The renin-angiotensin-aldosterone-system and left ventricular mass in young black and white adults: The African-PREDICT study

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Dissertation accepted in fulfilment of the requirements for the degree Master of Health Sciences 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.

DECLARATION BY AUTHORS

The following contributions were made:

Mr WL du Toit

Responsible for writing the proposal and ethics application of the study, performing extensive literature research, dataset cleaning and statistical analyses, design and planning of the research article, interpretation of the results and writing all sections of this dissertation.

Prof CMC Mels (supervisor), Prof AE Schutte, Dr LF Gafane-Matemane (co-supervisors) and Prof R Kruger (co-author)

The supervisor and co-supervisors, supervised the design, planning and writing of all sections of the dissertation with additional input from the co-author (Prof. R Kruger) in chapter 3. Furthermore, they provided guidance, intellectual input and critical evaluation of the dissertation.

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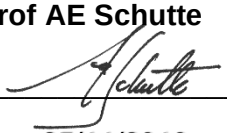
Sign:		
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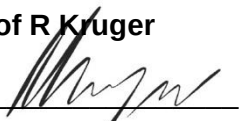
Sign:		
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PREFACE

This study, " The renin-angiotensin-aldosterone-system and left ventricular mass in young black and white adults: The African-PREDICT study " forms part of the dissertation for the degree Master of Health Science in Cardiovascular Physiology at the North-West University of Mr WL du Toit.

The dissertation is compiled in the article format as described and recommended by the North-West University. Following this format, the chapter outline is as follows:

- Chapter 1: Literature review
- Chapter 2: Methodology
- Chapter 3: Research manuscript
- Chapter 4: Concluding chapter

The manuscript is prepared for submission to the journal *Hypertension Research*. The referencing style for the chapters are prepared in accordance with the author instructions of this journal (see page 48). Furthermore, all imaging in the dissertation were compiled by WL du Toit using respective sources.

CONFERENCE PRESENTATION

Wessel L. du Toit, Aletta E. Schutte, Lebo F. Gafane-Matemane, Ruan Kruger, Catharina M.C. Mels. Does low socio-economic status predispose young adults to RAS-related increases in left ventricular mass? The African-PREDICT study. Medical Research Council Newton Project Workshop. Bakubung Bush Lodge, Pilanesberg National Park. 29 October 2019. Oral presentation.

SUMMARY

Motivation. The renin-angiotensin system (RAS) is a central regulatory component implicated in sodium and water homeostasis that affects blood volume and pressure. Dysregulation of this system results in increased blood pressure (BP) and may contribute to the development of left ventricular hypertrophy (LVH). In addition to the RAS and BP, factors such as increased age, sex, black ethnicity and a low socio-economic status (SES) also contribute to left ventricular remodelling. In the South African context low SES may be even more important as it affects 55.5% of the population with a large proportion (63.4%) of them being young and unemployed. It is therefore important to investigate RAS-related increases in left ventricular mass (LVM) along with the possible influence low SES may have in young South Africans.

Aim. This study investigated the relationship between LVMi (index) and the RAS components in young (20-30 years) healthy participants of the African-PREDICT study while taking factors such as SES, ethnicity and sex into consideration.

Methods. This study used cross-sectional data from 1 186 black and white men and women divided into low and high SES groups. Demographic data including age, sex, ethnicity, skill level (classified according to the South African Standard Classification of Occupation (SASCO), education and income were collected using various questionnaires. Socio-economic status was calculated using a point system adapted from the Kuppuswamy's Socioeconomic Status Scale. Anthropometric measurements and physical activity were measured. Cardiovascular measurements included clinic BP, 24h ambulatory BP, total peripheral resistance and echocardiography which were used to determine LVM - normalised for body surface area to derive LVMi. The RAS Fingerprint® was measured with an ultra-pressure-liquid chromatography tandem-mass spectrometry (LC-MS/MS) method. A wide range of other biochemical markers considered as cardiovascular disease risk markers were also analysed.

Results. Aligned with the aim of this study it was determined whether LVMi is associated with components of the RAS. LVMi associated inversely and independently with plasma renin activity (β=-0.168; P=0.017), angiotensin I (β=-0.155; P=0.028) and angiotensin II (β=-0.172; P=0.015), only in black women with low SES. No associations were evident between LVMi and components of the RAS in black women with high SES, or white women, black or white men, independent of SES.

Conclusion. This finding suggests that multiple factors may play a role in the development of increased LVM, including suppressed RAS, raised BP, female sex, black ethnicity and a low socio-economic environment.

CHAPTER 1

LITERATURE REVIEW

Background and problem statement

1. Introduction

The World Health Organisation (WHO) reports that the prevalence of hypertension is a particular concern in Africa with the highest prevalence across all WHO regions (1). The burden of hypertension is further emphasised in groups with low socio-economic status (SES) where the diagnosis, treatment, control and prevention is sub-optimal (2-9). Apart from its link to mortality, hypertension is also a major risk factor for cardiac hypertrophy which is associated with unfavourable outcomes such as sudden death or progression to overt heart failure (10-13).

Normally, cardiac hypertrophy is viewed as a compensatory mechanism for pathological stimuli such as hypertension, valvular defects and aortic regurgitation (10-17). Other factors known to be important in blood pressure (BP) regulation, such as renin-angiotensin system (RAS) dysregulation can also lead to cardiac remodelling. Physiologically, the RAS is essential for control of sodium and water balance that determines blood volume and pressure (19, 20). Dysregulation of this system is associated with oxidative stress, inflammation, endothelial dysfunction, raised BP, fibrosis and cardiac remodelling (19, 20).

Due to the cardiovascular disease (CVD) burden (21-23), it is important to investigate early CVD development by focusing on components such as the RAS and left ventricular geometry in a young apparently healthy population to gain this knowledge. By identifying early CVD risk factors, improvements can be made in lifestyle, diagnosis and treatment. This can ultimately lead to effectively controlling and preventing such consequences as raised BP, particularly in Africa were the influence of low SES greatly increase this risk. Hence the focus of this study is on the RAS and how it relates to left ventricular mass (LVM) in a young apparently healthy black and white cohort taking into account the health effects of low SES.

1.1. The renin-angiotensin system

The RAS is a hormonal proteolytic cascade that functions as an endocrine system (systemic RAS), but also has a local paracrine or autocrine action in tissues and organs such as the heart and kidneys (intrarenal RAS) (19, 20, 24). The RAS exerts its effects on the kidney and cardiovascular system mainly via the binding of angiotensin II (Ang II) to angiotensin II type 1 receptors (ATR1) (Figure 1) (19, 20). Under physiological conditions, once activated, the RAS will increase sodium and water retention in the kidney, vasoconstriction and ultimately increased blood volume and pressure (19, 20). Over-activation of the RAS has been linked to processes leading to increased oxidative stress, inflammation, endothelial dysfunction, raised BP and tissue remodelling such as cardiac hypertrophy (19, 20).

1.1.1. The classical renin-angiotensin system

The rate-limiting enzyme in the RAS cascade, renin, is synthesized from an enzymatically inactive biosynthetic precursor, prorenin, and is stored in the juxtaglomerular cells of the kidneys (19, 20, 25-28). Renin is secreted due to low renal perfusion pressure and low sodium delivery to the macula densa (19, 20, 25-28). Renin exerts its enzymatic action onto angiotensinogen, and during this reaction angiotensin I (Ang I) is formed (19, 20, 27, 28). The inactive Ang I is then hydrolyzed by angiotensin converting enzyme (ACE) and chymase to form Ang II, which is the primary active product of the RAS (Figure 1) (19, 20, 27, 28).

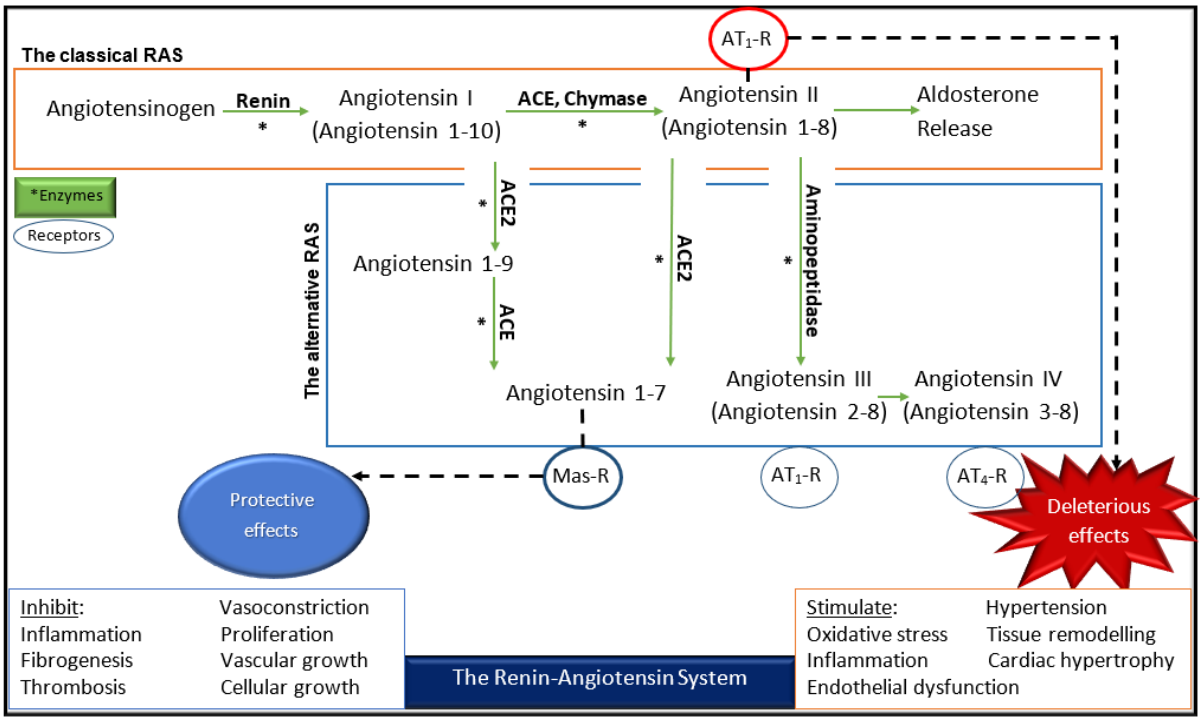

Figure 1. The Renin-Angiotensin System*

The metabolic pathway of the RAS. Dysregulation of the classical RAS causes deleterious effects such as oxidative stress, inflammation, endothelial dysfunction, raised BP and tissue remodelling such as cardiac hypertrophy through the effects of Ang II on its AT_1-R. On the other hand, the alternative RAS has protective effects through Mas receptor activation. This include inhibitory effects on inflammation, fibrogenesis, thrombosis, vasoconstriction and proliferation.
ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; AT_1-R, angiotensin II type 1 receptor; AT_4-R, angiotensin II type 4 receptor; Mas-R, Mas receptor.

* Figure 1 was compiled from sources (28, 29).

Angiotensin II has both physiological and pathophysiological effects. Angiotensin II maintains vascular tone which is important in modifying minute-to-minute changes in BP and blood volume, by regulating aldosterone release for adequate sodium reabsorption and thus water retention (19). On the other hand, the pathophysiological effects of Ang II are exerted through constant binding of Ang II to ATR1 (Figure 2) (19). This persistent binding increases oxidative stress by activating NADPH oxidase, leading to the formation of reactive oxygen species (ROS) (19, 29). Furthermore, Ang II indirectly decreases nitric oxide bioavailability through the binding of ROS such as superoxide to nitric oxide (19, 29). In turn, a decrease in nitric oxide bioavailability and increased endothelin may lead to vasoconstriction, platelet aggregation and the release of plasminogen activator inhibitor (PAI-1) (1, 6). Inflammation is also stimulated through various mechanisms related to increasing vascular permeability and activating signalling pathways associated with cytokine release and upregulation of adhesion molecules

(19, 29). In addition, via stimulating the production of ROS via NADPH oxidase, Ang II plays a role in low-density lipoprotein peroxidation and the up-regulation of lectin-like oxidised low-density lipoprotein receptor (1, 6). Finally, Ang II promotes tissue remodelling through the activation of matrix metalloproteinases causing matrix deposition and cardiac hypertrophy by activating the mitogen-activated protein kinase and growth factor pathways (Figure 2) (19). Ultimately, the Ang II-mediated volume retention, vasoconstriction and pathophysiological effects increase BP and thus contribute to the development of hypertension–mediated target organ damage such as left ventricular hypertrophy (LVH) (19, 28-30).

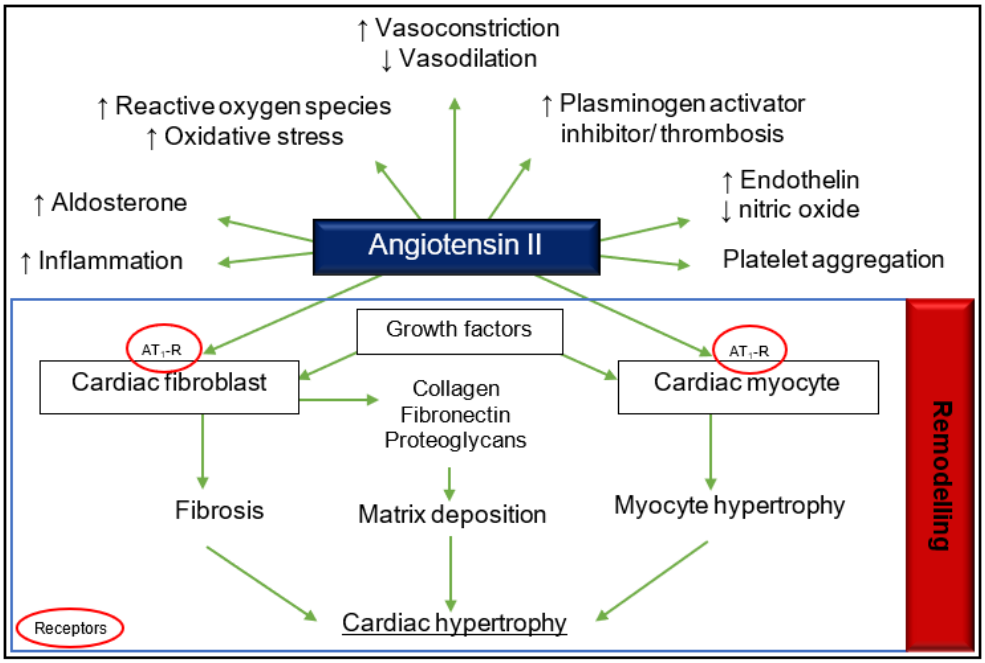

Figure 2. Pathophysiological effects of angiotensin II*

Angiotensin II increases aldosterone, oxidative stress, endothelin, decreases nitric oxide bioavailability and causes vasoconstriction, platelet aggregation and the release of plasminogen activator inhibitor. Inflammation is also stimulated. Tissue remodelling is promoted through the stimulation of cardiac fibroblasts, cardiac myocytes and matrix metalloproteinases causing matrix deposition and cardiac hypertrophy.
AT_1-R, angiotensin II type 1 receptor.

* Figure 2 was compiled from source (31).

1.1.2. The alternative renin-angiotensin system

The alternative branch of the RAS counter-regulates the action of Ang II (27, 30) by exerting cardiovascular protective effects. This pathway of the RAS starts with the removal of the amino acid from the N-terminus of Ang II via the action of aminopeptidases forming angiotensin III (or angiotensin 2-8/Ang III) and angiotensin IV (or angiotensin 3-8/Ang IV) (Figure 1) (27, 30).

The effects of Ang III and Ang IV on cardiovascular function are not well known. Most of the protective effects of this pathway are attributable to angiotensin 1-7 (Ang 1-7), which can be formed through two pathways (27, 29, 32). The first and the most prominent pathway is the conversion of Ang II to Ang 1-7 via the action of angiotensin converting enzyme 2 (ACE2) (27, 29, 32). The second pathway is the conversion of Ang I to angiotensin 1-9 (Ang 1-9) via the action of ACE2 which is then further metabolised to Ang 1-7 by ACE (Figure 1) (27, 29, 32). Ang 1-7 oppose many actions of Ang II on ATR1, especially vasoconstriction and proliferation (27, 29, 32). Furthermore, Ang 1-7 acting on the Mas receptor exerts inhibitory effects on inflammation, and vascular and cellular growth mechanisms (27, 29, 32). This inhibitory effect occurs due to the reduction in key signalling pathways and molecules thought to be relevant for fibrogenesis and thrombosis such as transforming growth factor beta (TGF-β), Smad2/3, extracellular signal-regulated kinase1/2 (ERK1/2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-$_K$B) (Figure 1) (27, 29, 30, 32).

1.2. Cardiac hypertrophy

An increase in LVM is an independent predictor of cardiovascular morbidity and mortality in populations with and without hypertension and is a common finding in patients with CVD or with cardiovascular risk factors (33-35). Increased LVM is a result of physiological adaptations to correct or compensate for increased wall stress and to maintain cardiac output (34, 35). The compensatory responses ultimately alter the myocardium, causing changes in ventricular mass as well as in myocardial cellular structure that leads to the development of fibrosis and cardiac remodelling (34).

1.2.1. Physiological cardiac hypertrophy

In an attempt to classify cardiac hypertrophy, the European Association of Cardiovascular Imaging and the American Society of Echocardiography defined normal relative wall thickness (RWT) as the ratio of twice the posterior wall thickness to the left ventricular internal diastolic diameter with values ranging from 0.32 to 0.42 (17, 36). When incorporating RWT cut-off

values with left ventricular dilatation and LVM to classify LVH, new concepts such as physiologic hypertrophy surfaces (Figure 3) (37).

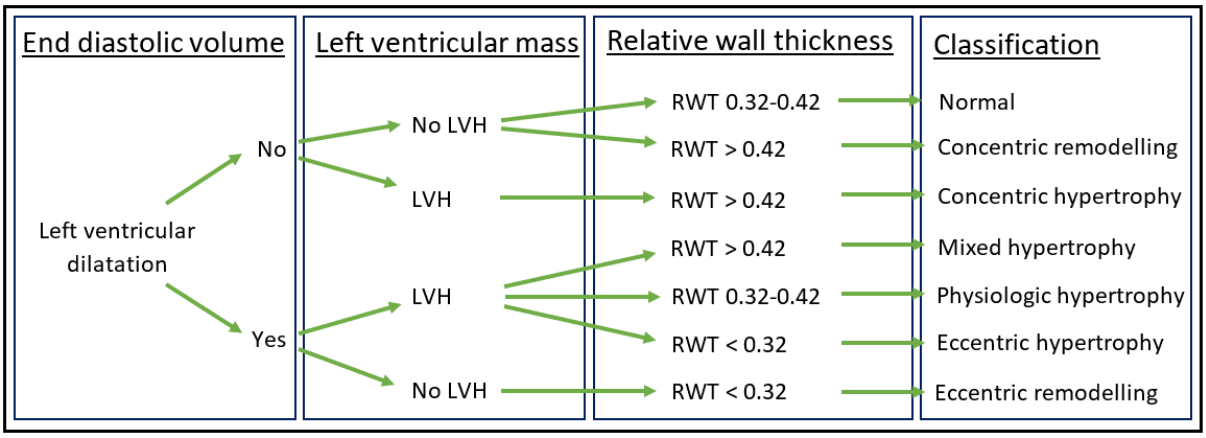

Figure 3. Cardiac hypertrophy classification*

Patterns of left ventricular remodelling based on left ventricular dilation, left ventricular mass and relative wall thickness. LVH, left ventricular hypertrophy; RWT, relative wall thickness.

*Figure 3 was compiled by me using source (17, 37).

Physiological hypertrophy is different in its structural and molecular profile to pathological hypertrophy associated with fibrosis and pathological growth (11, 14, 16, 17, 37-39). In physiological hypertrophy the cardiac structure is normal with normal or enhanced cardiac function (11, 14, 16, 17, 37, 39). Furthermore, physiological hypertrophy in response to exercise training can be subdivided as concentric or eccentric (11, 14, 16, 17, 37-39). Concentric hypertrophy usually develops with isometric or static exercise which involves the development of muscular tension against resistance with little movement, since pressure load on the heart develops rather than volume load (11, 14, 16, 17, 38, 39). Conversely, eccentric hypertrophy usually develops to increase the venous return to the heart and is associated with isotonic exercise which involves the movement of large muscle groups (11, 14, 16, 17, 38, 39).

1.2.2. Pathological cardiac hypertrophy

When the heart faces a chronic hemodynamic burden, such as chronic pressure or volume overload, the heart compensates by augmenting muscle mass (using the frank-starling

mechanism to increase cross bridge formation) and by recruiting neuro-hormonal mechanisms to increase contractility and normalize the pressure or volume overload (14, 16, 17). This ultimately leads to an increase in myocardial muscle mass which is defined as cardiac hypertrophy or pathological hypertrophy (13, 14, 16). Cardiac hypertrophy can be subdivided as concentric or eccentric, based on the changes in shape that are dependent on the initial stimulus (pressure or volume overload) (Figure 4) (10-17, 37). Pathological stimuli causing pressure overload such as hypertension and valvular defects (aortic stenosis) produces an increase in systolic wall stress that results in concentric hypertrophy (increase in ratio of wall thickness to chamber dimensions) (Figure 4) (10-17, 37). Concentric hypertrophy is viewed as a feedback loop that develops in which sarcomeres hypertrophy thereby increasing wall mass to normalize the excess pressure (Figure 4) (10-15, 17, 37). On the other hand, stimuli causing volume overload such as aortic regurgitation produces an increase in diastolic wall stress and results in eccentric hypertrophy (decrease in ratio of wall thickness to chamber dimension) (Figure 4) (10-17, 37). In eccentric hypertrophy the volume overload stimulates the replication of sarcomeres in series, elongating individual myocytes; thus increasing cell length and ultimately increasing the total ventricular volume compensating for the volume overload (Figure 4) (10-14, 16, 17, 37).

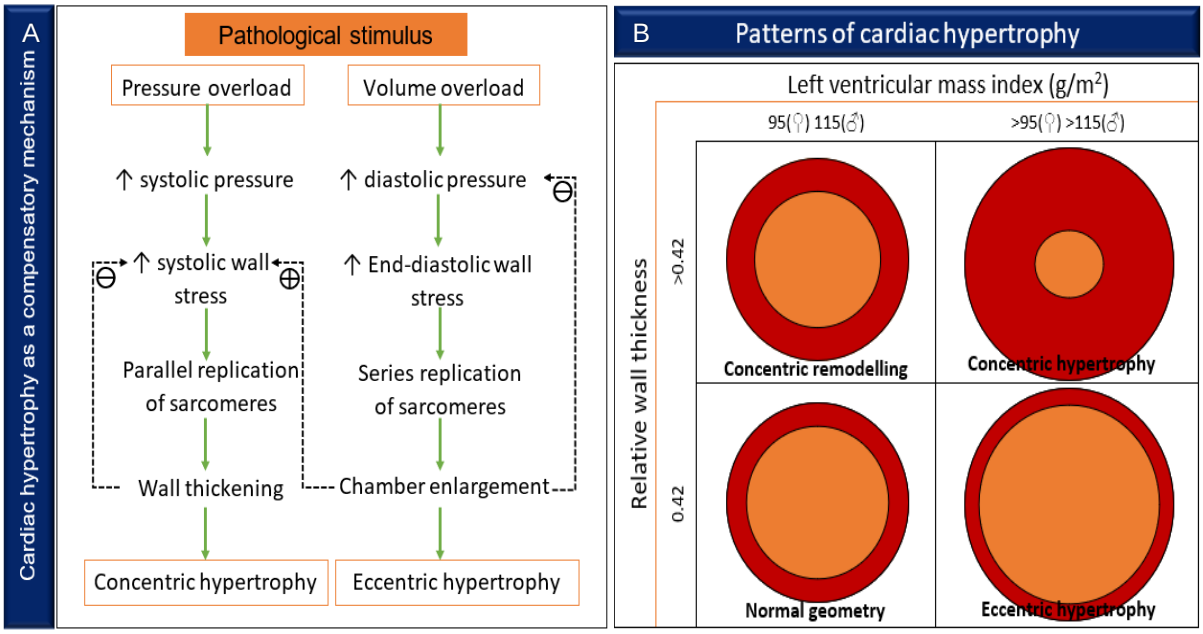

Figure 4. Cardiac hypertrophy as a compensatory mechanism (A) and patterns of cardiac hypertrophy (B)*

Stimuli causing pressure overload produces an increase in systolic wall stress that results in concentric hypertrophy (increase in ratio of wall thickness to chamber dimensions). In concentric hypertrophy the pressure overload stimulates sarcomeres to hypertrophy increasing wall mass to normalize the pressure. Stimuli causing volume overload produces an increase in diastolic wall stress and results in eccentric hypertrophy (decrease in ratio of wall thickness to chamber dimension). The volume overload stimulates the replication of sarcomeres in series, elongating individual myocytes thus increasing cell length and ultimately increasing the total ventricular volume compensating for the volume overload.

*Figure 4 was compiled from sources (10, 12, 13, 17).

Under normal conditions the cardiac myocytes are surrounded by extracellular matrix proteins and collagen fibres which provide a supporting framework for the transmission of mechanical force (10, 11, 15, 16, 37). However, in response to pathological stimuli such as pressure or volume overload, extracellular matrix proteins and cardiac fibroblasts accumulate disproportionately and excessively to compensate for the excess mechanical force (10, 11, 15, 16, 37). This leads to mechanical stiffness due to the accumulation of fibrosis, which contribute to cardiac hypertrophy and may lead to systolic and diastolic dysfunction (10, 11, 15, 16, 37). Furthermore as the cardiac muscle mass increases, the coronary reserve decreases and the oxygen requirement increases, which can lead to ischemia and death (10, 11, 13-16, 37). Cardiac hypertrophy is compensated growth in response to pathological stimuli. Consequently the hypertrophied heart may eventually decompensate leading to left ventricular dilation and heart failure (11-13, 16, 37).

1.3. The renin-angiotensin system and cardiac structure

The effects of a dysregulated RAS on LVM are entwined in several mechanisms (Figure 5). A previous study designed to evaluate the effects of renin levels on LVM in essential hypertension concluded that the degree of LVH is similar in low, normal and high renin hypertensives and is proportional to the degree of hypertension (40). A study focusing on the relation between plasma renin levels and end organ damage in an urbanized African population demonstrated low renin to be adversely associated with renal function (41). In this study the low renin group also reported increased total peripheral resistance, as opposed to the high renin group (41). The increased total peripheral resistance in the low renin group may have played a part in the development of low renin hypertension and may therefore contribute to subsequent cardiac remodelling (41). An increased LVMi (index) was also evident in Afro-Caribbeans with low plasma renin activity (PRA) and higher aldosterone compared to their white counterparts (42). In addition, it was demonstrated that increased BP over time suppresses renin in a black South African cohort (43). Furthermore, cardiac hypertrophy and fibrosis can be stimulated through binding of renin/prorenin to the soluble (pro)renin receptor (44, 45). The (pro)renin receptor is a 350–amino acid transmembrane protein consisting of a large N-terminal extracellular domain, a single transmembrane protein, and a short cytoplasmic domain (24, 45). The extracellular domain is cleaved to generate a soluble form of the (pro)renin receptor (24, 45). The activation of the (pro)renin receptor causes intracellular induction of the mitogen-activated protein kinase pathways, leading to increased cell proliferation and up-regulation of profibrotic genes that cause cardiac hypertrophy and fibrosis (Figure 5) (44, 45).

On the other hand, both high Ang II and aldosterone are associated with cardiac remodelling, collagen turnover and the formation of fibrous tissue (Figure 5) (46-51). Aldosterone which interacts with the mineralocorticoid receptors in the heart promotes myocardial fibrosis (Figure 5) (47-49, 51, 52). Similarly, Ang II, acting via ATR1, induces myocardial fibrosis, myocyte and fibroblast cell growth; thus leading to cardiac remodelling (Figure 5) (29, 47-49, 51, 53). In

addition, aldosterone mediates and exacerbates the deleterious effects of Ang II (47-49). Despite most of the results being on hypertensive subjects, it may be possible that cardiac structural changes precede hypertension (54, 55). This was demonstrated in a study conducted on a young population (aged 7 to 18 years old; 71.6% African American and 62.7% boys) where it was found that the African-American children had significantly lower serum aldosterone concentrations and PRA compared to their white counterparts (54). It was concluded in this afore-mentioned study that the aldosterone-to-renin ratio was positively correlated to LVMi, suggesting early cardiac remodelling (54). Another study demonstrated a positive association between aldosterone and LVMi in African-American boys (aged 15 to 19) (55). It is important to note that the latter two studies share a common feature in that the participants were not diagnosed with hypertension and that the black population seemed to be at higher risk of developing increased LVM at a younger age.

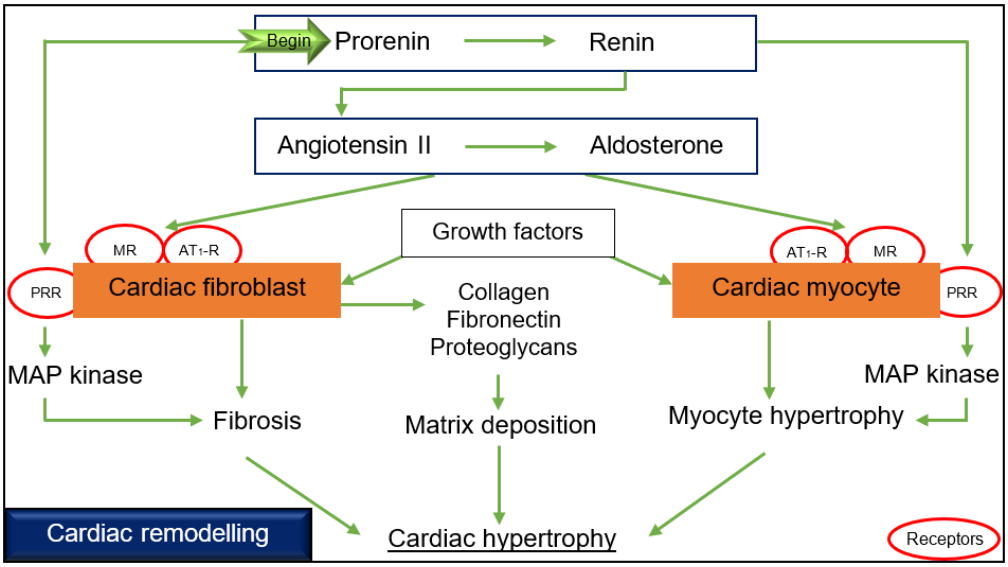

Figure 5. The renin-angiotensin system and cardiac structure*

The effects of a dysregulated RAS on LVM are entwined in several mechanisms. The activation of the (pro)renin receptor by prorenin/renin causes intracellular induction of the mitogen-activated protein kinase pathways, leading to increased cell proliferation and up-regulation of profibrotic genes that cause cardiac hypertrophy and fibrosis. Furthermore, both Ang II acting an AT_1-R and aldosterone acting on mineralocorticoid receptors cause cardiac remodelling through stimulating cardiac fibroblasts and cardiac myocytes.
AT_1-R, angiotensin II type 1 receptor; MR, mineralocorticoid receptors; PRR, (pro)renin receptor; MAP kinase, mitogen-activated protein kinase.

*Figure 5 was compiled from sources (44, 45,47-49).

Angiotensin 1-7, which is present in the heart, opposes many of the adverse effects of Ang II on cardiac function, including hypertrophy, fibrosis and remodelling (27, 29, 32, 51, 53).

Furthermore, it was shown in animal models that a decrease in Ang 1-7 and ACE2 results in early cardiac hypertrophy, fibrosis and remodelling (27). Seeing that ACE2 holds a higher affinity for Ang II than Ang I to form Ang 1-7 (27, 30), it may be postulated that the lower levels of Ang II reported in the black population may have a negative impact on the protective branch of the RAS.

1.4. The renin-angiotensin system, cardiac hypertrophy and confounding factors

Ethnicity. Low renin and therefore low renin hypertension is highly prevalent in the black population as opposed to their white counterparts, as reported by several studies (28, 41, 43, 54, 56-59). The elderly black population and black women have lower levels of renin than the white population and black males respectively (43, 56, 57). In addition, it was indicated that hypertensive black men have lower Ang I, Ang II and Ang 1-5 (a metabolite of Ang 1-7) levels than white men (28). However, PRA (and therefore the downstream cascade) was found to be lower in the black population, regardless of hypertensive status (53). Lastly, black children also present with low renin levels as opposed to white children (54); thus suggesting that this phenomenon is independent of age. Data on the black population for the detailed peptides of the RAS Fingerprint®, including the alternative RAS pathway, is very limited (28). Additionally, since most studies focus on older and diseased populations, limited information is available on young populations. Genetic factors associated with ethnicity also affect left ventricular geometry or left ventricular remodelling (12, 13, 17, 60). In a study that focused on the effects of BP and its relation to left ventricular geometry concluded that elevated BP levels have stronger detrimental effects on the patterns of LVH in the black population than is the case with their young (aged 24-47) white counterparts (60). Black individuals might therefore be more susceptible than white individuals to BP-related adverse cardiac remodelling (12, 13, 17, 60). Furthermore, it is well known that both hypertension and LVH are more common in black individuals than in their white counterparts (12, 13, 17, 61-63).

Gender. Sex hormones play an important role in the pathogenesis of cardiovascular and kidney disease (64). Clinical studies have demonstrated that the components of the RAS are markedly affected by sex hormones (32). Premenopausal women are protected to some degree from the effects caused by a dysregulated RAS on the cardiovascular and renal system (64). In general, estrogen increases angiotensinogen and decreases renin levels, ACE activity, AT1 receptor density and aldosterone production (64-66). Estrogen also increases AT2 and Mas receptor density (64, 67). Furthermore, the ACE2 gene is located on the X chromosome (64, 67). This may explain in part the higher ACE2 activity and therefore Ang 1-7 in women, which have been shown to counter-regulate the effects of Ang II on ATR1 receptors (64, 67). Progesterone also competes with aldosterone for mineralocorticoid receptor binding (64). On the other hand, testosterone seems to increase renin levels, ACE activity and therefore Ang II (64). It therefore seems that men, compared to women, are at higher risk of developing subsequent complications caused by a dysregulated RAS. Furthermore, LVM, volume and cardiac linear dimensions are significantly larger in men than in women (12, 17, 38, 68-70). Regardless, it seems that women, particularly black women, are at higher risk of concentric hypertrophy than men (12, 13, 60).

Body composition and physical activity. Measures of body composition such as total body fat, lean body mass and body mass index are factors that affects the RAS and LVM (71-75). Furthermore, physical activity also effects the RAS (71-73) and cardiac structure (11, 14, 16, 17, 38, 39, 75) due to its association with lean body mass. In addition, physical activity is associated with a decrease in renal perfusion pressure, sodium delivery to the macula densa, activation of sympathetic nervous system and a reduction in hormone clearance rate, these changes cause the activation of the RAS (71). With regard to cardiac structure, physical activity such as isometric or isotonic exercises usually cause physiological hypertrophy to increase the venous return and the cardiac output to meet the oxygen demands of the body (11, 14, 16, 17, 38, 39). As a result, normalization of LVM is required to adjust for body composition (17, 38, 68, 69). Body height and body surface area are commonly used to index

LVM. (68, 69). Height is strongly associated with lean body mass; however it does not make allowance for obesity (68, 69). Obesity is associated with LVH and indexing left ventricular measurements by weight alone might fail to detect pathological levels of LVM (12, 38, 68, 69). Conversely, body surface area makes partial allowance for obesity as weight is used in conjunction with height to determine body surface area increasing its accuracy and allowing the detection of pathological LVM (68, 69).

Age. With ageing the systemic and intrarenal RAS are suppressed (76, 77). Compared to the young, older populations present with lower levels of plasma renin, ACE and aldosterone (76, 77). Furthermore, older populations show an impaired ability to trigger appropriate responses to RAS stimuli (76, 77). This may be attributed to the progressive functional deterioration and structural change in the kidney, which is demonstrated by the progressive decline in glomerular filtration rate (76, 77). This decline may be due to a decrease in the number of functional glomeruli and increase in the number of sclerotic glomeruli (76, 77). Furthermore, these changes alter the activity or responsiveness of the RAS (76, 77), and these RAS changes may predispose individuals (particularly the elderly) to fluid and electrolyte imbalances which may relate to an increased risk for the development of hypertension and compensatory structural changes in the heart, and vice versa (76, 77). Ageing also results in the progressive deterioration in the structure and function of the heart, which results in the increase of cardiac mass (78, 79). The increasing cardiac mass with age is due to the dysregulation of growth factor signalling pathways, calcium homeostasis, production of ROS, extracellular matrix remodelling and dysregulation of neuro-hormonal signalling pathways such as the RAS (78). It also seems that women are at greater risk of cardiac hypertrophy with aging as demonstrated by a study focused on subjects free of hypertension and coronary heart disease (78). This afore-mentioned study found an increase in LVMi only for women with the progression of age (79, 80). Furthermore, it is well known that age is associated with the structural changes in the arterial system (79, 81-83). This includes hypertrophy, extracellular matrix accumulation, calcium deposits, decreased release of vasodilators, increased release

of vasoconstrictors and increase in vascular stiffness due to the increase in collagen production and the decrease of elastin (79, 81-83). Arterial stiffness causes increased systolic BP and pulse pressure, which in turn can lead to cardiac hypertrophy and impaired cardiac function (79).

Salt intake. A high sodium diet also plays a part in cardiac remodelling in addition to Ang II and aldosterone (48, 49, 51, 84, 85). Furthermore, acute sodium loads are known to suppress PRA and therefore cause a downstream dysregulated RAS and elevated BP (48, 49, 51, 84, 85). However, in the young adults of the African-PREDICT study, LVMi was already demonstrated to be positively associated with a high sodium excretion (reflecting a high sodium diet) in participants who presented with masked hypertension compared to true normotensives (86). This indicates that a higher salt intake may contribute to increased LVM which is potentially driven by early hypertension development (86). Therefore, more focus needs to be placed on the early phases of hypertension development and the components implicated in this process such as the RAS.

Socio-economic status. Literature on SES and how it relates and affects the RAS is limited. However, the RAS can be indirectly influenced by hypertension (87), which is associated with low SES (88). Dietary sodium intake also influences and activates the RAS (89, 90), placing groups of low SES in which sodium intake is higher at greater risk (91-93). Furthermore, black individuals retain more sodium which can increase fluid volume and thus lead to cardiac hypertrophy (94). Demographic factors also modulate the manner in which the ventricles respond to an elevation in BP (2-9, 13), placing black Africans at a greater risk of developing cardiac hypertrophy as they often have a lower SES (8, 95). This was also demonstrated by a study aimed at determining the relationship between SES, ethnicity and LVM (90). This afore-mentioned study demonstrated that in adults free of clinically overt CVD, SES was independently and inversely associated with LVM among hypertensive and normotensive black individuals (90). Similarly, a study conducted in Angola concluded that groups of low SES were more affected by hypertension, smoking and LVH (96). Furthermore, low SES at

childhood is associated with LVM and impaired diastolic performance more than three decades later even after adjustment for conventional cardiovascular risk factors at childhood and adulthood (97). This suggests that low SES not only affects current health but can also lead to adverse health outcomes with ageing.

1.5. Motivation

A dysregulated RAS is linked to cardiovascular consequences such as hypertension and LVH (28, 30, 98). However, the majority of studies on the RAS were performed on older populations (76, 77) and populations with hypertension (19, 40, 46, 65). Furthermore, limited information is available on SES and its relation to the RAS. Whether the same observations between the RAS and cardiovascular consequences will be made in a young population is uncertain. Advanced technology such as the RAS Fingerprint® provides increased precision necessary for angiotensin peptide analysis which is important in profiling young healthy populations where angiotensin peptide concentrations are expected to be low (99-101). A clear profile of the RAS in young populations is necessary to understand early hypertension development; therefore more focus needs to be placed on the youth. It is also important to establish the relationship between RAS components and LVM in a young normotensive cohort, before the onset of hypertension. This knowledge may contribute to a better understanding of early hypertension development in black and white populations and may aid in the development of new and cost-effective therapeutic strategies for raised BP, particularly in a low SES setting (8, 59, 95).

2. Aim and objectives

The aim of the study is to investigate the relationship between LVMi and the RAS components in young (20-30 years) healthy participants of the African-PREDICT study while considering SES, ethnicity and sex.

The following objectives were formulated:

- To determine the relationship between LVMi with PRA-S (Surrogate), Ang I, ACE-S (Surrogate), Ang II and aldosterone in this study population.
- To determine whether the relationships between LVMi and the RAS components are dependent on SES, ethnicity and sex.

3. Hypotheses

The following hypotheses were formulated taking into consideration the literature and the objectives of the study:

- Left ventricular mass index will be positively associated with PRA-S, Ang I, ACE-S, Ang II and aldosterone.
- The relationship between LVMi and RAS components will be dependent on SES, ethnicity and sex.

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CHAPTER 2

METHODOLOGY

1. Methodology

1.1. Study design and participants

The African Prospective study on Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT study) is a longitudinal study conducted in and around the Potchefstroom area of the North West Province (Figure 1) (1). The African-PREDICT study is designed to investigate early cardiovascular disease- (CVD) related pathophysiology and to identify early markers or predictors of CVD by tracking young apparently healthy black and white South Africans over time. This will enable the implementation of prevention programs in the long term. Details of the study protocol were previously published (1).

Figure 1. Maps indicating South Africa and the North-West Province*

* Figure 1 was compiled by using Google Maps.

Participants were invited to take part in the study through various strategies such as advertisements in newspapers, on notice boards, by health screening in public places and also by direct recruitment at workplaces. Participants were recruited on a voluntary basis and therefore constitute a convenience or availability sample, stratified into different ethnic (black and white), sex and socio-economic class groups (low, mid, high). Potential participants who expressed interest in participating in African-PREDICT underwent two phases, which are the screening (to determine whether they met the inclusion criteria for participation in the African-PREDICT study, Table 1) and the research study phases. Eventually, apparently healthy black

and white participants (N=1202) between the ages of 20 and 30 years were included in the African-PREDICT study from 2013 to 2017.

Table 1. Inclusion criteria and exclusion criteria of the African-PREDICT study

Inclusion criteria	Exclusion criteria
• Office BP<140/90 mmHg • Age of 20-30 years • Self-reported black and white ethnicity • Evenly distributed males and females (self-reported) • Not using chronic medication (self-reported) • Not pregnant or lactating females (self-reported)	• Diagnosed Type 1 or 2 Diabetes Mellitus. • Elevated glucose >5.6 mmol/L and confirmed glycated haemoglobin (HbA1c) ≥ 6.5%, Microalbuminuria > 30 mg/ml in spot morning urine or proteinuria, HIV infected, recent surgery or trauma (within the past three months), previous history of chronic diseases, stroke, angina pectoris or myocardial infarction

1.2. Data collection

1.2.1. Organisational procedures

Screening commenced in November 2012 and those participants that met the criteria of inclusion (Table 1) were contacted to take part in the African-PREDICT study. Detailed information was provided in advance, namely the processes involved in the screening and research phase. During 2013-2017 (baseline research phase) all research measurements and sampling took place at the Hypertension Research and Training Clinic (building F12 on the Potchefstroom campus, North-West University) under the supervision of a registered research nurse. A maximum of 4 participants were accommodated per day to ensure quality of the detailed measurements. Transport to and from the clinic was provided for individuals that had no means of transport. Participants arrived approximately 8 a.m. at the Hypertension Research and Training Clinic, and it was requested of them that they fasted overnight for at least 8 hours. The measurement procedures were again explained to the participants as captured in the informed consent form. The participants were also granted the opportunity of asking questions, and after written consent had been obtained, the measurements commenced. The measurements were taken in a private temperature-controlled room to ensure the accuracy of the measurements. After completion of all the measurements and

biological sampling, participants received a light meal that excluded caffeine. The participants of the study also received a grocery voucher as a token of appreciation for their time, and at approximately 1 p.m. participants were transported to their homes.

1.2.2. Questionnaire data

The questionnaires (Figure 2) were completed with the aid of the research nurse, trained research assistants and trained postgraduate students. General Health and Demographic Questionnaires were completed online on a web-based program and included demographic information, employment information, education, income, alcohol use, tobacco use, medication use (including hormonal contraceptive use) and family history of CVD.

Socio-economic status (SES) was calculated using a point system adapted from Kuppuswamy's Socioeconomic Status Scale 2010 (2) for a South African environment. Participants were scored in three categories: skill level (classified according to the South African Standard Classification of Occupation (SASCO)), education and income. These three factors were scored and used to categorise participants into low, middle and high socio-economic groups, and were determined as a continuous variable, namely SES score. Lower SES was shown to be an independent predictor of increased left ventricular mass (LVM) among hypertensive and normotensive blacks (3, 4). Furthermore, lower SES may influence the renin-angiotensin system (RAS) indirectly due to its association with hypertension (5). Interaction for ethnicity, sex and SES on the relationships between LVM index (LVMi) with the RAS were tested (Table S1), and based on these findings and the literature (3-5), participants were grouped to compare black and white men and women with low vs high SES.

Figure 2. Questionnaires

Images illustrating questionnaire data obtainment. A and B online web-based questionnaires.

1.2.3. Body composition and physical activity assessments

Anthropometric measurements (Figure 3) were done by a trained researcher who used the standard procedures as indicated by the International Society for the Advancement of Kinanthropometry (ISAK) (6). Measurements were done in a private, temperature-controlled room to obtain height (m) determined by the SECA 213 Portable Stadiometer (SECA, Hamburg, Germany), weight (kg) using the SECA 813 Electronic Scales (SECA, Hamburg, Germany) and waist circumference (cm) (Lufkin Steel Anthropometric Tape; W606 PM;Lufkin, Apex, USA). The body mass index (BMI) (weight (kg) / height (m^2)) was then calculated. Body surface area was calculated using the Mosteller equation (7). In addition to being thorough in the description of the study population, measures of body composition such as total body fat, lean body mass and body mass index are factors that affect LVM; therefore these anthropometric measurements are included in this study (8, 9).

ActiHeart physical activity monitor (CamNtech Ltd., England, UK). The ActiHeart device was fitted by trained researchers in a temperature-controlled private room. This is a compact, chest-worn monitoring device that records heart rate, inter-beat-interval and physical activity in one combined unit. It is designed for capturing heart rate variability data and for calculating and measuring activity energy expenditure. After being fitted with the ambulatory blood pressure (BP) apparatus, participants were also fitted with an ActiHeart physical activity monitor and the device was worn for a maximum of 7 consecutive days. Physical activity

effects cardiac structure (9) and the RAS (10-12) and is therefore included as a covariate in this current study to take into account the possible effects thereof on LVM and the RAS.

Figure 3. Anthropometric measurements

Images illustrating anthropometric measurements. A, height; B, neck circumference; C, waist circumference.

1.2.4. Cardiovascular measurements

Brachial blood pressure measurements (Figure 4A) were taken using the validated (13) Dinamap Procare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA). The participants were fitted with an appropriately sized brachial cuff before commencement of the measurement. Participants should not have exercised, smoked or eaten for the last 30 minutes prior to measurement being taken. The measurement was taken on the left and right arm in duplicate after the participants had been in a quiet state for 5 minutes (seated with the arm supported at heart level). Systolic BP, diastolic BP and heart rate were captured for each measurement. Blood pressure not only affects LVM (14, 15), but also RAS (16). It is therefore important to evaluate the effects thereof as a covariate in this current study.

Ambulatory blood pressure measurements (ABPM) were done using a 24h ABPM apparatus (Card(X)plore, Meditech, Budapest, Hungary). An appropriately sized cuff was fitted on the

participant's non-dominant arm. The participants were given instructions to ensure the successful inflation of the device across the 24h time period. The device measured BP in 30-minute intervals during daytime (6 a.m. to 10 p.m.) and hourly during the night (10 p.m. to 6 a.m.). Participants completed an ambulatory diary card for the time period of measurements. The participants of this current study population had a mean successful cuff inflation rate of 88%. As mentioned, BP not only affects LVM (14, 15), but also the RAS (16). Furthermore, ambulatory BP is demonstrated to be a stronger predictor than brachial BP, it also reveals participants presenting with masked hypertension, which was therefore included in this study (17).

Total peripheral resistance (Figure 4B) was tested by a trained researcher (including myself) using the validated (18-20) Finometer device (Finapres Measurement Systems, Amsterdam, The Netherlands). Each participant was requested to lie in the Fowler's position with their arm at heart level in a temperature-controlled room. A finger cuff was placed on the middle phalanx of the left hand's middle finger, and a brachial cuff was connected to the upper arm. A 2-minute calibration is performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial arterial pressure (19). Continuous measurement of resting cardiovascular variables was performed for a 5-minute period. Beatscope® software was used to calculate cardiovascular measurements such as total peripheral resistance (21). A low renin state (a dysregulated RAS) is associated with increased total peripheral resistance; therefore total peripheral resistance is included in the study as a covariate (22).

Echocardiography measurements (Figure 4C,D) were used to evaluate and calculate left ventricular geometry due to its sensitivity and reliability (23). A standard transthoracic echocardiogram was performed for each participant, while using the General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway), with a 2.5–3.5 MHz transducer and a single ECG lead (24). Each participant was scanned in a private and darkened room, resting in a partial left decubitus position with the head of the examining table slightly elevated. Left ventricular dimensions were measured by one clinical technologist specialist according to the

guidelines of the American Society of Echocardiography (24, 25). Echocardiography data were analysed using the EchoPAC software (GE, version 10.8.1) to determine measures of left ventricular structure. From these dimensions, we determined the interventricular septum thickness at end-diastole (IVSd), the left ventricular posterior wall thickness at end-diastole (LVPWd), the left ventricular internal diameter at end-diastole (LVIDd) and relative wall thickness (RWT) (defined as the ratio of twice the posterior wall thickness and LVIDd). Left ventricular mass was calculated by means of the corrected Devereux formula [LVM = 0.8 (1.04 $(IVSd + LVID + LVPWd)^3 - (LVID)^3 + 0.6)$] and was normalised for body surface area, from which LVMi (index) was derived (26). Left ventricular mass index was demonstrated to increase the predictive value of left ventricular hypertrophy significantly better than non-indexed LVM and those indices without weight (27, 28). Left ventricular mass is indexed in an attempt to adjust for variation in body composition since a complex relationship exists between body size, body composition and cardiac changes (27, 28).

Figure 4. Cardiovascular measurements

Images illustrating cardiovascular measurements. A, Brachial BP monitoring; B, total peripheral resistance; C and D, echocardiography measurements.

1.2.5. Biological sampling and biochemical analyses

In the early morning fasting blood samples (fasted overnight for an 8-hour period) were taken from the brachial vein branches by a registered nurse using a sterile winged butterfly infusion set and syringe in a temperature-controlled private room. Participants were requested to provide a spot urine and 24h urine sample. Each participant was given instructions and the necessary equipment to obtain a successful 24h urine sample on a day that was convenient for them (which was noted). The first urine of the day was to be discarded and all urine passed thereafter was to be collected in the provided container, including the first urine of the following morning (day two). The start and finish times were recorded. The 24h urine collection was executed in accordance with the Pan American Health Organisation/World Health Organisation protocol for population level sodium determination in 24h urine samples (29). Incomplete urine collections were defined as a volume <300mL/24h and/or a 24h creatinine excretion of <4mmol/>25mmol (women) and <6mmol/>30mmol (men) (30). The research assistant (that included me, trained in handling the biological samples) secured the samples in a closed container. The biological samples were immediately transferred to the onsite Research Laboratory where the blood samples were centrifuged and aliquoted into cryovials for storage in a biofreezer at –80ºC (Figure 5A,B). The urine samples were aliquoted and placed in a −80 °C freezer.

Serum samples were analysed for gamma-glutamyltransferase (enzymatic colorimetric assay), the lipid profile (total cholesterol (enzymatic, colorimetric method), high-density lipoprotein cholesterol (homogeneous enzymatic colorimetric assay), low-density lipoprotein cholesterol (homogeneous enzymatic colorimetric assay) and triglycerides (enzymatic colorimetric test), creatinine (kinetic colorimetric assay - Jaffé method), C-reactive protein (particle-enhanced turbidimetric assay) using the Cobas Integra® 400 plus (Roche, Basel, Switzerland). Sodium fluoride plasma samples were used to determine glucose levels (enzymatic reference method) using the Cobas Integra® 400 plus (Roche, Basel, Switzerland). Urinary (24h) sodium and potassium were measured by means of ion-selective

electrode potentiometry on the Cobas Integra® 400 plus (Roche, Basel, Switzerland) from which sodium excretion and 24h sodium-potassium ratio were calculated (31). Estradiol, progesterone and cortisol were measured using electrochemiluminescence method on the e411 (Roche, Basel Switzerland) in serum samples (Figure 5C,D). For the above-mentioned biochemical analysis, both intra and inter assay coefficient of variation (CV) ≤ 4.1%. Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany) apparatus (intra assay CV ≤ 10.8%). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate estimated glomerular filtrate rate (eGFR) from serum creatinine (32). Gamma-glutamyltransferase (33), cholesterol (34, 35), glucose (36), creatinine (37), C-reactive protein (38) and cotinine (39) are associated with CVD, including hypertension and increased LVM. These covariates may also influence the RAS, both directly and indirectly, through its link with hypertension (40-47). Furthermore, both sodium and potassium are associated with CVD (48-50), and the RAS and are linked to key processes involved in the system (51, 52). Sex hormones have also been demonstrated to play an important role in the pathogenesis of cardiovascular and kidney disease (53, 54) which may affect the components of the RAS (32). Lastly, cortisol is added as a covariate due to its association with stress (55, 56). During stress both the hypothalamic-pituitary-adrenal axis and the sympathetic-adrenal-medullary axis are activated which in turn influences cardiac structure (55) and the RAS (56).

Table 2. Summary of biochemical analyses

Sample type	Variables	Apparatus	Method of analysis
Serum samples	Gamma-glutamyltransferase	Cobas Integra® 400 plus (Roche, Basel, Switzerland)	Enzymatic colorimetric assay
	Total cholesterol		Enzymatic, colorimetric method
	High-density lipoprotein cholesterol		Homogeneous enzymatic colorimetric assay
	Low-density lipoprotein cholesterol		Homogeneous enzymatic colorimetric assay
	Triglycerides		Enzymatic colorimetric test
	Creatinine		Kinetic colorimetric assay - Jaffé method
	C-reactive protein		Particle enhanced turbidimetric assay
Sodium fluoride	Glucose levels		Enzymatic reference method

plasma samples			
Urine 24h samples	Sodium and Potassium		Ion-selective electrode potentiometry
Serum samples	Estradiol, Progesterone Cortisol	e411 (Roche, Basel Switzerland)	Electrochemiluminescence method Electrochemiluminescence method Electrochemiluminescence method
	Cotinine	Immulite (Siemens, Erlangen, Germany)	Chemiluminescence method
	The renin-angiotensin system	RAS Fingerprint® (Attoquant Diagnostics, Vienna, Austria).	Ultra-pressure-liquid chromatography-tandem mass spectrometry

Components of the RAS were analysed using the RAS Fingerprint® (Attoquant Diagnostics, Vienna, Austria). Analyses were performed using ultra-pressure-liquid chromatography-tandem mass spectrometry (LC-MS/MS) generated multiplex parameters consisting of the precisely quantified concentrations of 10 angiotensin (Ang) peptide metabolites (Ang I (CV=11.2%), Ang II (CV=7.3%), Ang 1-9, Ang 1-7, Ang 1-5, Ang 2-8, Ang 3-8, Ang 3-7, Ang 2-7, Ang 2-10) and aldosterone (CV=9.6%). Equilibrium angiotensin levels were further used to calculate the validated (57) surrogate markers, plasma renin activity (PRA-Surrogate) (Ang I + Ang II) and angiotensin converting enzyme (ACE-Surrogate) (Ang II/Ang I). Analytical techniques such as immunoassays are not ideal for the quantification of the RAS components due to the dynamic nature of the RAS components; very short angiotensin metabolite half-lives, rapid on-going angiotensin generation and antibody cross-reactivity (sharing molecular sequences with corresponding upstream metabolites including the highly abundant angiotensinogen) (58-60). Furthermore, poor specificity and sensitivity of these methods is one of the main reasons behind contradictory findings on angiotensin data among different studies (58-60). The RAS Fingerprint® technology uses LC-MS/MS, a special optimized angiotensin protease inhibitor cocktail (Circulating Angiotensin Levels), and adheres to sophisticated sample analytical protocols to thoroughly control the entire process from sampling down to signal detection; thus assuring the integrity and reproducibility of angiotensin

data (58-60). Furthermore, different panels exist for circulating plasma angiotensin levels, endogenous tissue angiotensin levels and plasma equilibrium levels increasing the reliability of the analysis (58-60). Hence the RAS Fingerprint® exhibited unopposed precision in determining multiple peptides in the pico-molar concentration range in biological samples which is important in profiling young healthy populations where angiotensin peptide concentrations are expected to be low (58-60).

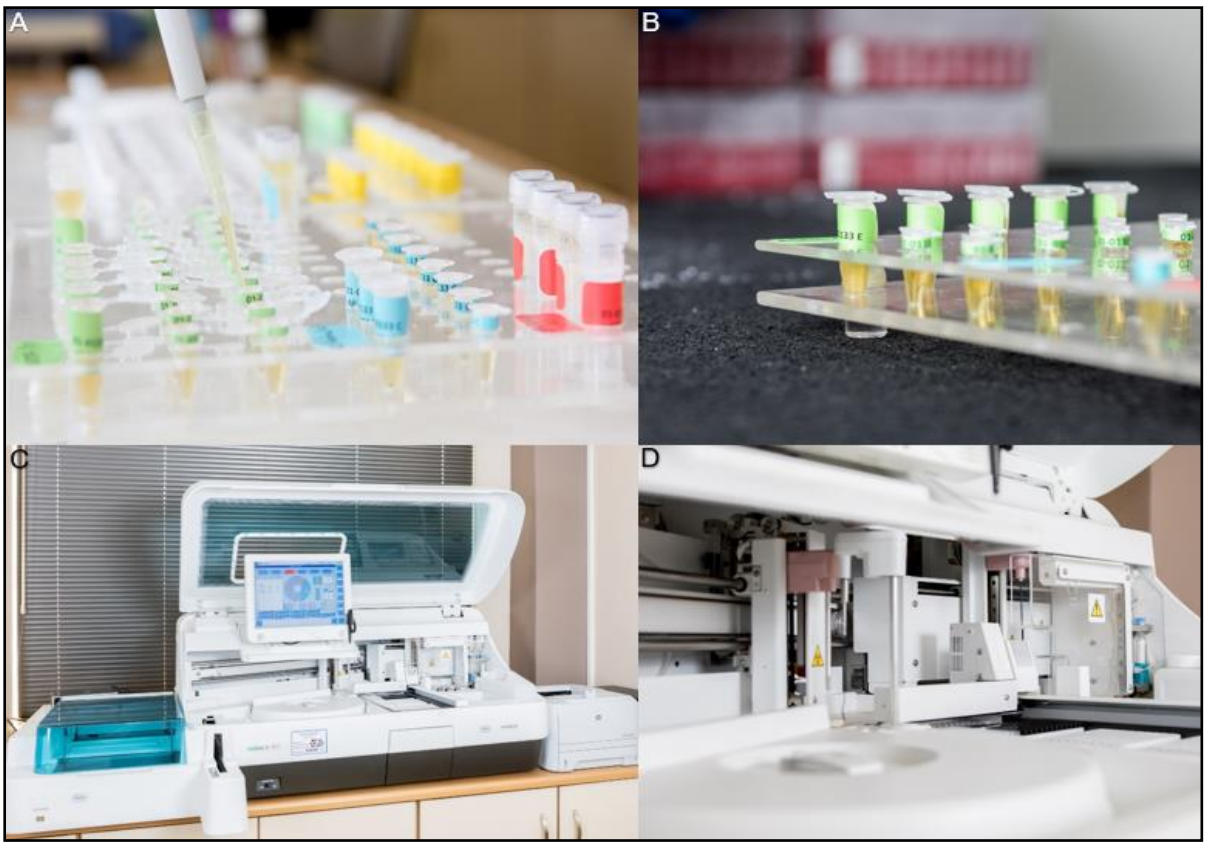

Figure 5. Biological sampling and biochemical analyses

Images illustrating biological sampling and biochemical analyses. A and B, aliquoting samples; C and D, biochemical analyses.

1.2.6. Data management

Data were anonymised and captured into REDCap (Research Electronic Data Capture, see http://projectredcap.org (61)), a password-protected database which is stored online and offline to ensure the safety of the data and privacy of the participants. Questionnaires were stored in a locked storage room in the Hypertension Research and Training Clinic.

1.2.7. Statistical analyses

Statistica 13.3. (Tibco software, Palo Alto, CA, USA) was used for all data analyses. Statistical analyses are explained in detail in Chapter 3.

2. Ethical Considerations

Information with regard to the ethical considerations, protocol, procedures and precautions of this MHSc study, are in coherence to those of the African-PREDICT study and no re-consent was needed, as all these measurements were described in detail within the original informed consent signed by all participants. Furthermore, the use of existing data from this study and the relevant above-mentioned methodology would not expose the participants to any additional mental, physical or emotional risks. The African-PREDICT study (NWU-0000-12-A1) and this MHSc study (NWU-00032-19-A1) were evaluated and approved by the Health Research Ethics Committee of the North-West University and adhere to the principles set out in the Declaration of Helsinki. This study had no direct benefit for the participants. However, the knowledge obtained during the study will add to the body of literature surrounding RAS and how it relates to LVM in the young normotensive black and white population (benefit of health promotion). Furthermore, all images used in this chapter are used with the consent of the applicable individuals.

3. Student contributions

I was involved in the research measurement phase of two studies conducted by the Hypertension in Africa Research Team (HART) while I was working in the Hypertension Research and Training Clinic. In the African-PREDICT study I aided in the collection of data by operating the Finometer device (FMS, Finapres Measurement Systems, Amsterdam, The Netherlands) and the SphygmoCor XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) - measures pulse wave velocity, a marker of arterial stiffness. Furthermore, I did laboratory work which included centrifugation and aliquoting of samples into individually marked cryovial tubes. With regard to the other HART study, the EndoAfrica study, I assisted the registered

nurse with the collection, preparation and long-term storage of blood samples (laboratory work) in the early morning (8 a.m.). I was also involved in sample sorting for shipment for the PURE study (another HART study). This involved placing cryovials into appropriately marked bags and capturing it in an excel sheet. Additionally, I contributed in taking BP during the May Measurement Month, a global awareness campaign initiated by the International Society of Hypertension. With regard to the dissertation, I was responsible for writing the proposal and ethics application, performing extensive literature research, dataset cleaning and statistical analyses, design and planning of the research article, interpretation of the results and writing all sections of the dissertation. Lastly, I presented the results of the study at the Medical Research Council Newton Project Workshop on 29th of October 2019 at Bakubung Bush Lodge, Pilanesberg National Park.

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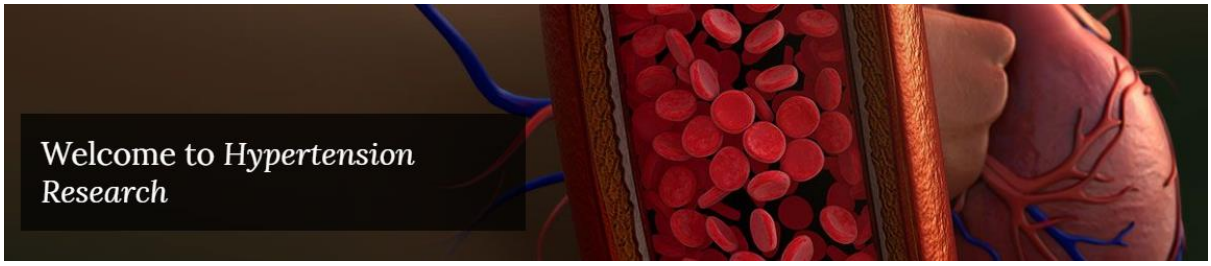
CHAPTER 3

DOES LOW SOCIO-ECONOMIC STATUS PREDISPOSE YOUNG ADULTS TO RAS-RELATED INCREASES IN LEFT VENTRICULAR MASS?

THE AFRICAN-PREDICT STUDY



SUMMARY OF AUTHOR INSTRUCTIONS: *Hypertension Research*

Article type specification

- Articles should be no more than 5000 words including abstract but excluding references and should not include more than six display items (tables and/or figures)

Preparation of article

House style:

- Do not make lines thinner than 1pt
- Use SI units throughout
- Spaces, not commas, should be used to separate thousands
- Abbreviations should be preceded by the words for which they stand in the first instance of use
- Text should be double spaced with a wide margin

Article sections:

- Title page: a concise but informative title, the first and last names and other initials of all authors, as well as their affiliations (but not degrees). Full contact details for the corresponding author
- Abstract: not more than 250 words. Three to five keywords, which may or may not appear in the title, should be given in alphabetical order
- Introduction
- Methods
- Results
- Discussion
- Acknowledgments
- Conflict of Interest
- References: list the first six authors followed by et al. In the text of the manuscript, references to the literature should be numbered consecutively. (Example of references: Glodny B, Pauli G. Medullopressin: a new pressor activity from the renal medulla. *Hypertens Res.* 2005; 28: 827–836.)
- Supplementary information

Does low socio-economic status predispose young adults to RAS-related increases in left ventricular mass?

The African-PREDICT study

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Abstract

Raised blood pressure (BP), with the renin-angiotensin system (RAS) as a central regulatory component, is one of the most important contributors to early development of left ventricular hypertrophy (LVH). Factors such as increased age, sex, black ethnicity and a low socio-economic status (SES) also contribute to left ventricular remodelling. To better understand the early factors contributing to left ventricular mass (LVM), we investigated the relationship between LVM index (LVMi) and the components of the RAS in young healthy adults while taking SES, ethnicity and sex into account. We grouped 1 186 black and white men and women (aged 20-30 years) according to low vs high SES. By using a standard echocardiogram, we determined LVMi. Ultra-pressure-liquid chromatography tandem-mass spectrometry (LC-MS/MS) was used to measure the RAS Fingerprint®. In black women with low SES, LVMi associated inversely with plasma renin activity (β=-0.168; P=0.017), angiotensin I (β=-0.155; P=0.028) and angiotensin II (β=-0.172; P=0.015). No associations were evident between LVMi and the RAS components in black women with high SES, or white women, black or white men, independent of SES. This finding suggests that multiple factors may play a role in the development of increased LVM, including suppressed RAS, raised BP, female sex, black ethnicity and a low socio-economic environment.

Key words: ethnicity, left ventricular mass, renin-angiotensin system, sex, socio-economic status

Introduction

According to the South African poverty trend report (2006 to 2015) 30.4 million (55.5%) of South Africans, particularly black South Africans, are living in poverty (1). Unfavourable socio-economic conditions such as low income, unemployment and low education are predisposing factors towards adverse health outcomes as demonstrated by the link between low socio-economic status (SES) and cardiovascular disease (CVD) (1-4). With a large proportion (63.4%) of the South African youth (aged 15–34 years) being unemployed (5), it is imperative to investigate the influence of low SES on cardiovascular health at this young age.

As a CVD risk factor, low SES is associated with hypertension-related target organ damage such as left ventricular hypertrophy (LVH) (6, 7). Hypertension and LVH are both more common in black populations than in their white counterparts (8-11), but it is not known whether this is dependent on SES. In addition, black populations frequently have suppressed renin-angiotensin system (RAS) activity, also referred to as the low renin phenotype (12-16). Physiologically the RAS is essential for the control of plasma volume as well as sodium and potassium homeostasis (17-19), with a dysregulated system leading to angiotensin II (Ang II)-mediated volume retention, vasoconstriction and fibrosis (17-19). This will in turn increase blood pressure (BP) and contribute to the development of hypertension-mediated target organ damage such as LVH (17-19).

Most of the studies focusing on the relationship between hypertension, left ventricular mass (LVM) and the RAS were conducted in older hypertensive patients (20, 21), with a limited focus on SES, ethnicity, age and sex. We therefore investigated the relationship between LVM index (LVMi) and components of the RAS in young adults (20-30 years) whilst considering SES, ethnicity and sex.

Methods

Study design and population

This study forms part of the African Prospective study on Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) and utilised existing baseline data. Detail on the methodology of this study was previously published (22). In short, the study has a longitudinal design, aimed at investigating early CVD-related pathophysiology by tracking young (aged 20–30 years) apparently healthy black and white adults over time (22). Participants were recruited on a voluntary basis from the North West Province of South Africa and during screening procedures were considered for inclusion if their clinic BP was <140/90 mmHg (23), HIV uninfected, not diagnosed with chronic diseases or using medication for chronic diseases (self-reported), nor pregnant or lactating women (self-reported). This cross-sectional study included the full baseline cohort of 1 202 young adults. Upon removal of participants with missing data for the RAS (N=11) and left ventricular dimensions (N=5), we included 1 186 black and white adults (N=573 men and N=613 women). This study (NWU-00032-19-A1) was approved by the Health Research Ethics Committee of the North-West University and adhered to the principles set out in the Declaration of Helsinki. All participants provided written informed consent.

Questionnaires

Demographic data were collected using a General Health and Demographic Questionnaire. Data obtained included age, sex, ethnicity, education level, employment information, household income, smoking, alcohol consumption and medication use including hormonal contraceptive use. From the demographic information, SES was calculated using a point system adapted from Kuppuswamy's Socioeconomic Status Scale 2010 (24) for a South African environment. Participants were scored in three categories: skill level (classified according to the South African Standard Classification of Occupation (SASCO)), education and income. These three factors were used to categorise participants into SES classes (low, middle, high) and to determine the SES score.

Anthropometric and physical activity measurements

Anthropometric measurements were taken by an anthropometrist in accordance with the guidelines of the International Society for the Advancement of Kinanthropometry (ISAK) (25) to obtain height (m), determined by the SECA 213 Portable Stadiometer (SECA, Hamburg, Germany), weight (kg), using the SECA 813 Electronic Scales (SECA, Hamburg, Germany) and waist circumference (cm), using the Lufkin Steel Anthropometric Tape (W606 PM; Lufkin, Apex, USA). Body mass index (BMI) (weight (kg) / height (m^2)) and body surface area (26) were then calculated. The ActiHeart (CamNtech, Cambridge, UK) device was used to capture Activity Energy Expenditure (AEE) for a maximum of 7 consecutive days.

Cardiovascular measurements

Clinic BP was measured at the left and right brachial artery in duplicate using Dinamap Procare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA) with an appropriately sized cuff and the participant in the upright sitting position. Participants were requested to rest for a 5-min period before and between each measurement and not to have exercised, smoked or eaten for the last 30 minutes prior to commencement of measurements. The mean of the two left measurements was used in this study.

Ambulatory BP measurements (ABPM) were obtained over 24 hours using the Card(X)plore (Meditech, Budapest, Hungary) apparatus with an appropriately sized cuff. The device measured BP in 30-minute intervals during daytime (6 a.m. to 10 p.m.) and hourly during the night (10 p.m. to 6 a.m.). The mean successful inflation rate over the 24h time period was 88%. Participants also completed an ambulatory diary card.

A continuous measurement of resting cardiovascular variables was performed using the Finometer device (Finapres Measurement Systems, Amsterdam, The Netherlands) (27-29). This was done for a 5-minute period after a 2-minute calibration to adjust the finger arterial pressure with the brachial arterial pressure to provide an individual subject-level adjustment (28). Total peripheral resistance was computed using the Beatscope® software.

Transthoracic echocardiography was performed according to the guidelines of the American Society of Echocardiography using the General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway), with a 2.5–3.5 MHz transducer and a single ECG lead (30, 31). Left ventricular dimensions were measured, and analysed using the EchoPAC software (GE, version 10.8.1). From the dimensions the interventricular septum thickness at end-diastole (IVSd), the left ventricular posterior wall thickness at end-diastole (LVPWd), the left ventricular internal diameter at end-diastole (LVIDd) and relative wall thickness (RWT) (defined as the ratio of twice the posterior wall thickness and LVIDd) were determined. Left ventricular mass was calculated by the corrected Devereux formula and was normalised for body surface area, from which LVM index (LVMi) was derived (32).

Biochemical analyses

A registered nurse obtained early-morning blood samples and a spot urine sample from fasted participants. A 24h urine sample was also collected in accordance with the Pan American Health Organisation/World Health Organisation protocol for population level sodium determination (33). The biological samples were immediately prepared and aliquoted into cryovials and stored at -80ºC until analysis. The RAS Fingerprint® (Attoquant Diagnostics, Vienna, Austria) was used to analyse the components of the RAS using ultra-pressure-liquid chromatography-tandem mass spectrometry (LC-MS/MS) generated multiplex parameters consisting of the precisely quantified concentrations of 10 angiotensin (Ang) peptides (Ang I (coefficient of variation (CV)=11.2%), Ang II (CV=7.3%), Ang 1-9, Ang 1-7, Ang 1-5, Ang 2-8, Ang 3-8, Ang 3-7, Ang 2-7, Ang 2-10) and aldosterone (CV=9.6%). The validated (34) surrogate markers, plasma renin activity (PRA-Surrogate) (Ang I + Ang II) and angiotensin converting enzyme (ACE-Surrogate) (Ang II/Ang I) were calculated using equilibrium angiotensin levels. For the alternative RAS peptides, data from most of the participants were below the lowest level of quantification (LLOQ) (Table 1).

Table 1. Summary of participants below the lowest level of quantification

	% of participants below the LLOQ			
	N=1186	%		%
The classical RAS	Plasma renin activity-S	0.00	**The alternative RAS**	Angiotensin 1-9, pmol/L	99.9
	Angiotensin I (1-10), pmol/L	6.00		Angiotensin 1-7, pmol/L	96.9
	Angiotensin converting enzyme-S	0.00		Angiotensin 1-5, pmol/L	82.3
	Angiotensin II (1-8), pmol/L	0.00		Angiotensin III (2-8), pmol/L	94.5
	Aldosterone, pmol/L	4.90		Angiotensin IV (3-8), pmol/L	70.6
				Angiotensin 3-7, pmol/L	100
				Angiotensin 2-7, pmol/L	100
				Angiotensin 2-10, pmol/L	93.0

S, surrogate.

The Cobas Integra® 400 plus (Roche, Basel, Switzerland) was used to analyse gamma-glutamyl transferase (GGT), the lipid profile (total cholesterol, high-density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides), creatinine, high sensitivity C-reactive protein in serum samples, glucose levels in sodium fluoride plasma samples, as well as sodium and potassium levels in 24h urine, from which the sodium-potassium ratio were calculated (35). Glomerular filtration rate was estimated (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (35, 36). Estradiol, progesterone and cortisol were analysed using the e411 (Roche, Basel Switzerland) in serum samples. For the above-mentioned biochemical analysis, both intra and inter assay CV ≤ 4.1%. Cotinine was analysed from serum samples using the Immulite (Siemens, Erlangen, Germany) apparatus with intra assay CV ≤ 10.8%.

Statistical analyses

Statistical analyses were performed with Statistica 13.3 (Tibco, Palo Alto, CA, USA). Variables were tested for normality and logarithmically transformed if skewed. Logged variables included weight, waist circumference, LVMi, total peripheral resistance, cortisol, GGT, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, C-reactive protein, cotinine and the renin-angiotensin-aldosterone system peptides. Normally distributed variables were reported as mean and standard deviation, and logarithmically transformed variables were presented by the geometric mean, 5th and 95th percentile intervals. Interactions of ethnicity, sex and SES on the relationships between LVMi and the

RAS were tested (Table S1). Based on these findings and the literature (37-39), participants were grouped to compare black and white men and women with low vs high SES. Based on the median SES score of 21, participants were clustered into low and high SES groups. The characteristics between groups were compared using the Chi-square test to compare categorical variables, and independent *t*-tests to compare continuous variables. Single and multivariable adjusted regression analyses were performed to determine associations between LVMi (dependent variable) and the RAS components as main independent variables in separate models. Various potential confounders were considered for inclusion in the model. Covariates included in the model were age, 24h systolic BP, total cholesterol, GGT, C-reactive protein and estradiol based on variables that were significantly correlated with the dependent and main independent variables using Pearson correlations.

Results

We firstly compared the characteristics of participants with low vs high SES. This was done for black women (Table 2) and white women (Table S2). No differences in BP or left ventricular dimensions were observed between the low vs high SES black or white women (P≥0.149). Regarding the RAS profile, aldosterone (P=0.016) was lower in black women with low SES than in their high SES counterparts. In the white women no difference was found in the RAS profile between low vs high SES groups (P≥0.295). When comparing other biochemical characteristics, black women with low SES presented with higher cortisol (P=0.001), GGT (P=0.003), and 24h urinary sodium-potassium ratio (P<0.001) than did their high SES counterparts.

In the comparison between low and high SES black (Table S3) and white men (Table S4), we found that for both black and white men with low SES, clinic diastolic BP (P=0.035 and P=0.006) and 24h diastolic BP (P=0.001 and P=0.009) were lower than those with high SES. No differences in left ventricular dimensions were observed in either black or white men with low vs high SES. With regard to the RAS, black men with low SES presented with higher ACE-

S (P=0.028) and lower aldosterone (P<0.001) than did black men with high SES. White men (low vs high SES) showed no differences between the RAS peptides (all P≥0.610).

Table 2. Characteristics of black women according to socio-economic status

	Black women		
	Low SES	**High SES**	**P-value**
N	209	97	
Age, years	24.1 ± 3.33	25.7 ± 2.99	**<0.001**
Socio-economic status score	15.0 ± 3.31	24.9 ± 2.98	**<0.001**
Anthropometric measurements			
Height, m	1.59 ± 0.06	1.60 ± 0.06	0.265
Weight, kg	66.0 (46.5; 97.1)	66.5 (47.0; 98.3)	0.791
Body mass index, kg/m^2	26.9 ± 6.13	26.8 ± 6.30	0.947
Body surface area, m^2	1.72 ± 0.21	1.73 ± 0.21	0.647
Waist circumference, cm	78.3 (62.5; 102)	77.7 (60.2; 103)	0.699
Cardiovascular measurements			
Clinic systolic blood pressure, mmHg	115 ± 10.3	113 ± 10.5	0.269
Clinic diastolic blood pressure, mmHg	78.5 ± 7.78	77.9 ± 7.80	0.551
24h Systolic blood pressure, mmHg	113 ± 8.23	113 ± 8.84	0.743
24h Diastolic blood pressure, mmHg	68.14 ± 5.53	68.3 ± 5.4	0.860
Interventricular septal thickness, cm	0.79 ± 0.15	0.78 ± 0.15	0.351
Left ventricular posterior wall thickness, cm	0.83 ± 0.14	0.82 ± 0.13	0.418
Left ventricular internal diameter, cm	4.37 ± 0.41	4.42 ± 0.36	0.336
Relative wall thickness, cm	0.38 ± 0.07	0.37 ± 0.07	0.252
Left ventricular mass index, g/m^2	63.4 (46.1; 92.5)	62.6 (43.4; 88.1)	0.588
Total peripheral resistance, mmHg/ml/s	1.22 (0.80; 2.07)	1.17 (0.73; 1.83)	0.220
Biochemical measurements			
Estradiol, pg/ml	78.5 ± 85.0	91.5 ± 102	0.263
Progesterone, ng/ml	1.50 ± 3.90	2.30 ± 5.58	0.167
Cortisol, nmol/L	354 (171; 757)	289 (149; 647)	**0.001**
Gamma-glutamyl transferase, U/L	22.8 (9.00; 62.7)	18.4 (8.30; 59.7)	**0.003**
Total cholesterol, mmol/L	3.49 (2.23; 5.09)	3.71 (2.25; 5.35)	0.055
HDL cholesterol, mmol/L	1.11 (0.61; 1.86)	1.19 (0.53; 1.89)	0.089
LDL cholesterol, mmol/L	2.23 (1.14; 3.70)	2.35 (1.31; 4.04)	0.230
Triglycerides, mmol/L	0.65 (0.34; 1.33)	0.61 (0.32; 1.34)	0.277
Glucose, mmol/L	4.09 ± 0.96	4.42 ± 0.78	**0.003**
C-reactive protein, mg/L	1.78 (0.16; 15.7)	1.72 (0.16; 13.2)	0.854
Cotinine, ng/ml	2.08 (1.00; 184)	1.75 (1.00; 170)	0.400
24h Urinary sodium, mmol/L	128 ± 64.7	122 ± 59.7	0.510
24h Urinary sodium-potassium ratio	4.32 ± 1.77	3.52 ± 1.25	**<0.001**
Glomerular Filtration rate, ml/min/1.73m^2	121 ± 15.7	120 ±1 15.8	0.616
Renin-angiotensin system*			
Plasma renin activity-S	63.8 (13.2; 279)	65.5 (10.0; 267)	0.827
Angiotensin I (1-10), pmol/L	16.9 (3.10; 81.8)	17.1 (3.10; 73.0)	0.959
Angiotensin converting enzyme-S	2.71 (1.40; 5.10)	2.77 (1.50; 5.30)	0.626
Angiotensin II (1-8), pmol/L	45.8 (9.70; 188)	47.4 (6.10; 197)	0.761
Aldosterone, pmol/L	67.8 (13.9; 268)	86.5 (20.4; 346)	**0.016**
Lifestyle			
Activity Energy Expenditure, kCal	484 ± 247	461 ± 204	0.474
Self-reported smoking, n (%)	20 (9.57)	9 (9.28)	0.936
Self-reported alcohol use, n (%)	110 (53.1)	41 (43.2)	0.107
Hormonal contraceptive use female, n (%)	112 (53.9)	30 (32.6)	**<0.001**

Data are presented as mean ± standard deviation; or geometric mean with 5th and 95th percentile intervals. *Renin-angiotensin system are presented as geometric mean with 5th and 95th percentile intervals. S, surrogate; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Bold values denote P<0.05.

Aligned with our aim, we sought to determine whether LVMi is associated with the RAS components. We performed analyses in all subgroups split for ethnicity, sex and SES. In black women with low SES, single regression analyses (Figure 1 and Table S5) indicated negative correlations between LVMi with PRA-S (r=-0.14; P=0.044) and Ang II (r=-0.15; P=0.030), with a borderline correlation with Ang I (r=-0.11; P=0.099).

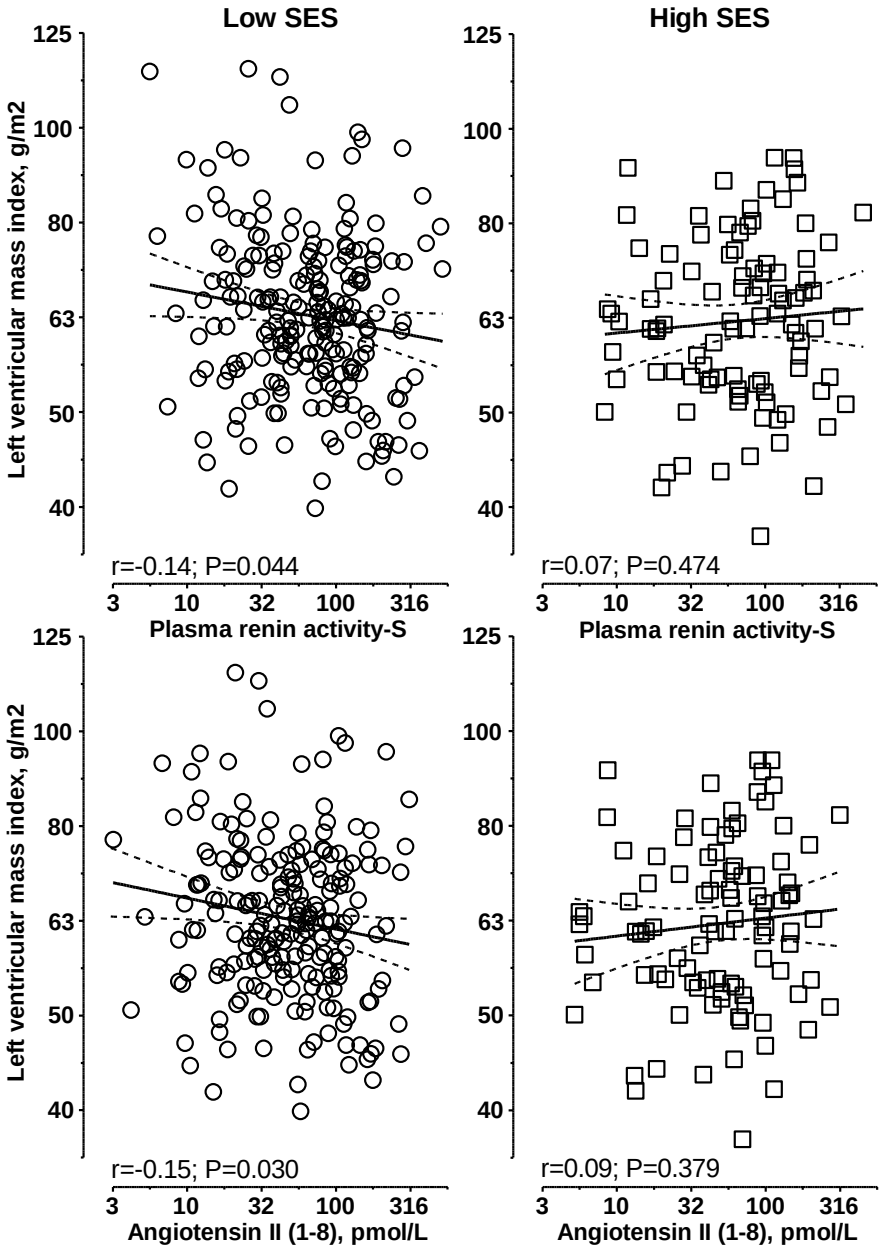

Figure 1. Single regression analysis of left ventricular mass index with plasma renin activity-S and angiotensin II in black women according to socio-economic status

In multivariable adjusted regression analyses performed in black women with low SES (Table 3), independent negative associations were indicated between LVMi with PRA-S (β=-0.168; P=0.017), Ang I (β=-0.155; P=0.028) and Ang II (β=-0.172; P=0.015). No associations were found between LVMi and the RAS in black women with high SES or in white women (low or high SES).

Table 3. Multiple regression analyses of left ventricular mass index with the renin-angiotensin system in black women according to socio-economic status

	Black women			
Dependent variable: Left ventricular mass index	**Low SES (N=209)**		**High SES (N=97)**	
	Adj. R^2; P-value	**β-value; P-value**	**Adj. R^2; P-value**	**β-value; P-value**
Plasma renin activity-S	R^2=0.085; P=0.001	**β=-0.168; P=0.017**	R^2=-----; P=0.846	β=0.055; P=0.646
24h Systolic BP, mmHg		**β=0.236; P=0.001**		β=-0.051; P=0.703
γ-glutamyl transferase, U/L		**β=0.148; P=0.046**		β=0.120; P=0.323
Angiotensin I (1-10), pmol/L	R^2=0.081; P=0.002	**β=-0.155; P=0.028**	R^2=-----; P=0.867	β=0.010; P=0.936
24h Systolic BP, mmHg		**β=0.244; P=0.001**		β=-0.066; P=0.621
γ-glutamyl transferase, U/L		**β=0.149; P=0.046**		β=0.125; P=0.300
Ang converting enzyme-S	R^2=0.058; P=0.010	β=-0.009; P=0.901	R^2=-----; P=0.691	β=0.140; P=0.219
24h Systolic BP, mmHg		**β=0.230; P=0.002**		β=-0.079; P=0.534
γ-glutamyl transferase, U/L		β=0.136; P=0.070		β=0.120; P=0.314
Angiotensin II (1-8), pmol/L	R^2=0.087; P=0.001	**β=-0.172; P=0.015**	R^2=-----; P=0.823	β=0.078; P=0.516
24h Systolic BP, mmHg		**β=0.233; P=0.001**		β=-0.044; P=0.741
γ-glutamyl transferase, U/L		**β=0.146; P=0.048**		β=0.116; P=0.337
Aldosterone, pmol/L	R^2=0.059; P=0.009	β=-0.041; P=0.560	R^2=-----; P=0.860	β=-0.033; P=0.778
24h Systolic BP, mmHg		**β=0.238; P=0.001**		β=-0.069; P=0.589
γ-glutamyl transferase, U/L		β=0.139; P=0.064		β=0.133; P=0.278

Covariates: age, 24h systolic BP, total cholesterol, gamma glutamyl transferase, C-reactive protein, estradiol. S, surrogate; BP, blood pressure; γ, gamma. Values in bold indicate statistical significance (P<0.05).

With regard to men, no associations between LVMi and the RAS were found in black men (low or high SES). White men with high SES presented with a positive association between LVMi and aldosterone in single regression (r=0.15; P=0.034) analysis. However, this association lost significance in multivariate analysis (Adj. R^2=0.031; β=0.132; P=0.060).

Sensitivity analyses

We additionally performed a sensitivity analysis in black women with low SES and included 24h urinary sodium excretion as an additional covariate. This was done since sodium is known to suppress the RAS (40) and can contribute to raised BP (41). After the additional adjustment

for 24h urinary sodium excretion, the association between LVMi with Ang I lost significance (Adj. R^2=0.068; β=-0.151; P=0.062), whereas the independent associations between LVMi with PRA-S (Adj. R^2=0.072; β=-0.164; P=0.042) and Ang II (Adj. R^2=0.073 β=-0.169; P=0.036) remained significant.

Discussion

In young healthy adults, we investigated the relationship between LVMi and the components of the RAS while taking SES, ethnicity and sex into account. Our study showed that in black women with low SES, LVMi associated inversely and independently with the components of the RAS (renin activity as well as Ang I and Ang II). No associations were evident between LVMi and the RAS in black women with high SES, white women (low and high SES) or in black and white men (low and high SES).

Our finding of an inverse association between LVMi with the RAS components in black women with low SES is in contradiction to previous findings indicating that increased LVMi is associated with over-activation of the RAS (17-19, 42). Activation of the RAS is controlled by renin release, which is stimulated by several factors, including a decrease in renal perfusion pressure and sodium delivery to the macula densa (15, 17-19, 42). With an over-activity of the RAS, increased Ang II may lead to vasoconstriction, fibrosis and the release of aldosterone, thereby mediating sodium and volume retention (17-19, 42). If sustained over time, this may increase BP and contribute to the development of hypertension-mediated LVH (17-19, 42).

The mechanism at play to explain the inverse association between LVMi with renin activity and its downstream consequents observed in the black women with low SES may involve the interplay of various factors. With an increase in salt intake (and low potassium intake), the RAS may be suppressed (15). Higher plasma sodium levels may result in increased plasma volume loading and cardiac output with resultant raised blood pressure, which is known to be a primary contributor to increased LVM over time (43). Suppression of the RAS in this group of women may be further amplified by factors related to low socio-economic environments

such as increased stress (raised cortisol) (44-46), increased alcohol consumption (raised GGT) (47, 48) and poor diet (high sodium-potassium ratio) (49-51) – which were all evident in this group. These behavioural risk factors may be attributed to limited financial means to purchase healthy foods, or a lack of knowledge on cardiovascular-related health behaviours. A meta-analysis demonstrated that education (amongst other SES determinants such as occupation and income) indicated the strongest association with prevalence of hypertension (52). It was further found that higher education and income are associated with a healthier lifestyle including more exercise, preventive medical care as well as healthy eating habits, with less alcohol intake and smoking (52, 53).

In the other groups investigated in this study no associations were evident between LVMi and the RAS. Whether this could be attributed to the young and apparent healthy nature of the study population, is unknown. Whether this observation will remain as this study population age, warrants further investigation in the follow-up phase of the study.

Several methodological strengths and limitations of the study should be considered. The cross-sectional design prevents us from inferring causal relationships. We could also not rule out the possibility of residual confounding due to unmeasured covariates. A major strength of our study is the fact that we included a young apparently healthy population with a detailed RAS estimation using a sensitive LC-MS/MS method.

In conclusion, the association between LVMi and a suppressed RAS in young black women with low SES may reflect how multiple factors, including BP, within a low socio-economic environment, may all contribute to the development of increased LVM.

Recommendations for future studies

In order to prevent dysregulation of the RAS and improve cardiovascular health, factors such as SES, the RAS, BP and the influence thereof on microvascular and macrovascular changes should be a focus of future research. This may include longitudinal studies to determine how

the RAS, including the alternative RAS changes over time and how these changes associate with changes in hemodynamic variables, such as cardiac output, vascular resistance, but also subclinical organ damage such as arterial stiffness and LVM. Furthermore, the black population seems to be vulnerable to suppression of the RAS regardless of the fact that BP is still within normal ranges (1-4). Hence future research needs to establish optimal ranges for RAS components, electrolytes (sodium, potassium) and BP in populations of African ancestry. Lastly, low SES and CVD are strongly associated (52, 53). Due to the challenging nature to improve SES in low-middle income countries and the CVD burden, future research needs to focus on the improvement of SES as a preventative strategy against the development of CVD. This may include educational programs and skills development. In turn, higher education and skills may increase employment opportunities and income, which may lead to healthier behaviours and a decrease in CVD (52, 53).

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Conflict of Interest

The authors declare no conflict of interest.

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Table S1. Interactions of sex and socio-economic status on the relationships between left ventricular dimensions with the renin-angiotensin systems split according to ethnicity

		Left ventricular mass index new, g/m^2		Relative wall thickness, cm	
		P-values		P-values	
Ethnicity	Plasma renin activity-S	0.156		0.459	
	Angiotensin I (1-10), pmol/L	0.870		0.691	
	Angiotensin converting enzyme-S	**0.004**		0.668	
	Angiotensin II (1-8), pmol/L	0.059		0.526	
	Aldosterone, pmol/L	0.432		0.667	
		Grouped according to ethnicity			
		Black	White	Black	White
		P-values	P-values	P-values	P-values
Sex	Plasma renin activity-S	0.984	0.945	0.983	0.830
	Angiotensin I (1-10), pmol/L	0.734	0.763	0.832	0.748
	Angiotensin converting enzyme-S	0.364	0.500	0.442	0.646
	Angiotensin II (1-8), pmol/L	0.935	0.854	0.921	0.900
	Aldosterone, pmol/L	0.884	**0.009**	0.856	0.422
Socio-economic status	Plasma renin activity-S	0.902	0.328	**0.033**	0.645
	Angiotensin I (1-10), pmol/L	0.764	0.563	0.052	0.646
	Angiotensin converting enzyme-S	0.532	0.262	0.935	0.870
	Angiotensin II (1-8), pmol/L	0.963	0.371	**0.034**	0.652
	Aldosterone, pmol/L	0.537	0.685	0.442	0.666

S, surrogate. Values in bold indicate statistical significance (P<0.05).

Table S2. Characteristics of white women according to socio-economic status

	White women		
	Low SES	**High SES**	**P-value**
N	84	223	
Age, years	22.6 ± 2.78	25.1 ± 2.92	**<0.001**
Socio-economic status score	16.20 ± 2.81	26.10 ± 3.02	**<0.001**
Anthropometric measurements			
Height, m	1.66 ± 0.06	1.67 ± 0.06	0.200
Weight, kg	67.2 (50.3; 112)	66 (50.5; 94.3)	0.506
Body mass index, kg/m^2	25.2 ± 6.84	24.2 ± 4.93	0.152
Body surface area, m^2	1.77 ± 0.24	1.76 ± 0.19	0.566
Waist circumference, cm	76.7 (64.2; 103)	75.1 (63.4; 95.5)	0.226
Cardiovascular measurements			
Clinic systolic blood pressure, mmHg	112 ± 11.7	110 ± 9.52	0.149
Clinic diastolic blood pressure, mmHg	76.2 ± 6.87	75.2 ± 7.12	0.250
24h Systolic blood pressure, mmHg	113 ± 9.71	112 ± 7.95	0.603
24h Diastolic blood pressure, mmHg	67.2 ± 6.09	67.6 ± 5.41	0.576
Interventricular septal thickness, cm	0.78 ± 0.13	0.76 ± 0.14	0.401
Left ventricular posterior wall thickness, cm	0.81 ± 0.12	0.80 ± 0.14	0.490
Left ventricular internal diameter, cm	4.60 ± 0.39	4.62 ± 0.36	0.657
Relative wall thickness, cm	0.35 ± 0.06	0.35 ± 0.06	0.348
Left ventricular mass index, g/m^2	65.5 (51.0; 88.4)	64.8 (44.7; 89.0)	0.690
Total peripheral resistance, mmHg/ml/s	1.07 (0.67; 2.25)	1.09 (0.71; 1.74)	0.764
Biochemical measurements			
Estradiol, pg/ml	79.1 ± 104	92.4 ± 103	0.328
Progesterone, ng/ml	1.22 ± 3.22	2.60 ± 5.11	0.025
Cortisol, nmol/L	475 (206; 1181)	402 (160; 1089)	0.075
Gamma-glutamyl transferase, U/L	12.7 (5.20; 40.7)	12.0 (5.00; 32.1)	0.475
Total cholesterol, mmol/L	3.67 (2.17; 5.66)	4.00 (2.28; 6.14)	**0.026**
HDL cholesterol, mmol/L	1.12 (0.69; 1.79)	1.35 (0.77; 2.27)	**<0.001**
LDL cholesterol, mmol/L	2.41 (1.33; 4.08)	2.42 (1.26; 4.26)	0.886
Triglycerides, mmol/L	0.76 (0.36; 1.84)	0.75 (0.32; 1.66)	0.748
Glucose, mmol/L	4.14 ± 1.20	4.29 ± 0.93	0.247
C-reactive protein, mg/L	1.11 (0.05; 10.9)	0.86 (0.09; 8.58)	0.168
Cotinine, ng/ml	2.53 (1.00; 267)	2.10 (1.00; 241)	0.427
24h Urinary sodium, mmol/L	116 ± 58.6	107 ± 50.7	0.179
24h Urinary sodium-potassium ratio	3.03 ± 1.78	2.85 ± 2.09	0.508
Glomerular Filtration rate, ml/min/1.73m^2	120 ± 18.1	115 ± 19.1	**0.046**
Renin-angiotensin system*			
Plasma renin activity-S	122 (39.4; 359)	122 (38.5; 307)	0.945
Angiotensin I (1-10), pmol/L	34.7 (10.0; 113)	35.1 (8.00; 104)	0.908
Angiotensin converting enzyme-S	2.46 (1.30; 4.30)	2.42 (1.10; 4.90)	0.766
Angiotensin II (1-8), pmol/L	85.1 (28.7; 267)	84.7 (28.1; 226)	0.960
Aldosterone, pmol/L	166 (36.7; 994)	192 (24.4; 996)	0.295
Lifestyle			
Activity Energy Expenditure, kCal	474 ± 255	408 ± 204	**0.032**
Self-reported smoking, n (%)	17 (20.2)	33 (14.8)	0.250
Self-reported alcohol use, n (%)	42 (50.0)	120 (53.8)	0.551
Hormonal contraceptive use female, n (%)	39 (46.4)	87 (39.2)	0.251

Data are presented as mean ± standard deviation; or geometric mean with 5th and 95th percentile intervals. *Renin-angiotensin system are presented as geometric mean with 5th and 95th percentile intervals. S, surrogate; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Bold values denote P<0.05.

Table S3. Characteristics of black men according to socio-economic status

	Black men		
	Low SES	**High SES**	**P-value**
N	217	76	
Age, years	23.8 ± 2.92	26.0 ± 2.79	**<0.001**
Socio-economic status score	14.7 ± 3.14	24.8 ± 2.90	**<0.001**
Anthropometric measurements			
Height, m	1.70 ± 0.07	1.71 ± 0.06	0.210
Weight, kg	61.3 (47.4; 83.2)	68.9 (51.2; 96.6)	**<0.001**
Body mass index, kg/m^2	21.6 ± 3.53	24.1 ± 4.73	**<0.001**
Body surface area, m^2	1.71 ± 0.18	1.82 ± 0.19	**<0.001**
Waist circumference, cm	74.6 (63.7; 90.8)	80.1 (67.1; 95.3)	**<0.001**
Cardiovascular measurements			
Clinic systolic blood pressure, mmHg	124 ± 11.4	123 ± 12.4	0.753
Clinic diastolic blood pressure, mmHg	80.7 ± 8.7	83.2 ± 8.55	**0.035**
24h Systolic blood pressure, mmHg	119 ± 7.87	121 ± 9.37	0.177
24h Diastolic blood pressure, mmHg	68.8 ± 6.08	71.7 ± 6.31	**0.001**
Interventricular septal thickness, cm	0.90 ± 0.18	0.89 ± 0.18	0.860
Left ventricular posterior wall thickness, cm	0.91 ± 0.15	0.92 ± 0.14	0.852
Left ventricular internal diameter, cm	4.69 ± 0.41	4.74 ± 0.39	0.337
Relative wall thickness, cm	0.39 ± 0.08	0.39 ± 0.07	0.690
Left ventricular mass index, g/m^2	82.8 (54.3; 116)	79.3 (53.8; 115)	0.137
Total peripheral resistance, mmHg/ml/s	1.48 (0.95; 2.87)	1.39 (0.87; 3.02)	0.174
Biochemical measurements			
Estradiol, pg/ml	43.0 ± 15.6	43.4 ± 13.5	0.836
Progesterone, ng/ml	0.22 ± 0.51	0.17 ± 0.10	0.332
Cortisol, nmol/L	396 (200; 671)	391 (225; 782)	0.786
Gamma-glutamyl transferase, U/L	21.9 (8.00; 62.4)	27.6 (10.0; 90.2)	**0.008**
Total cholesterol, mmol/L	3.00 (1.64; 4.82)	3.40 (1.95; 5.50)	**0.004**
HDL cholesterol, mmol/L	1.01 (0.55; 1.72)	1.07 (0.60; 1.98)	0.210
LDL cholesterol, mmol/L	1.80 (0.83; 3.49)	2.12 (1.11; 4.41)	**0.004**
Triglycerides, mmol/L	0.65 (0.29; 1.44)	0.65 (0.23; 1.90)	0.966
Glucose, mmol/L	3.56 ± 1.05	3.93 ± 1.09	**0.009**
C-reactive protein, mg/L	0.50 (0.07; 5.41)	0.73 (0.09; 6.32)	**0.042**
Cotinine, ng/ml	11.5 (1.00; 468)	3.56 (1.00; 293)	**0.001**
24h Urinary sodium, mmol/L	132 ± 64.0	137 ± 58.0	0.557
24h Urinary sodium-potassium ratio	4.97 ± 2.55	3.80 ± 1.66	**<0.001**
Glomerular Filtration rate, ml/min/1.73m^2	127 ± 15.4	120 ± 17.9	**0.001**
Renin-angiotensin system*			
Plasma renin activity-S	60.9 (10.8; 246)	66.2 (10.1; 277)	0.517
Angiotensin I (1-10), pmol/L	15.6 (3.10; 71.1)	18.8 (3.10; 103)	0.173
Angiotensin converting enzyme-S	2.82 (1.40; 6.50)	2.43 (0.90; 5.90)	**0.028**
Angiotensin II (1-8), pmol/L	43.9 (7.10; 175)	45.7 (5.80; 176)	0.758
Aldosterone, pmol/L	56.9 (13.9; 213)	87.7 (22.8; 312)	**<0.001**
Lifestyle			
Activity Energy Expenditure, kCal	378 ± 188	361 ± 153	0.505
Self-reported smoking, n (%)	106 (48.6)	18 (24.0)	**<0.001**
Self-reported alcohol use, n (%)	142 (65.7)	36 (48.7)	**0.009**

Data are presented as mean ± standard deviation; or geometric mean with 5th and 95th percentile intervals. *Renin-angiotensin system are presented as geometric mean with 5th and 95th percentile intervals. S, surrogate; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Bold values denote P<0.05.

Table S4. Characteristics of white men according to socio-economic status

	White men		
	Low SES	**High SES**	**P-value**
N	70	210	
Age, years	22.7 ± 2.44	25.5 ± 2.86	**<0.001**
Socio-economic status score	15.7 ± 2.94	26.3 ± 3.17	**<0.001**
Anthropometric measurements			
Height, m	1.79 ± 0.06	1.79 ± 0.06	0.744
Weight, kg	82.8 (61.6; 119)	84.9 (63.2; 112)	0.301
Body mass index, kg/m^2	26.3 ± 5.22	26.8 ± 4.76	0.436
Body surface area, m^2	2.04 ± 0.19	2.07 ± 0.21	0.303
Waist circumference, cm	87.1 (70.0; 109)	88.7 (74.1; 110)	0.337
Cardiovascular measurements			
Clinic systolic blood pressure, mmHg	123 ± 8.66	125 ± 10.1	0.114
Clinic diastolic blood pressure, mmHg	77.8 ± 7.50	80.7 ± 7.44	**0.006**
24h Systolic blood pressure, mmHg	123 ± 7.19	124 ± 7.59	0.420
24h Diastolic blood pressure, mmHg	68.2 ± 4.99	70.3 ± 6.14	**0.009**
Interventricular septal thickness, cm	0.86 ± 0.15	0.90 ± 0.17	0.078
Left ventricular posterior wall thickness, cm	0.97 ± 0.12	0.94 ± 0.15	0.199
Left ventricular internal diameter, cm	5.08 ± 0.49	5.06 ± 0.44	0.683
Relative wall thickness, cm	0.39 ± 0.07	0.38 ± 0.07	0.337
Left ventricular mass index, g/m^2	80.5 (58.7; 108)	79.5 (57.8; 106)	0.636
Total peripheral resistance, mmHg/ml/s	1.11 (0.70; 2.20)	1.02 (0.63; 1.86)	0.112
Biochemical measurements			
Estradiol, pg/ml	36.1 ± 11.9	34.8 ± 17.9	0.554
Progesterone, ng/ml	0.22 ± 0.11	0.19 ± 0.10	**0.023**
Cortisol, nmol/L	411 (271; 628)	354 (191; 661)	**0.004**
Gamma-glutamyl transferase, U/L	17.0 (5.90; 52.7)	19.2 (6.90; 50.8)	0.188
Total cholesterol, mmol/L	3.52 (1.98; 6.41)	3.84 (2.01; 6.08)	0.092
HDL cholesterol, mmol/L	0.83 (0.48; 1.43)	0.93 (0.51; 1.57)	**0.025**
LDL cholesterol, mmol/L	2.30 (1.02; 4.42)	2.57 (1.09; 4.71)	0.079
Triglycerides, mmol/L	0.91 (0.34; 3.31)	0.85 (0.34; 2.09)	0.453
Glucose, mmol/L	3.94 ± 1.21	4.38 ± 1.13	**0.005**
C-reactive protein, mg/L	0.68 (0.07; 7.21)	0.64 (0.07; 5.54)	0.703
Cotinine, ng/ml	8.17 (1.00; 461)	3.90 (1.00; 301)	**0.025**
24h Urinary sodium, mmol/L	129 ± 48.8	115 ± 50.2	**0.048**
24h Urinary sodium-potassium ratio	3.13 ± 1.27	3.04 ± 2.56	0.795
Glomerular Filtration rate, ml/min/1.73m^2	124 ± 20.1	113 ± 22.0	**<0.001**
Renin-angiotensin system*			
Plasma renin activity-S	134 (37.5; 310)	132 (42.4; 367)	0.894
Angiotensin I (1-10), pmol/L	33.9 (8.40; 106)	32.4 (7.60; 108)	0.677
Angiotensin converting enzyme-S	2.96 (1.50; 6.90)	3.00 (1.50; 6.30)	0.822
Angiotensin II (1-8), pmol/L	93.0 (25.9; 220)	97.4 (30.7; 276)	0.610
Aldosterone, pmol/L	118 (26.3; 323)	123 (31.5; 446)	0.711
Lifestyle			
Activity Energy Expenditure, kCal	383 ± 180	371 ± 175	0.661
Self-reported smoking, n (%)	28 (40.0)	51 (24.3)	**0.011**
Self-reported alcohol use, n (%)	37 (52.9)	129 (61.7)	0.191

Data are presented as mean ± standard deviation; or geometric mean with 5th and 95th percentile intervals. *Renin-angiotensin system are presented as geometric mean with 5th and 95th percentile intervals. S, surrogate; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Bold values denote P<0.05.

Table S5. Single regression analysis of left ventricular mass index with the renin-angiotensin system in black women according to socio-economic status

	Left ventricular mass index, g/m^2	
	Low SES N=209)	**High SES (N=97)**
Plasma renin activity-S	**r=-0.14; P=0.044**	r=0.07; P=0.474
Angiotensin I (1-10), pmol/L	r=-0.11; P=0.099	r=0.04; P=0.702
Angiotensin converting enzyme-S	r=-0.06; P=0.414	r=0.11; P=0.290
Angiotensin II (1-8), pmol/L	**r=-0.15; P=0.030**	r=0.09; P=0.379
Aldosterone, pmol/L	r=0.01; P=0.890	r=-0.01; P=0.922

S, surrogate. Values in bold indicate statistical significance (P<0.05).

CHAPTER 4

CONCLUDING CHAPTER

1. Introduction

Two hypotheses were set out in Chapter 1 taking into consideration the literature and the objectives of this study. In this chapter these hypotheses will be accepted or rejected, and briefly discussed, based on the results of this study.

2. Hypotheses

Hypotheses 1. *Left ventricular mass index (LVMi) will be positively associated with plasma renin activity (PRA-Surrogate), angiotensin I (Ang I), angiotensin converting enzyme (ACE-Surrogate), angiotensin II (Ang II) and aldosterone.*

According to literature increased LVMi is associated with over-activation of the renin-angiotensin system (RAS) (1-4). Increased RAS activity results in increased Ang II production, which leads to vasoconstriction, fibrosis and the release of aldosterone, thereby mediating sodium and volume retention (5). This may increase blood pressure (BP) and ultimately contribute to the development of hypertension-mediated cardiac hypertrophy (6-8). However, we did not observe the expected physiological pathway whereby activation of the RAS leads to Ang II-mediated vasoconstriction, fibrosis and aldosterone release, as no independent positive associations were found between LVMi and the RAS components in the study population. Instead inverse associations between LVMi and the RAS components were found in one specific sub-group – black women with a low SES. Hypotheses 1 is therefore rejected.

Hypotheses 2. *The relationship between LVMi and RAS components will be dependent on socio-economic status (SES), ethnicity and sex.*

Based on interaction terms we sought to determine whether LVMi is associated with the RAS components in all subgroups split for ethnicity, sex and SES. Only in black women with low SES, did LVMi associate inversely and independently with the components of the RAS (renin activity as well as Ang I and Ang II). The potential mechanism to explain the association between LVMi and a suppressed RAS may be due to negative feedback inhibition caused by increased BP (Figure 1) (9). The observation seen in black women with low SES is a reflection

of the typical sodium-volume overload phenotype which is commonly found in black populations (10-12). Physiologically, sodium works together with the RAS to ensure optimal blood pressure and volume to maintain adequate perfusion pressure in tissues (6-8). However, with increased salt intake, plasma sodium levels increase, resulting in increased plasma volume and cardiac output with consequently raised BP and suppression of the RAS (Figure 1) (10-14). The increased BP may ultimately lead to left ventricular hypertrophy (LVH) over time (10-12). The sodium-volume overload phenotype (15) may be augmented in the low socio-economic stetting by factors such as stress, alcohol consumption and poor diet (6-8, 10-14), explaining the inverse association between LVMi and the RAS components in black women with low SES (Figure 1). It is evident from these findings that SES, ethnicity and sex play an important role in the relationship between LVMi and RAS based on the inverse associations between LVMi and RAS components found in black women with low SES. Hence hypothesis 2 is accepted.

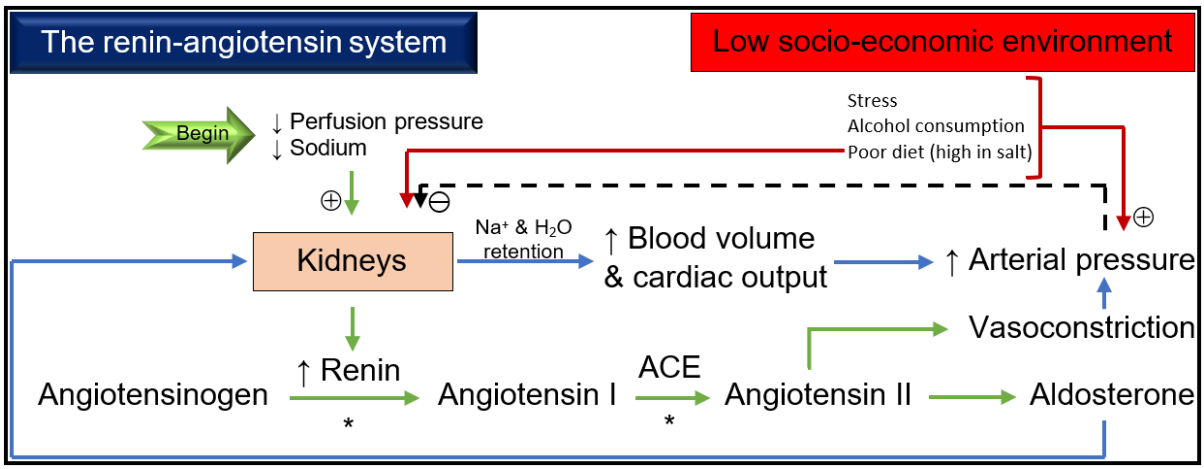

Figure 1. Suppression of the RAS*

Higher plasma sodium, increased plasma volume and cardiac output results in raised BP. The increased BP inhibit the RAS due to negative feedback inhibition. In the low socio-economic stetting factors such as increased stress, increased alcohol consumption and poor diet may further amplify suppression of the RAS and contribute to increased LVM.
ACE, angiotensin converting enzyme; Na^+, sodium; H_2O, water.

*Figure 1 was compiled from source (16).

3. Strengths and limitations

The cross-sectional design of the study prevented us from inferring causal relationships. Furthermore, we could not rule out the possibility of residual confounding due to unmeasured

covariates. With regard to the RAS, most of the alternative RAS peptides such as Ang 1-9, Ang 1-7, Ang 1-5, Ang 2-8, Ang 3-8, Ang 3-7, Ang 2-7 and Ang 2-10 were below the lowest level of quantification despite this sensitive method used; therefore relationships with LVMi could not be determined. A major strength of our study is the fact that more focus could be placed on the link between LVMi and RAS in a young and apparently healthy population before the onset of cardiovascular disease. Lastly, the RAS Fingerprint® provided increased precision for angiotensin peptide analysis which was important in profiling young healthy populations.

4. Recommendations for future studies

In order to prevent dysregulation of the RAS and improve cardiovascular health, factors such as SES, the RAS, BP and the influence thereof on microvascular and macrovascular changes (and vice versa), should be a focus of future research. This knowledge may contribute to a better understanding of the early phases in cardiovascular disease (CVD) development and may aid in the development of new therapeutic strategies to control CVD, especially in low SES settings. The following are recommendations for future studies:

- Longitudinal studies (including the African-PREDICT study) should be conducted to determine how the RAS, including the alternative RAS changes over time. In addition, it can also be determined whether changes in the RAS over time are associated with changes in hemodynamic variables, such as cardiac output, vascular resistance, but also subclinical organ damage such as arterial stiffness and LVM by reviewing longitudinal changes over time.
- The black population seems to be vulnerable to suppression of the RAS regardless of the fact that blood pressure is still within normal ranges (17-20). Hence future research needs to establish optimal ranges for RAS components, electrolytes (sodium, potassium) and BP in populations of African ancestry.
- Low SES and CVD are strongly associated (21, 22). Due to the challenging nature to improve SES in low-middle income countries and the CVD burden, future research needs

to focus on the improvement of SES as a preventative strategy against the development of CVD. This may include educational programs and skills development. In turn, higher education and skills may increase employment opportunities and income, which may lead to healthier behaviours and a decrease in CVD (21, 22).

5. Conclusion

In young black women with low SES, an independent inverse association between increased LVMi and suppressed RAS components were found. This inverse association between LVMi and RAS components may be amplified by female sex, black ethnicity and factors related to a low socio-economic environment. It is therefore important to establish preventive strategies in the low SES setting to prevent and manage health consequences such as a suppressed RAS, hypertension, and cardiac hypertrophy.

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APPENDICES

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02 May 2019

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North West University Health Research Ethics Committee (NWU-HREC) on 02/05/2019, the NWU 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 title: The renin-angiotensin-aldosterone-system and left ventricular mass in young black and white adults: The African-PREDICT study.
Study Leader/Supervisor (Principal Investigator)/Researcher: Prof CMC Mels
Student: WL du Toit

Ethics number:

N	W	U	-	0	0	0	3	2	-	1	9	-	A	1
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 02/05/2019 **Risk:** **Minimal**
Expiry date: 31/05/2020
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of an 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):

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:

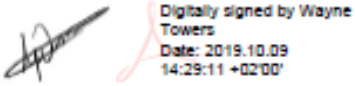
- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-HREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-HREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility the NWU-RERC and NWU-HREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected;*

- *it becomes apparent that any relevant information was withheld from the NWU-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.*

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

The NWU-HREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the NWU-HREC or the NWU-RERC for any further enquiries or requests for assistance.

Yours sincerely



Prof Wayne Towers
Chairperson NWU Health Research Ethics Committee

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22 November 2019

I, **Ms Cecilia van der Walt**, hereby declare that I took care of the **editing** of the **dissertation** of **Mr WL du Toit** titled *The renin-angiotensin-aldosterone-system and left ventricular mass in young black and white adults: The African-PREDICT study.*

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