The relationship of pulse pressure and pulse pressure amplification with the renin-angiotensin-aldosterone system in young adults: The African-PREDICT study

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Dissertation submitted in fulfilment of the requirements for the degree Master of Health Science in Cardiovascular Physiology at the North-West University

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* Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF does not accept any liability in this regard.
Preface

This sub-study falls within the larger African-PREDICT study. It forms part of the dissertation for the degree Master of Health Science in Cardiovascular Physiology at the North-West University. This dissertation was structured in article format as authorised and recommended by the North-West University. The manuscript was compiled to be published in the Blood Pressure Journal.

In accordance with the above-mentioned format, the chapter framework is as follows:

Chapter 1: Background and Motivation

Chapter 2: Methodology

Chapter 3: Research Article

Chapter 4: Summary of main results, limitations, conclusions and recommendations
Author contributions

Ms NL Mokae

MHSc. student, responsible for writing the research proposal and compiling the application to the Health Research Ethics Committee for approval. The student is also responsible for the compilation of this master’s dissertation which included the literature study, methodology, statistical analyses as well as the writing of the research article and final chapter. Performing electrocardiography measurements and basic biochemical analyses for the African-PREDICT research project.

Dr LF Gafane-Matemane

Study supervisor. Oversaw the writing of the proposal, ethics application and the compilation of the manuscript. Imparted advice regarding the initial planning, statistical analyses, the writing of the manuscript and directional information concerning the renin-angiotensin-aldosterone system.

Dr Y Breet

Study co-supervisor. Oversaw the writing of the proposal, ethics application and the compilation of the manuscript. Imparted advice regarding the initial planning, statistical analyses, the writing of the manuscript and directional information concerning pressure dynamics in the cardiovascular system.

NL Mokae        Dr LF Gafane-Matemane        Dr Y Breet
Summary

Motivation
There are number of factors that are known to contribute to the elevation of blood pressure (BP) and the subsequent increase in cardiovascular risk. One of the most prominent systems is the renin-angiotensin-aldosterone system (RAAS), which controls electrolyte and fluid volume. Components of the RAAS (prorenin, renin, angiotensinogen, angiotensins, angiotensin-converting enzyme (ACE) and aldosterone) have been linked to cardiac and vascular remodelling and subsequent cardiovascular disorders such as hypertension, atherosclerosis and cardiac hypertrophy. Pulse pressure (PP) has been established as a significant marker of cardiovascular risk. Furthermore, pulse pressure amplification (PPA), the difference between central PP and brachial PP, has in recent years shown the potential to be a risk factor for cardiovascular disease (CVD). It is thus clear that in order to understand the development and progression of hypertension and its associated risk, it is important to recognise that BP varies across the vasculature and this complexity may influence the relation with BP regulating pathways such as the RAAS. Previous studies investigating the associations between hemodynamic factors and the RAAS focused largely on older and high-risk populations. It is therefore unclear whether any adverse associations are already present between the RAAS and PP as well as PPA in young populations. It therefore, becomes imperative to investigate the link between PP and its amplification in young healthy populations in order to broaden understanding and identify possible areas of intervention to prevent the development of cardiovascular disease.

Aim
The main aim of this study was to investigate the relationship of PP and its amplification (PPA) with RAAS components including prorenin, renin, aldosterone and ACE in young black and white, men and women.
Methods

The study population consisted of 752 participants from the African-PREDICT study. Demographic information was obtained through the general health questionnaire. The following anthropometric measurements were also taken: height, weight, body mass index, waist circumference, weight to height ratio was then subsequently calculated. The ActiHeart device (CamNtech Ltd., England, UK) was used to calculate total energy expenditure (TEE) over a period of 7 days. Brachial blood pressure was measured with the Dinamap Procare 100 Vital signs Monitor (GE Medical Systems, Milwaukee, USA) with GE Critikon latex-free Dura-Cuffs (medium and large). The brachial artery was used on both left and right arms and the measurements were performed in duplicate at 5 minutes intervals. Brachial PP was then calculated by subtracting diastolic BP (DBP) from systolic BP (SBP) using the mean of both the right and left arms. The SphygmoCor XCEL device (SphygmoCor XCEL, AtCor Medical, Sydney, Australia) was used to produce an arterial waveform from which pulse wave analysis was used to obtain central SBP (cSBP) and central PP (cPP). PPA was the classified as bPP/cPP along with these pulse wave velocity (PWV) was also captured at the right carotid and femoral arterial pulse points. Twenty-four-hour BP measurements were also performed (heart rate (HR), DBP, SBP and PP). Masked hypertension was classified as clinical BP measurements within normal limits (<140/90 mm Hg) and 24-hour BP classed as hypertensive (SBP>140 mm Hg and/or a DBP>90 mm Hg). Dipper status was determined according to ambulatory BP with the formula used by American Heart Association. The following concentrations for biological and biochemical variables were determined: Serum creatinine, cotinine, C-reactive protein (CRP), total and high-density lipoprotein cholesterol, glucose and gamma glutamyltransferase (GGT) as well as urinary sodium, potassium and chloride, then the Na/K ratio was calculated. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula. Serum samples were analysed for total renin, aldosterone as well as ACE. EDTA samples was used for analysis of prorenin.

Results
Of the total population, 16.8% were found to have masked hypertension of this, 20.2% were white and 13.6% were black (p=0.02). The white group was older when compared to the black group (p<0.001). When looking at the RAAS components, the white group showed higher prorenin and aldosterone levels (both p<0.001), whereas the black group showed higher total renin (p=0.05), eGFR (p<0.001) and sodium-to-potassium ration (p<0.001). When looking at the cardiovascular measurements; the black group had higher cSBP (p<0.001) and DBP (p=0.002), on the other hand, the white group had a higher 24-hour SBP and 24-hour PP (both p<0.001) but a lower heart rate (p<0.001). No significant differences in PPA were observed. A lower percentage of the black group presented as nocturnal dippers compared to the white group (46.7% vs 64.3, p<0.001). Though, the white group had a higher TEE they presented with higher weight, BMI and waist circumference (all p<0.001) as compared to the black group. The white group also had higher glucose (p<0.001) and total cholesterol (p=0.001) levels.

When comparing the men and women within the black and white group, black men presented with higher office bPP, 24-hour PP, cSBP and cPP (all p<0.001) as well as PPA (p=0.007), but a lower 24-hour HR (p<0.001) as compared to black women. White men also had higher bPP and, 24-hour PP, cSBP, cPP and PPA (all p<0.001), but a lower HR (P<0.001). A higher percentage of black men (18.9% vs 10.21%) and white men (35.6% vs 7.92%) had masked hypertension (p=0.02 and p<0.001 respectively) as compared to their female counterparts. When comparing the RAAS components, black women had lower total renin (p<0.001), prorenin (p<0.001) and ACE (p=0.001) levels than the black men. White women had lower renin (p<0.001), prorenin (p=0.005) and eGFR (p=0.006) but had higher aldosterone levels (p<0.001) than black men. In the forward stepwise multiple regression analyses an association between cPP with ACE (β=0.10, p=0.001) was observed only in the total group. A negative association between total renin and bPP (β=-0.20, p=0.05), as well as a positive association between aldosterone and PPA (β=0.18, p<0.001) were observed in black women, whereas in white women only a negative association between ACE and PPA (β=-0.19, p<0.001) was observed.
Conclusions

cPP associated positively with ACE in the total group and PPA negatively with ACE in white women. PPA associated positively with aldosterone and negatively with renin in black women. Our results suggest that at a young age, the RAAS is adversely associated with haemodynamics and this may translate to increased ethnic and gender specific cardiovascular risk later in life.
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<tr>
<td>ACE</td>
<td>Angiotensin-converting enzyme</td>
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<td>African-PREDICT</td>
<td>African PRospective study on the Early Detection and Identification of Cardiovascular disease and HyperTension</td>
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<td>Ang II</td>
<td>Angiotensin II</td>
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<td>BP</td>
<td>Blood Pressure</td>
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<td>bPP</td>
<td>Brachial pulse pressure</td>
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<td>cPP</td>
<td>Central pulse pressure</td>
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<td>cSBP</td>
<td>Central systolic blood pressure</td>
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<td>CARDIA</td>
<td>Coronary artery risk development in young adults</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DBP</td>
<td>Diastolic blood pressure</td>
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<td>DHS</td>
<td>Dallas heart study</td>
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<td>HARVEST</td>
<td>Hypertension and ambulatory recording venetia study.</td>
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<td>HR</td>
<td>Heart rate</td>
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<td>MAP</td>
<td>Mean arterial pressure</td>
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<td>NCDs</td>
<td>Non-communicable diseases</td>
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<td>Renin-angiotensin-aldosterone system</td>
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<td>Pulse pressure amplification</td>
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<td>PRR</td>
<td>(pro)renin receptor</td>
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<td>SBP</td>
<td>Systolic blood pressure</td>
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Chapter 1

Background and Motivation
1. **Background**

Non-communicable diseases (NCDs) have been identified as the leading cause of disability and death worldwide (1). South Africa is no exception as NCD-related mortality has been on a steady increase in recent years as a result of various risk factors such as hypertension and unhealthy lifestyle habits (2). In developing countries, such as those in sub-Saharan Africa, the epidemiological transition has been implicated in the rising prevalence of hypertension and its associated cardiovascular disease (CVD) (3). The high prevalence of hypertension in both rural and urban areas of South Africa (4) poses a public health challenge to the already burdened acute and chronic healthcare services (5). Hypertension is defined as unusually high clinic blood pressure (BP), ≥140/90 mmHg (6). Even though there isn’t a specific cause for hypertension, it is clear that genetic and environmental factors contribute to the dysregulation of physiological mechanisms involved in the short- and long-term maintenance of BP, subsequently leading to chronically elevated BP (7, 8). When discussing pressure throughout the cardiovascular system it is essential to consider all its components such as pulse pressure (PP). Pulse pressure represents the force generated by the heart (9), and therefore allows for a better representation of the BP dynamic (9, 10). Pressure in the cardiovascular system is influenced by various regulatory pathways to allow optimal delivery of nutrients and oxygenated blood to the tissues. One of such controlling systems is the renin-angiotensin-aldosterone system (RAAS), involved in the regulation of BP via its effects on volume, electrolytes and vascular tone (11). It has been established that the RAAS has a significant impact on BP and the associated pathophysiological aspects leading to cardiovascular morbidity and mortality (12, 13). This study focused on the potential link between PP and the RAAS as well as more recent phenomenon termed pulse pressure amplification (PPA) in a young population.
2. Pressure across the cardiovascular system

Figure 1: Comparison of the walls of structurally different arteries (from Betts et al., 2013 (14))

The walls of both arteries and veins consist of three layers namely the tunica intima (innermost layer), tunica media (middle layer) and the tunica adventitia (outermost layer) (Figure 1) (15). The innermost layer is comprised of an endothelium, with connective tissue and a basal layer of elastic tissue separating it from the middle layer. The more muscular middle layer has concentric layers of vascular smooth muscle cells and an extracellular matrix full of elastin. The outermost layer of the blood vessel consists of fibroblasts, collagen, mast cells as well as nerve endings (16). The two essential components of the blood vessel wall that determine the compliance of the blood vessel are elastin and collagen (17). Collagen strengthens the blood vessel wall (18) while elastin enables the blood vessel to return to its shape (elastic recoil) following contraction or stretching (19). Elastin fibres function in such a way that during systole the arterial wall distends, increasing the volume of the lumen and during diastole the arterial wall recoils in order to maintain BP (18). The capacity of an artery to distend and contract with pulsation and relaxation is termed compliance (20). As the pressure wave travels down the arterial tree the pressure varies at different regions due to variations in the compliance of the blood vessels and a phenomenon termed wave reflection (21). Functionally, there are three major compartments of the arterial tree: large central arteries, muscular conduit arteries and
small arteries and arterioles (22). Each of these have distinct structural properties depending on the wall thickness as well as composition (23). For instance, the central arteries like the aorta and the proximal carotids which have thin walls and are more elastin, thereby providing a dampening effect on the pressure (24). Though muscular conduit arteries are internally smaller than central vessels they have a greater wall thickness and comprise of less elastin and more collagen (25). The decrease in diameter of conduit arteries results in a progressive increase in afterload, which leads to widening of PP (25).

2.1 Static versus pulsatile blood pressures

The importance of BP as a determinant of cardiovascular risk and the benefits of treating hypertension have been established. However, the precise component of BP that best predicts cardiovascular risk has recently been a subject of considerable debate (26-28). Systolic and diastolic BP can be described as points of inflection of BP, with systolic BP (SBP) being due to ventricular ejection and peripheral arterial resistance, while diastolic BP (DBP) is a result of wave reflection (29). Blood pressure can be divided into two other components; a steady component termed mean arterial pressure (MAP) and a pulsatile component termed PP (30). Mean arterial pressure is the average pressure across an individual’s arteries during one cardiac cycle presented as a function of the contractility of the left ventricle, heart rate (HR) and elasticity averaged over time (31). Pulse pressure is the difference between systolic and diastolic arterial BP which represents variation in BP with a normal range from 30 mmHg to 60 mmHg (32). The determinants of PP include left ventricular ejection fraction, compliance of the arterial system, early pulse wave reduction fraction and HR (33). Thus PP is predictive of two pathophysiological mechanisms where an increase in SBP influences the level of end-systolic stress and promotes cardiac hypertrophy (34) and a decrease in DBP alters coronary perfusion and therefore favours myocardial ischemia (35). Though the steady component of BP has been found to be a prognostic factor in cardiovascular mortality in both men and women, in women the pulsatile component was seen to be a cardiovascular risk factor independent of the steady component (30). Nevertheless, a study conducted in men of
European and South Asian ancestry found that MAP, rather than PP, correlated with stroke risk in south Asians whereas the opposite was found in the European population, suggesting that SBP may not be a reliable predictor of risk in populations of south Asian ancestry (68).

2.2 Central versus peripheral blood pressure

In clinical practice, predominantly systolic and diastolic pressures are reported (consistently measured in the brachial artery using cuff sphygmanometry) (10). This method is indirect and thus has a number of limitations on the information it provides. For example it does not report on the left ventricular ejection impedance or the relative contribution of BP on left ventricular ejection and distal vascular stiffness and resistance (9). It further lacks in depicting the role reflected pressure waves play in the generation of central SBP (36). Systolic BP has been found to be higher when measured at the brachial artery rather than the aorta due to the amplification of pressure that occurs (37). This amplification is as a result of the increase in stiffness as you move down the arterial tree from elastic blood vessels to more muscular blood vessels (38).

Central aortic pressure is classified as the pressure exerted at the level of the heart, the kidney and the brain (39). Physiologically the heart and large arteries are directly exposed to central SBP (cSBP) instead of brachial (peripheral) BP (40), thus rendering cSBP a superior predictive value of cardiovascular risk (37, 41). Similarly, aortic PP is a superior cardiovascular predictor to brachial PP (46). In addition, the assessment of cSBP has been proposed to improve stroke prediction in young populations (42). In hypertensives, central BP has been linked to changes in the structure of small arteries (43) and the narrowing of the retinal arteries (44). Evidence surrounding the significance of cSBP in improving cardiovascular risk analysis has not been consistent, with only some findings indicating that cSBP as compared to brachial BP associates with indices of preclinical target organ damage (40, 45). This variance in BP does not only manifest in the measured but also in the treatment of pathology, as discrepancies between brachial and cSBP responses to vasoactive drugs has been observed (46, 47). Antihypertensive drug classes seem to have varying effects on arterial stiffness, though ACE
inhibitors, calcium channel blockers, and mineralocorticoid receptor antagonist reduce arterial stiffness and central BP, beta blockers could have the opposite effect while lowering peripheral BP (48).

These recent findings regarding the significance of cSBP and PP thus necessitates that more studies be done on potentially innovative markers of cardiovascular risk (49, 50).

3. The renin-angiotensin-aldosterone system’s role in blood pressure control

The RAAS is a major hormonal system responsible for the regulation of fluid and electrolyte balance and its role in cardiovascular risk is known (51, 52). Renin secretion, the rate limiting step of the RAAS (53), is initiated in response to low perfusion pressure and high sodium concentration in the juxtaglomerular apparatus (54). Renin is formed from its stored precursor, prorenin (55, 56). Renin then cleaves the substrate angiotensinogen, to form angiotensin I which is converted to the octopeptide angiotensin II through the angiotensin converting enzyme (ACE) (6). Angiotensin II functions as a potent vasoconstrictor (55), increases intrinsic heart rate (57), increases water and sodium reabsorption and initiates the release of aldosterone (58-60). Aldosterone controls blood volume through its management of sodium and potassium concentrations (11). The RAAS is usually suppressed during increased sodium intake and elevated BP (61), by means of a negative feedback system serving a protective function, however, dysregulation of the RAAS has been linked to hypertension development and cardiovascular disease progression (61, 62). The concentrations of circulating RAAS components appears to be greatly affected by plasma oestrogen concentrations (63). Pre-menopausal women have been found to present with lower renin levels than menopausal women and men, which may be due to the role oestrogen plays in decreasing adrenalin and noradrenaline, consequently indirectly decreasing circulating renin levels (64). Estradiol has been found to attenuate the actions of ACE (65), Though, its impact on ang II is not well established (66) it is worth mentioning that the reaction to ang II mediated vasoconstriction of the aortic rings and mesenteric vessels appears weakened in female rats (67).
3.1 Prorenin

Prorenin is the precursor of the active enzyme renin which along with renin bind the (pro)renin receptor (PRR) in mice, with the ability to elicit both angiotensin-dependent and angiotensin-independent increases in pressure (68). Transgenic overexpression of the PRR in smooth muscle cells was seen to elevate BP and increase HR (69). Though there have been multiple controversies in animal models it has been established that elevated plasma prorenin levels are predictors of the development of diabetic nephropathy and retinopathy in humans (70). Studies have been conducted to investigate the PRR findings in mice in humans, and the findings have been inconclusive regarding whether prorenin-PRR interaction elicits effects that can lead to vascular pathology (70, 71). Prorenin, though the precursor to active renin, has been found to circulate in the plasma at higher levels than renin (71). The PRR has also been found to have a function in normal tissue function (72-75), and PRR deletion yielded a harmful phenotype (75). In vivo models on the PRR with prorenin overexpression yielded an ang II-dependent phenotype (69, 71), while ang II-independent effects required prorenin concentrations that weren’t physiologically possible, thus the PRR may not be an appropriate therapy target for cardiovascular and renal disease (75). Nevertheless, PRR has been linked to elevated systolic and diastolic BP in Japanese men (76)

3.2 Renin

The main source of circulating renin is the juxtaglomerular cells (77). Apart from the circulating renin-angiotensin system, various tissues like the heart, brain and kidneys have their own local renin-angiotensin systems (78). Renin has been found in mitochondrial intermembrane inclusion bodies and with aldosterone also produced in adrenal mitochondria, it has been proposed that mitochondrial renin plays a role in aldosterone control (78, 79). Neural, hemodynamic and hormonal factors control the release of renin into the circulation (80). Rises in renin levels occur due to a decrease in BP and blood sodium concentrations and increased activity of the renal beta adrenergic receptors during sympathetic activation (55). Therefore, renin has been found to associate negatively with BP (84). Active renin was shown to be lower
in blacks compared to whites, particularly in advanced adulthood (81). Though ethnic differences in renin levels have been reported, Tu et al., reported no ethnic difference in prorenin levels in a study conducted in young black and white population (81). One of the differences in the pathogenesis of hypertension between blacks and whites have been the low renin status often observed in black populations (6), though this difference in plasma renin activity has been found to not be consistent in children (82, 83).

### 3.3 Angiotensin-converting enzyme

Angiotensin-converting enzyme is located in various tissues and biological fluids (84, 85). It has two isoforms, somatic ACE and testis ACE, the former mainly distributed in epithelial and endothelial cells (85). In addition to its role in activating angiotensin, ACE also inhibits bradykinin, a potent vasodilator, further propagating vasoconstriction (86). This inhibition of bradykinin results in a change in haemodynamics by increasing systemic vascular resistance (87, 88). Angiotensin-converting enzyme blockade was shown to lower BP as well as reduce arterial wave reflection, consequently increasing PPA (55, 89, 90). Even though similar ACE plasma concentrations were seen in black and white populations, ACE was shown to associate negatively with BP in the black population but associated positively in the white population (91). Response to ACE inhibitors has also been found to differ between the two ethnicities, resulting in less efficacy in the black group (92).

### 3.4 Angiotensin II

Angiotensin II (Ang II), via activation of its receptors, angiotensin II receptor type 1 (AT1R) regulates BP by directly influencing vascular smooth muscle cells, sodium and volume homeostasis as well as aldosterone secretion (52). In addition to its actions BP and sodium reabsorption, Ang II contributes to reactive oxygen species formation as well as proinflammatory and proliferative processes in various cells types (93, 94). It promotes cell growth, cytokine production (95) and pathological conditions including oxidative stress, inflammation, endothelial dysfunction and tissue remodelling (52, 96).
In the vasculature, Ang II bind to receptors, prompting intracellular signal transduction cascades that lead to short-term vascular effects (contraction) and long term biological effects (cell hypertrophy, extracellular matrix deposition and inflammation) (97). Similar to ACE inhibition, Ang II inhibition by angiotensin receptor blockers is also a well-established therapeutic tool (98). Even though these are not often used concomitantly, a study conducted on treating resistant hypertension found that their parallel use yielded a decrease in BP via a decrease in the augmentation of pressure in the ascending aorta, with a larger decrease in cPP as opposed to bPP and a subsequent increase in PPA (89). Through the years the impact of the RAAS on the progression of CVD has been found to differ between black and white ethnicities (99, 100). Black hypertensive men were seen to have lower levels of ang I and ang II when compared to their white counterparts (6, 101), this aligns with the low renin levels often observed in black populations (6, 102).

3.5 Aldosterone

The main function of aldosterone is homeostatic control of blood volume through plasma sodium and potassium concentration regulation (103). Primary control of aldosterone production and secretion takes place in the kidneys, through the actions of ang II. Aldosterone regulates blood volume and pressure such that abnormalities affecting its synthesis contribute to the development of hypertension and congestive heart failure (104). High levels of aldosterone were found in patients with an acute myocardial infarction and was linked with worse outcomes (105, 106). Furthermore, elevated levels of aldosterone were also observed in patients with chronic heart failure, indicating that aldosterone plays a significant role on the progression of cardiovascular pathology (107-109). Arterial stiffness is a well-established risk factor for cardiovascular morbidity and mortality (36) and it is also a determinant of PP (23). ACE-mediated effects of the RAAS are associated with adverse vascular effects that lead to reduced arterial compliance (71). This association could be the underlying reason for the weak association found between high aldosterone and elevated PP in patients with primary hypertension (110).
4. Increased pulse pressure

The pathophysiological mechanism associated with an increased PP have not yet been well established, though hemodynamic stress, vascular inflammation and calcification as well as matrix remodelling seem to be involved in the pathophysiology as well as the adverse effects of increased PP (111). Furthermore, carotid and not brachial PP has been associated with carotid intima-media thickness (112, 113). A significant association was seen between parameters of PP and the extent of coronary atherosclerosis, suggesting that elevated PP may be a cause as well as effect of atherosclerosis which could cause a vicious cycle; PP augments the development of atherosclerosis, which then reduces the compliance of the arteries further, thereby enhancing pulse wave reflection, subsequently increasing PP (114). For the same mean SBP and DBP, central PP (cPP) is found to be physiologically lower than the brachial PP (bPP) (8). This is as a result of the increase in arterial stiffness as the distance increases from the more elastic larger central blood vessels to the more peripheral/brachial muscular blood vessels (38). These variations between cPP and bPP can be of clinical significance since aortic as opposed to brachial pressure determines left ventricular workload (115). Pulse pressure is therefore amplified between the aorta and brachial artery due to the increase in arterial stiffness resulting from decreases in the elastin to collagen ratio from the heart to the periphery (7, 116). This difference between bPP and cPP is termed pulse pressure amplification (PPA). Pulse pressure amplification is increasingly being regarded as an important risk factor for CVD. An inverse association has been observed between PP and brachial flow mediated dilation in middle-aged subjects with no prior heart conditions, indicating a mechanism by which elevated PP could contribute to cardiovascular disease (117). A Study found that, when participants were stratified according to brachial artery BP, those who have “high-normal” SBP and those with a normal brachial BP had aortic pressures similar to those with stage 1 hypertension (118). These findings imply that individuals with relatively high central pressures are not being treated, thus the necessity for inquiry into the clinical implications of PPA (31). In patients with chronic kidney disease (CKD) RAAS inhibition was found to attenuate the adverse hemodynamic effects associated with an elevated PP
Patients with CKD present with an adverse nocturnal pressure profile and an elevated PP which leads to more severe cardiac organ damage (120). Though treatment for CKD patients is more extensive, the control of BP is similar to those without CKD (120), therefore prescribing antihypertensive medications at bedtime can restore nocturnal dips in BP (121). In a diabetic population a stronger association was observed for nocturnal PP than diurnal PP with coronary heart disease and cardiovascular mortality (122). It is therefore clear that sympathetic activity as indicated by less dipping in BP and PP and cardiovascular risk.

5. Decreased pulse pressure amplification

As established above, the elastin to collagen ratio decreases as you move away from the heart to the periphery (24). This non-linear elasticity of the arteries results in a subsequent increase in arterial pressure (25) due to the fact that the PP is amplified between the aorta and brachial artery due to the increase in arterial stiffness (116, 45). Therefore, the difference between bPP and cPP is termed pulse pressure amplification (PPA), which is calculated by dividing peripheral PP with cPP (123), with normal values being around 14 mmHg (8, 124). This amplification is however not fixed as there are a number of factors that determine the PPA of an individual, namely; BP, body composition (i.e. height) and gender (125) as well as posture (116), exercise (126) and age (8). Different studies have also reported that acute changes in HR also influence pressure amplification (127, 128), and it is for this reason that peripheral PP is not an accurate depiction of cPP (124). Central pressures vary significantly from peripheral pressures during day and night hours, with central pressure being lower during the day and night when compared to peripheral pressures (129).

These variations between cPP and peripheral PP can be of clinical significance as aortic as opposed to brachial pressure determines left ventricular workload (115). Studies have also found that when participants were stratified according to brachial arterial BP, individuals who under the Cardiology and hypertension Society guidelines would be classified as pre-hypertensive (120/80-140/90) and those who had normal brachial BP had aortic pressures similar to those with stage 1 hypertension. Possibly indicating that individuals with relatively
low central pressures could be on treatment and those with high central pressures may not be on treatment, and this is precisely what necessitates inquiry into the clinical implications of PPA (42). In elderly hypertensive men and women, β-blockers were found to have an independent impact on PPA and glucose levels in men but not in women (130). With the progression of age, cellular, biochemical and enzymatic changes in the vasculature occur along with modification in how they are modulated, a phenomenon coined vascular aging (131). Pulse pressure amplification decreases with age due to an increase in early wave reflection and augmentation of systolic and thus pulse pressure (131), and it has been identified as a promising clinical tool in early identification of cardiovascular risk (132). A decrease in PPA has been implicated in arterial stiffness, organ damage as well as mortality (133). In men older than 40 years of age, a higher PPA yielded a better cardiovascular profile such as a thickening of the intima media and reduced pulse wave velocity (PWV) (134).

Pulse pressure amplification, rather than carotid-femoral PWV has be found in multiple studies conducted in an elderly population to associate with a higher prevalence of heart disease (135, 136). Even though this decline in PPA is often observed in older individuals a study conducted within the African-PREDICT study population observed a decrease in PPA in black adults who were less than 30 years of age (137). This has been attributed to the premature occurrence of vascular, a phenomenon termed early vascular aging (138). In a study conducted in an elderly Chinese population PPA was found to be significantly associated with cardiac target organ damage such as left ventricular hypertrophy and left ventricular diastolic dysfunction (139). It can thus be seen that age plays a significant role in arterial stiffness and the subsequent decrease in PPA.
6. Pulse pressure, pulse pressure amplification and the renin-angiotensin-aldosterone system

Table 1: A summary of main human studies linking PP, PPA and the RAAS with gender and ethnicity.

<table>
<thead>
<tr>
<th>RAAS</th>
<th>Gender</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse pressure</td>
<td>• Banerjee et al., 2005 found that in patients with chronic kidney disease, elevated PP associated with poorer outcomes and RAAS inhibition was found to be protective (119).&lt;br&gt;• Aldosterone has been found to have a positive association with PP (110).</td>
<td>• PP is found to be a risk factor for cardiovascular mortality in women 55 years and older independent of MAP (30).</td>
</tr>
<tr>
<td>Pulse pressure amplification</td>
<td>None</td>
<td>• In men older than 40 years of age, a higher PPA yielded a better cardiovascular profile (134).&lt;br&gt;• In elderly hypertensives a negative association between β-blockers and PPA in men but not in women (130).</td>
</tr>
</tbody>
</table>

It has been established that in high risk populations the RAAS associates adversely with PP (119). Of note is the influence of gender and ethnicity, with women and Europeans more prone to suffer PP-mediated cardiovascular risk as compared to men and South Asians, respectively (134, 135). On the other hand, to the best of our knowledge, not studies had been undertaken to investigate the link between the RAAS and PPA. Similar to PP, significant gender and ethnic
difference have been observed on the relation of PPA with cardiovascular risk. Men presented with a favourable PPA profile and responded well to therapeutic intervention on PPA as compared to women. In addition, Asians and blacks showed an adverse association of PPA with target organ damage and earlier decrease in PPA, respectively (136, 137)(Table 1).

7. Motivation
It has been established that the RAAS has a significant impact on BP and the associated pathophysiological mechanisms leading to cardiovascular morbidity and mortality (12); and it is clear that ethnic and gender differences affect this associations. However, data on the link between the RAAS and haemodynamic factors such as PP and its amplification remains scant, particularly in young healthy populations (139). Many of the studies conducted on PP and PPA have been carried out in older populations or those with pre-existing CVD (137, 138), and did not include various RAAS components. It is thus becoming imperative to investigate if at the age (20-30 years of age) of peak cardiovascular health, there could already be adverse associations between the RAAS and PP as well as PPA. Angiotensin II and aldosterone have been implicated in early vascular aging, which can also be linked to PP via arterial stiffness (89, 140), however, the direct link between PP, its amplification and the RAAS remains unknown to the best of the researchers’ knowledge.

8. Aims and objectives
The main aim of this study was to investigate the relationship of PP and its amplification (PPA) with RAAS components including prorenin, renin, aldosterone and ACE in young black and white, men and women.

Objectives:

- Determine whether an interaction of sex or ethnicities on the relationships of PP and PPA with the RAAS components (prorenin, renin, aldosterone, ACE)
• To compare groups based on the interaction terms results in terms of pulse pressure (central, clinic/brachial and 24-hour PP), PPA, components of the RAAS (prorenin, renin, aldosterone, angiotensin and ACE) as well as PPA.

• To investigate the associations of pulse pressure (central, clinic/brachial and 24-hour PP) with the RAAS (prorenin, renin, aldosterone, angiotensin and ACE) in the total group, blacks and whites and men and women separately.

• To investigate the associations between PPA and RAAS (prorenin, renin, aldosterone, angiotensin and ACE) in the total group, blacks and whites and men and women separately.

9. Hypotheses

Based on the literature the following has been hypothesised:

• Renin, prorenin and ACE will be comparable between ethnicities, while aldosterone will be higher in whites compared to blacks.

• PP will be higher in the black population and PPA lower.

• With regards to the second objective the following hypotheses were made:
  
  ▪ Renin will be negatively associated with cPP and positively associated with PPA in the total group, both blacks and whites, men and women.
  
  ▪ Prorenin will associate positively with cPP and negatively with PPA in the total group, both blacks and whites, men and women.
  
  ▪ ACE will be positively associated with cPP and negatively with PPA in the total group, both blacks and whites, men and women.
  
  ▪ Aldosterone will be negatively associated with cPP and positively with PPA in the total group, both blacks and whites, men and women.
10. References


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122. Bouhanick B, Chamontin B. Should pulse pressure and day/night variations in blood pressure be seen as independent risk factors requiring correction or simply as markers to be


Chapter 2
Methodology
1. Study design and participant recruitment

This dissertation forms part of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT), as a sub-study. The African-PREDICT study, is an on-going study that recruited 1202 white and black participants between the ages of 20-30 years to perform follow-up measurements over the following 10-20 years. The study aims to offer insight into the underpinning pathophysiological mechanisms involved in cardiovascular diseases as well as into predictors of cardiovascular disease in an apparently healthy South African young adult population. This will aid in the development of innovative preventative tools.

Recruitment of participants for the African-PREDICT study took place continuously throughout baseline data collection, where advertisements in the local newspaper, notice boards (originally approved by the HREC), and health screening days in public places, direct recruitment at the workplace and radio were used to invite people to participate in the study with emphasis on the fact that participation is voluntary. The recruitment mediators comprised of field workers and registered nurses. Once through the screening process (either at their place of work or at the Hypertension Research Clinic (building F11) of the North-West University, Potchefstroom campus), successful participants were given formal letters to confirm their participation in the study. A small number of students and colleagues who complied with the necessary inclusion criteria participated voluntarily in the African-PREDICT study in order to benefit themselves by gaining valuable information with regards to their health status, without any specific invitation or creating a power relationship.

2. Literature Databases

The following databases were used:


Science Direct: http://www.sciencedirect.com/

Google Scholar: https://scholar.google.co.za
This study made use of already existing data from the African-PREDICT study. The following methodology was for the African-PREDICT study.

3. Study Design

This sub-study made use of existing data from the African-PREDICT (African Prospective study on Early Detection and Identification of Cardiovascular disease and HyperTension) study. The African-PREDICT study is an on-going study that recruited 1202 white and black participants between the ages of 20-30 years to perform follow-up measurements over the following 10-20 years. The African-PREDICT study commenced in 2012 and advanced measurements subsequently followed in February 2013. The African-PREDICT study was approved by the Health Research Ethics Committee (HREC) of the North-West University in 2012 (NWU-00001-12-A1) with endorsement from the National and Provincial Department of Health. Additionally, all procedures complied with the Declaration of Helsinki (2008) and conformed to the Medical Research Council guidelines of good clinical practice. This specific sub-study included only 800 participants with cross sectional data. The following inclusion criteria applied, Table 1:
Table 1: Inclusion criteria and justification

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Apparently healthy individuals</td>
<td>1. The African-PREDICT study aims to track young healthy individuals over a period of 10-20 years.</td>
</tr>
<tr>
<td>2. Between 20-30 years of age</td>
<td>2. As the African-PREDICT study aims to track and evaluate the development and early stages of hypertension, individuals in the age group 20-30 years, proved the ideal population as they are adults that are considered to be in good cardiovascular health.</td>
</tr>
<tr>
<td>3. Black or white ethnicity</td>
<td>3. Black individuals in South Africa are at increased risk for the development of hypertension and are therefore included in this study. White individuals are used as a comparison group. Other ethnicities are excluded as the black population has shown to be particularly at risk.</td>
</tr>
<tr>
<td>4. Equal distribution of men and women.</td>
<td>4. Both men and women are included to allow for the determination of gender differences.</td>
</tr>
<tr>
<td>5. Office brachial blood pressure of &lt;140mmHg and 90mmHg</td>
<td>5. As the aim of this study was to observe the development of hypertension,</td>
</tr>
</tbody>
</table>
Involvement in this study was on a voluntary basis and participants were informed they can withdraw anytime should they wish do so. Participants of this study obtained a token of gratitude in the form of a R50 grocery shopping voucher. Screening commenced as of November 2012 with research measurements beginning in February 2013. Black and white participants from a range of different socio-economic environments were invited to participate in the health screening at the Hypertension clinic on the Potchefstroom Campus in building F11, with transport to and fro provided for. Participants that met the required inclusion criteria during the screening visit were then invited to voluntarily take part in the research measurements and if they indicated they wished to continue an appointment was made. Participants had all procedures explained to them by a staff member before written informed consent was obtained to participate in the screening and research measurement stages of the African-PREDICT study. Participants could also ask questions about anything they were uncertain of. Participants that did not meet the inclusion criteria at screening were provided with feedback, counselling and referral to the necessary, appropriate health services. We will apply for ethics approval for this study.

| 6. HIV uninfected | individuals already identified as hypertensive were excluded. |
| 7. No previous diagnosis or medication for a chronic disease | 6/7. Individuals with a self-reported previous diagnosis of disease were excluded as the aim of this study was to track seemingly healthy individuals. |
| 8. Pregnant or lactating women were not included in the study | 8. Pregnant or lactating women were excluded due to the known influence of pregnancy hormones on the cardiovascular system |
In the present sub-study, those who did not have data for components of the RAAS and PP (N=457) were further excluded.
4. **Logistical procedures and Data Collection Methods**

Black and white participants from a range of different socio-economic environments were invited to participate in the health screening at the Hypertension clinic on the North-West University Potchefstroom Campus. Participants had all procedures explained to them by a staff member before written informed consent was obtained to participate in the screening and research measurement stages of the African-PREDICT study. Those who met the required inclusion criteria during the screening visit were then invited to voluntarily take part in the research measurements and if they indicated they wished to continue an appointment was made. Participants were asked to fast overnight for at least 8 hours prior to attending the research measurements day. Participants were asked to arrive at 07h45. Upon arrival they were asked to provide a spot urine sample. Then the participant was taken to a private room where a registered research nurse took blood samples. Participants were then rotated around a number of stations aided by the nurse. In between the stations participants then had to complete questionnaires. All procedures were performed according to good clinical practice.

4.1 **General Health Questionnaire**

The questionnaire was completed online on a web-based program, involving demographic information, employment information, alcohol and tobacco use, medication use, and family history. The socioeconomic status (SES) of a participant was obtained from three categories included in the biographical questionnaire, namely skills level, education, and household income. Points were given for each of these categories, and the total number of points were used to determine whether a participant had a low, middle, or high SES. This classification was adapted from Patro *et al.*, (1). The questionnaires were stored within a locked storage room in the Hypertension Clinic, with only the project leader, data manager and Head of the Hypertension Clinic allowed access to the documents. Names were linked to participant numbers to ensure anonymous data analysis.
4.2 Cardiovascular Measurements

With the participant in recumbent position, the SphygmoCor XCEL device (SphygmoCor XCEL, AtCor Medical, Sydney, Australia) was used to produce an arterial waveform which gave an estimated central SBP, central pulse pressure (cPP) and augmentation index (AIx) reading, attained through the built-in generalised transfer function. The supine brachial systolic and diastolic blood pressures were measured with the Dinamap Procare 100 Vital signs Monitor (GE Medical Systems, Milwaukee, USA) with GE Critikon latex-free Dura-Cuffs (medium and large). The brachial artery was used on both left and right arms and the measurements were performed in duplicate at 5 minutes intervals. Brachial PP and mean arterial pressure (MAP) were then calculated from diastolic BP (DBP) and systolic BP (SBP) using the mean of both the right and left arms. The SphygmoCor XCEL device (SphygmoCor XCEL, AtCor Medical, Sydney, Australia) was used to produce an arterial waveform from which pulse wave analysis was used to obtain central SBP (cSBP) and central PP (cPP). Pulse pressure amplification (PPA) was classified as the ratio of the amplitude of the PP between a distal and proximal location (bPP/cPP). Pulse wave velocity (PWV) was captured at the right carotid and femoral arterial pulse points. The femoral artery wave form was taken with an appropriately sized cuff placed around the thigh, and the carotid arterial waveform was captured simultaneously through applanation tonometry. The infantometer was used to measure distance between the pulsated sites, and 80% of these distances were used as the pulse wave travelled distance (2). All 24-hour cardiovascular measurements were performed with use of a validated CardioXplore device (CardioXplore, MediTech, Budapest, Hungary), programmed to take recordings every 30 min during the day (06:00 to 22:00) and every hour during the night (22:00 to 06:00). The ambulatory BP monitoring (ABPM) was fitted to each participant at approximately the same time every morning, using an appropriately sized cuff. Percentage dipping for BP and HR were calculated as follows: % dipping=(day-night)/day)100. Despite the inclusion criteria of normal BP, a significant number of African-PREDICT participants were found to have masked hypertension, classified as clinical BP measurements.
within normal limits (<140/90 mm Hg) and 24-hour BP classed as hypertensive (SBP>140 mm Hg and/or a DBP>90 mm Hg).

4.3 Anthropometric Measurements
Trained anthropometrists measured weight (kg) to the nearest 0.01 kg (SECA electronic scales, SECA, Birmingham, UK), and height (m) to the nearest 0.1 cm (SECA stadiometer, SECA, Birmingham, UK). Waist circumference were measured three times using a non-flexible tape measure (Holtain, Crymych, UK), and recorded to the nearest 0.1 cm. The median of the three recordings was used in subsequent analyses. Body mass index (BMI) was calculated using the standard weight (kg)/height (m²) calculation, and waist-to-height ratio was calculated using waist circumference (cm)/height (cm).

4.4 Physical Activity Measurements
With the use of an accelerometer device, a compact chest-worn device that registers heart rate, inter-beat-interval and physical activity in one combined unit was used to quantitatively measure physical activity. The ActiHeart device (CamNtech Ltd., England, UK), designed to calculate total energy expenditure (TEE), was worn for a maximum of 7 days. To ensure privacy, the ActiHeart device was fitted by trained researchers in a temperature controlled private room.

4.5 Biological sampling and biochemical analyses
Participants were asked to fast overnight for at least 8 hours prior to attending the research measurements day. Early in the morning venous blood samples were collected from the brachial vein branches, using a sterile needle, by a qualified nurse in a temperature controlled private room. Serum samples were analysed for C-reactive protein (CRP), creatinine, total cholesterol, glucose and gamma glutamyltransferase (GGT) (Cobas Integra 400plus, Roche, Basel, Switzerland). Ethylenediaminetetraacetic acid (EDTA) whole blood was used for analysis of glycated haemoglobin (HbA1c) (Cobas Integra 400plus, Roche, Basel, Switzerland). Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany)). Serum samples were analysed for renin (Quantikine ELISA
kit (R&D systems, Minneapolis, MN USA) analysed on Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA), and aldosterone (RIA Aldosterone Kit (Beckman Coulter, Immunotech, Radiova, Czech Republic)) as well as ACE (Quantikine ELISA kit (R&D systems, Minneapolis, MN USA) analysed on Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA)). EDTA samples was used for analysis of prorenin 1 and 2 (Human Prorenin ELISA Kit (Biovendor-Laboratorni medicina, Karasek, Czech Republic)). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) Formula. The protocol for 24-hour urine collection follows that of the PAHO/WHO protocol for population level sodium determination in 24-hour urine samples (3). Incomplete urine collections were defined as a volume less than 300 mL per 24 hours and/or, a 24-hour creatinine excretion of less than 4 mmol or higher than 25 mmol in women, and less than 6 mmol or more than 30 mmol in men. The samples were prepared according to standardised protocols and stored at -80°C until the time of analysis. Urinary sodium, potassium and chloride were measured by means of ion- selective electrode potentiometry on the Cobas Integra® 400 plus (Roche, Basel, Switzerland), these were then used to calculate the Na/K ratio and creatinine concentrations were measured using the Creatinine Jaffé Gen.2 reagent (Roche, Basel, Switzerland) from the 24hr urine sample obtained.

5 Data management

The African-PREDICT study makes use of the Research Electronic Data Capture (REDCap) system for data capturing. REDCap is a free, secure, web-based and user-friendly electronic database software that can be developed quickly and is also customisable for studies. It is intended for collecting along with tracking information and data from research studies. Additionally, REDCap can also be used to schedule patient visits (4). A data manager was appointed for this study and trained to fulfil this duty. In REDCap, all laboratory specimens, evaluation forms, reports, data and other records are identified only by the participant number to maintain subject confidentiality, along with this all the data is backed up on password protected hard drives. Data accuracy for statistical analyses is ensured through importing the
data directly into Statistica from automatically generated Excel sheets. As explained during the initial screening as well as in the participant leaflet attached to the informed consent form, voluntary participation included consent that all personally identifiable information as well as sensitive personally identifiable information would be captured as part of the study with the understanding that this information would be stored and handled very securely and coding would take place promptly. Furthermore, the minimum amount of personally identifiable information was captured within the study and only information that directly contributed towards the aims of the study were captured.

6 Statistical Analyses
Data analysis was done with Statistica v13.2 (TIBCO Statistica ™). Ethnicity and sex interactions on the associations between RAAS, PP and PPA were performed. All continuous variables were checked for normality by visual inspection and the Kolmogorov-Smirnoff test, and those with non-Gaussian distributions were logarithmically transformed. Comparisons were performed by making use of T-tests and Chi-square analysis. In addition, we performed single, partial and multiple regression analyses between PP, PPA and components of the RAAS. The following was included in multiple regression models: age, gender, ethnicity, body composition (BMI and height), cotinine, GGT, glucose, HbA1c, socioeconomic status, total cholesterol: high density lipids, eGFR, NA/K ratio, TEE.

7 Ethical Considerations
This MHSc dissertation was assessed and approved by the Health Research Ethics Committee (HREC) of the North-West University. The African-PREDICT study which this MHSc study forms a part of was approved by the Health Research Ethics Committee (HREC) of the North-West University in 2012 (NWU-00001-12-A1) with endorsement from the National and Provincial Department of Health. Additionally, all procedures complied with the Declaration of Helsinki (2008) and conformed to the Medical Research Council guidelines of good clinical practice. described in detail within the original informed consent signed by all participants.
8 Student Involvement

As an MHSc student I was involved in the electrocardiography measurements and ensuring the data is stored correctly. Additionally, I was also involved in laboratory work for this study on allocated data collection days. Upon completion of blood and urine collection I contributed to the centrifuging and aliquoting of blood samples into individually marked cryovial tubes for proper storage in the -80°C bio-freezers.
9 References


Chapter 3

Research Article
Summarised author Instructions for *Blood Pressure* journal

**Original Studies**

Manuscripts may not exceed 6000 words and should contain a maximum of 6 tables and/or figures. Each figure may contain a maximum of 4 panels (parts labelled A, B, C, and D). The word count includes all text in the manuscript beginning with the title page and ending with the last reference. It does not include supplemental digital content.

**Conflicts of interest**

Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. All relevant conflicts of interest and sources of funding should be included on the title page of the manuscript with the heading “Conflicts of Interest and Source of Funding”.

**Ethics committee approval**

It must be stated clearly in your submission in the Methods section that you conducted studies on human participants with the approval of an appropriate named ethics committee. Please also look at the latest version of the Declaration of Helsinki.

**PRESENTATION OF PAPERS**

**Title page**

The title page should carry the full title of the paper and a short title to be used as a ‘running head’ (and which should be so identified). The first name, middle initial and last name of each author should appear. If the work is to be attributed to a department or institution, its full name should be included. Any disclaimers should appear on the title page, as should the name and address of the author responsible for correspondence concerning the manuscript and the name and address of the author to whom requests for reprints should be made. Finally, the title page should include a statement of conflicts of interest and source of funding, and when none state “none declared”.

Abstracts
The second page should carry a structured abstract of no more than 250 words. The abstract should state the Objective(s) of the study or investigation, basic Methods (selection of study subjects or laboratory animals; observational and analytical methods), main Results (giving specific data and their statistical significance, if possible), and the principal Conclusions. It should emphasise new and important aspects of the study or observations.

Keywords
The abstract should be followed by a list of 3–10 keywords or short phrases which will assist the cross-indexing of the article and which may be published. When possible, the terms used should be from the Medical Subject Headings list of the National Library of Medicine (http://www.nlm.nih.gov/mesh/meshhome.html).

Text
Full papers of an experimental or observational nature may be divided into sections headed Introduction, Methods (including ethical and statistical information), Results and Discussion (including a conclusion), although reviews may require a different format.

Acknowledgements
Acknowledgements should be made only to those who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions.

References
References should be numbered consecutively in the order in which they first appear in the text. They should be assigned Arabic numerals, which should be given in brackets. References should include the names of all authors when six or fewer; when seven or more, list only the first six names and add et al. References should also include full title and source information. Journal names should be abbreviated as in MEDLINE (NLM Catalogue, http://www.ncbi.nlm.nih.gov/nlmcatalog).

Tables
Each table should be typed on a separate sheet in double spacing. Each table to be assigned an Arabic numeral, e.g. (Table 3) and a brief title. Vertical rules should not be used. Place explanatory matter in footnotes, not in the heading.
Figures

Shading is acceptable, but pattern fills within MS Office may not be used. If created in MS Office simply save as an Office document and submit the file. If created in another design program such as Illustrator, save as an EPS or PDF* and submit the file. If you are uncertain about creating the postscript file, send the original application file. Ensure all fonts and imported images are included. If specialty software is used, select from the following options:

If a figure has been published before, the original source must be acknowledged and written permission from the copyright holder for both print and electronic formats should be submitted with the material. Permission is required regardless of authorship or publisher, except for documents in the public domain.

Figures may be reduced, cropped or deleted at the discretion of the editor. Colour illustrations are acceptable but authors will be expected to cover the extra reproduction costs.

Units of measurement

Measurements of length, height, weight, and volume should be reported in metric units (metre, kilogram, or litre) or their decimal multiples. Temperatures should be given in degrees Celsius. Blood pressures should be given in millimetres of mercury.

Abbreviations and symbols

Use only standard abbreviations. Avoid abbreviations in the title and abstract.
The relationship of pulse pressure and pulse pressure amplification with the renin-angiotensin-aldosterone system in young adults: The African-PREDICT study.

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Conflict of interest and source funding

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Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF does not accept any liability in this regard.

**Conflict of interest**

Author reports no conflict of interest
Abstract:

**Objectives:** The renin-angiotensin-aldosterone system (RAAS) controls haemodynamic homeostasis and has been linked to elevated blood pressure (BP). Our study explored associations of pulse pressure (PP) and its amplification (PPA) with the RAAS in young black and white, men and women. **Methods:** This study consisted of 752 participants (390 black and 362 white) aged 20-30 years. Cardiovascular measurements included office, ambulatory and central BP and PP. Prorenin, renin, angiotensin-converting enzyme (ACE), aldosterone and estimated glomerular filtration rate (eGFR) were determined. **Results:** Whites had higher prorenin and aldosterone (p<0.001) whereas blacks had higher renin (p=0.05) and eGFR (p<0.001). Blacks had a higher cSBP (p<0.001) and DBP (p=0.002) whereas whites had higher ambulatory SBP and PP (both p<0.001). All cardiovascular measurements except heart rate (p<0.001) were higher in black and white men (all p≤0.03) when compared to their female counterparts. White women had lower renin, prorenin and eGFR (all p≤0.006), and higher aldosterone than men (p<0.001). After multiple adjustments for covariates, ACE associated positively with cPP in the total group and negatively with PPA in white women, while in black women PPA associated positively with aldosterone and brachial PP negatively with renin (all p≤0.001). **Conclusion:** cPP associated positively with ACE in the total group and PPA negatively with ACE in white women. PPA associated positively with aldosterone and negatively with renin in black women. Our results suggest that that at a young age, the RAAS is adversely associated with haemodynamics and this may translate to increased ethnic and gender specific cardiovascular risk later in life.

**Key words:** central pulse pressure, angiotensin-converting enzyme, pulse pressure amplification, cardiovascular risk.
Introduction

Hypertension is one of the leading contributors to the non-communicable disease burden plaguing developing countries in sub-Saharan regions, including South Africa (1). Dysregulation in mechanisms such as the renin-angiotensin-aldosterone system (RAAS) and the autonomic nervous system have been associated with chronically elevated blood pressure (BP) (2). The RAAS is responsible for maintaining hemodynamic homeostasis (3), and dysfunction of this system is associated with hypertension, cardiac hypertrophy and atherosclerosis (4). The main effector molecule of the RAAS is angiotensin II (Ang II), which results from the cleavage of angiotensin I by angiotensin-converting enzyme (ACE). Angiotensin II functions as a potent vasoconstrictor (16), which increases intrinsic heart rate (HR) (17) and initiates the release of aldosterone by the adrenal glands. Aldosterone controls blood volume and BP through its direct effects on sodium and potassium concentrations (18-20). In addition to formation of Ang II (5), ACE inhibits bradykinin, a potent vasodilator, further propagating vasoconstriction (6). Consequently, ACE blockade lowers BP and reduces arterial wave reflection (7, 8), thereby increasing amplification of the pressure wave (9).

There is considerable controversy regarding the contribution of various pressure components across the vascular tree to cardiovascular risk (10). The superiority of central blood and pulse pressure to brachial components in the prediction of cardiovascular events is known, though this is more evident in older groups as compared to younger populations (11). The difference between brachial pulse pressure (bPP) and central pulse pressure (cPP) is termed pulse pressure amplification (PPA) and it is increasingly being regarded as an important risk indicator for cardiovascular disease (CVD) (12). These variations between cPP and bPP can be of clinical significance since aortic as opposed to brachial pressure determines left ventricular workload (13), and therefore cardiovascular risk. Additionally, it is clear that the development of cardiovascular pathologies is no longer limited to the elderly, and can occur in younger populations, however, little information on the pathways involved (14, 15) is
available. Therefore, the aim of the study was to explore associations of PP and its amplification (PPA) with the RAAS in a young apparently, healthy population.

Materials and Methods

Study Design and Population

This sub-study forms part of African-PREDICT (African Prospective study on Early Detection and Identification of Cardiovascular disease and HyperTension) study which included 1202 black and white apparently healthy participants aged 20 to 30 years, who will be followed up over a period of 10-20 years at 5-year intervals. The study aimed for an equal distribution of men and women. Exclusion criteria were: Individuals with an office bPP of > 140 mmHg and 90 mmHg, pregnant or lactating women, individuals who were on chronic medication or have been previously diagnosed with a chronic condition and HIV infected individuals. In the present sub-study, those who did not have data for components of the RAAS and PP (N=450) were further excluded.

This sub study was approved by the Health Research Ethics Committee of the North-West University (NWU-00064-18-S1) and aligned with the Declaration of Helsinki criteria for human research (revised 2004).

General Health Questionnaire

An online web-based program was used to collect demographic information, as well as data regarding alcohol and tobacco use, medication use, and family history. The socioeconomic status (SES) of a participant was obtained from three categories included in the biographical questionnaire, namely skills level, education, and household income. Points were given for each of these categories, and the total number of points were used to determine whether a participant fell in the low, middle, or high SES. This classification was adapted from Patro et al., (16).
Cardiovascular Measurements

With the participant in the recumbent position, the SphygmoCor XCEL device (SphygmoCor XCEL, AtCor Medical, Sydney, Australia) was used to produce an arterial waveform which gave an estimated central systolic BP (cSBP) and cPP. The supine bSBP and bDBP were obtained with the Dinamap Procare 100 Vital signs Monitor (GE Medical Systems, Milwaukee, USA), which was subsequently used to calculate the mean arterial pressure (MAP) and bPP. Pulse pressure amplification was classified as the ratio of the amplitude of the PP between a distal and proximal location (bPP/cPP). Pulse wave velocity (PWV) was captured at the right carotid and femoral arterial pulse points. The infantometer was used to measure distance between the pulsated sites, and 80% of these distances were used as the pulse wave travelled distance (17).

Twenty-four-hour BP measurements were performed with use of a validated CardioXplore devices (CardioXplore, MediTech, Budapest, Hungary), programmed to take recordings every 30 min during the day (06:00 to 22:00) and every hour during the night (22:00 to 06:00). The Ambulatory Blood Pressure Monitoring (ABPM) device was fitted to each participant at approximately the same time every morning, using an appropriately sized cuff. Dipper status (percentage blood pressure decreases at night) was determined according to the formula used by American Heart Association (uses SBP): Blood pressure dip = [1-(SBPsleeping/SBPawake)] * 100% (18). Masked hypertension was classified as clinical BP measurements within normal limits (<140/90 mm Hg) and 24-hour BP classed as hypertensive (mean SBP≥130 mm Hg and/or a mean DBP≥80 mm Hg) according to ESC ESH 2018 guidelines.

Anthropometric Measurements

Trained anthropometrists measured weight (kg) to the nearest 0.01 kg (SECA electronic scales, SECA, Birmingham, UK), and height (m) to the nearest 0.1 cm (SECA stadiometer, SECA, Birmingham, UK). Waist circumference (WC) was measured three times using a non-flexible tape measure (Holtain, Crymych, UK), and recorded to the nearest 0.1 cm. The median
of the three recordings was used in subsequent analyses. Body mass index (BMI) was calculated using the standard weight (kg)/height (m$^2$) calculation, and waist-to-height ratio was calculated using waist circumference (cm)/height (cm).

**Physical Activity Measurements**

An accelerometry (ACTi Heart) device worn on the chest, that registers HR, inter-beat-interval and physical activity in one combined unit was used to quantitatively measure physical activity. The ActiHeart device (CamNtech Ltd., England, UK), designed to calculate total energy expenditure (TEE), was worn for a maximum of 7 days.

**Biological sampling and biochemical analyses**

Participants were asked to fast overnight for at least 8 hours prior to attending the research measurements day. Blood samples were collected early in the morning by a qualified nurse from the brachial vein branches, using a sterile needle. Participants were also asked to collect their urine for 24 hours. The samples were prepared according to standardised protocols and stored at -80°C until the time of analysis.

Serum samples were analysed for creatinine, C-reactive protein (CRP), total and high-density lipoprotein cholesterol, glucose and gamma glutamyltransferase (GGT) (Cobas Integra 400plus, Roche, Basel, Switzerland). Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany)). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula.

Urinary sodium, potassium and chloride were measured by means of ion- selective electrode potentiometry on the Cobas Integra® 400 plus (Roche, Basel, Switzerland), these were then used to calculate the Na/K ratio and creatinine concentrations were measured using the Creatinine Jaffé Gen.2 reagent (Roche, Basel, Switzerland) from the 24-hour urine sample obtained. Serum samples were analysed for renin (Quantikine ELISA kit (R&D systems, Minneapolis, MN USA) analysed on Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA), and aldosterone (RIA Aldosterone Kit (Beckman Coulter, Immunotech, Radiovia, Czech Republic)) as well as ACE (Quantikine ELISA kit (R&D systems, Minneapolis, MN USA))
analysed on Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA)). EDTA samples was used for analysis of prorenin (Human Prorenin ELISA Kit (Biovendor-Laboratorní medicina, Karasek, Czech Republic)).

**Statistical analyses**

Data analysis was performed with Statistica v13.2 (TIBCO Statistica ™). There was an interaction of ethnicity on the association between PPA and aldosterone (p=0.01) and no interaction of sex on the associations between PP, PPA and the RAAS (all p≤ 0.74) (Supplementary Table 1). The data is therefore presented for the total group and subsequently by ethnicity (black versus white). Additionally, based on the literature we further explored in the men and women within the black and white group. All continuous variables were checked for normality by visual inspection and the Kolmagorov-Smirnoff test, and those with non-Gaussian distributions were logarithmically transformed (including CRP, GGT, aldosterone and prorenin). Independent T-tests and Chi-square analyses were performed in order to compare characteristics of the black and white populations as well as men and women. Single and partial regression analyses were performed to investigate the associations of PP and PPA with components of the RAAS (prorenin, renin, aldosterone and the ACE), in black (men and women) and white (men and women) groups separately. The partial correlations were adjusted for age, sex and BMI. Forward stepwise multiple regression analyses were performed with either PP or PPA as the dependent variables, respectively in all models, while RAAS components were included in models one at a time as main independent variables. After testing for a priori factors other independent variables included in the model were age, gender, ethnicity, BMI, cotinine, GGT, glucose, CRP, socioeconomic status, lipids, eGFR, NA/K ratio, TEE. Height and HR were additionally included as confounders in the model with PPA as dependent variable.

**Results**

Table 1 illustrates Characteristics of the total population and comparison between blacks and whites. The white group was older when compared to the black group (p<0.001). With regards
to components of the RAAS the white group had higher prorenin and aldosterone levels (both p<0.001), whereas the black group presented with higher total renin (p=0.05) and eGFR (p<0.001) as compared to whites. The black group had an elevated cSBP (p<0.001) and DBP (p=0.002) whereas the white group showed a higher 24-hour SBP and 24-hour PP (p<0.001). No significant differences in PPA were observed. Heart rate was lower in the white group (p<0.001). Of the total population, 16.8% were found to have masked hypertension with 20.2% comprising of the white population as compared to 13.6% of the black population (p=0.02). A lower percentage of the black group presented as nocturnal dippers compared to the white group (46.7% vs 64.3.5, P<0.001). The white group had a higher TEE, weight, BMI and waist circumference (all p<0.001) as compared to the black group. Urinary sodium-to-potassium ratio was higher in blacks as compared to whites (p<0.001), while glucose (p<0.001) and total cholesterol (p=0.001) were higher in the white group compared to the black group.
Table 1: Characteristics of the total population and comparison between blacks and whites

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Blacks</th>
<th>Whites</th>
<th>*P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>752</td>
<td>390</td>
<td>362</td>
<td></td>
</tr>
</tbody>
</table>

**Demographics**

| Age (years)                       | 24.9 ± 3.04 | 24.6 ± 3.18 | 25.3 ± 2.84 | <0.001 |
| Women n (%)                       | 440 (58.5)  | 238 (61.0)  | 202 (55.8)  | 0.12    |

**Socioeconomic status**

| Low SES (n (%))                  | 283 (37.6)  | 223 (78.8)  | 60 (21.2)   | <0.001  |
| Middle statuses (n (%))          | 192 (25.5)  | 104 (54.2)  | 88 (45.3)   |         |
| High SES n (%)                   | 277 (36.8)  | 63 (22.7)   | 214 (77.3)  |         |

**Anthropometric measurements**

| Weight (kg)                      | 71.8 ± 17.7 | 66.7 ± 15.0 | 77.4 ± 18.6 | <0.001  |
| Body mass index (kg/m²)          | 25.5 ± 5.74 | 25.1 ± 5.95 | 25.9 ± 5.49 | 0.04    |
| Waist circumference (cm)         | 80.5 ± 13.5 | 77.9 ± 11.8 | 83.3 ± 14.5 | <0.001  |
| Waist to height ratio            | 0.48 ± 0.08 | 0.48 ± 0.08 | 0.48 ± 0.08 | 0.39    |

**Cardiovascular measurements**

| Office SBP (mmHg)                | 115 ± 12.3  | 116 ± 12.1  | 115 ± 12.5  | 0.14    |
| Office DBP (mmHg)                | 78.6 ± 8.55 | 80 ± 8.49   | 78 ± 8.52   | 0.002   |
| Office PP (mmHg)                 | 38.1 ± 8.44 | 37.7 ± 8.36 | 38.5 ± 8.51 | 0.63    |
| 24-hour SBP (mmHg)               | 117 ± 9.45  | 115 ± 9.16  | 118 ± 9.65  | <0.001  |
| 24-hour DBP (mmHg)               | 68.9 ± 5.87 | 69 ± 5.95   | 69 ± 5.78   | 0.51    |
| 24-hour HR (b/min)               | 75.0 ± 10.7 | 76 ± 10.6   | 74 ± 10.6   | 0.001   |
| Central SBP (mmHg)               | 109 ± 10.1  | 111 ± 9.7   | 107 ± 10.2  | <0.001  |
| Central PP (mmHg)                | 34.1 ± 6.13 | 33.8 ± 6.12 | 34.5 ± 6.13 | 0.14    |
| Pulse pressure amplification     | 1.13 ± 0.22 | 1.13 ± 0.22 | 1.13 ± 0.22 | 0.74    |
| Pulse wave velocity (m/s)        | 6.27 ± 0.89 | 6.31 ± 0.92 | 6.28 ± 0.89 | 0.71    |
| Dipper status n (%)              | 406 (55.2)  | 174 (46.7)  | 232 (64.3)  | <0.001  |
| Masked Hypertension n (%)        | 125 (16.8)  | 52 (13.6)   | 73 (20.2)   | 0.02    |

**Renal variables**

| Total renin (pg/ml)              | 760 ± 332  | 787 ± 363  | 732 ± 294  | 0.03    |
| Prorrenin (pg/ml)                | 823 (12.2; 16645) | 744 (12.2; 12143) | 916 (18.4; 16645) | <0.001  |
| Aldosterone (pg/ml)              | 67.7 (3.8; 820) | 50.6 (3.80; 729) | 92.5 (7.46; 820) | <0.001  |
| ACE (pg/ml)                      | 155 ± 41.2  | 154 ± 41.42 | 156 ± 41.0  | 0.43    |
| 24-hour urinary Na/K             | 3.64 ± 1.72 | 4.24 ± 1.79 | 3.09 ± 1.47 | <0.001  |
| eGFR (ml/min/1.73m²)             | 121 ± 22.6  | 136 ± 17.3  | 106 ± 16.2  | <0.001  |

**Biochemical measurements**

| Glucose (mmol/L)                 | 4.66 ± 0.74 | 4.42 ± 0.81 | 4.93 ± 0.53 | <0.001  |
| TC:HDL                           | 4.27 ± 1.01  | 3.84 ± 0.84 | 4.72 ± 0.99 | <0.001  |
| C-reactive protein (mg/L)        | 1.12 (0.03; 42.5) | 1.26 (0.03; 41.8) | 0.99 (0.03; 42.5) | 0.02    |
| GGT (units)                      | 21.7 (4.5; 227) | 29.9 (4.50; 227) | 18.9 (5.20; 222) | 0.25    |
| Cotinine (pg/ml)                 | 53 ± 113     | 56.3 ± 117  | 48.5 ± 107  | 0.35    |

**Lifestyle factors**

<p>| Smoking n (%)                    | 171 (23.1)  | 96 (25.0)  | 75 (21.1)  | 0.20    |</p>
<table>
<thead>
<tr>
<th>Alcohol use n (%)</th>
<th>438 (59.7)</th>
<th>223 (59.0)</th>
<th>215 (60.4)</th>
<th>0.70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy expenditure</td>
<td>2373 ± 528</td>
<td>2184 ± 390</td>
<td>2375 ± 526</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Difference between the black and white groups. Values are arithmetic mean ± standard deviation; geometric mean (5th and 95th percentile interval) for logarithmically transformed variables. Abbreviations: N, number of participants; SES, socioeconomic status; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, Pulse pressure; ACE, angiotensin-converting enzyme; Na/K, sodium-to-potassium ratio; eGFR, estimated glomerular filtration rate; TC, total cholesterol; HDL, High density lipoprotein; GGT, gamma glutamyl transferase.

When comparing the men and women within the black and white group (Table 2), black men presented with higher office bPP, 24-hour PP, cSBP and cPP (all p<0.001) as well as PPA (p=0.007), but a lower 24-hour HR (p<0.001) as compared to black women. White men also had higher bPP and 24-hour PP, cSBP, cPP and PPA (all p<0.001), but a lower HR (P<0.001). A higher percentage of black men (18.9% vs 10.21%) and white men (35.6% vs 7.92%) had masked hypertension (p=0.02 and p<0.001 respectively) as compared to their female counterparts. When comparing the RAAS components, black women had lower total renin (p<0.001), prorenin (p<0.001) and ACE (p=0.001) levels than the black men. White women had lower renin (p<0.001), prorenin (p=0.005) and eGFR (p=0.006) but had higher aldosterone levels (p<0.001) than black men. Body mass index, waist circumference and weight-to-height ratio (all p<0.001) were higher in black women than their male counterparts whereas the white females had lower weight, BMI, waist circumference and weight-to-height ratio than their male counterparts (all p<0.001).
Table 2: Comparison between men and women within the black and white groups

<table>
<thead>
<tr>
<th></th>
<th>Blacks</th>
<th>Women</th>
<th><em>P</em>-value</th>
<th>Whites</th>
<th>Women</th>
<th><em>P</em>-value</th>
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<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td></td>
<td>Men</td>
<td>Women</td>
<td></td>
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<tr>
<td>N</td>
<td>152</td>
<td>238</td>
<td></td>
<td>160</td>
<td>202</td>
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**Demographics**

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<tbody>
<tr>
<td>Age (years)</td>
<td>24.4 ± 3.06</td>
<td>24.7 ± 3.26</td>
<td>0.28</td>
<td>25.5 ± 2.89</td>
<td>25.2 ± 2.80</td>
<td>0.27</td>
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**Socioeconomic status**

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<tbody>
<tr>
<td>Low SES (n (%))</td>
<td>94 (61.8)</td>
<td>129 (54.2)</td>
<td></td>
<td>25 (15.6)</td>
<td>35 (17.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>Middle statuses (n (%))</td>
<td>36 (23.7)</td>
<td>68 (24.6)</td>
<td>0.33</td>
<td>38 (23.8)</td>
<td>50 (24.8)</td>
<td></td>
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<tr>
<td>High SES n (%)</td>
<td>22 (14.7)</td>
<td>41 (17.2)</td>
<td></td>
<td>97 (60.6)</td>
<td>117 (57.9)</td>
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**Anthropometric measurements**

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</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>63.9 ± 12.7</td>
<td>68.5 ± 16.0</td>
<td>0.003</td>
<td>87.0 ± 15.3</td>
<td>69.8 ± 17.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.1 ± 4.03</td>
<td>25.0 ± 6.19</td>
<td>&lt;0.001</td>
<td>27.4 ± 5.01</td>
<td>24.7 ± 5.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>75.1 ± 9.98</td>
<td>79.7 ± 12.6</td>
<td>&lt;0.001</td>
<td>90.7 ± 12.6</td>
<td>77.4 ± 13.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist to height ratio</td>
<td>0.11 ± 0.06</td>
<td>0.50 ± 0.08</td>
<td>&lt;0.001</td>
<td>0.51 ± 0.07</td>
<td>0.46 ± 0.08</td>
<td>&lt;0.001</td>
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**Cardiovascular measurements**

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<tbody>
<tr>
<td>Office SBP (mmHg)</td>
<td>122 ± 12.4</td>
<td>113 ± 10.6</td>
<td>&lt;0.001</td>
<td>123 ± 9.49</td>
<td>109 ± 11.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Office DBP (mmHg)</td>
<td>81 ± 8.76</td>
<td>79 ± 8.16</td>
<td>0.002</td>
<td>81 ± 8.20</td>
<td>75 ± 8.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Office PP (mmHg)</td>
<td>41.37 ± 8.95</td>
<td>35.3 ± 7.02</td>
<td>&lt;0.001</td>
<td>43.5 ± 7.77</td>
<td>34.5 ± 6.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24-hour SBP (mmHg)</td>
<td>119 ± 8.37</td>
<td>113 ± 8.60</td>
<td>&lt;0.001</td>
<td>124 ± 7.30</td>
<td>113 ± 8.67</td>
<td>&lt;0.001</td>
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<tr>
<td>24-hour DBP (mmHg)</td>
<td>69.6 ± 6.19</td>
<td>68.3 ± 5.75</td>
<td>0.03</td>
<td>70 ± 5.76</td>
<td>68 ± 5.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24-hour HR (b/min)</td>
<td>68.2 ± 8.19</td>
<td>80.9 ± 8.94</td>
<td>&lt;0.001</td>
<td>70.4 ± 9.87</td>
<td>76.8 ± 10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24-hour PP (mmHg)</td>
<td>50.2 ± 6.67</td>
<td>44.5 ± 5.73</td>
<td>&lt;0.001</td>
<td>53.3 ± 6.83</td>
<td>45.2 ± 5.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>113 ± 9.82</td>
<td>109 ± 9.21</td>
<td>&lt;0.001</td>
<td>111 ± 8.95</td>
<td>103 ± 9.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>35.8 ± 5.76</td>
<td>32.56 ± 6.02</td>
<td>&lt;0.001</td>
<td>37.4 ± 5.71</td>
<td>32.2 ± 5.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse pressure amplification</td>
<td>1.17 ± 0.23</td>
<td>1.11 ± 0.21</td>
<td>0.007</td>
<td>1.18 ± 0.22</td>
<td>1.09 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse wave velocity (m/s)</td>
<td>6.75 ± 0.90</td>
<td>6.01 ± 0.80</td>
<td>&lt;0.001</td>
<td>6.67 ± 0.79</td>
<td>5.97 ± 0.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dipper status n (%)</td>
<td>65 (44.5)</td>
<td>109 (47.6)</td>
<td>0.82</td>
<td>99 (61.9)</td>
<td>133 (66.2)</td>
<td>0.69</td>
</tr>
<tr>
<td>Masked Hypertension n (%)</td>
<td>28 (18.9)</td>
<td>24 (10.21)</td>
<td>0.02</td>
<td>57(35.6)</td>
<td>16 (7.92)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Renal variables**

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<tr>
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<tbody>
<tr>
<td>Total renin (pg/ml)</td>
<td>879 ± 325</td>
<td>727 ± 375</td>
<td>&lt;0.001</td>
<td>836 ± 274</td>
<td>650 ± 284</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prorenin (pg/ml)</td>
<td>952 (12.2;12143)</td>
<td>637 (15.0;13164)</td>
<td>&lt;0.001</td>
<td>1078 (25.0;13164)</td>
<td>804 (18.4;16645)</td>
<td>0.005</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)40</td>
<td>64.7 (5.01;337)</td>
<td>51.4 (3.80;729)</td>
<td>0.62</td>
<td>80.2 (17.4;587)</td>
<td>104 (7.46;820)</td>
<td>0.004</td>
</tr>
<tr>
<td>ACE (pg/ml)</td>
<td>162 ± 43.9</td>
<td>148 ± 38.9</td>
<td>0.001</td>
<td>169 ± 43.8</td>
<td>146 ± 35.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24-hour urinary Na/K</td>
<td>4.46 ± 1.82</td>
<td>4.11 ± 1.76</td>
<td>0.12</td>
<td>3.14 ± 1.41</td>
<td>3.05 ± 1.52</td>
<td>0.61</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>136 ± 16.7</td>
<td>136 ± 17.6</td>
<td>0.88</td>
<td>103 ± 16.6</td>
<td>108 ± 15.7</td>
<td>0.006</td>
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**Biochemical measurements**

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</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.46 ± 0.30</td>
<td>5.49 ± 0.31</td>
<td>0.57</td>
<td>5.29 ± 0.25</td>
<td>5.30 ± 0.27</td>
<td>0.90</td>
</tr>
<tr>
<td>TC:HDL</td>
<td>3.79 ± 0.85</td>
<td>3.88 ± 0.83</td>
<td>0.31</td>
<td>4.83 ± 0.99</td>
<td>4.64 ± 0.98</td>
<td>0.067</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.68 (0.03;34.5)</td>
<td>1.8 (0.03;41.7)</td>
<td>&lt;0.001</td>
<td>0.91 (0.03;41.1)</td>
<td>1.06 (0.05;42.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>GGT (units)</td>
<td>27.7 (4.5227)</td>
<td>22.9 (7.1193)</td>
<td>0.002</td>
<td>26.1 (8.10222)</td>
<td>14.7 (5.2090.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cotinine (pg/ml)</td>
<td>105 ± 150</td>
<td>24.0 ± 70.3</td>
<td>&lt;0.001</td>
<td>72.9 ± 131</td>
<td>30.6 ± 84.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Lifestyle factors**


<table>
<thead>
<tr>
<th>Smoking n (%)</th>
<th>69 (45.4)</th>
<th>28 (11.8)</th>
<th>&lt;0.001</th>
<th>46 (28.8)</th>
<th>34 (16.8)</th>
<th>0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol use n (%)</td>
<td>48 (32.0)</td>
<td>123 (68.0)</td>
<td>0.002</td>
<td>106 (66.3)</td>
<td>109 (54.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total energy expenditure</td>
<td>2222 ± 324</td>
<td>2158 ± 428</td>
<td>0.18</td>
<td>2611 ± 437</td>
<td>2186 ± 518</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Difference between the black and white groups. Values are arithmetic mean ± standard deviation; geometric mean (5th and 95th percentile interval) for logarithmically transformed variables. Abbreviations: N, number of participants; SES, socioeconomic status; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, Pulse pressure; ACE, angiotensin-converting enzyme; Na/K, sodium-to-potassium ratio; eGFR, estimated glomerular filtration rate; TC, total cholesterol; HDL, High density lipoprotein; GGT, gamma glutamyl transferase
Supplementary Table 2A represents the unadjusted correlations between PP (bPP, 24-hour PP and cPP) and PPA with components of the RAAS. Before any adjustments bPP associated positively with renin (r= 0.09, p=0.018) and ACE (r=0.13, p<0.001) in the total group and with renin (r=0.18, p<0.001) and ACE (r=0.18, p=0.001) in the white group, while a negative association with aldosterone (r=-0.12, p=0.022) was also observed in the white group (Supplementary table 2A). Twenty-four-hour PP associated positively with renin (r=0.13, p<0.001) and ACE (r=0.15, p<0.001) in the total group and in the white (r=0.21, p<0.001) group. Central PP was positively associated with ACE (r=0.17, p<0.001) in the total group and with renin (r=0.15, p=0.01) in the white group and PPA associated positively with aldosterone in the black group (r=0.13, p=0.013) (Supplementary table 2A). Supplementary Table 2C represents the unadjusted correlations between PP (bPP, 24-hour PP and cPP) and PPA with components of the RAAS in men and women within the black and white groups. 24-hour PP associated positively with ACE (r=0.14, 0.04) and PPA associated negatively with ACE (r=-0.16, p=0.02) in the white women, whereas PPA associated positively with aldosterone (r=0.25, p<0.001) in the black women. In the white men cPP associated positively with renin (r=0.17, p=0.037) (Supplementary table 2C).

After adjusting for age, sex and BMI, the positive association between aldosterone (r=0.09, p=0.02) and 24-hour PP along with the positive association between ACE (r=0.11, p=0.003) and cPP remained in the total population. The positive association between aldosterone (r=0.12, p=0.01) and PPA in the black group also persisted. In the white group, cPP associated positively with prorenin (r=0.11, p=.047) and ACE (r=0.13, p=0.01) (Supplementary Table 2B). 24-hour PP associated positively with ACE (r=0.14, p=0.04) and PPA associated negatively with ACE (r=-0.16, p=0.02) in the white women, whereas PPA associated positively with aldosterone (r=0.25, p<0.001) in black women. In the white men cPP associated positively with renin (r=0.17, p=0.037) (Supplementary table 2D).
After forward stepwise multiple regression analysis to determine independent relations between PP and PPA with components of the RAAS (Table 3), a positive association between cPP and ACE was observed only in the total group (β=0.10, p=0.001). In black women a negative association between total renin and bPP (β=-0.20, p<0.001) was observed along with a positive association between aldosterone and PPA (β= 0.18, p=0.05) whereas in the white female group a negative association between ACE and PPA was observed (β=-0.19, p<0.001) (Table 4) .
Table 3: Forward stepwise multiple regression analyses of PP and PPA with components of the RAAS in the total group, and black and white groups

<table>
<thead>
<tr>
<th></th>
<th>Brachial pulse pressure</th>
<th>24-hour pulse pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N=752)</td>
<td>Black (N=390)</td>
</tr>
<tr>
<td></td>
<td>Adjusted R2 β ± SE</td>
<td>Adjusted R2 β ± SE</td>
</tr>
<tr>
<td>Renin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log aldosterone</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log prorenin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ACE</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Central pulse pressure</th>
<th>Pulse pressure amplification</th>
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<tbody>
<tr>
<td></td>
<td>Adjusted R2 β ± SE</td>
<td>Adjusted R2 β ± SE</td>
</tr>
<tr>
<td>Renin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log aldosterone</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log prorenin</td>
<td>0.22 0.05 ± 0.04*</td>
<td>0.32 0.11 ± 0.07*</td>
</tr>
<tr>
<td>ACE</td>
<td>0.23 0.10 ± 0.04***</td>
<td>0.32 0.07 ± 0.06*</td>
</tr>
</tbody>
</table>

- did not enter model; independent variables included age, gender, ethnicity, BMI, cotinine, GGT, Glucose, CRP, socioeconomic status, lipids, eGFR, NA/K ratio, TEE, height and HR. *P>0.05, ** P<0.05, ***P≤0.001.
Table 4: Forward stepwise multiple regression analyses of PP and PPA with components of the RAAS in men and women within black and white groups

<table>
<thead>
<tr>
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<th>Brachial pulse pressure</th>
<th>24-hour pulse pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted R^2</td>
<td>β ± SE</td>
</tr>
<tr>
<td>Renin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log aldosterone</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log prorenin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ACE</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Central pulse pressure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adjusted R^2</td>
<td>β ± SE</td>
</tr>
<tr>
<td>Renin</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Log aldosterone</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>Log prorenin</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>ACE</td>
<td>-</td>
<td>NS</td>
</tr>
</tbody>
</table>

* did not enter model, NS, did enter the model but was not significant; independent variables included age, gender, ethnicity, BMI, cotinine, GGT, Glucose, CRP, socioeconomic status, lipids, eGFR, NA/K ratio, TEE, height and HR. *P>0.05, ** P<0.05, ***P≤0.001.
Discussion

The main aim of this study was to investigate the association of pulse pressure (b PP, c PP and 24-hour PP) and its amplification (PPA) with RAAS components including prorenin, renin, aldosterone and ACE in young South African adults. The prominent findings of the study are the independent positive association between c PP and ACE in the total group, the negative association between total renin and b PP along with a positive association between aldosterone and PPA in the black women and the negative association between ACE and PPA observed in the white women.

Angiotensin converting enzyme inhibitors attenuates Ang II-mediated vasoconstriction (18) and can also decrease BP by inhibiting the degradation of bradykinin (19). In patients with chronic heart failure, preservation of bradykinin has been found to be a contributing factor in the long term effects of ACE inhibition on systemic haemodynamics, this can be a possible explanation for the observed clinical differences between ACE inhibitors and angiotensin blockers in the treatment of heart failure (20). Thus, association of ACE with an increase in c PP in the total group suggests that ACE may have a more substantive control of central pressure than brachial pressure in this young population, however the exact mechanisms could not be established. A study conducted in patients with mild hypertension found that the ACE inhibitor enalapril reduces wave reflection resulting in a decrease in aortic systolic pressure but none found with the peripheral pressure measurements (21). Morgan et al., conducted a study to investigate the impact of different hypertension treatments on central and brachial BP in elderly patients with systolic hypertension and found that ACE inhibitors had an impact less than that of calcium blockers and diuretics on brachial SBP but had a greater impact on central aortic pressure (22).

Pulse pressure is the difference between systolic and diastolic arterial BP which represents variation in BP (23) and it is a well-established significant independent marker of cardiovascular risk (24). The pathophysiological mechanism associated with an increased PP may include hemodynamic stress, vascular inflammation and calcification as well as matrix
remodelling (25). Our results in a young population echo some aspects of Morgan et al., (21) even though the study was conducted in older participants. However, in another study conducted by Deary et al., in younger groups with diastolic hypertension, ACE inhibitors were found to control brachial arterial BP more effectively (26). The difference in our findings may be as a result of our study’s demographic; young apparently healthy individuals. With the progression of age, cellular, biochemical and enzymatic changes in the vasculature occur along with modification in the way in which they are modulated, a phenomenon coined vascular aging (27). These changes have also been seen to occur prematurely in early vascular aging (28) and it has been identified as a promising clinical tool in early identification of cardiovascular risk (26). A decrease in PPA has been implicated in arterial stiffness and organ damage as well as mortality in older individuals (29). Pulse pressure amplification decreases with age due to an increase in early wave reflection and augmentation of systolic and thus PP (30). It has been reported that the most effective tool in maintaining a normal pulse pressure (around 40mmHg) is through increasing compliance which can further explain the positive association between cPP in the total group and ACE as well a negative association between PPA and ACE in white women (29). This is because arterial stiffness leads to an increased wave reflection during systolic ejection adding to the forward wave which results in an augmented SBP and a widening PP (31). With abovementioned changes in haemodynamics (increase vascular resistance through bradykinin degradation and increased BP through Ang II stimulation) caused by ACE, other contributing factors unaccounted for in this study include in central venous pressure and cardiac output (32), both hemodynamic parameters (33).

A study conducted in children found that between the ages 12 through 18 boys had higher serum ACE activity and a positive association was seen between serum ACE activity and BP in boys while in the girls a negative association was observed (35). In the present study we also found that women had lower plasma ACE concentrations than men (Table 1), along with a positive association between PPA and aldosterone in black women and a negative
association between ACE and PPA in white women. When matched for body height, it has been found that the timing of left ventricular ejection and arterial wave reflection differ between men and women (36). This may indicate that other mechanisms affect central and aortic structure as well as function, this may be due to women especially having smaller and stiffer blood vessels, causing early return of the reflected arterial wave and a subsequently increased PP and greater augmentation index (37). In post-menopausal women, the attenuation of PPA caused an increased aortic stiffness related cardiovascular risk (38). PPA is determined by BP, body composition, gender (39), exercise (40) and age (41). In our study group there were significant differences in PWV, a marker of arterial stiffness (42) but no significant differences in age, suggesting the mechanisms underpinning the associations found in the women may be specifically due to gender difference between men and women and estradiol levels (not measured) between black and white women. Both black and white women did however present with a lower PPA when compared to their male counterparts, which aligns with the findings of Pichler et.al. who found that women presented with a lower PPA than men, where age, female gender and mean arterial pressure were found to associate inversely with PPA (39). This can explain the lack of findings in the male group.

Circulating RAAS components are greatly affected by plasma oestrogen concentrations (43). Which can be seen in pre-menopausal women who tend to present with lower renin levels than men, this may be due to the role oestrogen plays in decreasing adrenalin and noradrenaline, consequently indirectly decreasing circulating renin levels (44). In the current study lower total renin concentrations were observed in women when compared to the men which is in line with the literature. Estradiol has been found to attenuate the actions of ACE (45). Even though the impact of estradiol on Ang II is not clear (46), the reaction to Ang II mediated vasoconstriction of the aortic rings and mesenteric vessels appears weakened in female rats (47). Response to ACE inhibitors has also been found to differ between blacks and whites, with indications of less efficacy in blacks (48).The negative association between PPA and ACE seen in the white women and the positive association observed between PPA
and aldosterone in the black women may offer insight into how gender may eventually have an impact on cardiovascular risk. The black women presented with lower aldosterone levels when compared to their male counterparts whereas the white women presented with higher aldosterone levels when compared to the white men. Therefore, the positive association between PPA and aldosterone found in black women suggests that a suppressed RAAS may serve a protective role. This is also supported by the negative association between bPP and renin also in black women which is indicative of the negative feedback mechanism by which the circulating RAAS as initiated by renin secretion regulates BP (7, 49).

These findings should be interpreted within the context of the strengths and limitations of the study. This study is cross-sectional in design as a result causality cannot be inferred. In terms strengths having young participants with no pre-existing chronic disease enables investigation of the physiologic mechanisms which can aid in understanding of the potential cardiovascular risk. To the best of our knowledge this is the first study to look into the relationship between the impact of the RAAS on different components of pressure in a young South African population.

In conclusion, we found that already in a young, healthy population, ACE associates with cPP, suggesting that ACE could lead to early changes in central haemodynamics. We also found a negative association between PPA and ACE in the white women and a positive association between PPA and aldosterone in black women, which may indicate an ethnicity-specific ACE-mediated changes in arterial compliance and a potential cardioprotective effect of oestrogen in black women.

Acknowledgements

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Medical Research Council (SAMRC) with funds from National Treasury under its Economic Competitiveness and Support Package; the South African Research Chairs Initiative (SARChI) of the Department of Science and Technology and National Research Foundation of South Africa; the Strategic Health Innovation Partnerships (SHIP) Unit of the SAMRC with funds received from the South African National Department of Health, GlaxoSmithKline R&D, the UK Medical Research Council and with funds from the UK Government’s Newton Fund; as well as corporate social investment grants from Pfizer (South Africa), Boehringer-Ingelheim (South Africa), Novartis (South Africa), the Medi Clinic Hospital Group (South Africa) and in kind contributions of Roche Diagnostics (South Africa).

Any opinion, findings and conclusions or recommendations stated in this manuscript are those of the authors and therefore, the NRF do not accept any liability in regard.
References


### Supplementary tables

#### Supplementary Table 1: Interaction of ethnicity and gender on the associations of PP and PPA with components of the RAAS

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<td></td>
</tr>
<tr>
<td>Independent Variable:</td>
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<td>p</td>
<td>P</td>
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<tr>
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<tr>
<td>Aldosterone</td>
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<td>Prorenin</td>
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<tr>
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</tbody>
</table>

Abbreviations: ACE, Angiotensin-converting enzyme. Bold text indicates p<0.05
Supplementary Table 2A: Pearson’s correlations of PP and PPA with components of the RAAS in the total group, and in the black and white groups

<table>
<thead>
<tr>
<th></th>
<th>Brachial pulse pressure</th>
<th>24hr Pulse pressure</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Total (N=752)</td>
<td>Black (N=390)</td>
</tr>
<tr>
<td></td>
<td>r</td>
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<tr>
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<td>Aldosterone</td>
<td>-0.01</td>
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<tr>
<td>Prorenin</td>
<td>0.02</td>
<td>0.60</td>
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<td>ACE</td>
<td>0.13</td>
<td>&lt;0.001</td>
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<th>Central pulse pressure</th>
<th>Pulse pressure amplification</th>
</tr>
</thead>
<tbody>
<tr>
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<td>r</td>
<td>P</td>
</tr>
<tr>
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<td>0.09</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>-0.04</td>
<td>0.28</td>
</tr>
<tr>
<td>Prorenin</td>
<td>0.01</td>
<td>0.80</td>
</tr>
<tr>
<td>ACE</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: ACE, Angiotensin-converting enzyme. Bold text indicates p<0.05
Supplementary Table 2B: Partial correlations of PP and PPA with components of the RAAS in the total group, and in the black and white groups

<table>
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<tr>
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<th>Brachial pulse pressure</th>
<th>24hr Pulse pressure</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total (N=752)</td>
<td>Black (N=390)</td>
</tr>
<tr>
<td>Renin</td>
<td>r: -0.03, P: 0.41</td>
<td>r: -0.07, P: 0.15</td>
</tr>
<tr>
<td>Log Aldosterone</td>
<td>r: 0.20, P: 0.68</td>
<td>r: 0.07, P: 0.19</td>
</tr>
<tr>
<td>Log Prorenin</td>
<td>r: 0.20, P: 0.53</td>
<td>r: 0.02, P: 0.76</td>
</tr>
<tr>
<td>ACE</td>
<td>r: 0.03, P: 0.43</td>
<td>r: 0.01, P: 0.83</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th></th>
<th>Central pulse pressure</th>
<th>Pulse pressure amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Black</td>
</tr>
<tr>
<td>Renin</td>
<td>r: -0.03, P: 0.49</td>
<td>r: -0.48, P: 0.35</td>
</tr>
<tr>
<td>Log Aldosterone</td>
<td>r: -0.03, P: 0.45</td>
<td>r: -0.07, P: 0.18</td>
</tr>
<tr>
<td>Log Prorenin</td>
<td>r: 0.03, P: 0.46</td>
<td>r: -0.04, P: 0.42</td>
</tr>
<tr>
<td>ACE</td>
<td>0.11, P: 0.003</td>
<td>0.10, P: 0.06</td>
</tr>
</tbody>
</table>

Abbreviations: ACE, Angiotensin-converting enzyme. Bold text indicates p<0.05. Adjusted for age, sex and BMI.
### Supplementary Table 2C: Pearson’s correlations of PP and PPA with components of the RAAS in men and women within the black and white groups

<table>
<thead>
<tr>
<th></th>
<th>Blacks</th>
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<th>24hr Pulse pressure</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brachial pulse pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blacks</td>
<td></td>
<td>Whites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin</td>
<td>-0.06</td>
<td>0.44</td>
<td>0.06</td>
<td>0.33</td>
<td>0.08</td>
<td>0.29</td>
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<td>0.76</td>
<td>-0.07</td>
<td>0.38</td>
<td>-0.04</td>
</tr>
<tr>
<td>Log Aldosterone</td>
<td>0.03</td>
<td>0.69</td>
<td>0.10</td>
<td>0.12</td>
<td>-0.03</td>
<td>0.75</td>
<td>-0.07</td>
<td>0.34</td>
<td>0.08</td>
<td>0.31</td>
<td>0.08</td>
</tr>
<tr>
<td>Log Prorenin</td>
<td>0.02</td>
<td>0.84</td>
<td>-0.01</td>
<td>0.94</td>
<td>-0.03</td>
<td>0.73</td>
<td>-0.01</td>
<td>0.92</td>
<td>0.08</td>
<td>0.34</td>
<td>0.06</td>
</tr>
<tr>
<td>ACE</td>
<td>0.02</td>
<td>0.83</td>
<td>0.03</td>
<td>0.64</td>
<td>0.06</td>
<td>0.42</td>
<td>0.02</td>
<td>0.80</td>
<td>0.03</td>
<td>0.72</td>
<td>0.04</td>
</tr>
</tbody>
</table>

|                        | Central pulse pressure |                       |                  |                       |                    |                       |                       |                       |                       |                       |                       |                       |
|                        | Blacks          |                       | Whites          |                       |                    |                       |                       |                       |                       |                       |                       |                       |
| Renin                  | -0.04           | 0.62                  | -0.07           | 0.28                  | **0.17**           | **0.03**             | 0.02                | 0.75                  | -0.03                | 0.71                  | 0.11                 | 0.11                  | -0.06                | 0.46                  | -0.03                | 0.69                  |
| Log Aldosterone        | 0.03            | 0.68                  | -0.12           | 0.06                  | -0.02              | 0.82                  | 0.02                | 0.79                  | -0.002               | 0.98                  | 0.22                 | 0.001                 | -0.02                | 0.83                  | -0.09                | 0.22                  |
| Log Prorenin           | 0.07            | 0.38                  | 0.06            | 0.36                  | -0.01              | 0.85                  | -0.08               | 0.25                  | -0.02                | 0.79                  | -0.02                | 0.79                  | -0.02                | 0.85                  | 0.04                 | 0.53                  |
| ACE                    | 0.05            | 0.55                  | 0.10            | 0.14                  | 0.02               | 0.85                  | **0.24**           | **0.001**            | 0.02                 | 0.77                  | -0.07               | 0.27                  | 0.06                 | 0.48                  | **-0.15**            | **0.03**              |

Abbreviations: ACE, Angiotensin-converting enzyme. Bold text indicates p<0.05.
### Supplementary Table 2D: Partial correlations of PP and PPA with components of the RAAS in men and women within the black and white groups

#### Brachial pulse pressure

<table>
<thead>
<tr>
<th></th>
<th>Blacks</th>
<th>Whites</th>
<th>Blacks</th>
<th>Whites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Renin</td>
<td>-0.05</td>
<td>0.52</td>
<td>0.07</td>
<td>0.27</td>
</tr>
<tr>
<td>Log Aldosterone</td>
<td>0.01</td>
<td>0.93</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Log Prorenin</td>
<td>-0.02</td>
<td>0.80</td>
<td>-0.02</td>
<td>0.81</td>
</tr>
<tr>
<td>ACE</td>
<td>-0.01</td>
<td>0.89</td>
<td>0.02</td>
<td>0.73</td>
</tr>
</tbody>
</table>

#### Central pulse pressure

|                  | Blacks                 | Whites                 | Blacks                 | Whites                 |
|                  | r         | p       | r         | p       | r         | p       | r         | p       | r         | p       | r         | p       |
| Renin            | -0.03    | 0.76    | -0.06    | 0.39    | **0.17** | **0.037** | 0.06     | 0.39    | -0.03    | 0.74    | 0.10     | 0.13    | -0.06    | 0.46    | -0.03    | 0.70    |
| Log Aldosterone  | 0.01     | 0.87    | -0.12    | 0.06    | -0.02    | 0.82    | 0.03     | 0.73    | -0.02    | 0.83    | **0.22** | **0.001** | -0.02    | 0.83    | -0.09    | 0.22    |
| Log Prorenin     | 0.07     | 0.40    | 0.11     | 0.10    | -0.02    | 0.85    | -0.08    | 0.24    | -0.06    | 0.47    | -0.06    | 0.38    | -0.02    | 0.85    | 0.06     | 0.44    |
| ACE              | 0.05     | 0.52    | 0.12     | 0.08    | 0.02     | 0.85    | **0.25** | **<0.001** | -0.06    | 0.48    | -0.09    | 0.18    | 0.06     | 0.48    | -0.16    | **0.02** |

#### Pulse pressure amplification

|                  | Blacks                 | Whites                 | Blacks                 | Whites                 |
|                  | r         | p       | r         | p       | r         | p       | r         | p       | r         | p       |
| Renin            | -0.03    | 0.76    | -0.06    | 0.39    | **0.17** | **0.037** | 0.06     | 0.39    | -0.03    | 0.74    | 0.10     | 0.13    | -0.06    | 0.46    | -0.03    | 0.70    |
| Log Aldosterone  | 0.01     | 0.87    | -0.12    | 0.06    | -0.02    | 0.82    | 0.03     | 0.73    | -0.02    | 0.83    | **0.22** | **0.001** | -0.02    | 0.83    | -0.09    | 0.22    |
| Log Prorenin     | 0.07     | 0.40    | 0.11     | 0.10    | -0.02    | 0.85    | -0.08    | 0.24    | -0.06    | 0.47    | -0.06    | 0.38    | -0.02    | 0.85    | 0.06     | 0.44    |
| ACE              | 0.05     | 0.52    | 0.12     | 0.08    | 0.02     | 0.85    | **0.25** | **<0.001** | -0.06    | 0.48    | -0.09    | 0.18    | 0.06     | 0.48    | -0.16    | **0.02** |

Abbreviations: ACE, Angiotensin-converting enzyme. Bold text indicates p<0.05. Adjusted for age, sex and BMI.
Chapter 4

Summary of main results, limitations, conclusions and recommendations
1. Introduction
This chapter serves as a summary of the findings of the study. The hypotheses made in the first chapter are revisited and compared with the main findings and the literature and conclusions are made. Recommendations will also be offered for future studies on the association of pulse pressure and pulse pressure amplification with the renin-angiotensin-aldosterone system (RAAS).

2. Interpretation of the main findings and a comparison with the relevant literature
In this section, the results of the current study are discussed based on the original hypotheses as set out in Chapter 1, and with reference to the relevant literature. Initially, hypothesis 3.1-3.3 were set for the black and white groups separately, however, after extensive statistical explorations, we opted to also investigate the associations in the total group along with the black (men and women separately) and white (men and women separately) groups.

*Hypothesis 1: Renin, prorenin and ACE will be comparable between ethnicities, while aldosterone will be higher in whites compared to blacks.*

In this study, total renin was lower in the white group as compared to the black group, whereas prorenin was found to be lower in the black group as compared to the white group. There were no significant differences in ACE values observed between the two-ethnic group. The white group was found to have higher aldosterone levels as compared to the black group.

Black populations usually present with low plasma renin levels and activity compared to their white counterparts (1, 2). Thus, our finding of lower renin levels in the white group does not align with literature and there are two possible explanations. Firstly, previous studies measured active plasma renin, where else in our study, total renin (including active and inactive components of renin) was measured. Secondly, most of the studies were often conducted in older groups or those with existing hypertension (2-4). Notwithstanding standing the latter, a study conducted in a young healthy population reported that blacks had lower plasma renin concentrations when compared to whites (3) which is seen as an indication of
expanded extracellular volume but prorenin levels similar to the white group (3, 4). Furthermore, it was found that the black group had lower prorenin levels when compared to the white group. One of the proposed explanations for low renin concentration accompanied by higher prorenin levels which was observed in the white group may be a low prorenin-to-renin conversion rate (5, 6). It has also been reported that in individuals with low renin levels around 98% of the total renin is inactive (6). Therefore, it can be assumed that the higher renin levels along with lower prorenin levels observed in the black group may be due to the absence of any conversion rate issues. Regardless of differences in plasma renin activity, blacks and whites present with similar ACE levels (26), which is in agreement with the lack of difference in ACE levels between out black and white groups. Aldosterone has been shown to be higher in whites as compared to blacks regardless of age (children and adults) (7, 8). Thus, our finding of higher aldosterone levels in the white group aligns with the literature. Blacks have been reported to present with an upregulated response to aldosterone proposed to be due to a pre-existing heightened sodium retention (8). Which can explain the lower levels observed in the black group when compared to the white group. Differences in response to ACE inhibitor monotherapy has been observed between the blacks and whites, with the blacks having a poor response (9, 10). ACE has been shown to associate negatively with BP in blacks but associate positively with BP in whites even though no differences in ACE plasma concentrations were reported (11). This suggests that with regard to ACE a difference in ACE activity may exist but not in its synthesis hence the similar ACE levels.

As renin and prorenin levels were not comparable between the two ethnic groups and no significant differences in ACE levels were found we cannot completely accept the hypothesis. We however did indeed find that the white group had higher aldosterone levels when compared to their black counterparts, therefore the hypothesis is partially accepted.
Hypothesis 2: PP will be higher in the black population and PPA lower.

We found that the black group had a lower 24-hour PP when compared to the white group, no significant differences in brachial pulse pressure (bPP), pulse pressure (cPP) and PPA were found between the two groups.

Breet et al., found a declined PPA in black normotensives who were younger than 30 years of age, indicating the possibility of early aging of the vasculature (12). With the progression of age, due to changes in the vasculature, the blood vessels stiffen (11). As result associations observed with PPA are more prominent in elderly patients (13, 14). Our study populations consisted of participants with no pre-existing chronic illness and as a result we observed no significant differences. In our sample 20.2% of the white group were classed as having masked hypertension (normal clinical blood pressure measurements but elevated 24-hour BP). Therefore, this can explain the lower 24-hour PP observed in the black group. PP as an established marker of cardiovascular risk is impacted by a decrease in DBP as well as an increase in SBP (15). Though there were no significant differences in 24-hour DBP observed between the two races, the black group had a significantly lower SBP. This aligns with the literature on the determinants of PP. Most of our black group is of a lower socioeconomic status as compared to the white group. Individuals from a lower socioeconomic status have been found to have a vulnerability to the adverse outcomes of psychosocial stress (16). Prolonged psychosocial stress leads to increased activity of the sympathetic nervous system (17), which has been observed to associate with elevation in BP (18). Thus, it would be expected to find a higher BP and subsequently PP in the black group which is not the case in our study. Therefore, the observation of a lower 24-hour PP in the black group may also be a chance finding.

There were no significant differences in PPA between the black and white groups and a lower PP (24-hour) was observed in the black group, therefore the hypothesis is rejected.
Hypothesis 3.1: Renin will be negatively associated with cPP and positively associated with PPA in the total group, both blacks and whites, men and women.

No association was observed between renin and cPP in both ethnic groups, there was also no significant association between renin and PPA in both ethnic groups and genders. We did however find that renin associated negatively with brachial pulse pressure (bPP) in the black women. Black groups often present with a low renin status which has been seen to have an adverse relationship with blood pressure (19-21). Indeed, the women in the current study presented with lower renin levels than their male counterpart, however the women were found to have better cardiovascular profile (lower brachial SBP, brachial SBP and brachial PP) than the men. Many studies conducted with renin are conducted in either the elderly or participants with a compromised cardiovascular profile, as the RAAS dysregulation is often associated with cardiovascular risk (4, 22-24). The apparently health profile of our study groups may perhaps be the reason that no association between cPP and PPA with renin were observed in both ethnic groups. The negative association found between renin and bPP may also be attributed to negative feedback mechanism exerted by blood pressure on renin secretion.

Therefore, the hypothesis is rejected as there were no significant results observed between renin and cPP in the total group and both blacks and whites, males and females. Though, a negative association was found between renin and bPP in the black women.

Hypothesis 3.2: Prorenin will associate positively with cPP and negatively with PPA in the total group, both blacks and whites, men and women.

We did not observe any association between prorenin and cPP and PPA in both blacks and whites, men and women. Prorenin has been suggested to have a role in vascular pathology which could result in subsequent rises in BP (25, 26). It has been found that prorenin has angiotensin dependent and non-angiotensin dependent effects on the cardiovascular system in mice (27) though the findings in humans are inconclusive (28). The controversy stems from the fact that angiotensin dependent effects appears to demand abnormally high levels of
prorenin (25). This may explain the absence of the anticipated association of PP and its amplification with prorenin. Additionally, prorenin concentrations can be assumed to be within normal limits based on the kit insert, though no clear cut off values have been established, and that could explain the lack of impact on the vasculature at this young age.

Due to the lack of significant results found with prorenin and central pulse pressure in neither the black or the white group, the hypothesis is therefore rejected.

**Hypothesis 3.3: ACE will positively associate with cPP and with PPA in the total group, both blacks and whites, men and women.**

There was no association observed between ACE and cPP as well as PPA in white men. A positive association was observed between ACE and PPA in white women but none with cPP. It has been reported that ACE associates positively with BP in whites (29, 30). Gender differences in serum-ACE activity in children and adolescents (31). Women have been found to have especially smaller and stiffer blood vessels which increases PWV causing early return of the reflected arterial wave and a subsequently increased PP and greater augmentation index (32). This may explain the lower PPA observed in the white women with no known pre-existing cardiovascular disease implicated. A difference in the timing of left ventricular ejection and arterial wave reflection has been seen between men and women (33), indication varying mechanism affect central and aortic structure and function are implicated in men and women (34). Many of the studies looking into ACE are often done to investigate the effectivity and mechanism of action of ACE inhibitors (35, 36), therefore there is limited information on the direct association ACE has with BP in healthy individuals.

Therefore, the hypothesis is partially accepted as there were no significant results observed between ACE and central pulse pressure in the white group, but a positive association was observed between ACE and PPA in the white women.
Hypothesis 3.4: Aldosterone will negatively associate with central pulse pressure (cPP) and positively with pulse pressure amplification (PPA) in the total group, both blacks and whites, men and women.

No significant associations were observed between aldosterone and cPP in the white men and women. Aldosterone is responsible for sodium reabsorption, and therefore aldosterone excess has been proposed to have a role in states of low renin hypertension (30). It is worth mentioning that the aldosterone-to-renin ratio, a marker of inappropriate aldosterone activity (37), was not included in our study as the population consisted of healthy individuals therefore we cannot state whether the high aldosterone observed in the white group is pathological. The cut off values for hyperaldosteronism are classified according to aldosterone-to-renin ratio values (30 ng/dl) and therefore conclusions cannot be made in this regard (38). The impact of aldosterone on BP is often observed in the presence of pre-existing disease as is seen with all components of the RAAS due to the system exacerbating pressure elevation (39). Aldosterone has been found to decrease the compliance of arteries (40), and thus a negative association with PPA was hypothesised (37).

Though no associations were observed in white men and women we did however, find a positive association between aldosterone and PPA in black women. The black women had lower aldosterone levels when compared to their male counterparts. Ang II has been seen to associate with adverse vascular effects that lead to reduced arterial compliance (26). Therefore, the positive association between PPA and aldosterone found in black women suggests that the low aldosterone levels seen in black women could indicate that aldosterone is not a contributing factor to reduced arterial compliance in this group.

Due to the lack of significant results found between aldosterone and cPP in the white group, the hypothesis is therefore partially accepted.
3. Discussion of main findings in the total group

A positive association between ACE and cPP was observed in the total group, while a negative association was observed with PPA in white women. ACE has an established role in the elevation of BP, seen through the impact of ACE inhibitors in the lowering of BP in hypertensives (41-43). Angiotensin-converting enzyme inhibitors have been found to have greater control of central pressure as compared to brachial pressure in elderly patients (42). Thus, the association found with cPP and not with bPP in the total group aligns with the literature in this regard. Angiotensin converting enzyme functions as a vasoconstrictor through initiating the production of ang II (a vasoconstrictor) (19), along with the metabolism of bradykinin, a potent vasodilator (44). One of the main determinants of PP is the compliance of the arterial system (45), ACE regulates BP by increasing arterial stiffness through it stimulation of vasoconstriction and inhibition of vasodilation (41, 44). The positive correlation between ACE and cPP may therefore be due to the impact the ACEs vasoconstrictive functions have on the augmentation pressure (42) Thus, our finding does indicate that ACE plays a substantial role in the control of BP, more so central pressure (42, 43, 46), even in young populations, irrespective of ethnicity.

4. Limitations and confounding

This was a cross-sectional study, consequently no overt causative conclusions can be made regarding the correlation between cPP and ACE. Additionally, the exact mechanisms through which the aforementioned correlation still remain unclear. Our study is not illustrative of the diverse South African population. Our study sample was made up of young apparently healthy participants which limited the ability to explore the impact of age-related structural changes can have on the relationship between RAAS components and pressure components.

5. Conclusion

We found that already in a young, healthy population, ACE associates with cPP. This could indicate that ACE could lead to early changes in central haemodynamics indicating early cardiovascular compromise. This finding is important in that ACE inhibitors are an integral tool
in the treatment of hypertension, hence understanding the physiological mechanisms at play in its regulation of BP may aid in improvement of treatment and preventative strategies. We also found a negative association between PPA and ACE in the white women and a positive association between PPA and aldosterone in the black women. This is important as provides some insight into how the gender differences observed in circulating RAAS components relate to the cardiovascular system. Multiple factors can impact haemodynamics and central aortic function, understanding of these relationships may be of use to clinicians in order to modify therapy for each patient so as to improve the control pulsatile components of pressure.

6. Recommendations

- Since African-PREDICT is a longitudinal study, there is an opportunity establish the role of ACE and other RAAS components in central haemodynamic regulation over time.
- The impact of age on the impact of RAAS function on central pressure should be determined in the follow-up stage of African-PREDICT.
- The inclusion of ACE-2, a proposed central regulator for cardiovascular function, to explore the mechanisms involved in the control of central pressure by ACE may assist with elucidation of the exact mechanism involved.
- Further investigation of the association between PP measurements and components of the RAAS in those with masked hypertension, prehypertension and non-dipping status
7. References


Appendices
ETHICS APPROVAL LETTER OF STUDY

Based on approval by the Health Research Ethics Committee (HREC) on 10/08/2018 after being reviewed at the meeting held on 10/08/2018, the Health Research Ethics Committee hereby approves your study as indicated below. This implies that the North-West University Research Ethics Regulatory Committee (NWU-RERC) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

| Study Leader/Supervisor (Principle Investigator)/Researcher: | Dr LF Gafane-Matemane |
| Student: | NL Mokae |
| Ethics number: | NWU - 00064 - 18 - A1 |
| Application Type: | Single study |
| Commencement date: | 10-08-2018 |
| Expiry date: | 31-08-2019 |

The study is approved for a period of one year. You may apply for an extension if this is required. The study will be monitored annually and if any adverse events occur, you will be contacted. The study will be reviewed in line with the study approval and if any issues arise, you will be contacted.

Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- The study leader must ensure that the stipulated monitoring is completed.
- The study leader must ensure that the study is conducted in line with the approved study protocol.
- The study leader must ensure that any adverse events are reported to the HREC.
- The study leader must ensure that any amendments to the study protocol are approved by the HREC before implementation.
- The study leader must ensure that any deviations from the study protocol are reported to the HREC.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- The study leader must ensure that the study is conducted in line with the approved study protocol.
- The study leader must ensure that any adverse events are reported to the HREC.
- The study leader must ensure that any amendments to the study protocol are approved by the HREC before implementation.
- The study leader must ensure that any deviations from the study protocol are reported to the HREC.

- The study leader must ensure that any unethical principles or practices of the study are revealed or suspected.
- It becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented.
- Submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or
- New institutional rules, national legislation or international conventions deem it necessary.

The HREC can be contacted for further information or any report templates via Ethics-HRECApply@nwu.ac.za or 018 299 1206.

Yours sincerely

Prof Wayne Towers
Chair NWU Health Research Ethics Committee
DECLARATION

I, C Vorster (ID: 710924 0034 084), Language editor and Translator and member of the South African Translators’ Institute (SATI member number 1003172), herewith declare that I did the language and technical editing of a dissertation written by NL Mokae (student number: 25130412) form the North-West University.

Title of the dissertation: The relationship of pulse pressure and pulse pressure amplification with the renin-angiotensin-aldosterone system in young adults: The African-PREDICT study

____________________________  _______________________
C Vorster                                Date

26 November 2018

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