness progression in a Han-Chinese population in Taiwan: A 4-year longitudinal follow-up. *Biomed Central: Cardiovascular Disorders*, **15**, 100-108.

Maeda, S., Tanabe, T., Otsuki, T., Sugawara, J., Ajisaka, R., & Matsuda, M. (2008). Acute Exercise Increases systemic arterial compliance after 6-month exercise training in older women. *Hypertension Research*, **31**, 377–381.

Maranon, R., & Reckelhoff, J. F. (2013). Sex and gender differences in control of blood pressure. *Clinical Science*, **125**, 311–318.

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

Messner, B., & Bernhard, D. (2014). Smoking and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **34**, 509–515.

Miladinović, A., Ajčević, M., Siveri, G., Liguori, L., Pascazio, L., & Accardo, A. (2021). Ambulatory blood pressure monitoring versus office blood pressure measurement: Are there sex differences? *Procedia Computer Science*, **192**, 2912–2918.

Milutinović, A., Šuput, D., & Zorc-Pleskovič, R. (2020). Pathogenesis of atherosclerosis in the tunica intima, media, and adventitia of coronary arteries: an updated review. *Bosnian Journal of Basic Medical Sciences*, **20**, 21–30.

Muniyappa, R., & Sowers, J. R. (2013). Role of insulin resistance in endothelial dysfunction. *Reviews in Endocrine & Metabolic Disorders*, **14**, 5–12.

Muñoz-Durango, N., Fuentes, C. A., Castillo, A. E., González-Gómez, L. M., Vecchiola, A., Fardella, C. E., & Kalergis, A. M. (2016). Role of the renin-angiotensin-aldosterone system beyond

blood pressure regulation: molecular and cellular mechanisms involved in end-organ damage during arterial hypertension. *International Journal of Molecular Sciences*, **17**, 797-814.

Nagao, T., Nogawa, K., Sakata, K., Morimoto, H., Morita, K., Watanabe, Y., & Suwazono, Y. (2021). effects of alcohol consumption and smoking on the onset of hypertension in a long-term longitudinal study in a male workers' cohort. *International Journal of Environmental Research and Public Health*, **18**, 11781-11792.

Nakamura, K., Fuster, J. J., & Walsh, K. (2014). Adipokines: A link between obesity and cardiovascular disease. *Journal of Cardiology*, **63**, 250–259.

Nethononda, R. M., Lewandowski, A. J., Stewart, R., Kylinterias, I., Whitworth, P., Francis, J., Leeson, P., Watkins, H., Neubauer, S., & Rider, O. J. (2015). Gender specific patterns of age-related decline in aortic stiffness: a cardiovascular magnetic resonance study including normal ranges. *Journal of Cardiovascular Magnetic Resonance*, **17**, 20-29.

Nevzati, E., Shafighi, M., Bakhtian, K. D., Treiber, H., Fandino, J., & Fathi, A. R. (2015). Estrogen induces nitric oxide production via nitric oxide synthase activation in endothelial cells. *Acta Neurochirurgica*, **120**, 141–145.

Noon, J. P., Trischuk, T. C., Gaucher, S. A., Galante, S., & Scott, R. L. (2008). The effect of age and gender on arterial stiffness in healthy Caucasian Canadians. *Journal of Clinical Nursing*, **17**, 2311–2317.

O'Brien, E. (2003). Ambulatory blood pressure monitoring in the management of hypertension. *Heart*, **89**, 571–576.

O'Brien, E., Parati, G., Stergiou, G., Asmar, R., Beilin, L., Bilo, G., Clement, D., de la Sierra, A., de Leeuw, P., Dolan, E., Fagard, R., Graves, J., Head, G. A., Imai, Y., Kario, K., Lurbe, E., Mallion, J.-M., Mancia, G., Mengden, T., … Zhang, Y. (2013). European society of hypertension position paper on ambulatory blood pressure monitoring. *Journal of Hypertension*, **31**, 1731–1768.

Pappaccogli, M., Di Monaco, S., Perlo, E., Burrello, J., D'Ascenzo, F., Veglio, F., Monticone, S., & Rabbia, F. (2019). Comparison of automated office blood pressure with office and out-off-office measurement techniques. *Hypertension*, **73**, 481–490.

Para, I., Albu, A., & Porojan, M. D. (2021). Adipokines and arterial stiffness in obesity. *Medicina*, **57**, 653-657.

Park, S., & Lakatta, E. G. (2012). Role of Inflammation in the pathogenesis of arterial stiffness. *Yonsei Medical Journal*, **53**, 258–261.

Park, W., Park, H.-Y., Lim, K., & Park, J. (2017). The role of habitual physical activity on arterial stiffness in elderly individuals: a systematic review and meta-analysis. *Journal of Exercise Nutrition & Biochemistry*, **21**, 16–21.

Parksook, W. W., Heydarpour, M., Gholami, S. K., Luther, J. M., Hopkins, P. N., Pojoga, L. H., & Williams, J. S. (2022). Salt sensitivity of blood pressure and aldosterone: interaction between the lysine-specific demethylase 1 gene, sex, and age. *The Journal of Clinical Endocrinology and Metabolism*, **107**, 1294–1302.

Peeters, S. A., Engelen, L., Buijs, J., Chaturvedi, N., Fuller, J. H., Jorsal, A., Parving, H.-H., Tarnow, L., Theilade, S., Rossing, P., Schalkwijk, C. G., & Stehouwer, C. D. A. (2017). Circulating matrix metalloproteinases are associated with arterial stiffness in patients with type 1 diabetes: Pooled analysis of three cohort studies. *Cardiovascular Diabetology*, **16**, 139-148.

Pereira, T., Correia, C., & Cardoso, J. (2015). Novel methods for pulse wave velocity measurement. *Journal of Medical and Biological Engineering*, **35**, 555–565.

Petrák, O., Štrauch, B., Zelinka, T., Rosa, J., Holaj, R., Vránková, A., Kasalický, M., Kvasnička, J., Pacák, K., & Widimský, J. (2010). Factors influencing arterial stiffness in pheochromocytoma and effect of adrenalectomy. *Hypertension Research: Official Journal of the Japanese Society of Hypertension*, **33**, 454–459.

Piano, M. R. (2017). Alcohol's effects on the cardiovascular system. *Alcohol Research : Current Reviews*, **38**, 219–241.

Pinto, E. (2007). Blood pressure and ageing. *Postgraduate Medical Journal*, **83**, 109–114.

Potenza, M. A., Addabbo, F., & Montagnani, M. (2009). Vascular actions of insulin with implications for endothelial dysfunction. *American Journal of Physiology-Endocrinology and Metabolism*, **297**, 568–577.

Primatesta, P., Falaschetti, E., Gupta, S., Marmot, M. G., & Poulter, N. R. (2001). Association between smoking and blood pressure. *Hypertension*, **37**, 187–193.

Reckelhoff, J. F. (2001). Gender differences in the regulation of blood pressure. *Hypertension*, **37**, 1199-1208.

Sayk, F., Becker, C., Teckentrup, C., Fehm, H.-L., Struck, J., Wellhoener, J. P., & Dodt, C. (2007). To dip or not to dip. *Hypertension*, **49**, 1070–1076.

Saz-Lara, A., Cavero-Redondo, I., Álvarez-Bueno, C., Notario-Pacheco, B., Ruiz-Grao, M. C., & Martínez-Vizcaíno, V. (2021). The acute effect of exercise on arterial stiffness in healthy subjects: a meta-analysis. *Journal of Clinical Medicine*, **10**, 291-304.

Schulz, E., Gori, T., & Münzel, T. (2011). Oxidative stress and endothelial dysfunction in hypertension. *Hypertension Research*, **34**, 665-673.

Schutte, A. E., Kruger, R., Gafane-Matemane, L. F., Breet, Y., Strauss-Kruger, M., & Cruickshank, J. K. (2020). Ethnicity and arterial stiffness. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **40**, 1044–1054.

Shariq, O. A., & McKenzie, T. J. (2020). Obesity-related hypertension: A review of pathophysiology, management, and the role of metabolic surgery. *Gland Surgery*, *9*(1), 80–93.

Sowers, J. R., Zemel, M. B., Zemel, P., Beck, F. W., Walsh, M. F., & Zawada, E. T. (1988). Salt sensitivity in blacks. Salt intake and natriuretic substances. *Hypertension*, *12*(5), 485–490.

Stewart, Arthur., Marfell-Jones, Mike., & International Society for Advancement of Kinanthropometry., J. H. (2011). *International standards for anthropometric assessment*. International Society for the Advancement of Kinanthropometry.

Sun, Z. (2015). Aging, Arterial Stiffness, and Hypertension. *Hypertension*, **65**, 252–256.

Tan, J. L., & Thakur, K. (2021). Systolic Hypertension. *In StatPearls*, **30**, 45-50.

Taniyama, Y., & Griendling, K. K. (2003). Reactive oxygen species in the vasculature. *Hypertension*, **42**, 1075–1081.

Tasnim, S., Tang, C., Musini, V. M., & Wright, J. M. (2020). Effect of alcohol on blood pressure. *The Cochrane Database of Systematic Reviews*, **7**, 1-140.

Tsai, J.-P., Wang, J.-H., Chen, M.-L., Yang, C.-F., Chen, Y.-C., & Hsu, B.-G. (2016). Association of serum leptin levels with central arterial stiffness in coronary artery disease patients. *Biomed Central: Cardiovascular Disorders*, **16**, 80-85.

Verdecchia, P., Schillaci, G., Guerrieri, M., Gatteschi, C., Benemio, G., Boldrini, F., & Porcellati, C. (1990). Circadian blood pressure changes and left ventricular hypertrophy in essential hypertension. *Circulation*, **81**, 528–536.

Wang, M., Kim, S. H., Monticone, R. E., & Lakatta, E. G. (2015). Matrix metalloproteinases promote arterial remodeling in aging, hypertension, and atherosclerosis. *Hypertension*, **65**, 698–703.

Wen, W., Luo, R., Tang, X., Tang, L., Huang, H. X., Wen, X., Hu, S., & Peng, B. (2015). Age-related progression of arterial stiffness and its elevated positive association with blood pressure in healthy people. *Atherosclerosis*, **238**, 147–152.

White, R. E. (2002). Estrogen and vascular function. *Vascular Pharmacology*, **38**, 73–80.

Wildman, R. P., Gu, D., Muntner, P., Huang, G., Chen, J., Duan, X., & He, J. (2005). Alcohol intake and hypertension subtypes in Chinese men. *Journal of Hypertension*, **23**, 737–743.

Wilkinson, I. B., MacCallum, H., Flint, L., Cockcroft, J. R., Newby, D. E., & Webb, D. J. (2000). The influence of heart rate on augmentation index and central arterial pressure in humans. *The Journal of Physiology*, **525**, 263–270.

Woollard, K. J., & Geissmann, F. (2010). Monocytes in atherosclerosis: subsets and functions. *Nature Reviews. Cardiology*, **7**, 77–86.

World Health Organisation (WHO). (2008). Waist circumference and waist–hip ratio. *Report of a WHO Expert Consultation*, **0**, 8–11.

Wu, C. Y., Yu, T. J., & Chen, M. J. (2000). Age related testosterone level changes and male andropause syndrome. *Chang Gung Medical Journal*, **23**, 348–353.

Younus, H. (2018). Therapeutic potentials of superoxide dismutase. *International Journal of Health Sciences*, **12**, 88–93.

Zhang, M., Liu, S., Yang, L., Jiang, Y., Huang, Z., Zhao, Z., Deng, Q., Li, Y., Zhou, M., Wang, L., Chen, Z., & Wang, L. (2019). Prevalence of smoking and knowledge about the hazards of smoking among 170 000 Chinese adults. *Nicotine & Tobacco Research*, **21**, 1644–1651.

Zieman, S. J., Melenovsky, V., & Kass, D. A. (2005). Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **25**, 932–943.

# APPENDICES

**Appendix 1:** Ethical clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Mr Wantonda Mukhovha

## HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

## CLEARANCE CERTIFICATE NO. M220737

| | |
|---|---|
| **NAME:** (Principal Investigator) | Mr Wantonda Mukhovha |
| **DEPARTMENT:** | Physiology<br>Human Nutrition Research Laboratory |
| **PROJECT TITLE:** | *The role of clinical and ambulatory arterial stiffness in the pathogenesis of left ventricular hypertrophy* |
| **DATE CONSIDERED:** | 29/07/2022 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **NOTE:** | If contact information regarding student study participants is required, please contact the Registrar's office - Nicolean.Potgieter@wits.ac.za |
| **SUPERVISOR:** | Prof Muzi Maseko |
| **APPROVED BY:** | Dr CB Penny, Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 29/11/2022 |

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

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

11/01/2023
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

**Appendix 2:** Change of title.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

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

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

25 October 2023
Person No: 1834012
TAA

Mr WP Mukhovha
250 Nkotoane Street Tsolo Section
Katlehong
Germiston
1431
South Africa

Dear Mr Wantonda Mukhovha

**Master of Science in Medicine: Change of title of research**

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

| | |
|---|---|
| From: | **The role of clinical and ambulatory arterial stiffness in the pathogenesis of left ventricular hypertrophy** |
| To: | **Is 24-hour augmentation index a better indicator of arterial stiffness compared to clinic augmentation index** |

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

**Appendix 3:** Plagiarism report

1834012_Mukhovha_Wantonda_MScDissertation.-2.docx

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**Appendix 4:** Standardised 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 |

| | | |
|---|---|---|
| | Unknown | |
| | Unknown | |
| | Unknown | |
| | Unknown | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Your grandfather (mother's side)** | Yes | | No | | Unknown | |
| | **Your grandmother (Mother's side)** | Yes | | No | | Unknown | |
| | **1 or more of your own children** | Yes | | No | | Unknown | |
| | **1 or more of your own grandchildren** | Yes | | No | | Unknown | |
| | **1 or more brothers or sisters of your father** | Yes | | No | | 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?** | | | | | | |
| | | | | | | | |
| **2.7** | **Do you currently exert any professional or occupational activity?** | **Yes** | | **No** | | | |
| | If yes: What is your present occupation?<br><br>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 below** | | | Yes | | No | | |
| | | | | | | | | |
| **END** | | | | | | | | |

**DECLARATION**

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

Signature: _____ Date: _____

D#: _____

**Appendix 5:** Informed Consent Form


**Human Nutrition Research Laboratory**
**South African Hypertension and Diet Study (SAHDS)**
**Written consent form**

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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** : _____