The cardiovascular profile of the low renin phenotype in a black South African population

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"Do not go where the path may lead; go instead where there is no path and leave a trail"

Ralph Emerson

PREFACE

The article-format has been chosen for this thesis. This is a format approved and recommended by the North-West University. The thesis consists of a literature overview, research methodology, three manuscripts submitted for publication to peer reviewed journals, namely the *Journal of Human Hypertension, Clinical and Experimental Hypertension,* and *Journal of Hypertension,* and a concluding chapter which summarises the main findings and recommendations.

The layout of the thesis is as follows:

Chapter 1: Broad literature overview leading to problem statement and detailed aim, objectives and hypotheses.

Chapter 2: Study design and research methodology followed to collect data for the SABPA and PURE studies used in this thesis.

Chapter 3: Research article prepared according to author's instructions for the *Journal of Human Hypertension.* Published in 2015.

Chapter 4: Research article submitted and under review at *Clinical and Experimental Hypertension Journal*.

Chapter 5: Research article submitted and under review at the *Journal of Hypertension*.

Chapter 6: Summary of the main findings, conclusions and recommendations.

The relevant references are provided at the end of each manuscript chapter according to the instructions for authors of the specific journal in which the papers were published or to which they have been submitted for publication. For the rest of the thesis, references are provided at the end of each chapter in *Vancouver* referencing style.

CONTRIBUTIONS OF AUTHORS

The promotor and co-promotors are included as co-authors of the manuscripts, as well as the collaborator who participated in data collection of the PURE study. The following researchers contributed to the manuscripts:

NAME	**ROLE IN THE STUDY**
Ms LF Gafane-Matemane	Responsible for conducting the literature search and collection of data. The candidate performed some biochemical analyses and all statistical analyses, designed, wrote and compiled the manuscripts.
Prof AE Schutte	**Promotor.** Supervised all stages of compiling the manuscripts, was responsible for collection of data and gave general professional input.
Prof R Schutte	**Co-promotor.** Provided recommendations on statistical analyses, writing of the manuscripts and interpretation of results.
Prof JM van Rooyen	**Co-promotor.** Provided recommendations on design of the manuscripts and interpretation of results.
Dr IM Kruger	**South African PURE study Project leader**. Provided professional advice on the manuscript presented in chapter 5.

Below is a statement from the authors confirming their individual contribution to the study and their permission that the manuscripts may form part of this thesis.

Hereby, I declare that I approved the aforementioned manuscripts and that my role in this study as stated above is representative of my actual contribution. I also give my consent that these manuscripts may be used as part of the Ph.D. thesis of Ms LF Gafane-Matemane.

Prof AE Schutte

Prof R Schutte

Prof JM van Rooyen

Dr IM Kruger

PUBLICATIONS AND CONFERENCE PRESENTATIONS RELATING TO THE THESIS

Publications

Gafane LF, Schutte R, Van Rooyen JM, Schutte AE. Plasma renin and cardiovascular responses to the cold pressor test differ in black and white populations: The SABPA study *J Hum Hypertens*, 2016; 30(5):346-51. *Original article*

Gafane L, Schutte A, Van Rooyen J, Schutte R. OS 32-08 Sympathetic nerve activity and the low renin phenotype: the SABPA study. *J Hypertens,* 2016 Sep; 34 Suppl 1:e391. doi: 10.1097/01.hjh.0000501003.23294.ed. *Abstract*

Conference presentations

Gafane LF, Schutte R, Van Rooyen JM, Schutte AE. Plasma renin and cardiovascular responses to the cold pressor test differ in black and white populations. The Physiology Society of Southern Africa (PSSA), Parys, Free State, South Africa, 06-09 September 2015. *Oral presentation*.

Gafane LF, Schutte R, Van Rooyen JM, Schutte AE. Renin predicts all-cause and cardiovascular mortality in Africans with low renin levels. The Stroke and Hypertension Congress (SAHS), Muldersdrift, Gauteng, South Africa, 19-21 August 2016. *Awarded with the Platinum Oral Award for the best oral presentation.*

Gafane LF, Van Rooyen JM, Schutte R, Schutte AE. Sympathetic nerve activity and the low renin phenotype: the SABPA study. The 26th Scientific meeting of the International Society of Hypertension (ISH), Coex, Seoul, South Korea, 24-29 September 2016. *Oral presentation*.

SUMMARY

The cardiovascular profile of the low renin phenotype in a black South African population

Motivation

Blood pressure is decreasing globally, however, the prevalence of hypertension continues to increase in Sub-Saharan African countries such as South Africa. Populations of African ancestry are more likely to suffer from cardiovascular outcomes such as cerebrovascular accidents and heart disease due to hypertension, as compared to whites. The mechanisms involved are not clear, particularly the role of the renin-angiotensin-aldosterone system (RAAS). The RAAS is the master regulator of water, electrolyte and blood pressure balance. The rate-limiting enzyme of this cascade, namely renin, is usually suppressed in Africans and as a result low-renin hypertension is common in this population group. Low-renin hypertension is not a diagnosis, but rather a description. A lower renin level indicates that renin secretion in the kidney is inhibited by increasing blood pressure, possibly due to volume-overload. Features of low-renin hypertension include blood pressure sensitivity to increased salt intake; a poor response to angiotensin blockade; and a positive response to calcium channel blockers, aldosterone blockade or diuretics. It is therefore questionable whether the activation of the RAAS has a causal role in the development of hypertension and its associated complications in Africans. Relationships between the components of the RAAS and cardiovascular disease are well established in other populations, however such information in Africans is scant, particularly a detailed physiological description of the low renin phenotype.

Aim

The overarching aim of this study was to examine the cardiovascular profile of a black South African population, and the associations of renin, aldosterone, and their ratio with cardiovascular

haemodynamics. In addition, the study aimed to determine if the low renin phenotype is associated with an increased risk for cardiovascular- and all-cause mortality. Firstly, the frequency of low renin levels in a black and white population was determined as well as the associations between renin and cardiovascular responses to a laboratory stressor, the cold pressor test (CPT). It was then explored whether aldosterone and renin relate to surrogate measures of sympathetic activity. Lastly, the prognostic value of renin and its interactions with systolic blood pressure (SBP) for all-cause and cardiovascular mortality was investigated.

Methodology

This thesis used data collected from the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) and Prospective Urban and Rural Epidemiology (PURE) studies. For the first objective, our study population consisted of 153 black and 188 white men and women (age range, 20 to 65 years) from the SABPA study. Haemodynamic measurements included blood pressure (BP), heart rate (HR), stroke volume, total peripheral resistance (TPR) and Windkessel arterial compliance. Active plasma renin levels were determined at rest and when a stressor (CPT) was applied. Reactivity was calculated for each participant as the percentage change from the resting value. For the second objective, black (N=162) and white (N=206) participants (also from the SABPA study) with similar age range as aforementioned were included. The study population was stratified by low and high renin status and the focus was on the low renin groups. Ambulatory BP and HR were measured and night-time dipping calculated. Biochemical analyses were done for plasma renin and aldosterone, and then the aldosterone-to-renin ratio (ARR) was calculated. Noradrenaline and creatinine were determined in urine and the noradrenaline:creatinine ratio was calculated. Lastly, from the PURE study, plasma renin was determined in 1502 black men and women from urban and rural areas in South Africa (age ≥ 35 years), and mortality was assessed over five years. The population was divided into low and high renin groups based on the cut-off from the Renin III CISBIO kit.

Results

Lower renin and elevated BP were apparent in blacks compared to whites at rest and during stress (both, *P*<0.001). When a stressor was applied, HR increased more in blacks (*P*<0.001), whereas stroke volume (*P*<0.001) and arterial compliance (*P*=0.013) decreased more in blacks compared to their white counterparts. There was a positive association between TPR reactivity and renin reactivity in blacks only (β=0.17; *P*=0.041), while in whites diastolic BP reactivity was positively associated with renin reactivity (β=0.21; *P*=0.005).

Furthermore, a high percentage of blacks exhibited a low renin status (80.9%) compared to whites (57.8%) (*P*<0.001). In univariate and after multivariate analyses the following significant associations were evident only in low-renin blacks: noradrenaline:creatinine ratio associated positively with aldosterone (β=0.32, *P*=0.001), 24-hour HR associated positively with renin (β=0.17, *P*=0.041), while HR dipping associated negatively with aldosterone (β=-0.30, *P*=0.001) and ARR (β=-0.23, *P*=0.010). No significant findings were obtained in whites in the low renin group.

Lastly, multivariable-adjusted Cox-regression analyses were performed. In the low renin group, SBP and renin*SBP interaction, but not renin, predicted both all-cause [(HR, 1.41; 95% CI, 1.07-1.87; *P*=0.014), (HR, 1.72, 95% CI, 1.05-2.83, *P*= 0.031)] and cardiovascular mortality [(HR, 1.87; 95% CI, 1.16-3.01; *P*=0.010), (HR, 2.40; 95% CI, 1.06-5.46; *P*=0.037)]. In the total group, renin and SBP*renin predicted all-cause, but not cardiovascular mortality [(HR, 1.33; 95% CI, 1.07-1.65; *P*=0.011), (HR, 1.30; 95% CI, 1.06-1.60; *P*=0.012)]. In the high renin group, neither renin, SBP nor the renin*SBP predicted all-cause or cardiovascular mortality.

Conclusion

The low renin phenotype (volume-loading hypertension) is eminent in the black South African population, and may be suggestive of an increased cardiovascular risk. Although blacks had supressed renin levels at rest and during acute stress, vascular resistance reactivity associated

positively with renin reactivity only in the black population. These results suggest that even at low renin levels a sympathetic response evoked by stress is linked to increased peripheral vascular responses, which may contribute to elevated BP in blacks. Furthermore, in a black low-renin population, the observed associations of surrogate indices of sympathetic nerve activity with components of the RAAS suggest that higher aldosterone levels relative to renin may have detrimental effects on the heart, and that the effects of aldosterone may be coupled to sympathetic dominance. Lastly, the interaction of renin with SBP is predictive of all-cause and cardiovascular mortality only in Africans with low renin levels.

Key words: black, renin, aldosterone, sympathetic nervous system, total peripheral resistance, hypertension, cardiovascular mortality

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LIST OF ABBREVIATIONS

AAMI	-	Association for the Advancement of Medical Instrumentation
ABPM	-	Ambulatory blood pressure monitoring
ACE	-	Angiotensin-converting enzyme
ACR	-	Albumin-to-creatinine ratio
Ang	-	Angiotensin
ANS	-	Autonomic nervous system
APA	-	Aminopeptidase A
ARB	-	Angiotensin Receptor Blocker
ARR	-	Aldosterone-to-renin ratio
AT_1R	-	Angiotensin II type 1 receptor
AT_2R	-	Angiotensin II type 2 receptor
BMI	-	Body mass index
CCB	-	Calcium channel blocker
CO	-	Cardiac output
CPT	-	Cold pressor test
CrCl	-	Creatinine clearance
CRP	-	C-reactive protein
CVD	-	Cardiovascular disease
Cwk	-	Windkessel arterial compliance
DBP	-	Diastolic blood pressure
ECG	-	Electrocardiogram
EDTA	-	Ethylene-diamine-tetraacetic acid
EnaC	-	Epithelial sodium channel
GFR	-	Glomerular filtration rate
GGT	-	Gamma glutamyl transferase
GRK4	-	G protein-coupled receptor kinase 4
HbA1c	-	Glycated haemoglobin

HDL	-	High density lipoprotein
HIV	-	Human immunodeficiency virus
HR	-	Heart rate
HT	-	Hypertension
IL-6	-	Interleukin-6
LDL	-	Low density lipoprotein
LVH	-	Left ventricular hypertrophy
MAP	-	Mean arterial pressure
MasR	-	*Mas* receptor
MrgD	-	*Mas*-related G-protein coupled receptor
PP	-	Pulse pressure
PRR	-	(Pro)renin receptor
PURE	-	Prospective Urban and Rural Epidemiology
RAAS	-	Renin-angiotensin-aldosterone system
SABPA	-	Sympathetic activity and Ambulatory Blood Pressure in Africans
SBP	-	Systolic blood pressure
SD	-	Standard deviation
SV	-	Stroke volume
TC	-	Total cholesterol
TNF-α	-	Tumour necrosis factor alpha
TPR	-	Total peripheral resistance

MEASURING UNITS

cm	-	centimetres
g/L	-	grams per litre
kg/m^2	-	kilograms per meter squared
kg	-	kilograms
mg/L	-	milligrams per litre

mL/min	-	millilitres per minute
mm	-	millimetres
mmHg	-	millimetre mercury
mmol/L	-	millimole per litre
ng/mL	-	nanograms per millilitre
U/L	-	Units per litre

CHAPTER 1

Introduction and literature overview

1. GENERAL INTRODUCTION

The burden of cardiovascular disease (CVD) is increasing worldwide, and is a major challenge in developing countries such as South Africa, which is plagued by a high prevalence of hypertension [1-5]. It is estimated that the prevalence of hypertension will be 29.2% by 2025 in the adult population and that 1.56 billion people in developing countries will be living with hypertension [1]. Hypertension is currently the major risk factor leading to cardiovascular damage and complications including coronary artery disease, heart failure, stroke and renal disease [6, 7]. The development of hypertension and its effects on the cardiovascular system are mainly attributable to the interplay between genetic and environmental factors, which consequently determine CVD development and progression [2, 8].

Poor diagnoses, inadequate treatment and the co-existence of hypertension with other morbidities such as diabetes contribute to extensive target organ damage and premature death due cardiovascular causes [8]. The focus of this thesis is to attempt to decipher some of the aspects pertaining to the low renin phenotype that often characterises hypertension in Africans. This may be achieved by determining the characteristics of some of the components of the renin-angiotensin-aldosterone system (RAAS) and contributing factors such sympathetic activation, as well as investigating the associations with the cardiovascular profile and determining if there is any cardiovascular risk associated with the low renin phenotype.

The RAAS is among the major pathways that regulate blood pressure and fluid balance [9]. Its pivotal role in the pathogenesis of hypertension and CVD is well documented with various mechanisms proposed [10, 11]. Downstream components of the RAAS, such as angiotensin II (Ang II) and aldosterone, are linked to pathophysiological pathways leading to disease development and progression to organ damage [12, 13]. Renin has been shown to predict cardiovascular outcomes, however its direct role as the rate limiting step of this pathway in increasing cardiovascular risk remains unclear [14-16]. Despite a higher prevalence of hypertension and severe cardiovascular outcomes, black populations are often characterised

by low circulating renin levels [2, 3, 17]. Investigations into contributions of the RAAS are mandatory owing to the significant increase in the prevalence of CVD and endpoints such as stroke and heart failure [3, 7, 18].

This chapter entails the applicable background and literature overview that is supplemented by the specific backgrounds given for each manuscript. Pathways responsible for regulation of blood pressure such as the nervous system and RAAS are discussed as well as their links to hypertension and CVD. This is followed by factors associated with hypertension in black populations including low-renin hypertension and its possible causes. Target organ damage, blockade of the RAAS as treatment for hypertension in relation to the topic of the thesis are covered and lastly the relationship between renin and mortality.

2. LITERATURE OVERVIEW

2.1. Blood pressure regulation

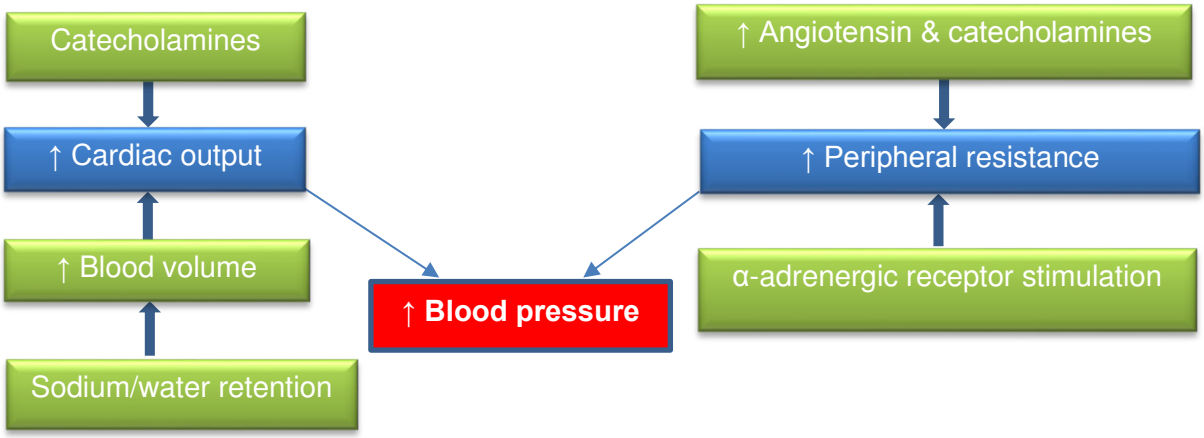

Figure 1: Schematic representation of basic blood pressure regulation. *Adapted from* Swales *et al.* [19].

Control of blood pressure is dependent on the actions of the cardiovascular, neural, endocrine and renal systems [20]. Therefore, understanding the combined role of these systems in the pathophysiology of hypertension requires knowledge of the individual contribution of each to chronic elevation of blood pressure. Various systems are briefly discussed; however, the focus

of this thesis is on the RAAS and related factors. Acute blood pressure regulation includes vasoconstriction and vasodilation which is attributable to local mechanisms, while chronically the number and calibre of blood vessels supplying the specific tissues are altered [20].

Maintenance of blood pressure is essentially dependent on the balance between cardiac output and peripheral vascular resistance which are controlled by the autonomic nervous system (ANS) [21]. Following secretion after stimulation of the sympathetic nervous system, catecholamines increase cardiac output. Additionally, increased water and sodium retention by the renal tubules increases blood volume, and ultimately cardiac output. Stimulation of α-adrenergic receptors increases peripheral vascular resistance, which together with high cardiac output result in elevated blood pressure [19, 22] (Figure 1).

It has been suggested that in the early stages of essential hypertension development, total peripheral resistance is not elevated and the increase in blood pressure is due to high cardiac output [23]. Consequently, peripheral vascular resistance in the arterioles may be increased as a compensatory mechanism to prevent transfer of the high blood pressure to the capillary bed, where it can cause fluid leakage and alter cell function [23]. Africans have a higher total peripheral resistance at rest and during stress compared to whites [24, 25], indicative of the pronounced role of the sympathetic nervous system in hypertension in black populations.

2.1.1. The nervous system

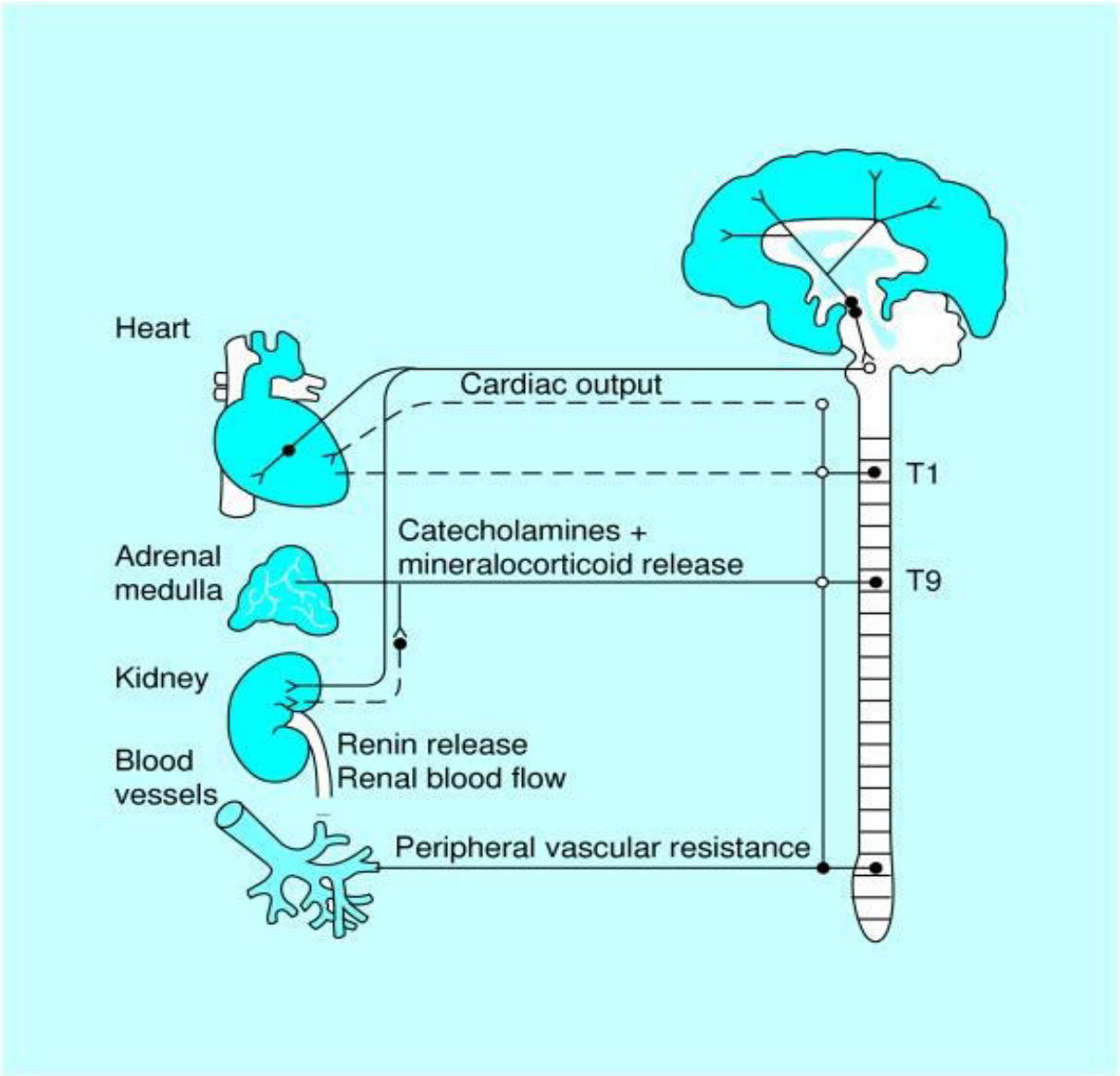

Figure 2: The autonomic nervous system and its control of blood pressure. *From* Swales and De Bono [26].

The autonomic nervous system is a collection of efferent and afferent neurons connecting the central nervous system and the internal effector organs such as the heart, liver and kidneys [27] (Figure 2). The ANS consists of two efferent pathways, namely, the sympathetic and parasympathetic nervous systems, which regulate both cardiac output and vascular resistance [27]. The main role in neural blood pressure control is played by the sympathetic nervous system, which is capable of both vasoconstriction and vasodilation, while the parasympathetic

nervous system contributes primarily to regulation of cardiac functions such as myocardial contractility [21]. Short term blood pressure regulation is maintained by the involvement of the sympathetic nervous system and RAAS, while long-term regulation is mostly dependent on the kidney [28, 29].

Raised blood pressure stimulates the baroreceptors in the carotid sinus and aortic arch, resulting in a reflex vagal bradycardia, mediated by the parasympathetic nervous system and inhibition of the sympathetic output from the central nervous system [29-31]. Cardiopulmonary receptors in the atria and ventricles likewise respond to increases in atrial filling by causing tachycardia via inhibition of the cardiac sympathetic nervous system, increasing atrial natriuretic peptide secretion and inhibiting vasopressin release [29-31]. Previous observations in Africans indicated a blunted baroreceptor sensitivity and depressed heart rate variability, supporting the sympathetic dominance in this population group, probably due to chronic stress [32, 33].

Heightened cardiovascular reactivity to stress has been shown to be one of the predominant factors linked to hypertension in black populations [22, 34]. In Africans, higher reactivity to stress in peripheral vascular resistance, blood pressure and heart rate compared to whites has been observed [24, 35]. There are two types of laboratory stressors that are commonly used to assess cardiovascular reactivity, namely, the STROOP Colour Word Conflict Test and the Cold Pressor Test (CPT) [24, 36]. The STROOP test is a mental stressor that stimulates mixed α-adrenergic and β-adrenergic receptors which results in myocardial reactions through central mechanisms [37].

The cold pressor test is a method used to study cardiovascular stress reactivity by immersing an individual's foot or hand in ice water for 1 minute. This results mostly in a peripheral vascular response with increased vascular resistance through α-adrenergic receptor stimulation [38]. The increased total vascular resistance increases ventricular afterload and blood pressure, thereby interfering with the heart's ability to increase stroke volume during

stress [22, 38]. In addition, the secretion of renin via stimulation of β-adrenergic receptors during sympathetic activation promotes vasoconstriction, further contributing to systolic and diastolic blood pressure elevation via the actions of Ang II [2].

2.1.2. The classic renin-angiotensin-aldosterone system

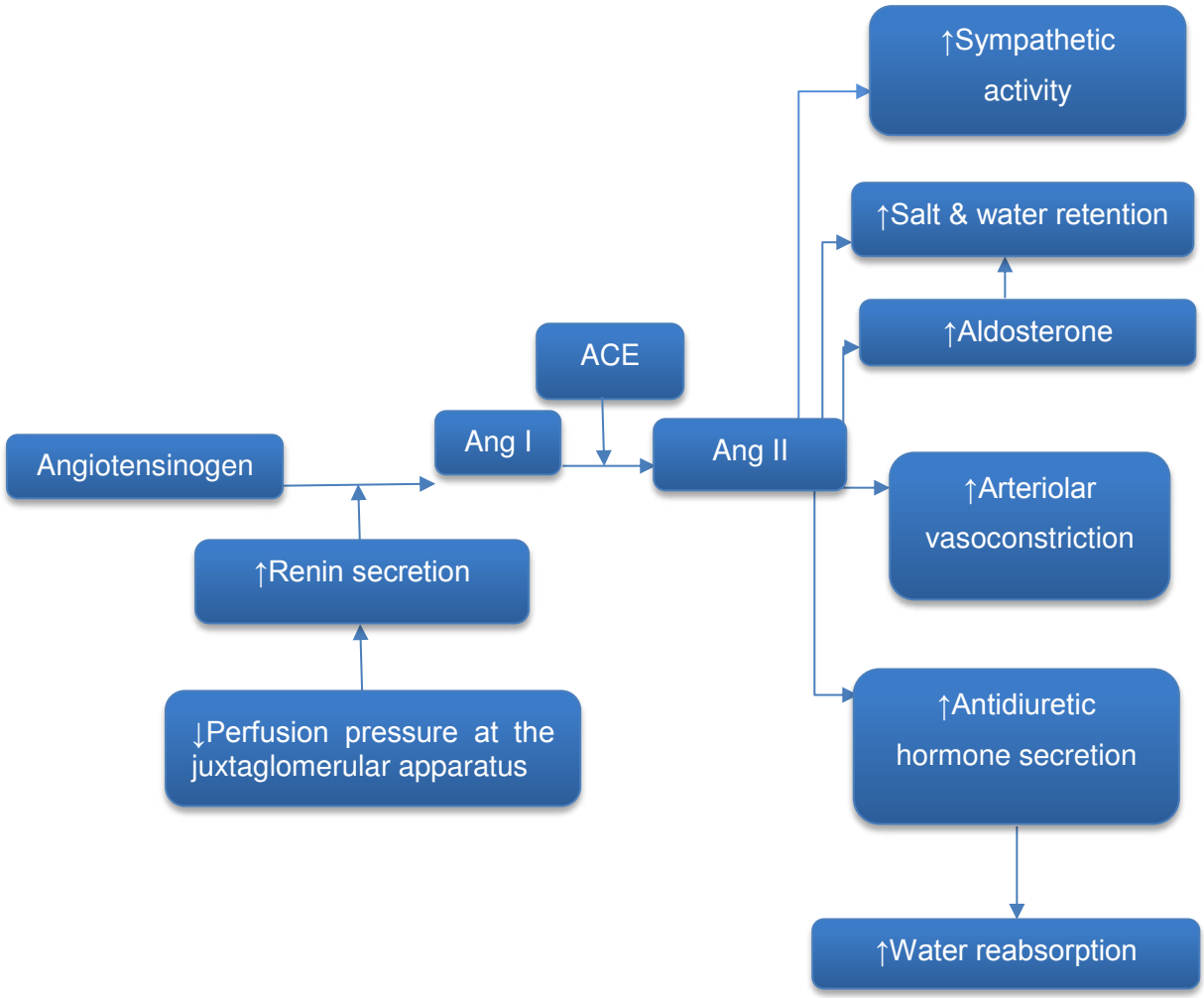

Figure 3: A simplified depiction of the classic renin-angiotensin-aldosterone system and its control of blood pressure. *Adapted from* Rad [39]. ACE, angiotensin-converting enzyme; Ang, angiotensin.

The RAAS plays a major role in the regulation of blood pressure, cellular growth and cardiovascular remodelling [9, 40, 41]. Its components have been linked to hypertension and

target organ damage leading to cardiovascular morbidity and mortality [42]. The RAAS is now recognised as a dual vasoactive system that acts as both a circulating endocrine system and a local tissue paracrine system [43, 44]. In the classical RAAS, the substrate angiotensinogen is degraded by renin into angiotensin I, which is then converted into Ang II by angiotensin converting-enzyme (Figure 3). Angiotensin II is the main effector molecule of the system that stimulates signal pathways in the heart, vasculature, kidneys, adipose tissue, pancreas and brain that results in physiological and pathophysiological effects attributable to the RAAS [11, 45, 46].

2.1.2.1. Renin

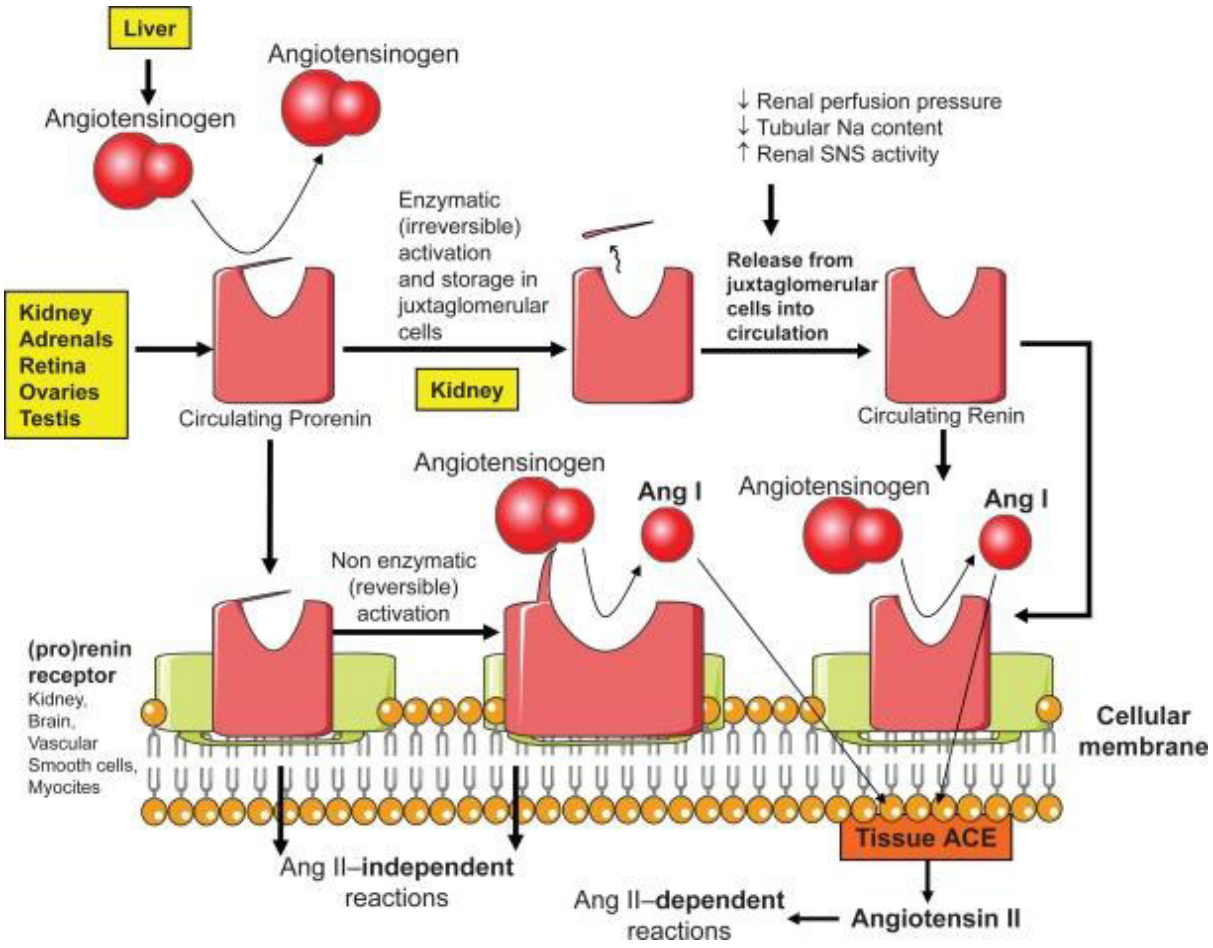

Figure 4: **Overview of prorenin, active renin and the (pro)renin receptor. *Adapted from* Verdecchia *et al.* [47].** Ang, angiotensin.

Renin, the initiator of the RAAS cascade of events was discovered in 1898 [48]. It is produced from circulating prorenin, its precursor protein [49] (Figure 4). Prorenin is activated by removal of the N-terminal by proteases in the kidney, then the active renin is stored and secreted at the juxtaglomerular apparatus [50]. The main function of renin in the classical RAAS is to cleave angiotensinogen which is mainly synthesized by the liver into angiotensin I [6, 50]. Prorenin is found mainly in the kidney and in other organs, including the brain and heart tissues [49]. These tissues are capable of secreting prorenin locally and into plasma [49].

Physiologically, prorenin is secreted constitutively and its plasma levels are usually correlated to that of renin, even though prorenin may be 10 times higher than circulating renin [51]. It has also been suggested that prorenin is 20% higher in blacks compared to whites [2]. In conditions such as pregnancy and diabetes, plasma prorenin is higher than renin, resulting in a higher prorenin-to-renin ratio [52, 53]. Prorenin was predictive of microvascular complications in diabetes and accounted for the increased intrarenal Ang-II production that may have contributed to diabetic nephropathy at low renin states [53, 54].

The (pro)renin receptor is expressed in organs including the kidney, vascular wall, brain and cardiac myocytes and has an affinity for both renin and prorenin, with a higher affinity for prorenin [55, 56] (Figure 4). Binding of prorenin and/or renin to the (pro)renin receptor has both angiotensin-dependent and angiotensin-independent effects [57]. In addition, (pro)renin receptor-bound renin has an increase in the catalytic efficiency of converting angiotensinogen to angiotensin I compared to free renin [9]. The angiotensin-independent effects include activation of the profibrotic and pro-inflammatory pathways that can increase cardiac and renal hypertrophy and fibrosis [52, 55, 58, 59]. The receptor is upregulated in hypertension and is linked to kidney diseases such as diabetic nephropathy [55, 57, 60].

2.1.2.2. Angiotensinogen

Angiotensinogen is the substrate for renin and the source of all angiotensin peptides [9]. Angiotensinogen is primarily synthesised by the liver; however other organs such as the brain,

kidney and the immune cells are capable of producing angiotensinogen [61, 62]. Alterations in angiotensinogen levels in the aforementioned tissues can affect the functioning of local RAS independent of circulating angiotensinogen [9]. Other factors such as oxidative stress can also affect the interaction between renin and angiotensinogen [63]. In the kidney, the production of angiotensinogen by the proximal tubules can be accelerated by Ang II that forms part of the local intrarenal RAAS. This feed forward mechanism may result in augmented sodium reabsorption and eventually hypertension [64].

Due to the role played by intrarenal angiotensinogen on sodium reabsorption and hypertension, urinary angiotensinogen can be utilised to assess intrarenal RAAS stimulation in hypertension and chronic kidney disease [9, 65, 66]. Urinary angiotensinogen was positively associated with blood pressure in a salt-sensitive, low renin group of black individuals, thus showing that even though circulating renin is suppressed, the amount of the substrate in tissue RAS may play a significant role in maintaining blood pressure [67].

2.1.2.3. Angiotensin-converting enzyme

Angiotensin-converting enzyme (ACE) cleaves the C-terminal dipeptide of angiotensin I to produce Ang II [68]. Angiotensin-converting enzyme is located in various tissues and biological fluids [69, 70]. It has two isoforms, somatic ACE and testis ACE, the former mainly distributed in epithelial and endothelial cells [70]. Its other roles include promoting degradation of bradykinin and reduction of nitric oxide bioavailability, both of which result in reduced vasodilation [71, 72]. Ethnic differences regarding the levels of ACE in plasma and its associations with measures of cardiovascular function has been reported. He *et al.* found similar ACE levels in both blacks and whites, however ACE associated negatively with blood pressure in blacks, while the association was positive in whites [73], suggesting variation in blood pressure regulation.

2.1.2.4. Angiotensin II

Angiotensin II regulates blood pressure by directly influencing vascular smooth muscle cells, sodium and volume homeostasis as well as aldosterone secretion [46]. Its actions involve direct elevation of blood pressure, increased sodium reabsorption, reactive oxygen species formation as well as proinflammatory and proliferative effects on various cells types [74, 75]. It promotes cell growth, cytokine production [76] and pathological conditions including oxidative stress, inflammation, endothelial dysfunction and tissue remodelling [46, 77].

The effects of Ang II are mediated through activation of specific receptors, and almost all the adverse effects on blood pressure regulation are attributable to angiotensin II receptor type 1 (AT_1R) [6]. It was recently shown that hypertensive African men exhibit lower levels of both angiotensin I and II as compared to their white counterparts, possibly due to suppression of renin secretion by high blood pressure [78]. Unlike AT_1R, consequences of angiotensin II receptor type 2 (AT_2R) activation are still a matter of investigation [76]. Most of the available evidence and suggestions indicate that AT_2R's actions mainly oppose the effects of AT_1R activation [45], specifically during pathological conditions [79].

Direct binding of the AT_2R protein on AT_1R, and its subsequent activation has also been shown to have the ability to protect organs such as the brain against ischemia [80, 81]. Stimulation of AT_2R promotes nitric oxide production that is enhanced by the presence of bradykinin, resulting in vasodilation [82]. The AT_2R was also shown to induce sodium excretion and this effect is mediated by endogenous Ang III, a by-product of Ang II breakdown by aminopeptidase [83]. Furthermore, activation of this receptor promotes the production of inhibitory G-protein and extracellular kinases that subsequently inhibit growth, while apoptosis is favoured [84]. Additionally, the inhibitory G-protein stimulates arachidonic-acid mediated hyperpolarisation and decreased membrane excitability [85].

2.1.2.5. Aldosterone

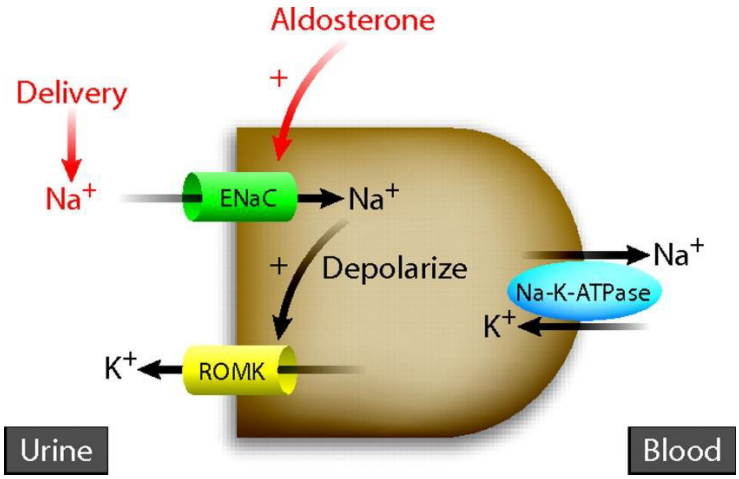

Figure 5: Aldosterone-mediated sodium reabsorption in the distal nephron. *From* Huang and Kuo [86].

Aldosterone is a mineralocorticoid hormone produced in the zona glomerulosa of the adrenal cortex. Its secretion is stimulated mainly by Ang II and potassium [87, 88]. The primary function of aldosterone in the kidney includes sodium reabsorption and potassium secretion by the renal tubular epithelial cells [20] (Figure 5). Sodium reabsorption at the distal nephron of the kidney is mediated by the epithelial sodium channel (ENaC) (Figure 5) [89]. In the highly regulated aldosterone-sensitive distal tubule, the transport of sodium is carried out in two phases, the early phase lasting 1-4 hours and the late phase after 4 hours. In the late phase, ENaC is upregulated and NA^+/-K^+-ATPase expression increased [90].

In addition to sodium and volume retention, aldosterone is associated with inflammation, oxidative stress, fibrosis and necrosis, especially in the heart and vasculature [87, 91]. Administration of amiloride, a potassium-sparing diuretic, not only reduces blood pressure by attenuating sodium reabsorption, but also directly improves vascular function by alleviating cell stiffness and swelling attributable to insertion of sodium channels [92, 93]. These regulatory effects seems to be mediated by aldosterone [94].

2.1.3. Recent developments in the renin-angiotensin system

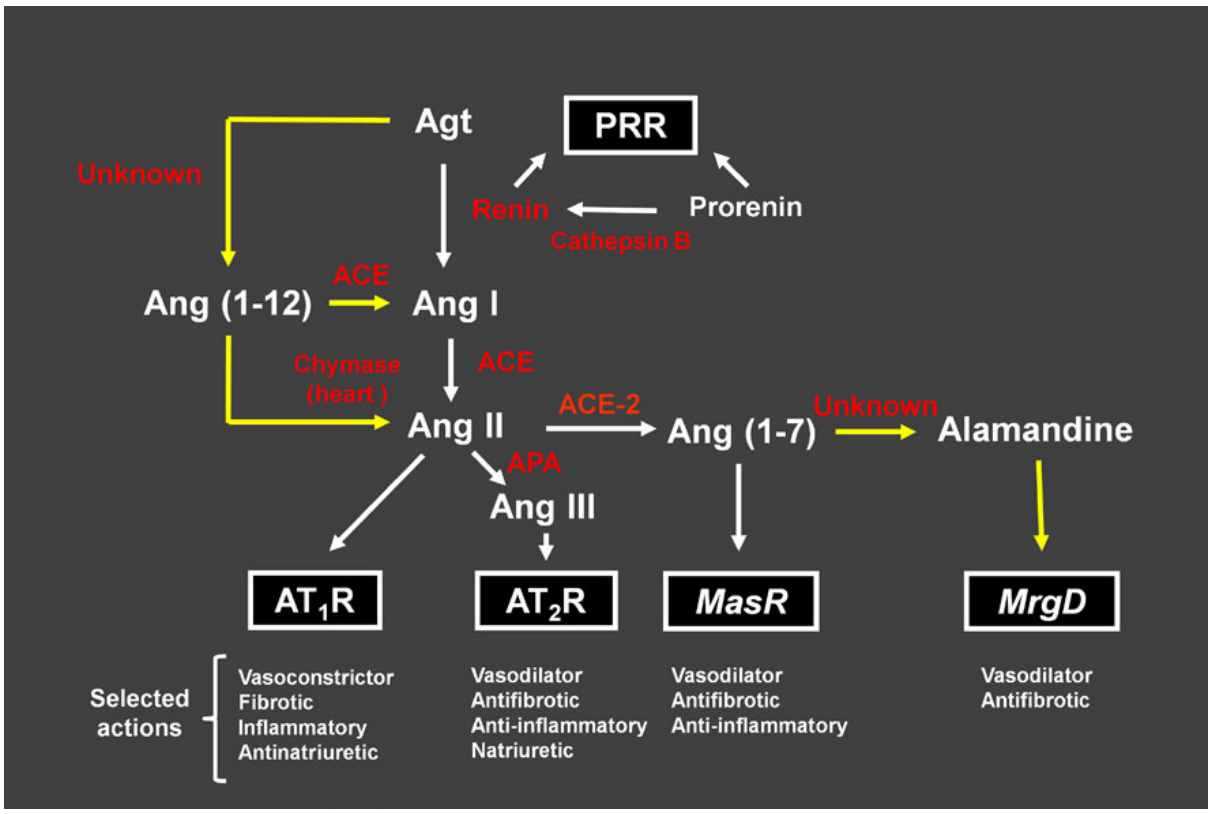

Figure 6: Overview of the recent advances in proteins, peptides, enzymes and receptors of the renin angiotensin system. ***From*** **Carey [40].** ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; Agt, angiotensinogen; APA, aminopeptidase A; PRR, (pro)renin receptor; AT_1R, angiotensin II type 1 receptor, AT_2R, angiotensin II type 2 receptor, *MasR*, *Mas* receptor; *MrgD*; *Mas*-related G-protein coupled receptor.

Explorations into the complexity of the role of the RAAS continue to be a novel part of research in cardiovascular and renal function as depicted in Figure 6. The highlights of these new discoveries are enzymes including angiotensin-converting enzyme 2 (ACE2) and chymase, peptides [Ang-(1-12), Ang-(1-9), Ang-(1-7)] and receptors [AT_2R, (pro)renin receptor, *Mas* receptor] [76]. Angiotensin-converting enzyme 2 is a carboxypeptidase that share some sequential similarities with ACE [95]. It increases the conversion of Ang I to Ang (1-9), which will subsequently be converted to Ang (1-7) [96], which possesses vasodilatory and growth inhibitory effects mediated by the *Mas* receptor [97].

Angiotensin-converting enzyme 2 can also hydrolyse Ang A, producing alamandine ([Ala-Ang (1-7), that varies from Ang (1-7) by the presence of an N-terminal [98]. Alamandine can also be generated from Ang (1-7), however, the enzyme responsible for this reaction is unknown. Additionally, alamandine reduces blood pressure and possesses vasodilatory and antifibrotic properties that may be through activation of the *Mas*-related receptor known as *Mas*-related G-protein coupled receptor (*MrgD)* [98, 99]. The AT_2R and (pro)renin receptor were discussed under renin and Ang II sections, respectively.

Chymase is a chymotrypsin-like enzyme present in the secretory granules of mast cells that is involved in the catalyses of Ang I to Ang II reaction in the heart and vasculature [100, 101]. Together with other enzymes, chymase may also contribute to the formation of Ang (1-12) from the substrate angiotensinogen [40]. Ang (1-12) is found in the digestive tract, aorta, heart and kidneys and it was validated as a potent vasoconstrictor and possible precursor of Ang II [102].

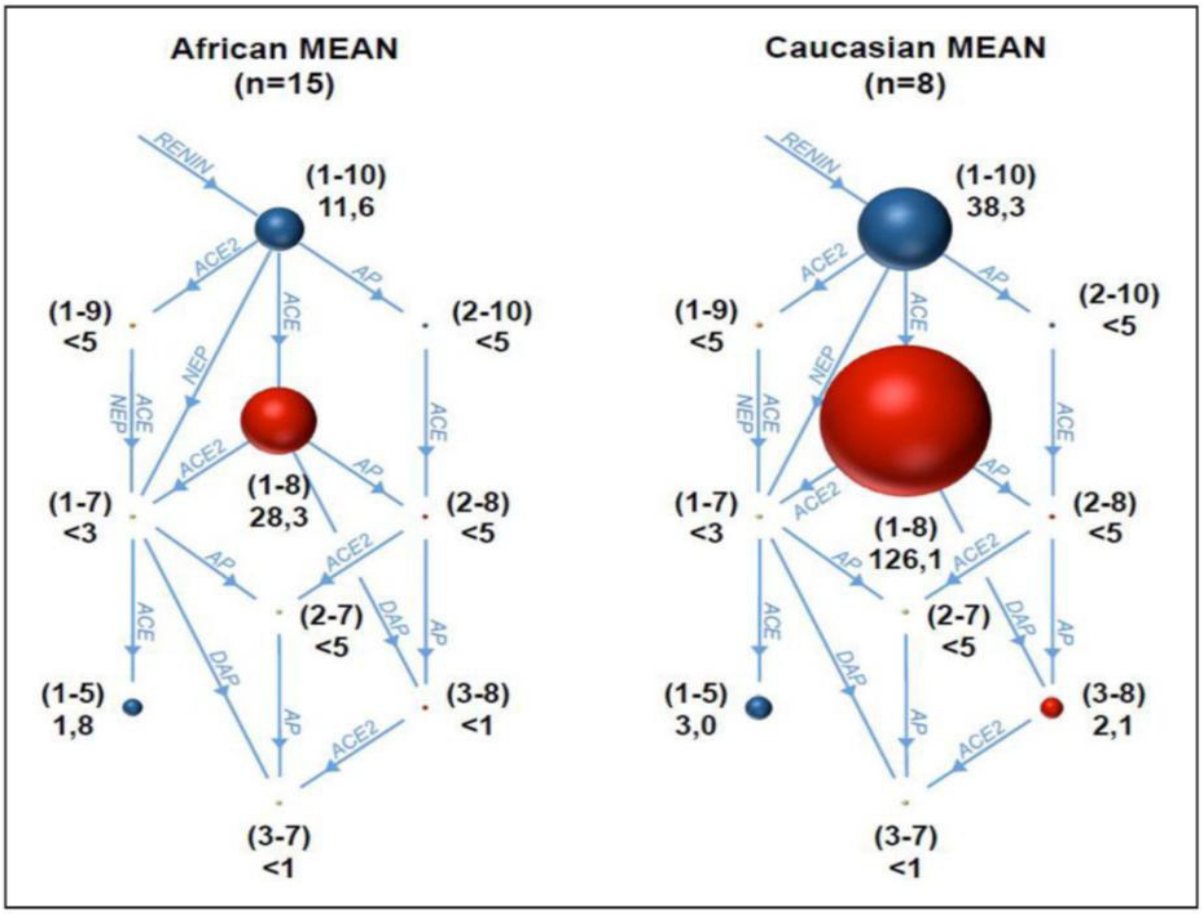

Figure 7: The Equilibrium RAS-Fingerprint of 15 hypertensive black (left) and eight hypertensive white (right) men. *From* Van Rooyen [78]. Sphere sizes, concentrations; blue arrows, enzymatic pathways. AP, aminopeptidase; NEP, neutral endopeptidase; DAP, di-aminopeptidase. The numbers in brackets are the sequence of the corresponding angiotensin metabolite.

In a recent study, including a small sample of hypertensive black and white men from South Africa, we have found that Ang (1-5), the downstream metabolites of Ang (1-7), were lower in blacks compared to whites [78] (Figure 7). Furthermore in Africans, urinary angiotensinogen is associated with blood pressure independent of the circulating RAAS and it seems that at low renin levels blood pressure is partly maintained by angiotensinogen [67,103]. The intrarenal RAAS was discovered and established as the key component in renal sodium excretion and blood pressure regulation [64]. Ang II formation within the renal tubules is accelerated via Ang II induced upregulation of the substrate angiotensinogen by means of a

positive feedback mechanism [9, 64]. This has been suggested as the mechanism through which intra-renal RAAS contributes to hypertension and renal damage [64].

Adipose tissue RAS is also regarded as an important factor in the development of hypertension [40]. The pathway in the synthesis of Ang II in adipocytes involves local cellular production of angiotensinogen which is in turn cleaved by chymase and cathepsins [43]. Since both the circulatory RAAS and adipose RAS are activated in obesity, it remains a challenge to determine if local adipose RAS plays a role in obesity-induced hypertension [40]. It was reported that a deficient adipose RAS prevents obesity-induced hypertension in mice, indicating that adipose RAS has a definite role in the mechanisms by which obesity causes hypertension [104].

2.1.4. The renal dopamine system

Dopamine is essential for the control of sodium balance and blood pressure through renal mechanisms [105]. The renal dopamine system is regarded as one of the main regulators of renal sodium excretion in cases of increased sodium intake [106]. Dopamine can be synthesised independent of sympathetic innervation [105, 107]. Alexander *et al*. found that high salt consumption results in a corresponding urinary sodium and dopamine excretion [108]. This is supported by findings that emphasise the role of the paracrine dopamine produced locally within the renal tubules in the control of sodium transport and excretion [109, 110]. The mechanisms of action include simultaneous inhibition of Na^+/K^+-ATPase activity and the Na^+/H^+ exchanger in the renal tubules, decreasing sodium transport and reabsorption [109, 111].

Dopamine can also regulate sodium and fluid balance via hunger and satiety centres in the hypothalamus and gastrointestinal tract [112, 113]. It modulates secretion of other hormones or messengers that are involved in control of the blood pressure and can either stimulate or inhibit dopamine's inhibitory effects on sodium transport [114, 115]. Intracellular sodium and

dietary sodium are the main stimulants for renal dopamine synthesis and secretion. This stimulatory effect was found to be impaired in prehypertensive and hypertensive Dahl salt-sensitive rats [116]. Furthermore, a decreased renal synthesis of dopamine and a defect in the D1 receptor-G protein-coupling have been linked to the development of hypertension [117].

In the proximal tubule, dopamine exerts its effects via the G-protein coupled receptor kinase 4 (GRK4), promoting sodium excretion which is enhanced by atrial natriuretic peptide [118, 119]. It seems that dopamine and GRK4 may have a role in low-renin and salt-sensitive hypertension. Variants of GRK4 are associated with sodium retention and hypertension in experimental models [120]. In human studies, GRK4 is associated with salt-sensitive hypertension and low aldosterone [121, 122], while in black populations GRK4 is additionally linked to impaired sodium excretion [122, 123]. These suggest that dopamine and GRK4 may be involved in the underlying mechanisms of volume-loading hypertension that result in supressed renin and aldosterone.

3. HYPERTENSION IN BLACK POPULATIONS

3.1. Prevalence

Hypertension is the most common reversible risk factor for cardiac and stroke events, left ventricular hypertrophy (LVH), renal disease and blindness [8]. Despite previous reports indicating that global blood pressure is declining, the prevalence of hypertension is still increasing in African countries [124]. This was confirmed in a black South African population of which 24% of the study participants with optimal blood pressure developed hypertension over five years [125]. Black hypertensive patients are more likely to encounter cerebral haemorrhage, kidney disease resulting in uraemia and congestive heart failure as compared to whites and Indians who are prone to coronary heart disease [126]. In addition, malignant hypertension is more common in individuals of African ancestry and has more severe

outcomes and the shortest survival period compared to other population groups [127]. The following section focuses on some of the factors predisposing black populations to the development and severe outcomes of hypertension, particularly “low-renin hypertension”, which is a common phenomenon in individuals of African descent.

3.2. Low-renin hypertension

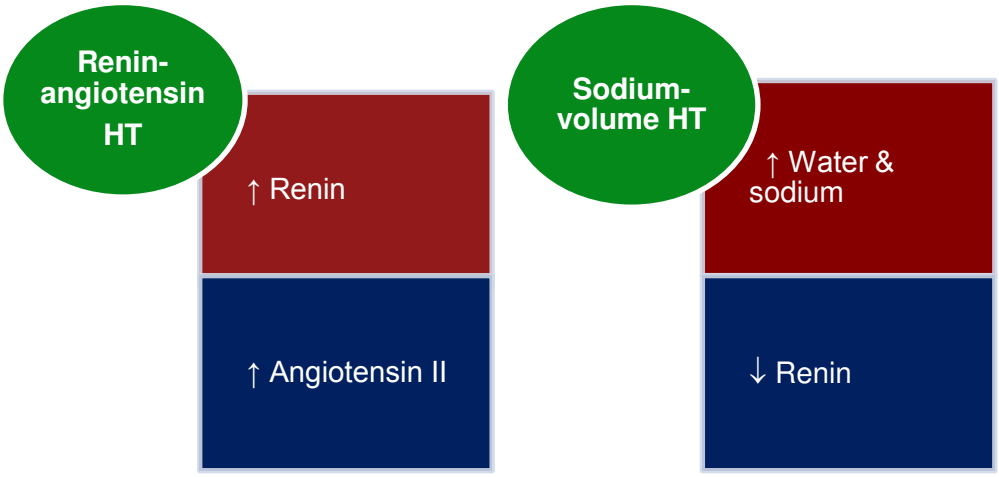

Figure 8: Two forms of renin-related hypertension. *Adapted from* Laragh *et al.* and Gordon *et al.* [128, 129]. HT, hypertension.

There are two types of renin-related hypertension (Figure 8). First, is the renin-angiotensin dependent hypertension which develops when there is not sufficient suppression of renin secretion according to sodium-volume content and is common in the medium to high renin hypertensive individuals [128, 130]. The second type is the arterial sodium-volume hypertension which is due to the kidney‘s reduced ability to excrete salt, resulting in a continuous systemic increase in sodium and water [128]. This type is characterised by a low renin status caused by suppression of renin secretion by elevated arterial blood pressure resulting from high content of sodium and resultant volume expansion [129].

The definition of low-renin hypertension should depend on the reference population and the type of assay used [129]. This is because low renin levels are a reflection of high perfusion pressure at the juxtaglomerular apparatus [131]. Since renin levels should ideally be low in

healthy populations, the problem arises when a low renin status is a result of sodium-volume overload in which both renin and aldosterone decrease in the presence of increased sodium intake, leading to low-renin hypertension [129]. Approximately 25-30% of people suffering from essential hypertension have the low renin variant [132-134].

The phenomenon of low-renin hypertension is common not only in populations of African ancestry, but in Asians as well as elderly individuals [131, 135, 136]. The ethnic distribution can be attributed to differences in sodium homeostasis and mineralocorticoid physiology, while other factors such as age and diabetes may also play a role [137, 138]. The three characteristics of low-renin hypertension include (1) significant sensitivity of blood pressure and plasma volume to increased salt intake (2) poor response to ACE inhibitors, angiotensin receptor blockers (ARBs), and (3) efficient response to calcium channel blockers (CCBs) or diuretics or aldosterone blockers [139-141].

According to the “Slavery Hypertension Hypothesis”, there may be a genotype that favours salt and water retention in black populations in the Western hemisphere, with the majority originating from West and Central Africa and to a lesser extent from South-eastern Africa [142, 143]. This genotype may explain the higher prevalence of hypertension in populations such as African Americans, sometimes even higher than black populations in Africa [143-146]. This hypothesis was based on the fact that modern African populations in the Western hemisphere may be genetically linked to those who survived and adapted, firstly, during the “Middle passage” (transportation of Africans across the Atlantic as slaves) with limited water and electrolytes, and secondly, to hot climates in the plantations, both of which resulted in excessive loss of salt and water [147, 148]. Therefore, a predisposition to retain sodium and water would have been favourable under such circumstances [143]. Even though this hypothesis was severely criticised [149, 150], it was still rendered testable and useful 16 years later [142, 151].

In Southern Africa, there are two main black African groups, the Sotho and Nguni groups, both of which originated from West Africa and formed part of the South-eastern African migration [152-154]. At the time of arrival, Southern Africa and the Kalahari Desert were inhabited by the San population, who led a hunter-gatherers lifestyle in hot climates and had low sodium intake [154, 155]. The Africans and the San shared the same geographical area for between 2000 and 3000 years and this may have resulted in genetic integrations between the two ethnic groups [154, 155]. The genetic mixture may explain the presence of a genetic variant of the EnaC in blacks and mixed ancestry populations, that might have originated from the San since it was absent in West Africans [156]. This variant is associated with hypertension in urban black African and mixed ancestry populations of South Africa [156]. It is probable that the adaptation that was required for survival in low sodium and water intake conditions may predispose to volume-loading hypertension with lifestyle changes to high sodium diets and other environmental exposures.

The tendency towards low-renin hypertension also extends beyond adult black populations. Li *et al*. found lower renin levels in black children compared to white children [157], supposedly due to increased sodium reabsorption, expanded extracellular volume, high adrenal sensitivity to Ang II, high sodium and lower potassium intake and /or increased activity of the distal renal tubule sodium channel [158-161]. Factors contributing to the aetiology of low-renin hypertension including aldosterone and salt-sensitivity are discussed in the following sections.

3.2.1. Aldosterone

Low-renin hypertension may be associated with high aldosterone levels as in Conn's syndrome or low aldosterone levels as in Liddle's syndrome as well as other forms of mineralocorticoid excess [131]. In addition, a variant of the aldosterone synthase gene has been associated with the initial rise in systolic blood pressure in newly diagnosed, untreated black hypertensives [162]. The aldosterone-to renin ratio (ARR) is an index of the extent of aldosterone activation in the context of renin that is used to examine primary aldosteronism

and may also be useful for studying low-renin hypertension [129, 163]. In black South Africans, ARR had a significant contribution to the association of urinary Na^+/K^+ and blood pressure [164]. This was also observed in African Americans whereby an association of ARR with blood pressure in cases of high dietary sodium may indicate a possible role of inefficient aldosterone suppression to salt-sensitivity in populations of African ancestry [163, 165].

The detrimental effects of aldosterone excess and increased dietary sodium on target organs and blood pressure are dependent on the interaction between aldosterone and sodium [166]. In mice, inefficient lowering of aldosterone relative to renin in the presence of sodium loading results in elevated blood pressure [165]. Tomaschitz *et al*. found that across a broad range of ARR values, inappropriately high levels of aldosterone relative to renin had a marked effect on both systolic and diastolic blood pressure [167]. Due to its sodium and water retaining effects [168], aldosterone hypersecretion, either in the form of primary aldosteronism or relative aldosterone excess, have a significant role in development low-renin hypertension [131, 169, 170]. Hyperaldosteronism causes excessive fluid retention, suppressing the renin-angiotensin pathway, meanwhile aldosterone may remain elevated with respect to renin levels, independent of the sodium content [171].

Black hypertensives may exhibit high aldosterone levels and low plasma renin activity, which is indicative of a possible variant of hyperaldosteronism that contributes to the high occurrence of low-renin hypertension [172, 173]. However, it has been shown that blacks as children and adults have lower aldosterone and renin as compared to whites [174, 175]. It seems that the deleterious effects of aldosterone may also be due to enhanced sensitivity of blood pressure to aldosterone in blacks [174]. Another contributing factor to the sodium-overload reflected by low-renin hypertension is inappropriate activation of ENaC by mineralocorticoids [156]. Genetic mutations may cause constitutive activation of the ENaC channel by removing or altering the amino acids of the beta-or gamma subunits. A variant of ENac, R563Q, has been found in individuals of African descent, but not whites, and is associated with low renin, low

aldosterone hypertension, suggesting an underlying predisposition to salt-sensitive hypertension [156].

3.2.2. Salt-sensitivity

Habitual intake of sodium-rich foods accompanied by impaired renal sodium regulation and increased vascular tone are the key factors in the development and progression of hypertension [106, 176]. Increased salt intake has been associated with hypertension in Africans, and it is linked to abnormal sodium transport mechanisms that promote salt retention that eventually suppresses renin secretion [126]. There are discrepancies regarding the short- and long-term effects of high sodium intake on blood pressure [171]. In otherwise healthy normotensive populations, there may not be any immediate detrimental changes in blood pressure in response to increased sodium intake as a result of compensatory sodium excretion by the kidney [177]. However, the blood pressure of certain individuals may fluctuate considerably with salt intake in a matter of days to weeks, and such individuals are regarded as “salt-sensitive” [171]. Different methods have been used to assess salt-sensitivity, the most common being a 10% increase or decrease in blood pressure in response to increased or decreased salt intake, respectively [178].

Alterations in sodium handling by the kidney seems to be the basis of salt-sensitive hypertension. Reduced nephron endowment, and consequently nephron deficiency can cause salt-sensitive hypertension, particularly in cases of high sodium intake [179]. Otani *et al*. found an increase in aldosterone secretion, higher blood pressure and urinary albumin excretion in stroke-prone spontaneously hypertensive rats offspring exposed to prenatal protein restriction and high sodium intake [180]. On the other hand, it was found that maternal ingestion of a high sodium diet modifies the systemic and renal RAAS in male offspring of Wistar rats as shown by lower plasma renin and aldosterone, but higher renal renin concentration [181]. Therefore, high maternal sodium intake hinders kidney development, resulting in offspring with low capacity to maintain sodium homeostasis, which in turn hampers sodium excretion,

promoting volume expansion that supresses the systemic RAAS. As expected, there is a causal relationship between chronically high salt intake and the development of hypertension when the kidney has an insufficient capacity to excrete salt [176].

4. TARGET ORGAN DAMAGE

4.1. Left ventricular hypertrophy

Pressure overload on the left ventricle in arterial hypertension can result in myocardial remodelling associated with cardiac hypertrophy and eventually heart failure [42]. Left ventricular hypertrophy can be a result of intrinsic factors that can cause pathological changes in cardiac structure and function and it is associated with cardiovascular morbidity and mortality [182]. The presence of early target organ damage such as LVH in arterial hypertension increases the risk of major cardiovascular events two- to five-fold [46]. Pathophysiological mechanisms underlying the relationship between LVH and CVD include abnormalities in the coronary arteries or platelets, a prothrombotic state, endothelial dysfunction and systemic inflammation that can result in atherosclerosis [183-185].

It has been observed that the RAAS and salt-sensitivity relate to increased afterload, leading to cardiac and vascular damage including LVH [42, 186, 187]. It was found that LVH was more pronounced in patients with renal artery stenosis as compared to those with primary hypertension with similar blood pressure levels [188]. Further associations of LVH with RAAS was found in clinical trials where calcium antagonists, ACE inhibitors and ARBs reduced left ventricular mass, rather than beta blockers and diuretics [189, 190]. However, in black children and adults, higher aldosterone levels relative to renin are positively associated with left ventricular mass and LVH due to the sodium retention and volume-mediated elevation in blood pressure [157, 191, 192].

4.2. Microalbuminuria

Microalbuminuria refers to the presence of relatively small amounts of albumin in urine [182] and it is a marker of generalised vascular dysfunction [193]. High rates of urinary albumin excretion are associated with target organ damage, renal disease as well as left ventricular dysfunction, stroke and myocardial infarction [194, 195]. The mechanisms linking microalbuminuria to cardiovascular morbidity and mortality are obscured, however the most generally accepted notion is that urinary albumin leakage probably reflects vascular damage, including endothelial dysfunction, low grade chronic systemic inflammation, which precedes renal and extra renal complications [196]. In blacks with low renin levels, renin was adversely associated with albumin-to-creatinine ratio, suggesting that factors associated with the low renin phenotype may pose a risk for cardiovascular damage even when the RAAS is suppressed [197]. Furthermore, urinary albumin excretion was associated with stroke and all-cause mortality in black South Africans [198].

5. THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM AS TARGET FOR HYPERTENSION TREATMENT

The main target in long-term treatment of hypertension is decreasing vascular resistance, however the underlying causes and co-morbid conditions should be considered since vascular tone alone may not be the driving force for elevated blood pressure [106]. In uncomplicated primary hypertension, treatment is initiated with a thiazide or thiazide-like diuretic, ACE inhibitor, ARB and/or CCB used as mono or combination therapy [199]. Black hypertensives have a poor response to antihypertensive drugs that target the RAAS; therefore diuretics and CCBs are recommended as first line treatment in blacks [200, 201]. This response may be explained by the phenotype of hypertension in black populations that is characterised by increased vascular resistance and low renin, volume-loading and salt-sensitive hypertension that may not be secondary to activation of RAAS [201]. Even though hypertension is likely to be identified and treated in black populations, controlling it remains a challenge [202].

Resistant hypertension is failure to achieve blood pressure control using ≥3 antihypertensive drugs from different classes including a diuretic or attaining blood pressure control using ≥4 agents [94, 203]. Approximately 15-20% of patients with resistant hypertension present with aldosterone excess [204, 205]. The discovery of amiloride-sensitive sodium channels in the endothelium and smooth muscle layer of the vasculature suggest that impaired sodium excretion may not be the sole pathway by which aldosterone contribute to resistant hypertension [206].

There seems to be a small proportion of patients with resistant hypertension who never achieve blood pressure control despite maximal medical treatment and this extreme phenotype is referred to as refractory hypertension [207]. It was initially indicated that this phenotype is characterised by increased heart rate and poor response to spironolactone compared to the controlled resistant hypertension group, suggesting that heightened sympathetic output, instead of aldosterone excess may be the contributing factor to the multi-drug failure [207]. In contrast, Calhoun *et al*. demonstrated that the underuse of mineralocorticoid receptor antagonist was among the causes of the high prevalence of refractory hypertension and recommended the use of spironolactone and long-acting thiazide diuretics to reduce the incidence, which is consistent with previous recommendations [203, 208, 209].

6. RENIN AND MORTALITY

Excessive activation of the RAAS is linked to factors that contribute to hypertension development and cardiac abnormalities associated with cardiovascular and renal diseases [12, 210]. High plasma renin was associated with all-cause, but not cardiovascular mortality in the Framingham cohort [211]. Several studies have indicated that renin is associated with cardiovascular events and mortality in patients with CVD and those on hypertension treatment [16, 212, 213]. Proposed mechanisms include aging of the RAAS, (pro)renin receptor activation and maintained activation of the sympathetic nervous system that may result in

vascular damage [213-215]. The relationship between high renin and mortality may also be attributable to the adverse effects of Ang II, the effector molecule of the RAAS. [216]. Ang II vascular remodelling and damage that is linked to cardiovascular diseases [46].

Plasma renin was associated with increased all-cause, but not cardiovascular mortality in the general population and a hypertensive cohort which was using ACE inhibitors, diuretics, CCBs and beta blockers [211]. Recently, in a prospective study, plasma renin was associated with long-term cardiovascular mortality in patients referred to coronary angiography with ongoing hypertension medication use including the above-mentioned medications as well as ARBs [212]. In addition, the afore-mentioned studies had populations consisting of between 3200 and 3408 mixed race men and women with a mean age between 59 and 63 years [211, 212]. It is probable that age and hypertension, a well-known risk factor for CVD [6], are important determinants of renin-mediated cardiovascular damage and death [212, 217].

Meanwhile, renin did not predict cardiovascular mortality after multiple adjustments for covariates in coronary heart failure patients with a mean age of 56 years [218], while Meade *et al.* did not find a relationship between renin and fatal cardiovascular events in industrial workers after 19 years of follow-up [219]. Black populations usually exhibiting a supressed RAAS and volume-loading hypertension [174, 220], however it is unclear if this phenotype predisposes to cardiovascular complications and eventually mortality as the previous studies reporting the prognostic role of renin for mortality were mostly based on white populations [14].

7. INTEGRATION OF CONCEPTS AND PROBLEM STATEMENT

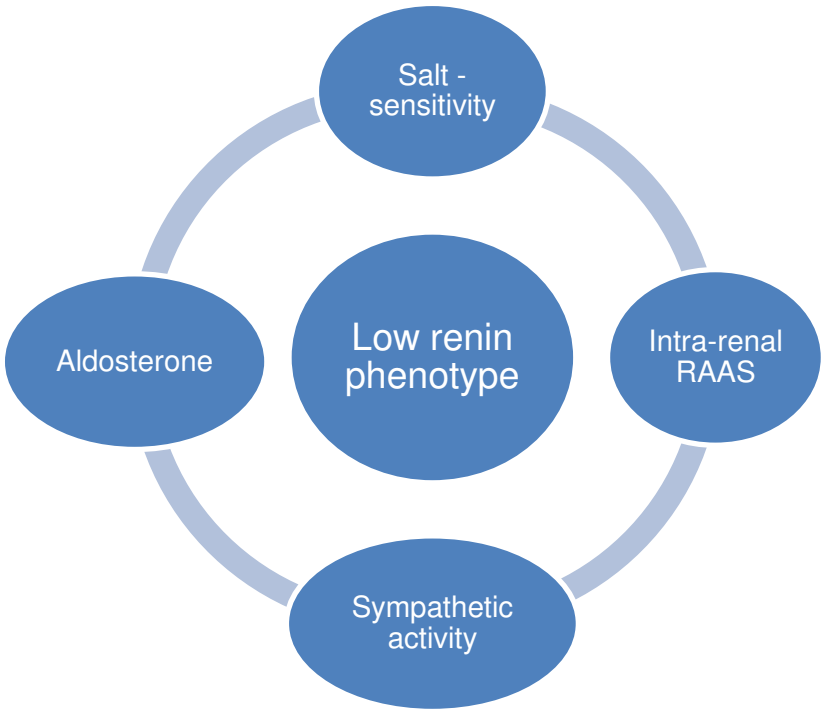

Figure 9: Features of the low renin phenotype in Africans

Hypertension is currently one of the major risk factors for strokes, kidney disease, coronary and hypertensive heart disease [221, 222]. Its prevalence is decreasing in the developed world, however, in Sub-Saharan countries such as South Africa, the increase is still alarming [5, 125]. Among the blood pressure regulating systems, the excessive activation of the RAAS is one of the known factors associated with the development of hypertension [14]. However, it is well-established that the RAAS is suppressed in blacks [2]. Therefore, the role of the RAAS in the development of hypertension in black populations that are commonly affected by low-renin hypertension is questionable. There are factors known to characterise low renin states in blacks (Figure 9).

Low-renin hypertension due to volume-loading has been attributed to salt-sensitivity resulting from alterations in sodium handling mechanisms that lead to excessive sodium and fluid

retention [131, 137]. Excess aldosterone for a given level of renin has also been linked to salt-sensitive hypertension and organ damage in blacks [157, 163, 192]. In a population of blacks with low renin it was also indicated that intrarenal RAAS has a significant relation with blood pressure and that it may function independent of the circulating RAAS [67]. The role of heightened sympathetic drive in hypertension in blacks should be considered as it is known that blacks have augmented cardiovascular reactivity to stress [24, 34]. Hamer *et al*. showed that plasma renin responses to stress associated with sub-clinical organ damage [223]. Extensive investigations into the possible mechanisms involved in the low renin phenotype characterising hypertension in blacks are urgently needed to prevent cardiovascular complications and improve treatment of hypertension in black populations.

8. MOTIVATION

This thesis consists of three original articles submitted for publication in peer-reviewed journals. The relevant backgrounds and motivations are included in the articles. This section includes a brief motivation for each article.

8.1. Chapter 3: Plasma renin and cardiovascular responses to the cold pressor test differ in black and white populations: The SABPA study

Suppressed renin and high cardiovascular reactivity to stress are among the predominant features of hypertension in black populations [2, 22, 34]. In the SABPA cohort, African men showed higher blood pressure and vascular resistance responses to a mental stressor compared to whites [24]. Additionally, Reimann *et al*. reported a higher cardiac and low parasympathetic outflow in blacks compared to whites in response to a cold stimulus [35]. Furthermore, renin reactivity to stress was adversely associated with a marker of subclinical organ damage [223]. However, the relation between renin and haemodynamic parameters in South Africans during stress is still unknown.

8.2. Chapter 4: Aldosterone and renin in relation to surrogate measures of sympathetic activity in blacks with low renin levels: The SABPA study

Blacks usually present with lower aldosterone levels compared to whites [24, 174]. However, even modest increases in aldosterone relative to renin have been shown to have adverse effects on blood pressure in blacks, even at low renin, salt-sensitive states [86, 154]. Reduced baro-receptor sensitivity and heart rate variability were recently observed in a black population [32, 33]. It is not clear if there is a link between renin and aldosterone, and measures of sympathetic activity in black populations.

8.3. Chapter 5: The low renin phenotype in Africans: Implications for all-cause and cardiovascular mortality

High renin has been associated with all-cause and cardiovascular mortality, particularly in populations of European origin [15, 16, 211]. Black populations usually experience severe cardiovascular outcomes due to hypertension while on the other hand this population group is usually affected by low renin, volume-loading hypertension [126, 127, 174, 220]. There is no evidence on the prognostic value of renin in black populations. In South Africa, strokes and heart diseases are among the leading causes of death [7]. Investigations into the role of blood pressure regulating pathways such as the RAAS are needed.

9. MOTIVATION FOR STUDY POPULATION DIVISION

The first division of the study was according to ethnicity, namely black and white adults from South Africa. The second division was based on renin status, low and high renin groups. The high frequency of low renin states in populations of African ancestry is well known [2, 171]. In both normotensives and hypertensives, low renin levels are more common in blacks compared

to whites [73, 220]. Even as children, blacks have lower renin levels compared to whites [174]. The low renin state is usually accompanied by higher blood pressure, possibly due to volume-loading [129]. Adverse associations have been reported between renin, aldosterone and target organ damage in blacks [191, 197]. It has also been shown that salt-sensitivity, one of the causes of low-renin hypertension is more common in blacks compared to other ethnicities [156, 173]. However, the cardiovascular profile of the low renin phenotype in South African blacks is not well characterised and it is not known if there is any cardiovascular risk associated with it. As a result, the study population was divided into blacks and whites, and low and high renin groups in the subsequent chapters.

10. RESEARCH QUESTIONS

Based on the literature review above, the following questions are pertinent:

- Is the prevalence of low renin status higher in black compared to white South Africans as expected?
- Are adverse associations evident between active plasma renin and cardiovascular responses to acute stress?
- Are active plasma renin, aldosterone and the ARR associated with ambulatory blood pressure and sympathetic activity?
- Will the low renin group have a higher cardiovascular risk for mortality compared to black individuals with normal renin levels?

11. RESEARCH AIM, OBJECTIVES AND HYPOTHESES

11.1. Aim

The central aim is to profile and analyse the low renin phenotype in black South Africans and how it relates to cardiovascular haemodynamics, as well as cardiovascular- and all-cause mortality.

11.2. Objectives

The detailed objectives are:

- To characterise black and white adults according to low and high renin levels.
- To explore associations between active plasma renin and cardiovascular responses to an acute stressor.
- To assess the associations of active plasma renin, aldosterone and ARR with ambulatory blood pressure and surrogate measures of sympathetic activity.
- To determine if the low renin phenotype is a predictor of cardiovascular mortality in black South Africans.

11.3. Hypotheses

- Low renin status is more prevalent in black compared to white adults.
- The low renin group will exhibit higher cardiovascular reactivity to stress and there will be adverse associations between active plasma renin and cardiovascular responses to stress.
- The low renin group will present with adverse associations of ambulatory blood pressure as well as surrogate measures of sympathetic activity with active plasma renin, aldosterone and ARR.

- Black South Africans with the low renin phenotype are at an increased risk for mortality compared to those with normal/high renin.

12. STRUCTURE OF THE THESIS

Chapter 1	General introduction, literature overview, aim, objectives and hypotheses
Chapter 2	Study design and research methodology
Chapter 3 Manuscript 1	Plasma renin and cardiovascular responses to the cold pressor test differ in black and white populations: The SABPA study
Chapter 4 Manuscript 2	Aldosterone and renin in relation to surrogate measures of sympathetic activity in blacks with low renin levels: The SABPA study
Chapter 5 Manuscript 3	The low renin phenotype in Africans: Implications for all-cause and cardiovascular mortality
Chapter 6	Summary of main findings, conclusions and recommendations

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CHAPTER 2

Study design and research methodology

1. STUDY DESIGN

Data from two studies were used for this thesis (Figure 1), including the **S**ympathetic activity and **A**mbulatory **B**lood **P**ressure in **A**fricans (SABPA) study, which is a target population comparative study. The second study is the South African leg of the multi-national **P**rospective **U**rban and **R**ural **E**pidemiology (PURE) study. Both studies were conducted in the North West province of South Africa. The main objective of the **SABPA** study was to assess the brain-heart link and neural response pathways to describe plausible mechanisms for cardiometabolic morbidity and mortality in a target population of school teachers from South Africa by evaluating psychological wellbeing and biological markers [1]. These included components of the renin-angiotensin-aldosterone system (RAAS), stress hormone profiling, catecholamine metabolites, oxidative stress, obesity, pro-thrombotic and pro-inflammatory, cardiovascular-, metabolic syndrome indicators and target end-organ damage [1]. The global **PURE** study was initiated to keep track of the development of chronic diseases of lifestyle in low-, middle- and high-income countries in both urban and rural dwelling participants [2].

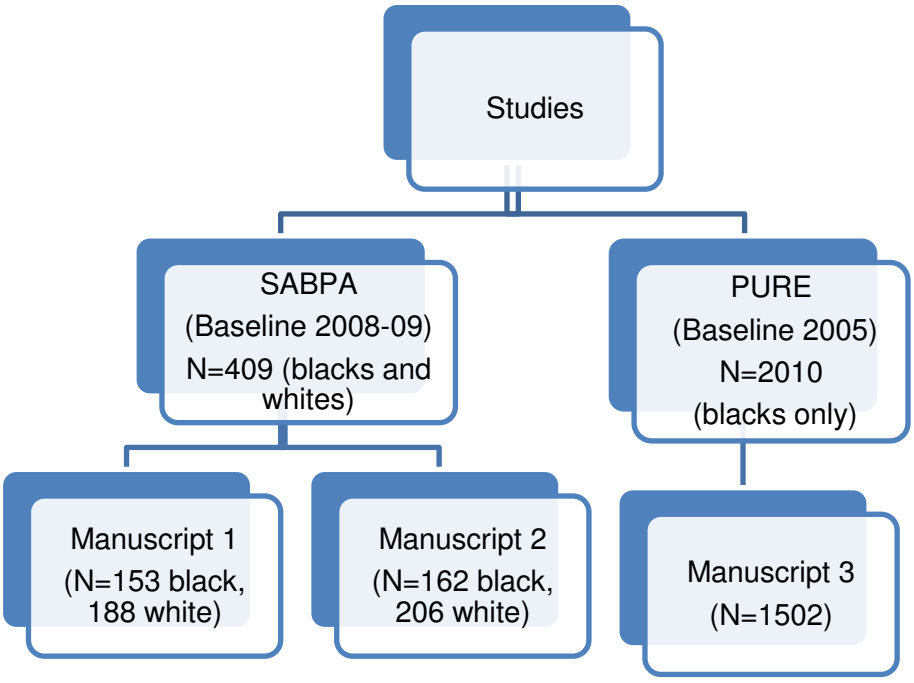

Figure 1: Studies used to compile manuscripts of this thesis.

1.1. Recruitment processes

SABPA study

Headmasters of schools were approached about the SABPA project in September 2007. A sample of black and white African teachers were recruited and screened by registered nurses for eligibility for inclusion in the study. Information sessions with the participants were held two months prior to the study (November 2007) and written informed consent was obtained.

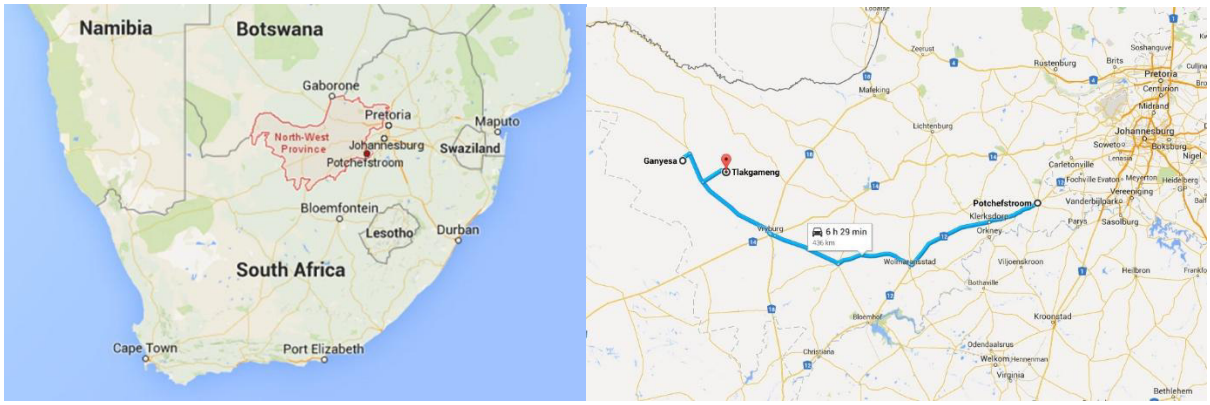

Figure 2: A map of South Africa showing the North West province (left) and the locations (right) from which the population for the PURE study was recruited.

PURE study

Four different resident areas were identified for participation. A rural community (A), namely Ganyesa (450 km from Potchefstroom), a deep rural community (B), namely Tlakgameng (35 km east of Ganyesa) (Figure 2). Community (C) was identified as the Ikageng Township bordering Potchefstroom, while community (D) was the informal settlements surrounding Ikageng. A household census regarding the number of people, their ages and health profiles was done in 1500 households in each community starting from a specific point. If a person declined participation or was not home, the next house was taken and a non-complier questionnaire was completed. Individuals between 35 years and 70 years from urban and rural areas described above were invited to participate in the study, and those who provided written

consent were enrolled. In those who declined to participate, a brief non-responder form was completed.

1.2. Participants

The **SABPA** participants included a homogenous sample of 201 black (99 women and 101 men) and 209 white (108 women and 101 men) urban teachers aged 20-65 years, and data collection was performed in 2008-2009. The **PURE** study population originally consisted of 2010 African volunteers aged 35 years and older from a sample of 6000 randomly selected households in both rural and urban areas of the North West Province. Baseline data collection occurred in 2005, and mortality data was collected from 2005 to 2010. All participants were from the North West province.

2. RESEARCH METHODOLOGY

2.1. Questionnaires

In the **SABPA** study general health, sociodemographic questionnaires, psychological distress and Berlin sleep apnoea questionnaires were administered. Trained African field workers conducted the interviews by making use of structured demographic, socio-economic, lifestyle and physical activity questionnaires that were developed and standardised for the international **PURE** study [2].

2.2. Anthropometric measurements

In all studies, weight, height, waist and hip circumferences of the participants were measured in triplicate by accredited anthropometrists according to standardised methods [3] with different calibrated instruments [**SABPA** (Precision Health Scale, A & D Company, Japan; Invicta Stadiometer, IP 1465, UK; Holtain non-stretchable metal flexible measuring tape),

PURE (Precision Health Scale, A & D Company, Japan; Leicester Height Measure, Seca, Birmingham, UK)].

2.3. Cardiovascular measurements

In the **SABPA** study, the 24-hour ambulatory blood pressure monitoring (ABPM) and electrocardiogram (ECG) recordings were conducted during the working week. The ABPM and two-lead electrocardiogram apparatus (Meditech CE120® Cardiotens, Budapest, Hungary) were attached on the participant's non-dominant arm. The ABPM apparatus was programmed to measure blood pressure at 30 minute intervals during the day (08:00 – 22:00) and every hour during the night (22:00 – 06:00), while the electrocardiogram recorded measurements every 5 minutes for 20 seconds. In both studies, brachial blood pressure measurements were performed in duplicate (5 minutes apart) while the participants were seated upright with the arm supported at heart level. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured with a mercury sphygmomanometer in the **SABPA** study (No. 1010-108 Diplomat-presameter®, Germany) while in the **PURE** study OMRON HEM-757 was used (Omron Healthcare, Kyoto, Japan). Appropriately sized cuffs were used for obese participants.

The **SABPA** study: The validated [4, 5] Finometer device (FMS, Finapres Measurement Systems, Amsterdam, The Netherlands) was connected, and after a ten minute resting period, a two-minute systolic calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial arterial pressure [5]. The highest precision in cardiovascular measurement can be achieved only after this calibration [5], and blood pressure measurements complied with the requirements of the Association for the Advancement of Medical Instrumentation (AAMI) [5, 6]. Continuous measurement of resting haemodynamic variables was performed in a five-minute period, and then the participant was exposed to the cold pressor test (CPT) for one minute. The cold pressor test is an experimental technique used to induce cold pain in humans [7] and its use has been extended to evaluate

cardiovascular reactivity to stress. The response to CPT may be mediated by the temperature component or the pain perception of the cold stimulus. The temperature raises blood pressure, mainly by increasing total peripheral resistance, while the cold pain may additionally activate central cardiac responses [8, 9]. It was performed by immersing the participant's right foot up to the ankle in ice water with a temperature of 4°C and the foot remained there until completion of the measurement (1 minute). The Beatscope® software was used to calculate SBP, DBP, HR and computed stroke volume (SV), total peripheral resistance (TPR), and Windkessel arterial compliance (Cwk) [10]. We used the average of 1 minute of the resting recording and the average of the last 20 seconds of the stressor recordings. Cardiovascular reactivity was calculated for each participant as the percentage change from the resting value.

The Laragh method of correcting for BP

The Poiseuille's equation can be applied to the control of normal BP and different forms of hypertension. The Poiseullie's equation: BP= CO*TPR may be utilized by substituting the essential elements of BP to have BP=V*R, where V is volume and R is renin-angiotensin. Therefore, in our study the sodium-volume component was calculated using blood pressure and plasma renin according to Laragh's derived equation [11]. For BP, we used SBP.

2.4. Assessment of outcome

The **PURE** study: The fieldworkers made quarterly follow-up visits to the participants' homes to retain participants and ensure their vital status. These visits were supervised by a senior researcher. In cases where a participant had died, the cause of death was obtained from family death certificates and verbal autopsy as coded by a physician according to the International Classification of Diseases codes for underlying causes. Cardiovascular mortality encompasses all fatal cardiac and stroke events and death indicated as "due to hypertension". Cardiac diseases that caused death included heart failure, myocardial infarction, congestive

heart failure or any other cardiac-related reason, while death due to stroke included any stroke or cerebrovascular incident.

2.5. Blood sampling and biochemical analyses

Participants were requested to be in a fasted state by not eating or drinking anything except water for approximately 8-10 hours prior to sample collection in the mornings. A registered nurse obtained a blood sample with a sterile winged infusion set from the antebrachial vein branches. Samples were prepared according to appropriate methods and stored at -80°C in the laboratory. In the rural areas (**PURE** study), samples were rapidly frozen and stored at -18°C (no longer than five days) until it could be transported to the laboratory facility and was then stored at -80°C until analysis. In both studies, sequential multiple analysers (Konelab 20i, ThermoScientific, Vantaa, Finland and Cobas Integra 400 plus Roche, Basel, Switzerland) were used to analyse total and high density lipoprotein (HDL) cholesterol, fasting glucose, creatinine, high sensitivity C-reactive protein (CRP) and gamma glutamyl-transferase (GGT) and percentage glycosylated haemoglobin (HbA1c). Percentage glycosylated haemoglobin in **PURE** was determined by using ion-exchange high-performance liquid chromatography (D-10 Haemoglobin testing system from Bio-Rad laboratories, Hercules, CA). In the **SABPA** study, an 8-hour morning spot urine sample was collected from which creatinine, sodium, potassium and noradrenaline were measured (Cobas Integra 400 plus, Roche, Basel, Switzerland & 3-Cat Fast Track kit, LDN, Nordhorn, Germany). Intra- and inter-assay coefficients for noradrenaline were 5.5% and 9.62%, respectively. In the **PURE** study spot urine samples were used to measure creatinine with a calorimetric method and albumin according to the turbidimetric method (Unicel DXC 800-Beckman and Coulter, Germany). Kidney function was assessed by using the estimated glomerular filtration rate (eGFR) (**SABPA)** and by estimating creatinine clearance (CrCl) according to the Cockcroft-Gault formula (**PURE**) [12]. Active plasma renin was analysed for both studies using the high sensitivity radioimmunometric assay and cross-reaction with prorenin was 0.4 % (Renin III

Generation, CIS Biointernational, Cedex, France). The source of reagents was mouse anti-human-active renin monoclonal antibody, inter-assay reproducibility: **SABPA:** 0.9-3.6 %; **PURE**: intra-assay variation=6.34%; inter-assay variation=4.52%). In the **SABPA** study, plasma aldosterone was analysed using a competitive radioimmunoassay, inter- and intrabatch variability: 3.4% and 1.64-6.08% respectively (Beckman Coulter, Brea, CA).

2.6. Human Immunodeficiency Virus testing

The South African National Department of Health protocol with pre- and post-counselling was followed to perform the human immunodeficiency virus (HIV) testing. In both studies, the HIV status of the participant was determined by rapid test cards (First Response, PMC Medical, India). If the first test tested positive confirmation was done with a different rapid test card (Pareeshak card test, BHAT Bio-tech, Bangalore, India). The results were communicated to the participants by two trained counsellors during individual sessions before departure from the collection site. Participants who tested positive for HIV were referred to the local clinic or hospital for CD4 cell counts.

2.7. Statistical analyses

Statistical analysis were performed using Statistica Version 13.

- Descriptive statistics were performed to check normality and to determine the means, standard deviations, standard errors, confidence intervals, minimum and maximum values.
- Independent t-tests and analysis of variance and covariance (ANOVA & ANCOVA) were used to compare means between groups.
- Chi square tests were performed to compare proportions.
- Single, partial and multiple regression analyses were done to investigate associations between relevant cardiovascular measures, renin and aldosterone.

- Cox regression analyses were used to determine hazard ratios for all-cause and cardiovascular mortality.

3. ETHICAL ASPECTS

Participants were given full information regarding the objectives and procedures of each study prior to participation. Participants also had the opportunity to ask questions. Where necessary, the information was conveyed in the participant's home language by trained African field workers fluent in English and Setswana. All participants signed an informed consent form prior to participation. The two studies complied with all applicable requirements of the international regulations, in particular, the Helsinki declaration of 1975 (and subsequent revisions) for investigation in human participants. The Health Research Ethics Committee (HREC) of the North-West University (Potchefstroom campus) approved these studies. An ethics application for this thesis was also approved by the HREC (Letter of approval attached as Annexure A).

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CHAPTER 3

Plasma renin and cardiovascular responses to the cold pressor test differ in black and white populations: The SABPA study

SUMMARY OF INSTRUCTIONS FOR AUTHORS

Journal title: *Journal of Human Hypertension (http://www.nature.com/jhh/about.html)*

Original articles: Include an extra table to be named 'Summary Table', with two parts: firstly, the heading 'What is known about topic', and then secondly: 'What this study adds'. This should be two or three bullet points for each, with one or two short sentence for each bullet point.

Essential title page information should carry the following

1) **Title:** Brief, informative, of 150 characters or less and should not make a statement or conclusion.
2) **Running title:** Consist of not more than 50 letters and spaces. It should be as brief as possible, convey the essential message of the paper and contain no abbreviations.
3) **Authors:** Full names of all the authors, highest academic degree obtained, and their affiliations, together with the name, full postal address, telephone and fax numbers and e-mail address of the author to whom correspondence and offprint requests are to be sent
4) **Conflict of interest**: Authors should disclose the sources of any support for the work, received in the form of grants and/or equipment and drugs.
5) **Word count:** The text limit is 4,000 words, not including the abstract, conflict of interest statement, summary table or references and a maximum of 35 references.

Abstract: Should not exceed 250 words and should be followed by keywords.

Article structure

Introduction: Be as brief as possible

Methods: This section should contain sufficient detail, so that all experimental procedures can be reproduced, and include references.

Results: This section should briefly present the experimental data in text, tables or figures. Tables and figures should not be described extensively in the text, either.

Discussion: Focus on the interpretation and the significance of the findings with concise objective comments that describe their relation to other work in the area. The final paragraph should highlight the main conclusion(s), and provide some indication of the direction future research should take.

Acknowledgements: Include sources of support including sponsorship.

References

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****Note:** some formatting was changed to maintain consistency throughout the thesis.

Plasma renin and cardiovascular responses to the cold pressor test differ in black and white populations: The SABPA study

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Abstract

Low plasma renin levels and augmented cardiovascular reactivity to stress are common in blacks and have been linked to the development of hypertension in this population. We (1) compared cardiovascular and plasma renin reactivity to a cold pressor test between a black and white population; and (2) investigated the associations between cardiovascular and plasma renin reactivity within the black and white populations. Our population consisted of 153 black and 188 white men and women (age range, 20 to 65 years). We measured blood pressure, heart rate, stroke volume, total peripheral resistance (TPR), Windkessel arterial compliance and determined plasma renin levels at rest and during the cold pressor test. Reactivity was calculated for each participant as the percentage change from the resting value. We found lower renin and elevated blood pressure (BP) in blacks compared to whites at rest and during stress (both, $P<0.001$). During stress, heart rate increased more in blacks ($P<0.001$), whereas stroke volume ($P<0.001$) and arterial compliance ($P=0.013$) decreased more in blacks compared to whites. TPR reactivity was positively associated with renin reactivity in blacks only ($\beta=0.17$; $P=0.041$), while in whites diastolic BP reactivity was positively associated with renin reactivity ($\beta=0.21$; $P=0.005$). Although blacks had supressed renin levels at rest and during acute stress, vascular resistance reactivity associated positively with renin reactivity only in the black population. These results suggest that low renin levels in blacks during rest and stress are linked to increased peripheral vascular responses to stress, which may contribute to elevated BP in blacks.

Key words: renin, total peripheral resistance, reactivity, cold pressor test, sympathetic activity

Introduction

The incidence of cardiovascular morbidity and mortality continues to increase in black South Africans, with hypertension being the most common cardiovascular risk factor.[1, 2] It is known that blacks tend to have a supressed renin-angiotensin-aldosterone system (RAAS) activity, including low renin status, and accordingly low-renin hypertension is common.[3] Furthermore, black populations have higher sympathetic nerve activity[4, 5] and peripheral resistance at rest and when the cardiovascular system is challenged[6, 7] when compared to whites.

The cold pressor test is a method used to study cardiovascular stress reactivity by immersing an individual's foot or hand in ice water for 1 minute. This results mostly in a peripheral vascular effect by stimulating α-adrenergic receptors which cause vasoconstriction and a subsequent elevation in total peripheral resistance, ventricular afterload and blood pressure, thereby interfering with the heart's ability to increase stroke volume during stress.[8-10] In addition, the secretion of renin during sympathetic activation promotes vasoconstriction, further contributing to systolic and diastolic blood pressure elevation via the actions of angiotensin II.[3] Renin can also result in blood pressure elevation by activation of the (pro)renin receptors in the vasculature, independent of other components of the RAAS.[11]

High plasma renin has detrimental effects on the vasculature such as activation of profibrotic and proinflammatory pathways through the (pro)renin receptors,[12, 13] however, in black populations low renin is positively associated with increased blood pressure and target organ damage.[3, 14-16] Despite the known low renin phenotype and increased cardiovascular reactivity in black populations, to the best of our knowledge no previous studies have investigated the specific haemodynamic and renin responses during a stressor in blacks. The aims of this study were (1) to compare cardiovascular and plasma renin reactivity to a cold pressor test between a black and white population; and (2) to investigate the associations between cardiovascular and plasma renin reactivity within the black and white populations.

Methods

Study design and population

The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study was conducted between February 2008 and May 2009. The study included 200 black (101 women and 99 men) and 209 white (108 women and 101 men) urban teachers in the North West Province of South Africa. The black group was mostly Setswana speaking. The reason for this selection was to have a homogeneous sample from a similar socioeconomic class. We invited eligible participants in the age range between 20 and 65 years. Exclusion was based on the following criteria: ear temperature > 37.5°C, vaccinated or donated blood in the previous 3 months before the study commenced, pregnancy, lactation, HIV infection, diabetes, any acute/chronic medication and psychotropic substance abuse or dependence. From 409 participants, we had renin data available for 153 black and 188 white participants.

Participants were fully informed about the objectives and procedures of the study before enrolment. Assistance was given to any participant who requested conveyance of information in their home language. All participants signed an informed consent form. The study complied with all applicable requirements of the international regulations, in particular, the Helsinki declaration of 1975 (as revised in 2008) for investigation of human participants. The Health Research Ethics Committee of North-West University (Potchefstroom campus) approved this study (NWU-00036-07-S6). Participants were transported at 16h30 to the Metabolic Unit Research Facility of the North-West University and they were familiarised with the experimental setup. After receiving a standardised dinner, participants were encouraged to go to bed at around 22h00. The participants woke up at 05h45 and the measurements commenced.

Questionnaires

We administered validated general health and sociodemographic questionnaires.

Anthropometric measurements

Weight, height, waist and hip circumferences were measured in triplicate by anthropometrists according to standardised methods with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, London, UK; Holtain non-stretchable metal flexible measuring tape). Body mass index (BMI) was calculated and expressed as kg/m^2.[17]

Cardiovascular measurements

The validated[18, 19] Finometer device (FMS, Finapres Measurement Systems, Amsterdam, The Netherlands) was connected, and after a 10 minute resting period, a 2-minute calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial arterial pressure.[19] The highest precision in cardiovascular measurement can be achieved only after this calibration,[19] and blood pressure measurements complied with the requirements of the Association for the Advancement of Medical Instrumentation (AAMI).[19, 20] Continuous measurement of resting cardiovascular variables was performed for a 5-minute period, after which the participant was exposed to the cold pressor test for 1 minute. It was performed by immersing the participant's right foot up to the ankle in ice water with a temperature of 4°C for 1 minute. The Beatscope® software was used to calculate systolic (SBP) and diastolic blood pressure (DBP), heart rate (HR) and computed stroke volume (SV), total peripheral resistance (TPR), and "Windkessel" compliance of the arterial system (Cwk).[21] Cwk is the "Windkessel" compliance of the arterial system also referred to as Windkessel compliance, or buffer compliance. As part of the nonlinear three-element model (aortic characteristic impedance, arterial compliance, and systemic vascular resistance), it is computed from an age-dependent, aortic pressure–area relationship and represents the lumped compliance of the entire arterial system.[21] We used the average of 1 minute of the resting recording and the average of the last 20 seconds of the stressor recordings. Cardiovascular reactivity was calculated for each participant as the percentage change from

the resting value to the stressor value. All measurements were performed at room temperature.

Biological sampling and biochemical analyses

Participants were requested to be in a fasted state by not eating or drinking anything except water for approximately 8-10 hours prior to sample collection in the mornings. An 8-hour morning spot urine sample was collected to measure norepinephrine using the 3-Cat Urine ELISA Fast Track kit (LDN, Nordhorn, Germany). A registered nurse obtained the first blood sample with a sterile winged infusion set from the antebrachial vein branches whilst the participant was in a supine position for a period of 30 minutes. Samples were prepared according to appropriate methods and stored at -80°C in the laboratory. Sequential multiple analysers (Konelab 20i, ThermoScientific, Vantaa, Finland; and Cobas Integra 400 plus, Roche, Basel, Switzerland) were used to analyse total and high density lipoprotein (HDL) cholesterol, fasting glucose, high sensitivity C-reactive protein (CRP), gamma-glutamyltransferase (GGT) and glycosylated haemoglobin (HbA1c). Serum creatinine was analysed using an enzymatic colorimetric test (Cobas Integra 400 plus, Roche, Basel, Switzerland). The Modification of Diet in Renal Disease (MDRD) formula was used to estimate glomerular filtration rate (GFR). Active plasma renin was analysed in duplicate using the high sensitivity radio-immunometric assay and cross-reaction with prorenin was 0.4% (Renin III Generation, CIS Biointernational, Cedex, France). The source of reagents was mouse anti-human-active renin monoclonal antibody (IBL Lab, 38T501, USA). Another blood sample to measure renin was collected 5-10 minutes after exposure to the cold pressor test and renin reactivity was calculated for each participant as the percentage change from the resting value to the stressor value.

Statistical analyses

We used Statistica Version 12 for all statistical analyses (Statsoft Inc., Tulsa, OK). Power analyses were performed for the SABPA study to obtain relevant effect sizes based on

differences in biological profiles and genotyping hypothalamic-pituitary adrenal (HPA) axis variation. Resulting sample sizes of 50–416 would enable explanation of biological differences and detection of single nucleotide polymorphisms (SNPs) with a statistical power of 0.8, and level of significance of 0.05. We tested for interaction of sex on the associations between total peripheral resistance and renin, SBP and renin as well as between DBP and renin, but none was significant (all $P \geq 0.06$). The distribution of HbA1c, GGT, total cholesterol, HDL-cholesterol and CRP were normalised by logarithmic transformation. The central tendency and spread of these variables were represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions were compared by using two-sided independent t-tests and Chi-square tests. In Figure 1 error bars represent the standard error of mean. We performed single, partial and multiple regression analyses to investigate associations between relevant cardiovascular variables and renin. In partial regression analyses we adjusted for age, BMI and sex. After considering several variables for inclusion in the multiple regression model, we finally included age, BMI, sex, GGT, total cholesterol: HDL-cholesterol ratio, antihypertensive medication, HbA1c, CRP and eGFR as covariates.

Results

Characteristics of the population

Table 1 lists the characteristics of the population stratified by ethnicity. Age was similar between the two groups, but the black group had a higher mean BMI (P=0.005) and a larger proportion (47.7%) was hypertensive (P<0.001) compared to whites (25.5%). Under resting conditions, SBP and DBP were higher in blacks compared to whites (P<0.001) (Table 1), while heart rate (P=0.16), stroke volume (P=0.59) and TPR (P=0.97) were not different (Figure 1). Cwk and renin were lower in the black compared to the white group (both P<0.001) (Figure 1).

Table 1: Characteristics of the population

	Black	White	*P*-value
N	153	188	
Age (years)	43.1 ± 7.71	43.9 ± 10.6	0.38
Women, n (%)	73 (47.7)	99 (52.7)	0.36
Anthropometric measurements			
Weight (kg)	80.2 ± 18.7	84.1 ± 21.7	0.077
Body mass index (kg/m^2)	29.7 ± 7.13	27.6 ± 6.09	0.005
Waist circumference (cm)	92.2 ± 15.7	92.7 ± 16.5	0.75
Resting cardiovascular measurements			
Systolic BP (mmHg)	140 ± 17.6	130 ± 13.3	<0.001
Diastolic BP (mmHg)	81.1 ± 9.83	76.3 ± 7.82	<0.001
Biochemical measurements			
Resting plasma renin (pg/ml)	4.19 ± 2.62	6.32 ± 3.07	<0.001
Glycosylated haemoglobin (%)	5.89 (5.10;7.50)	5.48 (5.00; 6.29)	<0.001
Gamma-glutamyltransferase (U/L)	44.4 (19.9; 177)	19.1 (7.00;75.9)	<0.001
TC: HDL	4.11 (0.32; 7.46)	4.71 (2.85; 7.86)	<0.001
C-reactive protein (mg/L)	3.80 (0.45;26.33)	2.05 (0.99;8.99)	<0.001
Serum creatinine (µmol/L)	75.1 ± 0.08	72.3 ± 0.08	<0.001
eGFR (ml/min/1.73m^2)	113 ± 27.6	95 ± 16.9	<0.001
Norepinephrine (ng/ml)	39.6 ± 40.8	45.6 ± 32.1	0.002
Lifestyle factors			
Smoking, n (%)	28 (18.3)	28 (15.0)	0.41
Hypertensive n (%)	73 (47.7)	48 (25.5)	<0.001
Alcohol use, n (%)	38 (24.8)	95 (50.8)	<0.001
Antihypertensive medication, n (%)	27 (17.6)	11 (5.9)	<0.001

Values are arithmetic mean ± standard deviation or geometric mean (5th and 95th percentile interval) for logarithmically transformed variables. Reactivity values are adjusted for baseline haemodynamic and plasma renin measurements. Abbreviations: BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; TC, total cholesterol.

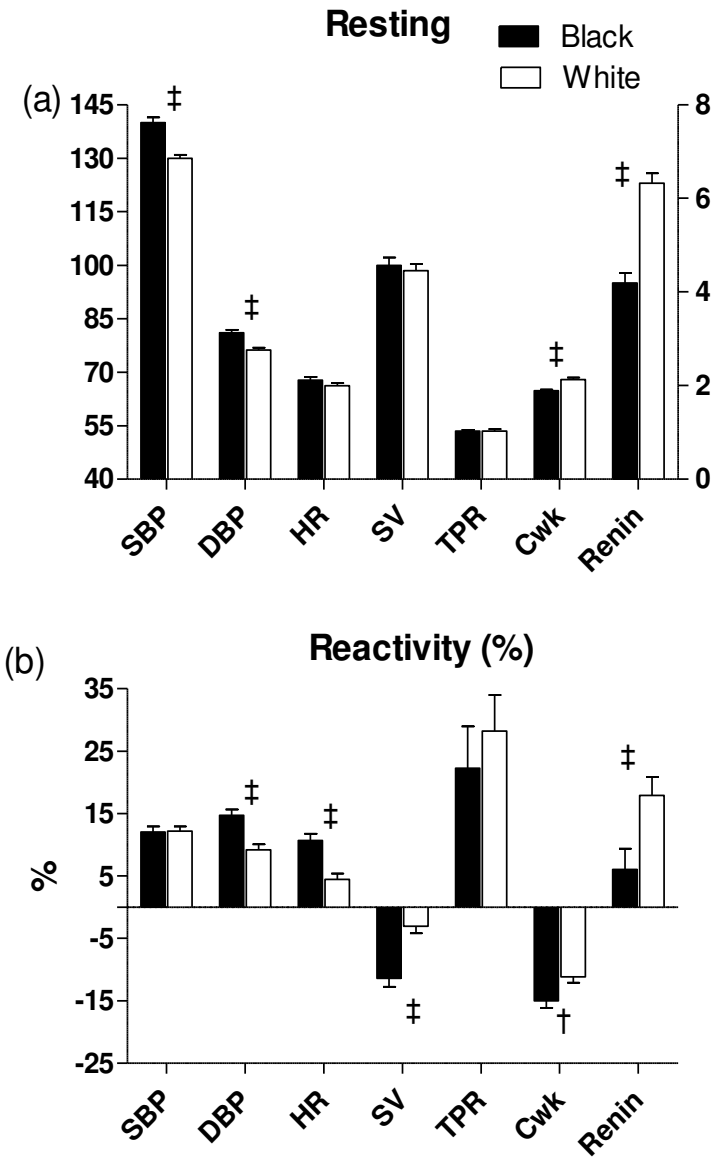

Figure 1: Comparison of haemodynamic variables and plasma renin between blacks and whites (a) Resting (b) Reactivity (%) from baseline during the cold pressor test.

*P<0.05; †, P≤0.01; ‡, P≤0.001. Reactivity values are adjusted for baseline haemodynamic and plasma renin measurements. Abbreviations: SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); SV, stroke volume; (ml); TPR, total peripheral resistance (mmHg/ml/s); Cwk, Windkessel arterial compliance (ml/mmHg); plasma renin (pg/ml).

In both groups during the cold pressor test, SBP, DBP, heart rate and TPR increased, while stroke volume and Cwk decreased (all *P*<0.001). Renin did not increase (*P*=0.67) in blacks during stress (Table 2). DBP and heart rate increased more in blacks (both *P*<0.001), whereas stroke volume (*P*<0.001) and Cwk (*P*=0.013) decreased more in blacks compared to whites (Figure 1).

Table 2: Changes from baseline within the black and white groups

		Black	
Variables	**Resting**	**Stressor**	***P*-value**
SBP (mmHg)	140 ± 17.6	155 ± 22.6	<0.001
DBP (mmHg)	81.1 ± 9.83	91.8 ± 12.4	<0.001
HR (bpm)	67.4 ± 10.4	73.9 ± 11.3	<0.001
SV (ml)	101 ± 26.1	88.3± 26.3	<0.001
TPR (mmHg/ml/s)	1.01 ± 0.29	1.22 ± 0.41	<0.001
Cwk (ml/mmHg)	1.89 ± 0.42	1.61 ± 0.48	<0.001
Renin (pg/ml)	4.17 ± 2.62	4.21 ± 2.29	0.67
	White		
SBP (mmHg)	130 ± 13.3	147 ± 15.9	<0.001
DBP (mmHg)	76.3 ± 7.82	83.9 ± 10.3	<0.001
HR (bpm)	66.2 ± 10.7	69.1 ± 12.5	<0.001
SV (ml)	98.5 ± 24.7	94.7 ± 24.7	<0.001
TPR (mmHg/ml/s)	1.02 ± 0.54	1.26 ± 1.06	<0.001
Cwk (ml/mmHg)	2.13 ± 0.52	1.89 ± 0.51	<0.001
Renin (pg/ml)	6.34 ± 3.07	6.72 ± 3.15	0.001

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SV, Stroke volume; Cwk, "Windkessel" compliance of the arterial system; TPR, total peripheral resistance.

Adjusted regression analyses

We determined the correlations between haemodynamic variables and renin under resting conditions and during the cold pressor test while adjusting for age, BMI and sex (Table 3). At

rest, SBP and DBP associated negatively with renin in both groups (both *P*<0.05), while TPR associated negatively with renin in blacks only (*P*=0.017). Cwk associated positively with renin in both blacks (*P*<0.001) and whites (*P*=0.018). When assessing similar correlations, but for the cold pressor test, DBP reactivity associated positively with renin reactivity in whites (*P*=0.010), while TPR reactivity associated positively with renin reactivity in blacks (*P*=0.041) only.

Table 3: Partial regression analysis between haemodynamic variables and plasma renin, adjusted for age, body mass index and sex

	Resting plasma renin (pg/ml)			
Resting variables	**Black**		**White**	
	r	*p*	*r*	*p*
SBP (mmHg)	**-0.33**	**<0.001**	**-0.29**	**<0.001**
DBP (mmHg)	**-0.22**	**0.01**	**-0.25**	**0.001**
HR (bpm)	0.15	0.07	-0.002	0.98
SV (ml)	0.05	0.56	0.03	0.73
TPR (mmHg/ml/s)	**-0.19**	**0.017**	-0.12	0.09
Cwk (ml/mmHg)	**0.36**	**<0.001**	**0.17**	**0.018**
	Plasma renin reactivity (%)			
Reactivity variables	**Black**		**White**	
	r	*p*	*r*	*p*
SBP (%)	0.09	0.32	0.06	0.40
DBP (%)	0.08	0.35	**0.19**	**0.010**
HR (%)	-0.08	0.35	-0.03	0.70
SV (%)	-0.09	0.25	-0.02	0.84
TPR (%)	**0.18**	**0.041**	0.02	0.84
Cwk (%)	-0.07	0.45	-0.14	0.063

Reactivity values are adjusted for baseline haemodynamic and plasma renin measurements. SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; TPR, total peripheral resistance; Cwk, "Windkessel" compliance of the arterial system. Bold values indicate statistical significance (p<0.05).

We performed forward stepwise multiple regression analyses (Table 4) to determine independent associations between haemodynamic variables and renin under resting conditions and during application of the cold pressor test. All of the above associations were confirmed. In addition, it is noteworthy to mention the borderline significant association between DBP reactivity and renin reactivity in blacks (*P*=0.060).

Table 4: Forward stepwise multiple regression analyses between haemodynamic variables and plasma renin

	Resting plasma renin (pg/ml)					
Resting variables	**Black**			**White**		
	[a]R^2	**β (95% C.I.)**	***P***	**R^2**	**β (95% C.I.)**	***P***
SBP (mmHg)	**0.30**	**-0.29 (-0.48; -0.16)**	**<0.001**	**0.27**	**-0.27 (-0.40;00.16)**	**<0.001**
DBP (mmHg)	**0.24**	**-0.19 (-0.34; -0.04)**	**0.012**	**0.29**	**-0.23 (-0.35; -0.10)**	**<0.001**
HR (bpm)	—	—	—	—	—	—
SV (ml)	—	—	—	—	—	—
TPR (mmHg/ml/s)	**0.16**	**-0.18 (-0.34; -0.04)**	**0.016**	0.09	-0.12 (0.26; 0.03)	0.11
Cwk (ml/mmHg)	**0.59**	**0.24 (0.14; 0.35)**	**<0.001**	**0.69**	**0.11 (0.02; 0.19)**	**0.014**
	Plasma renin reactivity (%)					
Reactivity variables	**Black**			**White**		
	[a]R^2	**β (95% C.I.)**	***P***	**R^2**	**β (95% C.I.)**	***P***
SBP (%)	0.05	0.10 (-0.06; 0.27)	0.22	—	—	—
DBP (%)	0.17	0.15 (-0.01; 0.30)	0.060	**0.07**	**0.21 (0.07; 0.37)**	**0.005**
HR (%)	—	—	—	—	—	—
SV (%)	0.02	-0.09 (-0.26; 0.07)	0.25	—	—	—
TPR (%)	**0.07**	**0.17 (0.01; 0.34)**	**0.041**	—	—	—
Cwk (%)	0.03	-0.09 (-0.26; 0.07)	0.28	0.01	-0.13 (-0.28; 0.02)	0.09

[a], Adjusted R^2; —, did not enter the model. Independent variables included in the model: age, body mass index, sex, gamma-glutamyltransferase, total cholesterol:high-density lipoprotein cholesterol ratio, antihypertensive medication, glycosylated haemoglobin, C-reactive protein, estimated glomerular filtration rate. Reactivity values are adjusted for baseline haemodynamic and plasma renin measurements. SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; TPR, total peripheral resistance, Cwk, "Windkessel" compliance of the arterial system. Bold values indicate statistical significance (p<0.05).

Sensitivity analysis

We investigated if the association found in blacks between TPR reactivity and renin reactivity was confounded by norepinephrine, and thus included norepinephrine in our multiple regression model. In doing so norepinephrine did not enter the model, but renin remained significant (R^2=0.07; β=0.17; *P*=0.049).

Discussion

We compared renin and cardiovascular responses to the cold pressor test between blacks and whites, and investigated if renin and cardiovascular reactivity are associated. The main finding of our study is that despite supressed levels at rest and during stress, renin was positively associated with total peripheral resistance in blacks at rest and when the cardiovascular system was challenged. In whites an increase in renin associated with an increase in diastolic BP. These results suggest a possible difference in the role of renin in mechanisms controlling blood pressure between blacks and whites, which may be influenced by the characteristics of our population such as hypertension, obesity and lifestyle factors.

The cardiovascular stress challenge results in activation of the sympathetic nervous system,[4, 9] as reflected by the increased vascular resistance, blood pressure, heart rate and a decrease in stroke volume and arterial compliance in our study. Changes in DBP, heart rate, stroke volume and arterial compliance were more pronounced in blacks compared to whites as previously shown in the same population.[5, 22] Vascular resistance reactivity was similar between the groups, however previous findings showed that total peripheral resistance tends to be higher in blacks compared to whites.[6] It is noteworthy that the changes in basic haemodynamics were accompanied by an increase in renin only in the white group (*P*=0.001 versus *P*=0.67 in blacks).

Our results on supressed renin during the cold pressor test are in contradiction with previous findings from the same study population which reported an increase in renin during a laboratory mental stressor in blacks which was associated with increased carotid intima-media

thickness.[16] In the previous study, Hamer *et al*. focused on recovery values and used the STROOP Colour Word Conflict Test which is known to elicit a mixed α-adrenergic and β-adrenergic response. The resultant effects include norepinephrine induced myocardial responses via central mechanisms and renin secretion from the juxtaglomerular apparatus by stimulation of β-adrenergic receptors. [23, 24] On the other hand, the cold pressor test has a predominant α-adrenergic response with a peripheral vasoconstrictive effect.[23, 24] Sympathetic nerve stimulation can also increase renin secretion by stimulating α-adrenergic receptors which cause constriction of the afferent arteriole, a fall in intrarenal blood pressure and eventually elevated renin secretion.[25] Blacks seem to have a higher density of α-adrenergic receptors responsible for higher peripheral vasoconstriction and BP reactivity.[9] Increased sodium retention, which is common in black hypertensives[27] may have played a role in suppression of renin secretion during a stressor[26] in the black group. Sensitivity analysis indicated that norepinephrine did not play a role in this association.

Although blacks tend to have low renin levels, their prorenin levels are reportedly higher than that of renin and also 20% higher in blacks compared to whites.[28, 29] The (pro)renin receptor is located in the vasculature[30] and it has been linked to elevated systolic and diastolic blood pressure in Japanese men.[12] Binding of renin and/or prorenin to this receptor can activate tissue RAS, resulting in pressure overload as well as end-organ damage in the heart and kidneys independent of the circulating RAAS.[28, 31-32] It was recently indicated that urinary angiotensinogen is positively associated with BP in a salt-sensitive, low renin group of blacks, thus showing that intrarenal RAS can contribute to BP control independent of the circulatory RAAS.[33] Also, in salt-sensitive individuals, the low renin phenotype is characterised by impairment of the compensatory mechanism for renal sodium reabsorption, renin production, formation of angiotensin II and constriction of the renal efferent arteriole.[27, 34] This suggests that since renin is suppressed in our black and mostly hypertensive population, other mechanisms such as vascular hyper-reactivity to stress,[5, 6, 22] (pro)renin receptor activation[30]

and local tissue RAS[33] may be responsible for the associations found between vascular resistance reactivity and renin reactivity.

Our study should be interpreted within the context of its limitations and strengths. We did not measure baseline and stressor reactivity for norepinephrine, prorenin, angiotensin II and aldosterone. By the time of the writing of this manuscript plasma aldosterone was not available, but it is planned for analysis in the near future. We did not assess salt-sensitivity. The higher vascular reactivity to a cold stimulus in blacks may also be due to an exaggerated perception of cold pain.[5] Some of the participants used antihypertensive medication such as beta blockers, diuretics and angiotensin receptor blockers (ARBs) that influence the RAAS and therefore could have affected our results. The medication was not taken on the day of the measurement and this was authorised by medical doctor. This was a cross-sectional study, therefore causality cannot be inferred, and our study population cannot be regarded as representative of the multi-ethnic South African population. However, to our knowledge, this is the first study to compare cardiovascular and plasma renin responses to a laboratory stressor between black and white South Africans. Our study was performed under well-controlled experimental conditions.

In conclusion, despite suppression of renin secretion during application of a stressor, total peripheral resistance reactivity was positively associated with renin reactivity only in the black population. These results suggest that although renin is suppressed in blacks during rest and stress, it may play a role in the underlying mechanisms that act on the peripheral vasculature to elevate blood pressure.

Summary Table

What is known about the topic

- Blacks tend to have a low renin status; consequently low-renin hypertension is common in this group.
- Blacks have increased cardiovascular reactivity to acute stressors as compared to whites.
- Low renin levels are associated with target organ damage and increased blood pressure in blacks.

What this study adds

- To the best of our knowledge, this is the first study to indicate that plasma renin is suppressed during acute stress in blacks compared to whites.
- Low renin is positively associated with total peripheral resistance in blacks only.
- Although renin is supressed in blacks during rest and stress, its ability to elicit responses in the peripheral vasculature remains active.

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Conflicts of interest

There are no conflicts of interest.

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CHAPTER 4

Aldosterone and renin in relation to surrogate measures of sympathetic activity in blacks with low renin levels:

The SABPA study

SUMMARY OF INSTRUCTIONS FOR AUTHORS

Journal title: *Clinical and Experimental Hypertension (http://www.tandfonline.com/loi/iceh20)*

Scope: a refereed journal in the English language for the rapid communication of new and significant data pertaining to human and animal hypertension of all types. Information concerning circulatory control, methodology, drugs or any other ancillary material relevant to hypertension is also acceptable.

Essential title page information

The title page must include the title, authors' names and addresses, and the phone and fax numbers and e-mail address of the corresponding author. Authors should also supply a shortened version of the title suitable for the running head, not exceeding 50 character spaces.

Abstract

An abstract as well as a list of three to six key (indexing) terms

Article structure

All parts of the manuscript should be typewritten, double-spaced, with margins of at least one inch on all sides. Number manuscript pages consecutively throughout the paper.

Acknowledgements

Acknowledgments should be gathered into a brief statement at the end of the text. All sources of financial sponsorship are to be acknowledged, including the names of private and public sector sponsors. This includes government grants, corporate funding, trade associations and contracts.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

References

References should be cited in text by reference number in parentheses. Multiple references within one set of parentheses should be set off by comma, but no space. Examples:

Journal: Flack JM, Gardin JM, Yunis C, Liu K. Static and pulsatile blood pressure correlates of left ventricular structure and function in black and white young adults: the CARDIA study. Am Heart J 1999; 138:856--864.

Book: Biggs R, MacFarlane R. Human Blood Coagulation and Its Disorders. Oxford: Blackwell Scientific Publications, 1957.

Chapter in Book: Kaplan NM, Ed. Primary hypertension: natural history and evaluation. In: Kaplan's Clinical Hypertension, 8th ed. Philadelphia: Lippincott Williams & Wilkins, 2002, 163.

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Tables and figures

A short descriptive title should appear above each table with a clear legend and any footnotes suitably identified below. All units must be included. Figures should be completely labelled.

****Note:** some formatting was changed to maintain consistency throughout the thesis.

Aldosterone and renin in relation to surrogate measures of sympathetic activity in blacks with low renin levels: The SABPA study

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Running title: Sympathetic drive and aldosterone

Word count: Abstract: 242

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Tables: 3

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Supplementary file: 1

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The authors report no conflicts of interest

Abstract

Hypertension, particularly in black populations, is often accompanied by augmented sympathetic activity and low renin levels, indicative of possible blood pressure (BP) dysregulation by the renin-angiotensin-system (RAS). The potential role of aldosterone in the functioning of the sympathetic nervous system in the context of low renin conditions is unclear. We therefore explored whether surrogate measures of sympathetic activity (noradrenaline, 24-hour heart rate (HR) and % dipping in night-time HR) relate to renin, aldosterone and aldosterone-to-renin ratio (ARR) in low-renin black and white Africans. We included black (N=162) and white (N=206) participants, stratified by low and high renin status, and focused on the low renin groups. We measured 24-hour BP, HR and calculated night-time dipping. We determined renin and aldosterone in plasma and calculated ARR. Noradrenaline and creatinine were determined in urine and noradrenaline:creatinine ratio calculated. More blacks had low renin (80.9%) compared to whites (57.8%) (P<0.001). In univariate and after multivariate analysis the following significant associations were evident only in low-renin blacks: noradrenaline:creatinine ratio associated positively with aldosterone (β=0.32, P=0.001), 24-hour HR associated positively with renin (β=0.17, P=0.041), while HR dipping associated negatively with aldosterone (β=-0.30, P=0.001) and ARR (β=-0.23, P=0.010). In a black low-renin population, the observed associations between surrogate measures of sympathetic activity and components of the RAS suggest that higher aldosterone levels relative to renin may have detrimental effects on the cardiovascular system and that the effects of aldosterone may be coupled to the effects of sympathetic nervous system.

Key words: Aldosterone, blacks, heart rate, hypertension, renin

Introduction

Hypertension is the most common risk factor for cardiovascular events and its prevalence continues to increase in sub-Saharan Africa [1, 2]. A suppressed renin-angiotensin-system (RAS) is one of the prominent features of hypertension [3, 4] especially in blacks. This may reflect a physiological response to increased blood pressure (BP) and sodium-volume overload attributable to aldosterone [5]. The aldosterone-to-renin ratio (ARR) is an index of the extent of aldosterone activation in the context of renin and has been used to screen for primary hyperaldosteronism, characterised by a reduction in renin as aldosterone increases [6, 7]. Even modest increases in aldosterone, particularly in the presence of high sodium intake and low renin levels, result in high BP in black populations [8, 9]. Recent evidence suggests that blacks have an increased mineralocorticoid receptor sensitivity compared to whites, which may augment aldosterone-mediated volume expansion with low-renin hypertension [4, 10].

In addition to the sodium and volume retention effects of aldosterone, it also influences the autonomic nervous system by, for example blunting the baroreflex response and potentiating the vasoconstrictor effects of noradrenaline [11, 12]. Blockade of aldosterone improves ambulatory HR variability and reduces HR, particularly in the early morning hours when sympathetic nerve activity is high [13]. It is therefore plausible that an interplay exists between aldosterone and sympathetic activity to increase blood pressure, particularly at low renin states. To address this, we determined if surrogate measures of sympathetic activity (noradrenaline, 24-hour HR and % dipping in HR) relate to renin, aldosterone and ARR in low-renin black and white Africans.

Materials and methods

Study design and population

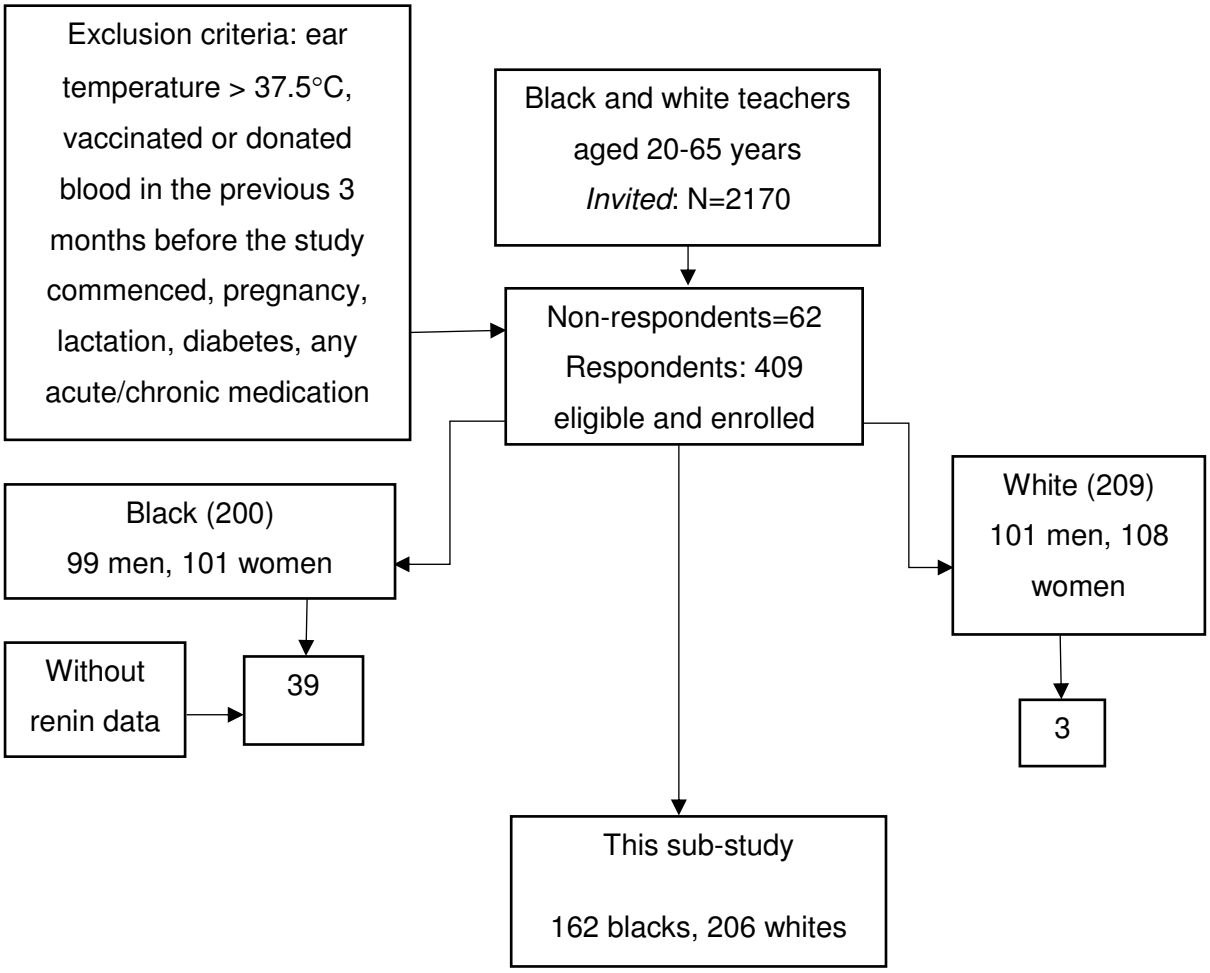

Figure 1: The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study population.

The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study was conducted between February 2008 and May 2009. The study included 200 black (49.3 % women) and 209 white (51.7% women) school teachers from the North West province of South Africa (Figure 1). The reason for this selection was to have a homogeneous sample from a similar socioeconomic class. We invited eligible participants in the age range between 20 and 65 years. Exclusion was based on the following criteria: ear temperature > 37.5°C, vaccinated or donated blood in the previous three months before the study commenced, pregnancy,

lactation, diabetes, any acute/chronic medication and psychotropic substance abuse or dependence [14]. For this sub-study we excluded 39 black and 3 white participants without renin values, with data therefore being available for 162 black and 206 white participants (Figure 1).

Participants were fully informed about the objectives and procedures of the study before enrolment. Assistance was given to any participant who requested conveyance of information in their home language. All participants signed an informed consent form. The study complied with all applicable requirements of the international regulations, in particular, the Helsinki Declaration of 1975 (as revised in 2008) for investigation of human participants. The Health Research Ethics Committee of the North-West University (Potchefstroom Campus) approved this study (NWU-00036-07-S6).

Questionnaires

We administered validated general health and sociodemographic questionnaires [14].

Anthropometric measurements

Weight, height, waist and hip circumferences were measured in triplicate by anthropometrists with calibrated instruments according to standardised methods (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, London, UK; Holtain non-stretchable metal flexible measuring tape). Body mass index (BMI) was calculated and expressed as kg/m^2 [15].

Cardiovascular measurements

The 24-hour ambulatory blood pressure (ABPM) and heart rate measurements were conducted during the working week. The ABPM apparatus (Meditech CE120® Cardiotens, Budapest, Hungary) was attached on the participant's non-dominant arm and programmed to measure BP at 30 minute intervals during the day (08:00 – 22:00) and every hour during the night (22:00 – 06:00). Percentage dipping for BP and HR were calculated as follows: %

dipping=(day-night)/day)100. HR is associated with muscle sympathetic nerve activity, which is the gold standard for assessing sympathetic outflow and plasma noradrenaline [16], while reduced HR dipping or a higher night-time HR can also represent a state of sympathetic overdrive [17]. We therefore included ambulatory heart rate and its dipping as well as noradrenaline as surrogate measures of sympathetic nerve activity. Hypertension was defined as ABPM ≥ 130/80 mmHg according to the European Society of Hypertension guidelines or the use of antihypertensive medication. The validated Finometer device [18, 19] (FMS, Finapres Measurement Systems, Amsterdam, Netherlands) was connected, and after a 10 minute resting period, a 2-minute calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial arterial pressure [19]. The highest precision in cardiovascular measurements can be achieved only after this calibration [19], and BP measurements complied with the requirements of the Association for the Advancement of Medical Instrumentation (AAMI) [19, 20]. Continuous measurement of resting cardiovascular variables was performed for a 5-minute period. The Beatscope® software was used to calculate cardiac output (CO), HR and computed stroke volume (SV) and total peripheral resistance (TPR). The sodium-volume component was calculated according to Laragh's volume equation (V=BP/R, V, volume; BP, blood pressure; R, renin) [21].

Biological sampling and biochemical analyses

Participants were requested to be in a fasted state by not eating or drinking anything except water for approximately 8-10 hours prior to sample collection in the mornings. An 8-hour morning spot urine sample was collected from which creatinine, sodium, potassium and noradrenaline were measured (Cobas Integra 400 plus, Roche, Basel, Switzerland; 3-Cat Fast Track kit, LDN, Nordhorn, Germany). A registered nurse obtained the first blood sample with a sterile winged infusion set from the antebrachial vein branches while the participant was in a supine position for a period of 30 minutes. Samples were prepared according to appropriate methods and stored at -80°C in the laboratory. Sequential multiple analysers (Konelab 20i, ThermoScientific, Vantaa, Finland; and Cobas Integra 400 plus, Roche, Basel, Switzerland)

were used to analyse total and high density lipoprotein cholesterol (HDL-c), fasting glucose, high sensitivity C-reactive protein (CRP), creatinine, serum sodium, potassium, gamma-glutamyltransferase (GGT) and glycosylated haemoglobin (HbA1c). Tumour necrosis factor-alpha (TNF-α) was analysed with a Quantikine high sensitivity enzyme linked immunosorbent assay (R&D Systems, Minneapolis, MN USA). Serum cotinine was analysed with a homogeneous immunoassay (Automized Modular, Roche, Basel, Switzerland). The Modification of Diet in Renal Disease (MDRD) formula was used to estimate glomerular filtration rate (eGFR) as a measure of renal function. We analysed active plasma renin using the high sensitivity radio-immunometric assay (Renin III Generation, CIS Biointernational, Cedex, France) with cross-reaction with prorenin being 0.4%. The source of reagents was mouse anti-human-active renin monoclonal antibody (IBL Lab, 38T501, USA). Plasma aldosterone was analysed using a competitive radioimmunoassay (Beckman Coulter, Brea, CA). We used the age-specific reference values from the renin III CISBIO kit to stratify the participants into low and high renin groups (20-40 years, mean 8.11 pg/ml; 40-60 years, mean 6.18 pg/ml) (Renin III Generation, CIS Biointernational, Cedex, France).

Statistical analyses

We used Statistica Version 12 for all statistical analyses (Statsoft Inc., Tulsa, OK). We found an interaction with ethnicity on the association between 24-hour HR and renin (P=0.018) and as a result performed all analyses in black and white groups separately. No sex interactions were observed. The distribution of renin, aldosterone, ARR, HbA1c, GGT, cotinine, total cholesterol, HDL-cholesterol, CRP, interleukin-6, creatinine and noradrenaline were normalised by logarithmic transformation. The central tendency and spread of these variables were represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions were compared by using independent t-tests and Chi-square tests, respectively. We performed single, partial and forward stepwise multiple regression analyses to investigate associations between relevant cardiovascular variables and renin, aldosterone and ARR, as well as between aldosterone and noradrenaline:creatinine ratio. In partial

regression analyses we adjusted for age, BMI and sex. Covariates included in the models were age, waist-to-hip ratio, sex, GGT, cotinine, urinary Na^+/K^+ ratio, total cholesterol: HDL-c ratio, antihypertensive medication, HbA1c, TNF-α, eGFR and TPR.

Results

Characteristics of the population

Low renin was more prevalent in the black group (80.9% vs. 58.7%, $P<0.001$). Table 1 compares the black and white low renin groups, which is the focus of the present study. The ethnic groups had similar mean ages ($P=0.055$) and sex distribution ($P=0.11$). Twenty-four hour, day- and night-time systolic and diastolic BP as well as HR were higher in the black group (all $P<0.001$). There was no difference in percentage dipping of SBP, DBP or HR between the blacks and whites (all $P\geq0.13$). Aldosterone was similar between the groups ($P=0.25$). However, blacks had lower renin ($P<0.001$) and a resultant higher ARR ($P=0.027$) and sodium-volume component ($P<0.001$), as well as a lower noradrenaline:creatinine ratio ($P=0.006$) compared to whites.

Table 1: Ethnic comparison between low renin groups

	Black (N=131)	White (N=119)	*P*
Age (years)	42.6 ± 7.56	44.8 ± 10.5	0.055
Women, n (%)	64 (48.9)	70 (58.8)	0.11
Anthropometric measurements			
Body mass index (kg/m^2)	**29.3 ± 6.81**	**27.4 ± 5.91**	**0.021**
Waist-to-hip ratio	0.86 ± 0.09	0.87 ± 0.08	0.76
Resting cardiovascular measurements[a]			
Sodium-volume component	**55.4 ± 40.8**	**35.3 ± 21.5**	**<0.001**
Cardiac output (l/min)	6.53 ± 1.74	6.44 ± 2.12	0.72
Heart rate (bpm)	67.3 ± 11.0	66.2 ± 10.9	0.37
Stroke volume (ml)	98.5 ± 25.5	98.0 ± 27.1	0.88
Total peripheral resistance (mmHg/ml/s)	1.06 ± 0.41	1.08 ± 0.64	0.77
Systolic BP			
24-hour (mmHg)	**132 ± 16.8**	**125 ± 12.9**	**<0.001**
Daytime (mmHg)	**137 ± 16.9**	**130 ± 12.9**	**<0.001**
Night-time (mmHg)	**123 ± 17.3**	**116 ± 14.8**	**<0.001**
Diastolic BP			
24-hour (mmHg)	**83.2 ± 11.2**	**77.0 ± 8.20**	**<0.001**
Daytime (mmHg)	**88.4 ± 11.2**	**81.8 ± 8.8**	**<0.001**
Night-time (mmHg)	**74.3 ± 12.1**	**67.7 ± 9.11**	**<0.001**
Hypertensive n (%)	**80 (61.1)**	**46 (38.7)**	**<0.001**
Antihypertensive medication, n (%)	**29 (22.1)**	**14 (11.8)**	**0.029**
Heart rate			
24-hour (bpm)	**78.4 ± 10.7**	**73.4 ± 9.70**	**<0.001**
Daytime (bpm)	**83.0 ± 11.2**	**78.0 ± 10.4**	**<0.001**
Night-time (bpm)	**70.4 ± 12.6**	**65.2 ± 9.62**	**<0.001**
% Dipping			
Systolic BP (mmHg)	10.1 ± 5.88	11.3 ± 6.71	0.13
Diastolic BP (mmHg)	16.0 ± 7.19	17.0 ± 7.65	0.26
Heart rate (bpm)	14.8 ± 11.3	16.3 ± 7.44	0.23
Biochemical measurements			
Aldosterone (pg/ml)	42.4 (10.2; 143)	47.0 (21.6; 135)	0.25
Renin (pg/ml)	**2.86 (0.95; 6.14)**	**4.01 (1.43; 7.24)**	**<0.001**
Aldosterone-to-renin ratio	**14.8 (3.63; 76.9)**	**11.7 (4.08; 37.5)**	**0.027**
Glycosylated haemoglobin (%)	**5.83 (5.10; 6.90)**	**5.47 (5.00; 6.10)**	**<0.001**
Total cholesterol: High density lipoprotein-c	**4.00 (2.33; 7.09)**	**4.57 (2.85; 7.17)**	**0.004**
C-reactive protein (mg/L)	**3.43 (0.41; 24.6)**	**2.16 (0.99; 10.7)**	**0.001**
Cotinine (ng/ml)	2.42 (1.00; 122)	1.86 (1.00; 211)	0.63
Gamma-glutamyltransferase (U/L)	**44.8 (20.0; 184)**	**18.2 (7.00; 76.0)**	**<0.001**
Tumor necrosis factor-α (IU/ml)	**2.82 ± 2.06**	**1.58 ± 1.64**	**<0.001**
Estimated glomerular filtration rate (ml/min/1.73m^2)	**112 ± 25.8**	**93.8 ± 16.7**	**<0.001**
Serum Na^+/K^+ ratio	32.7 ± 2.60	33.1 ± 2.98	0.26
Urinary Na^+/K^+ ratio	**5.57 ± 4.06**	**4.38 ± 2.86**	**0.008**
Urinary noradrenaline:creatinine ratio	**1.43 (0.91; 2.37)**	**1.57 (0.95; 2.79)**	**0.006**
Lifestyle factors			
Self-reported smoking, n (%)	20 (15.3)	17 (14.4)	0.84
Self-reported alcohol use, n (%)	**32 (24.4)**	**56 (47.5)**	**<0.001**

[a],Obtained from the Finometer device. Values are arithmetic mean ± standard deviation; geometric mean (5th and 95th percentile interval) for logarithmically transformed variables. Abbreviations: BP, blood pressure. Bold text indicate p<0.05.

Supplementary Table 1 compares the characteristics of the low and high renin groups within each ethnic group. In blacks, there was no difference in ambulatory BP and dipping between the low and high renin groups (all $P \geq 0.22$). Ambulatory and resting HR (all $P \leq 0.040$), and cardiac output ($P = 0.003$) were lower in the low renin group compared to the high renin group, while the sodium-volume component ($P < 0.001$) and TPR ($P = 0.026$) were higher in the low renin group. In addition, the black low renin group had lower aldosterone (42.2 vs. 70.4 pg/ml) ($P = 0.003$) and a higher ARR (14.8 vs. 8.49) ($P = 0.004$) and serum Na^+/K^+ ($P = 0.026$) compared to the high renin group. In whites, the low renin group also had a higher sodium-volume component ($P < 0.001$), as well as night-time systolic ($P = 0.019$) and diastolic BP ($P = 0.037$), and BP dipped less than in the high renin group (both $P \leq 0.006$). The white low renin group also had a higher ARR ratio (11.7 vs. 6.58) compared to the white high renin group ($P < 0.001$).

Regression analyses

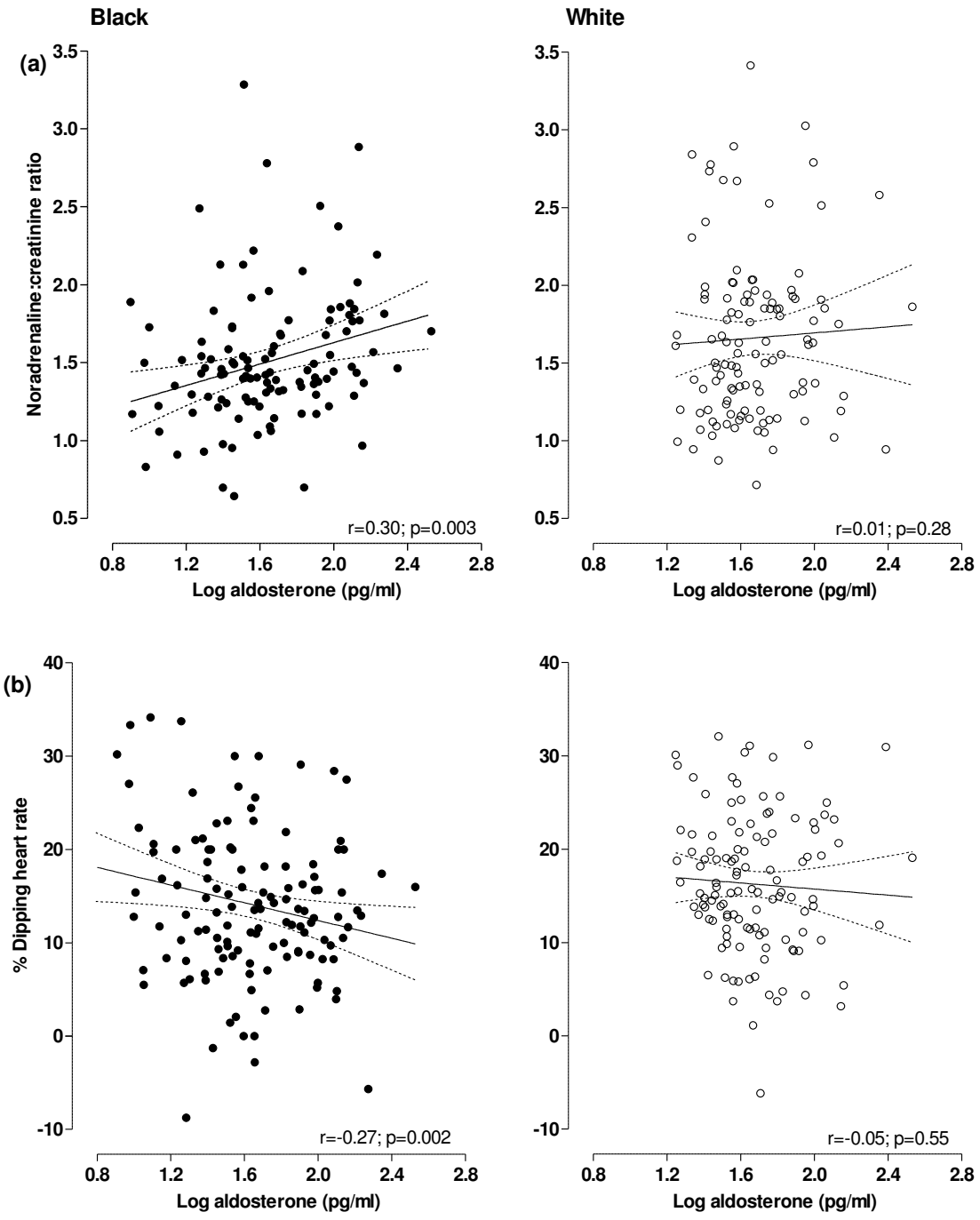

Figure 2: Associations between (a) log noradrenaline:creatinine ratio and log aldosterone; (b) night-time dipping in heart rate and aldosterone in black and white low renin groups. Solid and dashed lines represent the regression line and 95% CI boundaries, respectively.

We performed Pearson and partial correlations (adjusting for age, sex and BMI) to investigate the associations of surrogate measures of sympathetic activity (noradrenaline:creatinine ratio, 24-hour HR and night-time dipping in HR) with renin, aldosterone and ARR (Figure 2 and Supplementary Tables 2-3). In blacks, before and after full adjustment in multiple regression analysis, noradrenaline:creatinine ratio positively associated with aldosterone (β=0.32, *P*=0.001) (Table 2). In addition, 24-hour HR associated positively with renin (β=0.17, *P*=0.041), while night-time dipping in HR associated negatively with aldosterone (β=-0.30, *P*=0.001) and ARR (β=-0.23, *P*=0.010) (Table 3). Furthermore, 24-hour SBP (β=-0.18, *P*=0.019) and DBP (β=-0.18, *P*= 0.024) associated negatively with renin, while there was a borderline significant association of SBP (β=0.14, *P*=0.056) and DBP (β=0.15, *P*= 0.051) with ARR (Table 2). No associations were evident in the low renin white group.

Table 2: Independent associations of 24-hour BP and noradrenaline:creatinine ratio with renin, aldosterone and ARR in black and white low renin groups

Independent variables	Black (N=131)								
	24-hour SBP			24-hour DBP			Log noradrenaline:creatinine ratio		
	Adjusted R^2	β (95% CI)	*P*	Adjusted R^2	β (95% CI)	*P*	Adjusted R^2	β (95% CI)	*P*
Log renin	**0.32**	**-0.18 (-23.4; -2.35)**	**0.019**	**0.33**	**-0.18 (-15.2; -1.18)**	**0.024**	_	_	_
Log aldosterone	_	_	_	_	_	_	**0.21**	**0.32 (0.06; 0.36)**	**0.001**
Log ARR	0.31	0.14 (-0.05; 12.3)	0.056	0.30	0.15 (-0.03; 8.25)	0.051	0.16	0.17 (-0.02; 0.35)	0.060
	White (N=119)								
	24-hour SBP			24-hour DBP			Log noradrenaline:creatinine ratio		
	Adjusted R^2	β (95% CI)	*P*	Adjusted R^2	β (95% CI)	*P*	Adjusted R^2	β (95% CI)	*P*
Log renin	0.26	-0.09 (-16.3; 4.13)	0.24	0.30	-0.15 (-12.6; 0.24)	0.062	_	_	_
Log aldosterone	_	_	_	_	_	_	_	_	_
Log ARR	_	_	_	_	_	_	0.09	0.11 (-0.06; 0.27)	0.22

—, did not enter the model. Independent variables included in the model: age, waist-to-hip ratio, sex, gamma-glutamyltransferase, cotinine, urinary Na^+/K^+ ratio; total cholesterol:high-density lipoprotein cholesterol ratio, antihypertensive medication, glycosylated haemoglobin, tumour necrosis factor-α, estimated glomerular filtration rate, total peripheral resistance. ARR, aldosterone-to-renin ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure. Values in bold indicate $p<0.05$.

Table 3: Independent associations of 24-hour HR, night-time dipping in HR and BP with renin, aldosterone and ARR in black and white low renin groups

Black (N=131)	**24-HR**			**% HR**		
	Adjusted R^2	**β (95% CI)**	***P***	**Adjusted R^2**	**β (95% CI)**	***P***
Log renin	**0.17**	**0.17 (0.40; 15.2)**	**0.041**	_	_	_
Log aldosterone	_	_	_	**0.11**	**-0.30 (-0.12; -0.02)**	**0.001**
Log ARR	_	_	_	**0.10**	**-0.23 (-10.8; -1.66)**	**0.010**
	% SBP			**%DBP**		
	Adjusted R^2	**β (95% CI)**	***P***	**Adjusted R^2**	**β (95% CI)**	***P***
Log renin	_	_	_	_	_	_
Log aldosterone	0.02	0.12 (-0.90; 4.81)	0.18	0.10	0.16 (-0.08; 6.63)	0.058
Log ARR	_	_	_	_	_	_
White (N=119)	**24-HR**			**% HR**		
	Adjusted R^2	**β (95% CI)**	***P***	**Adjusted R^2**	**β (95% CI)**	***P***
Log renin	_	_	_	_	_	_
Log aldosterone	_	_	_	_	_	_
Log ARR	_	_	_	_	_	_
	% SBP			**%DBP**		
	Adjusted R^2	**β (95% CI)**	***P***	**Adjusted R^2**	**β (95% CI)**	***P***
Log renin	0.03	-0.15 (-11.3; 1.10)	0.11	_	_	_
Log aldosterone	_	_	_	_	_	_
Log ARR	_	_	_	_	_	_

–, did not enter the model. Independent variables included in the model: age, waist-to-hip ratio, sex, gamma-glutamyltransferase, cotinine, urinary Na^+/K^+ ratio; total cholesterol:high-density lipoprotein cholesterol ratio, antihypertensive medication, glycosylated haemoglobin, tumour necrosis factor-α, estimated glomerular filtration rate, total peripheral resistance. ARR, aldosterone-to-renin ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. Values in bold indicate $p<0.05$.

Sensitivity analysis

In order to assess if the positive association between renin and 24-hour HR were due to the use of antihypertensive medication, we individually adjusted for the different classes of antihypertensive drugs. By adjusting for diuretics (β=0.17, *P*=0.046), calcium channel blockers (β=0.18, *P*=0.043) and beta blockers (β=0.17, *P*=0.046), our results remained unchanged. The

information on the classes of antihypertensive drugs was obtained with the general health questionnaire. We further evaluated the influence of noradrenaline:creatinine ratio on the associations between 24-hour HR and renin, as well as for % dipping in HR with aldosterone by adjusting for noradrenaline:creatinine ratio. The latter remained significant (β=-0.23, *P*=0.012). The association between 24-hour HR and renin became non-significant (β=0.15, *P*=0.090). However, in the same analysis 24-hour HR associated positively with noradrenaline:creatinine ratio (β=0.25, *P*=0.007). We tested for an interaction of noradrenaline:creatinine ratio on the association between % dipping in HR and aldosterone and found that it existed (β=2.9, *P*<0.001). There was also an interaction between noradrenaline:creatinine ratio and aldosterone on the association with 24-hour SBP (β=0.70, *P*=0.037).

Discussion

The main finding of the present study is that in low-renin blacks, heart rate dipping and noradrenaline relate independently and adversely to aldosterone. In addition, 24-hour HR associated positively with renin. To the best of our knowledge, our study is the first to demonstrate that aldosterone may augment the effects of sympathetic drive in blacks with low renin. Previous observations in the SABPA cohort indicated blunted baro-receptor sensitivity and depressed heart rate variability, supporting the possibility of higher sympathetic activity in this population group [22, 23]. Our observed interaction of noradrenaline and aldosterone on the association with % dipping in HR suggests a possible interplay between the sympathetic nervous system and aldosterone at low renin states. In our study, blacks had a higher frequency of low renin compared to whites, with blacks even having lower renin levels than whites when comparing the low renin groups.

The higher prevalence of low renin levels in blacks is consistent with previous studies [24, 25]. Contrary to the common notion that blacks have lower aldosterone levels compared to whites [4, 10, 24], we found aldosterone levels to be similar in blacks and whites, which is also in agreement with previous findings [25, 26]. In addition, in our study, the mean renin concentration was below

the normal range, while aldosterone was within the normal range, resulting in a higher ARR in blacks. Excess aldosterone for a given level of plasma renin points to a possibility of autonomous aldosterone secretion and may indicate the presence of a variant of hyperaldosteronism, which has a significant role in salt/volume-related hypertension [27-29]. In the present study population with a high prevalence of hypertension (61% blacks, 39% whites), we observed a borderline significant association between ambulatory blood pressure and ARR in blacks. This confirms that slightly higher aldosterone levels in the context of renin contribute to increased blood pressure [8, 9].

A novel finding of this study is that despite similar aldosterone in low-renin blacks and whites, dipping of night-time HR was negatively associated with aldosterone and its ratio to renin in blacks only. A higher HR associates with total mortality and night-time HR predicts cardiovascular mortality in the general population [30]. In blacks, a more pronounced sympathetic drive as shown by an exaggerated cardiovascular reactivity to stress compared to whites contribute to elevation of BP through increased total peripheral resistance and HR [31]. It was recently suggested that sympathetic activation may not be a mechanism of hypertension in Africans, particularly in those with low angiotensin II [32], however our findings contradict this assumption. The positive association between ambulatory HR and renin may indicate β-adrenergic receptor stimulation at both the heart and kidney, resulting in increases in HR and renin secretion at the juxtaglomerular apparatus, respectively [33]. This was supported by our sensitivity analysis where the positive association between ambulatory HR and renin seemed dependent on noradrenaline. Previous findings in the same population under study indicated that even at suppressed renin levels, exposure to an acute stressor resulted in a positive association between total peripheral vascular resistance reactivity and renin reactivity in blacks, but not in whites [34]. The observed association of aldosterone with attenuated HR dipping may not only be an indication of possible synergy between aldosterone and sympathetic drive, but also the direct effects of aldosterone on the cardiovascular system via high density mineralocorticoid receptors [35, 36]. Aldosterone may

enhance the effects of catecholamines [12]. In the present study we found blacks to have lower noradrenaline, which was positively related to aldosterone. Experimental studies indicated that aldosterone prevents extraneuronal and myocardial uptake of noradrenaline and therefore may heighten its effects [12, 37], albeit lower levels. The increased aldosterone sensitivity in blacks may also explain the observed negative association with HR dipping despite having similar mean aldosterone levels compared to whites [10]. Furthermore, in this black population characterised by low renin levels, noradrenaline interacted with aldosterone on the positive association with ambulatory blood pressure.

Our study should be interpreted within the context of its strengths and limitations. Use of specific antihypertensive drugs, including beta blockers, calcium channels blockers and diuretics, may have influenced our findings regardless of adjustments in the multiple regression sensitivity analyses. We did not collect 24-hour urine to assess noradrenaline, salt intake and did not assess salt-sensitivity and angiotensin II. Microneurography and regional noradrenaline spillover are the gold standards [38] for studying sympathetic outflow and were not used in this study, however noradrenaline and its metabolites are commonly used to assess sympathetic activity [32, 39]. The noradrenaline:creatinine ratio was used in the present study. The high renin group in blacks was smaller when compared to the high renin group in whites and both low renin groups in blacks and whites. This was a cross-sectional study, therefore causality could not be inferred. This homogenous sample cannot be regarded as representative of the general South African population.

In conclusion, we confirmed a higher prevalence of low renin in blacks compared to whites, yielding a higher ARR in blacks. We found only in blacks with low renin that noradrenaline related positively with aldosterone, with aldosterone being further associated with less dipping in night-time HR. Our findings of an interaction between noradrenaline and aldosterone on the relationship with HR dipping, suggest that low-renin hypertension in blacks may be partly mediated by aldosterone's effects and its interrelation with the sympathetic nervous system.

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Disclosure of interest

The authors report no conflicts of interest.

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Supplementary information summary

Table 1 compares the basic characteristics of low and high renin groups in blacks and whites. Tables 2-3 indicate adjusted and unadjusted correlations of cardiovascular measurements (24-hour HR and BP), surrogate measures of sympathetic activity (NA:Creatinine ratio, 24-hour HR and its dipping) with components of the RAS (renin, aldosterone and ARR) in the low renin groups in blacks and whites.

Supplementary Table 1: Comparison between the low and high renin groups within the black and white populations

	Black			White		
	Low renin	**High renin**	***P***	**Low renin**	**High renin**	***P***
N	131	31		119	87	
Age (years)	42.6 ± 7.56	46.5 ± 7.78	0.012	44.8 ± 10.5	45.6 ± 11.3	0.62
Women, n (%)	64 (48.9)	12 (38.7)	0.31	70 (58.8)	36 (41.4)	0.013
Anthropometric measurements						
Body mass index (kg/m^2)	29.3 ± 6.81	31.9 ± 8.67	0.067	27.4 ± 5.91	27.9 ± 6.03	0.52
Waist-to-hip ratio	0.86 ± 0.10	0.89 ± 0.14	0.096	0.87 ± 0.09	0.89 ± 0.11	0.065
Cardiovascular variables						
Systolic BP						
24 hour (mmHg)	132 ± 16.8	133 ± 13.5	0.62	125 ± 13.0	123 ± 10.6	0.14
Daytime (mmHg)	137 ± 16.9	140 ± 13.8	0.39	130 ± 12.8	129 ± 11.1	0.45
Night-time (mmHg)	123 ± 17.3	124 ± 15.4	0.84	**116 ± 14.8**	**111 ± 11.5**	**0.019**
Diastolic BP						
24 hour (mmHg)	83.2 ± 11.2	83.1 ± 9.2	0.94	77.0 ± 8.21	76.3 ± 7.92	0.51
Daytime (mmHg)	88.4 ± 11.2	88.6 ± 13.1	0.91	81.8 ± 8.75	81.7 ± 8.90	0.98
Night-time (mmHg)	74.3 ± 12.1	72.9 ± 10.9	0.54	**67.7 ± 9.12**	**65.1 ± 7.92**	**0.037**
Heart rate						
24 hour (bpm)	**78.4 ± 10.9**	**85.6 ± 10.1**	**0.001**	73.4 ± 9.70	74.0 ± 10.8	0.66
Daytime (bpm)	**82.9 ± 11.2**	**91.5 ± 10.8**	**<0.001**	78.0 ± 10.4	79.1 ± 11.8	0.48
Night-time (bpm)	**70.4 ± 12.6**	**76.1 ± 10.5**	**0.016**	65.2 ± 9.62	64.4 ± 11.3	0.59
% Dipping						
Systolic BP (mmHg)	10.1 ± 5.88	11.6 ± 7.15	0.22	**11.3 ± 6.71**	**13.8 ± 5.57**	**0.004**
Diastolic BP (mmHg)	15.9 ± 7.19	15.9 ± 20.2	0.96	**17.0 ± 7.65**	**20.0 ± 7.60**	**0.006**
Heart rate (bpm)	14.8 ± 11.3	16.4 ± 8.99	0.47	16.3 ± 7.44	18.2 ± 10.6	0.12
Haemodynamic variables						
Sodium-volume component	**55.4 ± 40.4**	**16.9 ± 5.59**	**<0.001**	**39.3 ± 21.5**	**13.4 ± 4.40**	**<0.001**
Cardiac output (l/min)	**6.53 ± 1.74**	**7.60 ± 1.93**	**0.003**	6.44 ± 2.12	6.44 ± 1.71	0.99
Heart rate (bpm)	**67.4 ± 11.0**	**71.9 ± 11.2**	**0.040**	66.2 ± 10.9	66.5 ± 10.4	0.83
Stroke volume (ml)	98.5 ± 25.5	107 ± 26.3	0.10	98.0 ± 27.1	97.3 ± 20.0	0.85
TPR (mmHg/ml/s)	**1.06 ± 0.41**	**0.88 ± 0.26**	**0.026**	1.08 ± 0.64	0.98 ± 0.29	0.21
Biochemical measurements						
Aldosterone (pg/ml)	**42.4 (10.9; 143)**	**70.4 (13.4; 278)**	**0.003**	**47.0 (63.0; 135)**	**64.8 (47; 228)**	**0.001**
Renin (pg/ml)	**2.86 (0.95; 6.14)**	**8.30 (6.21; 15.9)**	**<0.001**	**4.01 (1.43; 7.24)**	**9.86 (6.32; 20.8)**	**<0.001**
Aldosterone-to-renin ratio	**14.8 (3.63; 76.8)**	**8.49 (1.44; 33.9)**	**0.004**	**11.7 (4.08; 37.5)**	**6.58 (1.87; 24.2)**	**<0.001**
Glycosylated haemoglobin	**5.8 (5.10; 6.90)**	**6.35 (5.10; 10.4)**	**0.006**	5.47 (5.00; 6.10)	5.53 (5.00; 6.30)	0.27
Total cholesterol:HDL-c	4.00 (2.33; 7.09)	4.45 (2.32; 11.5)	0.056	**4.57 (2.85; 7.17)**	**5.00 (2.88; 8.80)**	**0.036**
C-reactive protein (mg/L)	**3.43 (0.41; 24.5)**	**6.43 (1.33; 31.8)**	**0.010**	2.16 (0.99; 10.1)	1.89 (0.99; 8.70)	0.27
Tumor necrosis factor-α	**2.83 ± 2.06**	**3.75 ± 2.30**	**0.026**	**1.58 ± 1.64**	**2.34 ± 2.34**	**0.007**
eGFR (ml/min/1.73m^2)	112 ± 25.8	121 ± 34.3	0.085	93.8 ± 16.7	95.5 ± 17.6	0.49
Serum Na^+/K^+ ratio	**32.7 ± 2.60**	**31.6 ± 1.91**	**0.026**	33.2 ± 3.31	33.1 ± 3.04	0.87
Urinary Na^+/K^+ ratio	5.58 ± 4.06	5.03 ± 4.39	0.50	4.77 ± 5.09	3.68 ± 2.50	0.069
Urinary NA:Creatinine ratio	1.43 (0.91; 2.37)	1.48 (0.98; 2.23)	0.68	1.57 (0.95; 2.79)	1.59 (0.91; 2.77)	0.67
GGT (U/L)	44.80 (19.9; 120)	53.5 (22.5; 177)	0.21	18.2 (7.00; 76.00)	21.3 (8.00; 90.0)	0.12
Cotinine (ng/ml)	**2.42 (1.00; 122)**	**7.96 (1.00; 2.22)**	**0.004**	1.86 (1.00; 211)	1.70 (1.00; 145)	0.40
Smoking, n (%)	**20 (15.7)**	**10 (32.3)**	**0.030**	17 (14.4)	11(12.6)	0.71
Self-reported alcohol use, n	32 (24.4)	10 (32.3)	0.37	56 (47.5)	45 (51.7)	0.54
Hypertensive n (%)	80 (61.1)	23 (74.2)	0.17	46 (38.6)	36 (41.4)	0.69
Antihypertensive medication,	29 (22.1)	6 (19.4)	0.73	14 (11.8)	13(14.9)	0.50

Values are arithmetic mean ± standard deviation; geometric mean (5th and 95th percentile interval) for logarithmically transformed variables. Abbreviations: BP, blood pressure; HR, heart rate; SV, Stroke volume; CO, Cardiac output; TPR, total peripheral resistance; eGFR, estimated glomerular filtration rate; NA, noradrenaline; HDL-c, high density lipoprotein cholesterol; GGT, gamma-glutamyltransferase. Bold text indicate p<0.05.

Supplementary Table 2: Pearson and partial correlations of BP, HR and noradrenaline with renin, aldosterone and ARR in black and white low renin groups

Pearson correlations

	Black (N=131)							
Variables	**24-hour SBP**		**24-hour DBP**		**24-hour HR**		**NA:creatinine ratio**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Log renin	**-0.22**	**0.011**	**-0.18**	**0.030**	0.16	0.062	0.11	0.25
Log aldosterone	0.13	0.14	**0.17**	**0.047**	0.08	0.38	**0.30**	**0.001**
Log ARR	**0.24**	**0.006**	**0.26**	**0.003**	-0.06	0.77	**0.24**	**0.009**
	White (N=119)							
	24-hour SBP		**24-hour DBP**		**24-hour HR**		**NA:creatinine ratio**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Log renin	-0.05	0.059	-0.10	0.30	0.02	0.83	0.01	0.92
Log aldosterone	-0.04	0.66	0.01	0.89	0.10	0.29	0.01	0.28
Log ARR	-0.001	0.99	0.08	0.40	0.07	0.45	0.08	0.40

Adjusted for age, sex & BMI

	Black (N=131)							
Variables	**24-hour SBP**		**24-hour DBP**		**24-hour HR**		**NA:creatinine ratio**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Log renin	**-0.22**	**0.015**	**-0.22**	**0.015**	**0.20**	**0.022**	0.01	0.30
Log aldosterone	-0.005	0.96	0.04	0.65	0.09	0.30	**0.34**	**<0.001**
Log ARR	0.12	0.17	0.16	0.071	-0.04	0.67	**0.25**	**0.009**
	White (N=119)							
	24-hour SBP		**24-hour DBP**		**24-hour HR**		**NA:creatinine ratio**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Log renin	-0.13	0.16	**-0.19**	**0.038**	0.01	0.39	-0.03	0.76
Log aldosterone	-0.04	0.70	0.04	0.69	0.08	0.93	0.11	0.26
Log ARR	0.05	0.56	0.16	0.086	0.07	0.49	0.11	0.24

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure, HR, heart rate; NA, noradrenaline; ARR, aldosterone-to-renin ratio; BMI, body mass index. Bold text indicate $p<0.05$.

Supplementary Table 3: Pearson and partial correlations of percentage dipping in night-time BP and HR with renin, aldosterone and ARR in black and white low renin groups

Pearson correlations

Variables	**Black (N=131)**					
	% SBP		**% DBP**		**% HR**	
	r	*p*	*r*	*p*	*r*	*p*
Log renin	0.04	0.64	0.11	0.22	0.02	0.23
Log aldosterone	0.13	0.15	0.16	0.075	**-0.27**	**0.002**
Log ARR	0.08	0.34	0.07	0.42	**-0.24**	**0.006**
	White (N=119)					
	% SBP		**% DBP**		**% HR**	
	r	*p*	*r*	*p*	*r*	*p*
Log renin	-0.14	0.12	-0.0003	0.10	0.12	0.19
Log aldosterone	-0.05	0.58	-0.05	0.57	-0.05	0.55
Log ARR	0.05	0.57	-0.04	0.63	-0.13	0.16

Adjusted for age, sex & BMI

Variables	**Black (N=131)**					
	% SBP		**% DBP**		**% HR**	
	r	*p*	*r*	*p*	*r*	*p*
Log renin	0.03	0.70	0.08	0.35	-0.02	0.86
Log aldosterone	0.15	0.93	**0.20**	**0.027**	**-0.30**	**0.001**
Log ARR	0.10	0.23	0.11	0.21	**-0.22**	**0.015**
	White (N=119)					
	% SBP		**% DBP**		**% HR**	
	r	*p*	*r*	*p*	*r*	*p*
Log renin	-0.14	0.14	-0.04	0.63	-0.05	0.48
Log aldosterone	-0.04	0.67	-0.04	0.63	-0.05	0.56
Log ARR	0.06	0.55	-0.01	0.90	-0.09	0.33

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure, HR, heart rate; ARR, aldosterone-to-renin ratio; BMI, body mass index. Bold text indicates $p<0.05$.

CHAPTER 5

The low renin phenotype in Africans: Implications for all-cause and cardiovascular mortality

SUMMARY OF INSTRUCTIONS FOR AUTHORS

Journal title: *Journal of Hypertension*
(http://journals.lww.com/jhypertension/pages/aboutthejournal.aspx)

Scope: Most important and highly innovative papers from the current research

Essential title page information

Title: Full title of the paper, consisting of no more than 20 words; titles should be clear and brief, conveying the message of the paper.

Running head: Consist of not more than 40 characters, including spaces.

Authors: The last (family name) must appear in CAPITAL letters. The affiliations of all the authors; when authors are affiliated to more than one institution, their names should be connected using a,b,c, etc. These letters should follow the surname but precede the address.

Corresponding author: The name and address of the author responsible for correspondence concerning the manuscript, and the name and address of the author to whom requests for reprints should be made.

Conflict of interest: a statement on potential conflicts of interest: if authors have financial interests relevant to the research or constituting a conflict of interest, these must be stated.

Word count: word count: please list full word count (including references, but not tables and legends) as well as number of tables, figures and supplementary files.

Abstract

No more than 250 words. Include Objective(s), Methods, main Results, and the principal Conclusions.

Condensed abstract: should be supplied with the submission, and should consist of no more than 100 words, this abstract should briefly summarise the main findings of your study.

Keywords and abbreviations: 3–10 keywords. A short list of non-standard abbreviation definitions that may not be familiar to readers should be included in a separate mandatory document submitted with your paper.

Article structure

Full papers should include Introduction, Methods (including ethical and statistical information), Results and Discussion (including a conclusion).

Acknowledgements: should be made only to those who have made a substantial contribution to

References

Numbered consecutively in the order in which they first appear in the text. They should be assigned Arabic numerals, which should be given in brackets, e.g. [17]. References should include the names of all authors when seven or fewer; when eight or more, list only the first six names and add et al. References should also include full title and source information. Journal names should be abbreviated as MEDLINE.

Tables

Each table should be assigned an Arabic numeral, e.g. (Table 3) and a brief title. Vertical rules should not be used. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all non-standard abbreviations that are used in each table. Identify statistical measures of variations, such as standard deviation and standard error of the mean.

Figures

Artwork should be saved as TIFF, EPS, or MS Office (DOC, PPT, XLS) files. High resolution PDF files are also acceptable.

Supplementary data

Authors may submit supplementary data files via Editorial Manager to LWW journals that enhance their article's text to be considered for online posting.

The low renin phenotype in Africans:

Implications for all-cause and cardiovascular mortality

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Conflicts of interest: None declared

Abstract

Objective: Cardiovascular complications related to hypertension are among the leading causes of death in black populations, who usually exhibit low renin levels. Since the prognostic significance of renin in cardiovascular-related mortality in blacks is unknown, we determined whether renin and its interactions with blood pressure are predictive of all-cause and cardiovascular mortality in a black population with low vs. high plasma renin.

Methods: We measured active plasma renin in 1502 South Africans (age ≥ 35 years), and assessed mortality over five years. We divided the population into low (N=1002) and high renin (N=500) groups based on the cut-off of the Renin III CISBIO kit (Cedex, France).

Results: In multivariable-adjusted Cox-regression analyses performed in the low renin group, systolic blood pressure (SBP) and the interaction between renin and SBP, but not renin alone, predicted both all-cause [(HR, 1.41; 95% CI, 1.07-1.87; *P*=0.014), (HR, 1.72, 95% CI, 1.05-2.83, *P*= 0.031)] and cardiovascular mortality [(HR, 1.87; 95% CI, 1.16-3.01; *P*=0.010), (HR, 2.40; 95% CI, 1.06-5.46; *P*=0.037)]. In the total group, renin and its interaction with SBP predicted all-cause, but not cardiovascular mortality [(HR, 1.33; 95% CI, 1.07-1.65; *P*=0.011), (HR, 1.30; 95% CI, 1.06-1.60; *P*=0.012)]. No associations existed in the the high renin group.

Conclusions: The interaction of renin with SBP is predictive of all-cause and cardiovascular mortality only in Africans with low renin. The low renin volume-loading phenotype is eminent in Africans and predisposes them to increased risk for cardiovascular mortality.

Key words: renin; black; hypertension; cardiovascular mortality

Introduction

Renin is the rate-limiting step of the renin-angiotensin-aldosterone system (RAAS) which is central to the regulation of blood pressure, water and electrolyte balance [1]. Excessive activation of the RAAS is linked to hypertension development and cardiac abnormalities associated with cardiovascular and renal diseases [2, 3]. Hypertension is the most common risk factor for adverse cardiovascular outcomes in populations of African origin [4, 5]. Furthermore, low renin states characterised by a salt-sensitive and volume-loading hypertension phenotype are common in black populations as compared to other ethnicities [6-8]. In addition, an increase in total peripheral resistance in response to mental or physical stress is also common in blacks including those with low renin and angiotensin II [9, 10]. Therefore, antihypertensive medication not targeting the RAAS, namely diuretics and calcium channel blockers (CCBs) are commonly used as first line treatment in black populations [11]. On the other hand, renin-angiotensin dependent hypertension is caused by inappropriately high rates of renin secretion irrespective of the sodium-volume content and is common in individuals from European descent [12, 13].

High plasma renin was associated with all-cause, but not cardiovascular mortality in the Framingham cohort [14]. Several other studies have indicated that renin is associated with cardiovascular events and mortality in patients with cardiovascular disease and those on hypertension treatment [15-17]. Possible mechanisms include altered renin synthesis and dysregulated tissue RAAS associated with aging, (pro)renin receptor activation and maintained activation of the sympathetic nervous system, which may result in vascular damage [15, 17, 18].

Currently, South Africa has a very high prevalence (78%) of hypertension in older adults and a prevalence of 41%-48% was previously reported in black South Africans over a broad range of ages, including adolescents [19-21]. It is therefore no surprise that cerebrovascular accidents and heart disease form part of the leading causes of death [22]. We previously found that plasma renin associates adversely with cardiovascular haemodynamics and end-organ damage, particularly in blacks with low renin levels and increased blood pressure [23-25]. However, it is

not known how the low renin phenotype relates to all-cause and cardiovascular mortality in this population. We followed a South African cohort over five years to investigate the predictive value of renin and its interactions with blood pressure on all-cause and cardiovascular mortality.

Methods

Study design and population

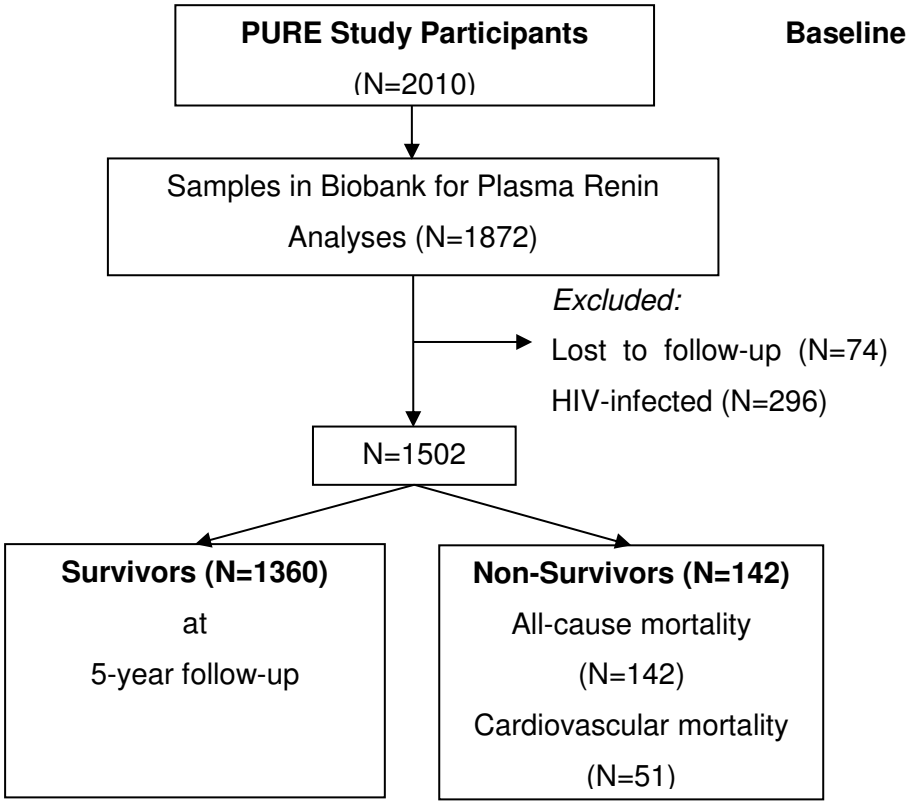

Figure 1: Participant Flow Chart

This study forms part of the multi-country Prospective Urban and Rural Epidemiology (PURE) study. The PURE study is a longitudinal study and was initiated to keep track of the development of chronic diseases of lifestyle in low-, middle-, and high-income countries in both urban and rural dwelling participants over a period of 12 years [26]. The baseline data collection of the South African leg of the PURE study in the North West province was conducted in 2005 and the first follow-up collection of data in 2010. The study population originally consisted of 2010 African

volunteers (1004 urban and 1006 rural) from a sample of 6000 randomly selected households. Participants were selected in a three stage sampling process: purposively selecting 4 communities, then, randomly selecting 1 500 households within each community, afterwards individuals within the households. Four different resident areas were identified for participation. A household census regarding the number of people per household, their ages and health profile was done to determine each member's eligibility for participation within the study. If a person declined participation or was not home, the next house was taken and a non-complier questionnaire was completed. The following inclusion criteria applied: migration stability, men and women aged ≥35 years, not intoxicated or suffering from cognitive disorders and women who were not pregnant. For this sub-study, after removing those lost to follow-up (n=74) and participants infected with human immunodeficiency virus (HIV) (n=296), a total of 1502 participants were included (Figure 1). We used the age-specific reference values from the Renin III CISBIO kit (20-40 years, mean 8.11 pg/ml; 40-60 years, mean 6.18 pg/ml) to stratify the participants into low (n=1002) and high renin (n=500) groups (Renin III Generation, CIS Biointernational, Cedex, France).

Participants were given full information regarding the objectives and procedures of the study prior to participation. The information was conveyed in the participant's home language by trained African field workers. All participants signed an informed consent form. In the case where a participant was illiterate, a thumb print was taken in the presence of a witness. The study complied with all applicable requirements of the international regulations, in particular the Helsinki declaration of 1975 (as revised in 2004) for investigation of human participants. The Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (Potchefstroom Campus) approved this study (04M10 and NWU-0006-10-A1).

Questionnaires

Trained African field workers conducted the interviews by making use of structured demographic, socio-economic, lifestyle and physical activity questionnaires that were developed and standardised for the international PURE study [26].

Anthropometric measurements

Weight, height and waist circumference of the participants were measured using calibrated instruments by accredited anthropometrists according to standardised methods [27] (Precision Health Scale, A & D Company, Japan; Leicester Height Measure, Seca, Birmingham, UK). Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

Blood pressure

After a 10 minute rest period, brachial blood pressure measurements were performed in duplicate (5 minutes apart) on the right upper arm, while the participants were seated upright with the right arm supported at heart level. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured with the validated OMRON HEM-757 (Omron Healthcare, Kyoto, Japan). Appropriately sized cuffs were used for obese participants. Hypertension was defined as clinic blood pressure of ≥140 and/or 90 mmHg or the use of antihypertensive drugs. The sodium-volume component was calculated according to Laragh's volume equation (V=BP/R, V, volume; BP, systolic blood pressure; R, renin) [28].

Biochemical analysis

Participants were requested to fast overnight by not eating or drinking anything except water for approximately 8-10 hours prior to sample collection in the mornings. A registered nurse obtained a blood sample by means of a sterile winged infusion set from the antebrachial vein. Samples were prepared according to appropriate methods and stored at -80°C in the laboratory. In the rural areas, samples were rapidly frozen and stored at -18°C (no longer than five days) until it could be transported to the laboratory facility where it was then stored at -80°C until analysis.

We used two sequential multiple analysers (Cobas Integra 400 plus Roche, Basel, Switzerland & Konelab 20i; Thermo Scientific, Vantaa, Finland) to analyse fasting serum samples for total cholesterol, glucose, creatinine, sodium, potassium, C-reactive protein (CRP) and gamma glutamyl-transferase (GGT). Urinary sodium and potassium were determined from overnight morning spot urine samples. Glycosylated haemoglobin (HbA1c) was determined by using ion-exchange high-performance liquid chromatography (D-10 Haemoglobin testing system from Bio-Rad laboratories, Hercules, CA). Diabetes was defined as HbA1c>6.5%. Creatinine clearance (CrCl) was estimated by using the Cockcroft-Gault formula [29]. The Elecsys 2010 analyser (Roche, Basel, Switzerland) was used to analyse interleukin-6 (IL-6). Active plasma renin was analysed in duplicate using a high sensitivity radio-immunometric assay and cross-reaction with prorenin was 0.4% (Renin III Generation, CIS Biointernational, Cedex, France). The source of reagents was mouse anti-human-active renin monoclonal antibody (IBL Lab, 38T501, USA). The intra-assay coefficient of variation was 6.34%, whereas the inter-assay coefficient was 4.52%.

The South African National Department of Health protocol was followed to perform HIV testing. The HIV status of the participant was determined using the First Response rapid test card (PMC Medical, India) and if the first test was positive, confirmation was done with the Pareeshak card test (BHAT Bio-tech, India).

Assessment of outcome

Trained field workers performed three monthly follow-up visits to the participant's homes under supervision of the senior researcher to verify the status of the participants. The cause of death was obtained from family members by means of official documents (death certificates) and verbal autopsy, and coded by a physician according to the International Classification of Diseases codes (10th revision) for the underlying causes. All-cause mortality included mortality due to any cause, whereas cardiovascular mortality included all fatal cardiac and stroke events and death noted as "due to hypertension". Cardiac-related illnesses that caused death included heart failure,

myocardial infarction, congestive heart failure, or any other cardiac-related reason. Death due to stroke included any stroke or cerebrovascular incident.

Statistical analyses

We used Statistica Version 13.0 for all statistical analyses (Statsoft Inc., Tulsa, OK). We tested for interactions of blood pressure (SBP, DBP, pulse pressure (PP)) and mean arterial pressure (MAP) on the relationship between renin and mortality (all-cause and cardiovascular). We found an interaction with SBP, PP and MAP for the relationship between renin and cardiovascular mortality (all $p \leq 0.038$) (Supplementary Table 1). As a result of the interaction with SBP and because elevated blood pressure suppresses renin, we made use of the interaction term between renin and SBP (renin*SBP) as one of our main independent variables in Cox proportional hazard models. The skewed distribution of renin, glucose, GGT, CRP, IL-6, creatinine clearance, sodium and potassium were normalised by logarithmic transformation. The central tendency and spread of these variables were represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions of high and low renin groups were compared by using two-sided independent t-tests and Chi-square tests. In all instances, $P < 0.05$ was regarded as being statistically significant. For survival curves, we used Kaplan Meier survival function estimates and the log-rank test. We used Cox proportional hazard regression to calculate standardised relative hazard ratios in the total group, and separately in the low and high renin groups to examine the prognostic significance of renin and its interaction with SBP. After considering and testing several variables for inclusion in the Cox regression model, we included the following covariates based on their univariate associations with renin and the endpoints: age, sex, locality (urban vs. rural), BMI, antihypertensive medication, diabetes status, glucose, total cholesterol, GGT, tobacco use, physical activity index, IL-6 and CrCl.

Results

In Table 1 we compare baseline characteristics across renin quartiles (quartiles 1-4: <2.22, 2.22-4.15; 4.16-8.26; >8.26 pg/ml). As the mean renin values in the quartiles increased from 1.53 pg/ml

(quartile 1) to 15.9 pg/ml (quartile 4), we found the proportion of men to increase and obesity measures to decrease (all *P-trend* <0.001). Sodium-volume component, systolic and diastolic blood pressure decreased with increasing renin (*P -trend* <0.001), while heart rate was the highest in the fourth quartile (*P -trend* <0.001). Markers of inflammation, GGT, all-cause and cardiovascular mortality increased with rising renin (all *P -trend* ≤ 0.049). We also compared the characteristics of the low (66% of the study population) and high renin groups (Table 2) and confirmed the findings from Table 1.

Table 1: Comparison of baseline characteristics across quartiles of renin

	Renin Q1 (N=373) <2.22 pg/ml	Renin Q2 (N=377) 2.22-4.15 pg/ml	Renin Q3 (N=377) 4.16-8.26 pg/ml	Renin Q4 (N=375) >8.26 pg/ml	*P* trend
Socio-demographics					
Age (years)	**51.5 ± 10.7**	**52.3 ± 10.3**	**50.2 ± 10.4**	**50.4 ± 10.0**	**0.018**
Gender, women, n (%)	**264 (70.8)**	**243 (64.5)**	**215 (57.0)**	**212 (56.5)**	**<0.001‡**
Locality, rural n (%)	180 (48.3)	189 (50.1)	192 (50.9)	173 (46.1)	0.56
Employment, n (%)	**29 (17.3)**	**45 (24.6)**	**43 (22.6)**	**24 (12.8)**	**0.018**
Educated, n (%)	221 (61.9)	229 (62.9)	233 (63.5)	224 (60.9)	0.91
Anthropometric measurements					
Body mass index (kg/m^2)	**26.4 ± 7.22**	**25.8 ± 7.05**	**23.9 ± 6.95**	**23.6 ± 6.92**	**<0.001‡**
Waist circumference (cm)	**82.5 ± 13.6**	**81.5 ± 12.7**	**79.0 ± 12.9**	**78.2 ± 12.9**	**<0.001‡**
Cardiovascular variables					
Systolic blood pressure (mmHg)	**141 ± 23.1**	**138 ± 26.6**	**133 ± 23.3**	**129 ± 21.7**	**<0.001‡**
Diastolic blood pressure (mmHg)	**91.1 ± 18.3**	**89.6 ± 15.0**	**86.9 ± 14.4**	**85.7 ± 13.3**	**<0.001‡**
Heart rate (bpm)	**69.5 ± 13.2**	**69.7 ± 14.3**	**75.8 ± 15.5**	**79.4 ± 18.0**	**<0.001‡**
Antihypertensive medication					
All, n (%)	72 (19.3)	54 (14.3)	66 (17.5)	77 (20.5)	0.13
Combinations, n (%)	8 (2.14)	7 (1.86)	9 (2.39)	12 (3.20)	0.66
ACE inhibitors, n (%)	17 (4.56)	14 (3.71)	15 (3.98)	24 (6.40)	0.29
Diuretics, n (%)	15 (4.02)	18 (4.77)	22 (5.84)	22 (5.87)	0.61
Beta blockers, n (%)	**12 (3.22)**	**4 (1.06)**	**5 (1.33)**	**3 (0.80)**	**0.035***
CCBs, n (%)	1 (0.27)	4 (1.06)	1 (0.27)	6 (1.60)	0.11
Non-Specified, n (%)	36 (9.65)	24 (6.37)	30 (7.96)	35 (9.33)	0.34
Biochemical variables					
Renin (pg/ml)	**1.53 (1.07; 2.13)**	**3.08 (2.31; 4.05)**	**5.81 (4.23; 7.95)**	**15.9 (8.71; 49.8)**	**<0.001‡**
*Sodium-volume component	**95.3 ± 28.8**	**45.7 ± 12.7**	**23.4 ± 6.40**	**9.25 ± 4.38**	**<0.001‡**
Fasting glucose (mmol/l)	4.80 (3.50; 6.20)	4.86 (3.50; 7.10)	4.85 (3.50; 6.10)	4.97 (3.50; 7.30)	0.29
Total cholesterol (mmol/l)	5.04 ± 1.34	5.22 ± 1.37	5.16 ± 1.32	5.10 ± 1.42	0.29
C-reactive protein (mg/L)	**2.86 (0.23; 24.1)**	**3.16 (0.27; 29.8)**	**2.54 (0.26; 31.4)**	**4.25 (0.25; 51.3)**	**<0.001‡**
Interleukin-6 (pg/ml)	**2.44 (0.75; 11.2)**	**2.57 (0.75; 12.8)**	**2.69 (0.75; 16.5)**	**3.57 (0.75; 27.3)**	**<0.001‡**
Gamma-glutamyltransferase (U/L)	**45.9 (19.6; 174)**	**49.8 (19.0; 230)**	**65.0 (19.9; 466)**	**69.0 (19.0; 459)**	**<0.001***
Creatinine clearance (ml/min)	97.1 (55.7; 173)	96.2 (53.6; 169)	95.3 (50.5; 164)	92.3 (55.7; 161)	0.32
Na+ /K+	**7.35 (3.94; 10.6)**	**7.76 (4.73; 10.5)**	**7.24 (2.74; 10.5)**	**7.41 (4.29; 10.6)**	**0.023**
Lifestyle factors and co-morbidities					
Self-reported smoking, n (%)	**176 (47.2)**	**186 (49.3)**	**209 (55.4)**	**207 (55.2)**	**0.049***
Self-reported alcohol use, n (%)	**116 (31.1)**	**128 (34.0)**	**166 (44.0)**	**179 (47.7)**	**<0.001‡**
Physical activity index	7.46 ± 2.00	7.23 ± 1.74	7.34 ± 2.13	7.10 ± 1.66	0.080
Diabetes, n (%)	17 (4.56)	19 (5.04)	14 (3.71)	28 (7.47)	0.11
Hypertension, n (%)	**238 (63.8)**	**208 (51.2)**	**191 (50.7)**	**177 (57.2)**	**<0.001‡**
Mortality					
All-cause, n (%)	**26 (6.97)**	**28 (7.43)**	**44 (11.7)**	**44 (11.7)**	**0.030***
Cardiovascular, n (%)	**7 (1.89)**	**11 (2.93)**	**21 (5.59)**	**12 (3.21)**	**0.040**
Cardiac, n (%)	3 (0.81)	4 (1.06)	6 (1.60)	8 (2.14)	0.42
Stroke, n (%)	2 (0.54)	6 (1.60)	8 (2.13)	3 (0.80)	0.19

*Sodium-volume component calculated according to Laragh's volume equation (V=BP/R). Values are arithmetic mean ± standard deviation; geometric mean (5th and 95th percentile interval) for logarithmically transformed variables. Abbreviations: ACE, angiotensin-converting enzyme; CCBs, calcium channel blockers. Bold text indicate p <0.05 across renin quartiles.‡p<0.001, †p<0.01, *p<0.05 difference between Q1 and Q4.

Table 2: Comparison of baseline characteristics between the low and high renin groups

	Low renin (N=1002)	High renin (N=500)	*P*
Socio-demographics			
Age (years)	51.2 ± 10.7	50.8 ± 9.71	0.49
Gender, women, n (%)	**650 (64.9)**	**284 (56.8)**	**0.002**
Locality, rural n (%)	505 (50.4)	263 (52.6)	0.42
Employment, n (%)	99 (20.8)	42 (16.7)	0.19
Educated, n (%)	605 (62.4)	302 (61.5)	0.75
Anthropometric measurements			
Body mass index (kg/m^2)	**25.7 ± 7.10**	**23.5 ± 6.97**	**<0.001**
Waist circumference (cm)	**81.4 ± 13.1**	**78.1 ± 12.9**	**<0.001**
Cardiovascular variables			
Systolic blood pressure (mmHg)	**138 ± 24.8**	**130 ± 22.1**	**<0.001**
Diastolic blood pressure (mmHg)	**89.4 ± 14.6**	**86.2 ± 13.5**	**<0.001**
Heart rate (bpm)	**71.1 ± 14.2**	**78.6 ± 17.8**	**<0.001**
Antihypertensive medication			
All, n (%)	**165 (16.5)**	**104 (20.8)**	**0.039**
Combinations, n (%)	**18 (1.80)**	**18 (3.60)**	**0.031**
ACE inhibitors, n (%)	**39 (3.89)**	**31 (6.20)**	**0.046**
Diuretics, n (%)	45 (4.49)	32 (6.40)	0.11
Beta blockers, n (%)	18 (1.80)	6 (1.20)	0.39
CCBs, n (%)	5 (0.50)	7 (1.40)	0.065
Non-Specified, n (%)	80 (7.98)	45 (9.00)	0.50
Biochemical variables			
Renin (pg/ml)	**2.71 (1.11; 5.94)**	**13.0 (6.54; 44.7)**	**<0.001**
*Sodium-volume component	**49.9 (20.3; 130)**	**9.90 (2.73; 21.9)**	**<0.001**
Fasting glucose (mmol/l)	4.85 (3.50; 6.70)	4.90 (3.50; 7.20)	0.41
Total cholesterol (mmol/l)	5.13 ± 1.35	5.15 ± 1.39	0.80
C-reactive protein (mg/L)	**2.90 (0.25; 28.7)**	**3.66 (0.25; 49.6)**	**0.006**
Interleukin-6 (pg/ml)	**2.53 (0.75; 13.1)**	**3.38 (0.75; 22.1)**	**<0.001**
Gamma-glutamyltransferase (U/L)	**50.3 (19.0; 235)**	**71.8 (19.1; 471)**	**<0.001**
Creatinine clearance (ml/min)	**97.1 (55.3; 169)**	**91.4 (53.4; 162)**	**0.005**
Urinary Na^+/K^+	7.48 (4.08; 10.6)	7.45 (4.22; 10.5)	0.79
Lifestyle factors and comorbidities			
Self-reported smoking, n (%)	**498 (49.7)**	**280 (56.0)**	**0.021**
Self-reported alcohol use, n (%)	**347 (34.6)**	**242 (48.4)**	**<0.001**
Physical activity index	**7.36 ± 1.96**	**7.13 ± 1.76**	**0.030**
Diabetes, n (%)	45 (4.49)	33 (6.60)	0.083
Hypertension, n (%)	**568 (56.7)**	**246 (49.2)**	**0.006**
Mortality			
All-cause, n (%)	**80 (7.98)**	**62 (12.4)**	**0.006**
Cardiovascular, n (%)	28 (2.81)	23 (4.61)	0.070
Cardiac, n (%)	**9 (0.90)**	**12 (2.40)**	**0.020**
Stroke, n (%)	11 (1.10)	8 (1.60)	0.42

*Sodium-volume component calculated according to Laragh's volume equation (V=BP/R). Values are arithmetic mean ± standard deviation; geometric mean (5th and 95th percentile interval) for logarithmically transformed variables. Abbreviations: ACE, angiotensin-converting enzyme; CCBs, calcium channel blockers. Bold text indicate p<0.05.

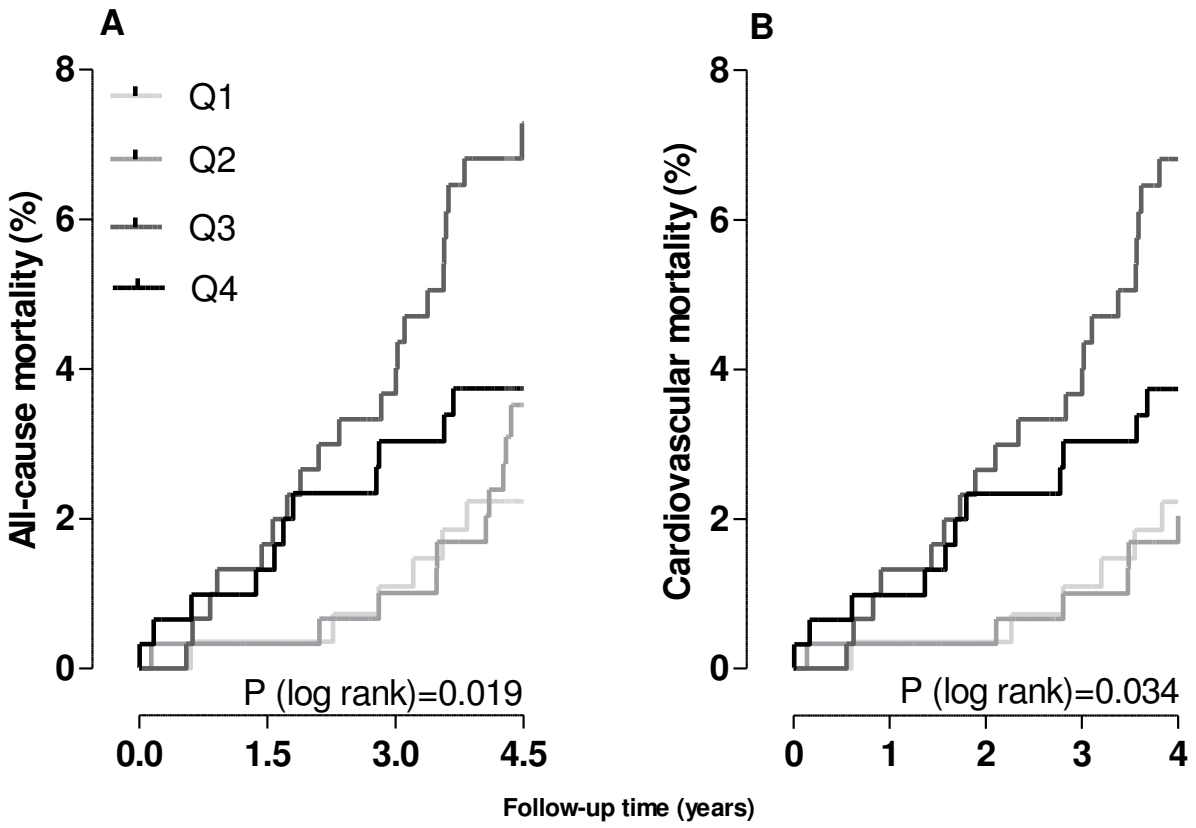

Figure 2: Kaplan-Meier plots showing (A) all-cause and (B) cardiovascular mortality across quartiles of plasma renin

In analyses of Kaplan-Meier estimates (Figure 2) the log-rank test was significant for both all-cause and cardiovascular mortality across quartiles of renin (both $P \leq 0.034$), with the highest all-cause and cardiovascular mortality indicated for quartile 3 (Figure 2).

Cox regression analyses

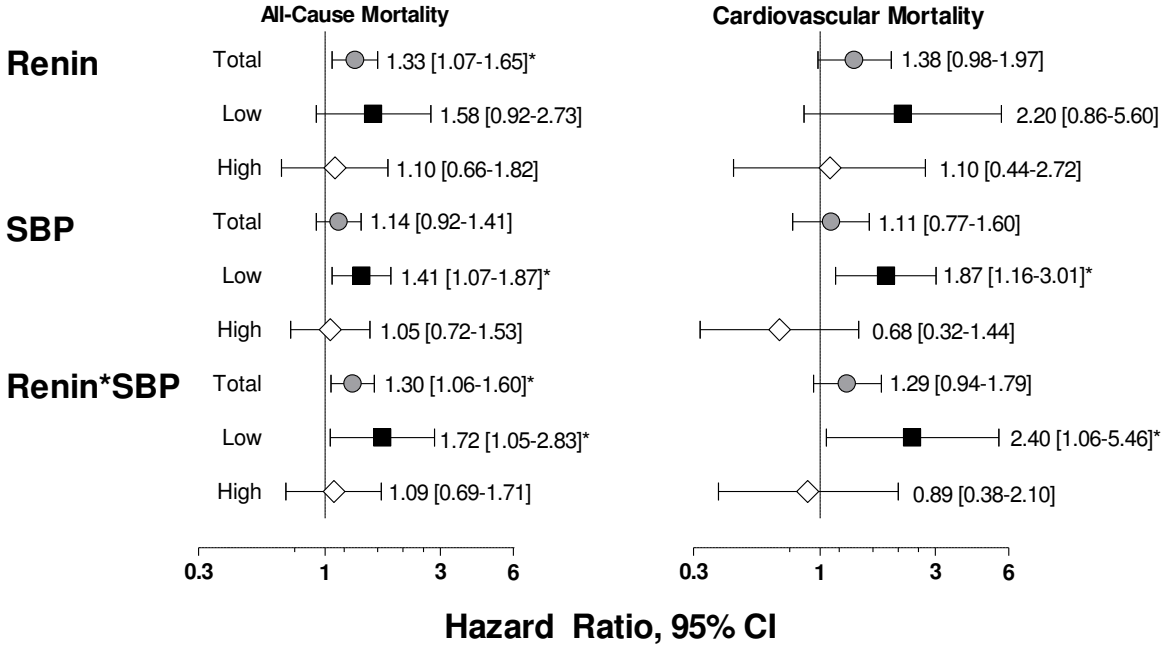

Figure 3: Multivariate Cox proportional hazard ratios of renin, SPB*renin and SBP with all-cause and cardiovascular mortality. ***p<0.001, **p<0.01, *p<0.5

We performed multivariate-adjusted Cox regression in the total group and separately in the low and high renin groups (Figure 3; Supplementary Tables 2-4), with either renin, SBP or renin*SBP as main independent variable. Only in the total group was renin positively associated with all-cause mortality (HR, 1.33; 95% CI, 1.07-1.65; *P*=0.011). In the total group, the standardised HR for renin*SBP were predictive of all-cause mortality (HR, 1.30; 95% CI, 1.06-1.60; *P*=0.012). Age, GGT and IL-6 were also associated with increased all-cause and cardiovascular mortality (Supplementary Table 2), while SBP was not associated with neither all-cause nor cardiovascular mortality. In the low renin group, SBP predicted both all-cause (HR, 1.41; 95% CI, 1.07-1.87; *P*=0.014) and cardiovascular mortality (HR, 1.87; 95% CI, 1.16-3.01; *P*=0.010). Furthermore, renin*SBP was predictive of all-cause (HR, 1.72, 95% CI, 1.05-2.83, *P*= 0.031) and cardiovascular mortality (HR, 2.40; 95% CI, 1.06-5.46; *P*=0.037), while there were no significant results for renin

alone. In the high renin group, neither renin, SBP nor the renin*SBP predicted all-cause or cardiovascular mortality.

Sensitivity analyses

In order to assess if the positive results observed in the low renin group were not due to the use of antihypertensive medication, we repeated the Cox regression analyses after removing all participants (N=296) using antihypertensive drugs. The associations between SBP and both all-cause (HR, 1.41; 95% CI, 1.05-1.89; *P*=0.022) and cardiovascular mortality (HR, 1.87; 95% CI, 1.05-3.32; *P*=0.032) as well as between renin*SBP and all-cause mortality (HR, 1.80, 95% CI, 1.04-3.11, *P*= 0.034) remained robust. The association between renin*SBP and cardiovascular mortality was borderline-significant (HR, 2.63; 95% CI, 0.91-7.63; P=0.075).

Discussion

We investigated whether renin and its interactions with blood pressure are predictive of all-cause and cardiovascular mortality over 5-years in a cohort of African ancestry, a population group prone to developing low-renin hypertension [6]. We found that only in Africans with low plasma renin, SBP, but particularly the interaction between SBP and renin, predicted both all-cause and cardiovascular mortality. In neither the total nor the high renin group did SBP or the SBP*renin interaction predict cardiovascular mortality.

We found it of interest that in the low renin Africans renin *per se* does not predict mortality, but SBP and its interaction with renin are associated with all-cause and cardiovascular mortality. Renin is secreted from the juxtaglomerular apparatus in response to volume depletion, activating a cascade of events leading to salt and water retention, volume expansion and increased blood pressure [30]; – which in turn results in renin suppression. Two patterns seems apparent, one of suppressed renin accompanied by high blood pressure and possibly high vascular resistance; and the other a co-existence of high renin with high heart rate, both observed previously and confirmed in the current population [10, 23, 25]. The former is a phenotype commonly reported in

populations of African ancestry, 80.9% in the SABPA study and 66% in the present study [23, 31]. The present study is the first to show that this phenotype is related to cardiovascular risk. The increased blood pressure is mainly attributed to sodium-volume overload due to abnormalities in renal sodium handling, salt-sensitivity or alterations in mineralocorticoid physiology [7, 32]. This is supported in the present study where the sodium-volume component was higher in the low renin group as compared to the high renin group. It was shown that blacks reabsorb more sodium in the proximal tubule and less in the distal renal tubules as compared to whites [33]. The distal tubules are highly regulated and sensitive to aldosterone, which promotes reabsorption of sodium via the epithelial sodium channel (ENaC) and Na^+-K^+-ATPase [34]. A mutation of the β-subunit in the ENaC was associated with the low-renin, low-aldosterone hypertension in Africans [7]. It was also recently shown that blacks have a higher sensitivity of blood pressure to aldosterone compared to whites [35], which may have also contributed to the prognostic value of the renin*SBP in our study.

Salt-sensitivity is an integral part of hypertension in Africans [36, 37]. It is well-known that salt-sensitivity relates to end-organ damage, such as left ventricular hypertrophy and urinary albumin excretion [38, 39]. In black South Africans with low renin levels, urinary albumin excretion was negatively associated with plasma renin, suggesting that the low-renin phenotype may predispose to kidney damage, which can also be a reflection of damage in the vascular tree [25]. Furthermore, microalbuminuria predicted cardiovascular mortality in another sub-study of the current population [40]. It is possible that pre-existing vascular damage and left ventricular dysfunction due to pressure and volume-overload may be mediating the renin-blood pressure-related mortality in the low renin group [15, 41].

Conflicting results have been reported on the relationship between plasma renin and mortality. Elevated plasma renin was associated with long-term cardiovascular mortality in patients referred to coronary angiography with ongoing hypertension medication use [15]. On the other hand,

Parikh *et al.* showed that renin predicts all-cause, but not cardiovascular mortality in a population consisting of both normotensives and hypertensives [14].This is consistent with our findings in the total group. We showed higher frequencies of all-cause and cardiovascular mortality in the upper quartiles of renin, and renin significantly predicted all-cause mortality in the total group. Our population had a high prevalence (54%) of hypertension, but also particularly elevated levels of GGT and IL-6, which have been shown to predict all-cause and cardiovascular mortality in other sub-studies of our population [42, 43]. Regardless of the higher rates of risk factors such as inflammation and mortality, our high renin group had significantly lower blood pressure than the low renin group (mean SBP 130 vs. 138 mmHg), despite similar ages. This may explain the absence of any association with blood pressure or its interaction with renin. However, in this high renin group, IL-6 predicted all-cause mortality (Supplementary Table 4).

There are specific limitations to our study. The number of deceased participants was not enough to do further subgroup, quartile or end point specific (cardiac, stroke) analyses. Other components of the RAAS, such as angiotensin II and aldosterone, were not available for analysis. We did not assess salt-sensitivity. The use of angiotensin-converting enzyme inhibitors, CCBs, diuretics and beta blockers may have influenced the results, but the use of hypertension treatment (18%, results not shown) did not predict mortality in any of the Cox regression analyses.

In conclusion, the interaction of renin with SBP and not renin *per se* is predictive of all-cause and cardiovascular mortality only in Africans with low renin levels, whereas in the total group renin and the SBP*renin interaction predicted only all-cause mortality. Volume-overload, potentially due to excessive salt intake, inadequate diuretic therapy and renal damage, is indicated as one of the causes of resistant hypertension [44]. This study lends further support to the use of diuretics and CCBs for lowering high blood pressures observed in the low renin phenotype, thereby reducing the vulnerability to heart failures, strokes and hypertensive kidney disease.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Interaction of renin with blood pressure on mortality

	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Log renin*SBP	0.70 (0.31-1.60)	0.40	**0.25 (0.08-0.78)**	**0.017**
Log renin*DBP	0.74 (0.27-1.99)	0.55	0.34 (0.07-1.51)	0.16
Log renin*MAP	0.67 (0.25-1.79)	0.43	**0.22 (0.05-0.92)**	**0.038**
Log renin*PP	0.84 (0.54-1.30)	0.43	**0.50 (0.26-0.93)**	**0.029**

HR, hazard ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure

Supplementary Table 2: Cox proportional hazard ratios with all-cause and cardiovascular mortality in the total group (N=1502)

	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Model 1: Renin, main independent variable				
Age	**1.31 (1.02-1.68)**	**0.032**	**1.50 (1.00-2.25)**	**0.048**
Sex	1.22 (0.72-2.050	0.46	1.15 (0.47-2.85)	0.75
Locality	0.92 (0.54-1.57)	0.77	1.40 (0.60-3.26)	0.44
Body mass index	0.88 (0.62-1.25)	0.47	1.48 (0.87-2.53)	0.15
Antihypertensive drugs	1.15 (0.67-1.97)	0.62	1.33 (0.57-3.07)	0.51
Log gamma-glutamyl transferase	**1.38 (1.14-1.67)**	**0.001**	**1.53 (1.10-2.12)**	**0.012**
Tobacco use	1.11 (0.75-1.65)	0.60	1.19 (0.60-2.34)	0.62
Physical activity index	**0.68 (0.50-0.92)**	**0.012**	**0.52 (0.32-0.84)**	**0.008**
Log glucose	0.79 (0.61-1.01)	0.063	0.89 (0.61-1.28)	0.52
Total cholesterol	**0.66 (0.51-0.85)**	**0.001**	**0.61 (0.39-0.95)**	**0.028**
Log interleukin-6	**1.34 (1.10-1.63)**	**0.004**	**1.53 (1.07-2.18)**	**0.020**
Log creatinine clearance	0.87 (0.65-1.17)	0.36	**0.60 (0.36-0.99)**	**0.047**
Diabetes status	0.89 (0.30-2.63)	0.83	1.28 (0.31-5.22)	0.73
SBP	1.22 (0.98-1.51)	0.079	1.20 (0.83-1.74)	0.34
Log renin	**1.33 (1.07-1.65)**	**0.011**	1.38 (0.97-1.97)	0.076
Model 2: SBP, main independent variable				
Age	1.27 (0.99-1.64)	0.060	1.44 (0.95-2.17)	0.084
Sex	1.27 (0.75-2.14)	0.38	1.23 (0.50-3.06)	0.65
Locality	0.93 (0.55-1.57)	0.78	1.38 (0.59-3.24)	0.45
Body mass index	0.87 (0.61-1.24)	0.48	1.44 (0.84-2.49)	0.19
Antihypertensive drugs	1.21 (0.71-2.07)	0.24	1.42 (0.62-3.27)	0.41
Log gamma-glutamyl transferase	**1.42 (1.18-1.71)**	**0.0002**	**1.57 (1.13-2.17)**	**0.007**
Tobacco use	1.08 (0.73-1.60)	0.69	1.12 (0.58-2.18)	0.73
Physical activity index	**0.70 (0.52-0.93)**	**0.015**	**0.55 (0.34-0.87)**	**0.011**
Log glucose	0.80 (0.62-1.05)	0.10	0.89 (0.61-1.30)	0.54
Total cholesterol	**0.68 (0.53-0.88)**	**0.003**	0.65 (0.42-1.01)	0.053
Log interleukin-6	**1.39 (1.13-1.70)**	**0.001**	**1.55 (1.07-2.23)**	**0.019**
Log creatinine clearance	0.86 (0.63-1.16)	0.33	0.60 (0.35-1.01)	0.054
Diabetes status	0.97 (0.33-2.85)	0.95	1.39 (0.34-5.62)	0.65
SBP	1.14 (0.92-1.41	0.24	1.11 (0.77-1.60)	0.59
Model 3: Renin*SBP, main independent variable				
Age	**1.35 (1.06-1.71)**	**0.014**	**1.52 (1.03-2.26)**	**0.033**
Sex	1.23 (0.73-2.07)	0.44	1.18 (0.48-2.91)	0.72
Locality	0.88 (0.52-1.48)	0.62	1.35 (0.59-3.11)	0.48
Body mass index	0.89 (0.63-1.27)	0.52	1.49 (0.87-2.54)	0.14
Antihypertensive drugs	1.19 (0.70-2.04)	0.52	1.37 (0.59-3.17)	0.47
Log gamma-glutamyl transferase	**1.38 (1.14-1.67)**	**0.001**	**1.52 (1.09-2.12)**	**0.013**
Tobacco use	1.10 (0.74-1.63)	0.64	1.15 (0.58-2.25)	0.69
Physical activity index	**0.69 (0.51-0.93)**	**0.013**	**0.53 (0.33-0.85)**	**0.009**
Log glucose	0.80 (0.62-1.04)	0.096	0.87 (0.59-1.28)	0.49
Total cholesterol	**0.65 (0.51-0.85)**	**0.001**	**0.61 (0.39-0.96)**	**0.032**
Log interleukin-6	**1.35 (1.11-1.64)**	**0.003**	**1.54 (1.08-2.19)**	**0.017**
Log creatinine clearance	0.88 (0.65-1.18)	0.38	**0.60 (0.36-0.99)**	**0.048**
Diabetes status	0.87 (0.29-2.59)	0.80	1.28 (0.31-5.21)	0.73
Log renin*SBP	**1.30 (1.06-1.60)**	**0.012**	1.29 (0.94-1.79)	0.12

Supplementary Table 3: Cox proportional hazard ratios with all-cause and cardiovascular mortality in the low renin group (N=1002)

	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Model 1: Renin, main independent variable				
Age	1.28 (0.90-1.83)	0.18	1.17 (0.64-2.13)	0.61
Sex	1.26 (0.60-2.64)	0.54	2.50 (0.65-9.61)	0.18
Locality	0.90 (0.44-1.85)	0.77	1.37 (0.43-4.30)	0.59
Body mass index	0.98 (0.61-1.56)	0.93	**2.38 (1.14-4.96)**	**0.021**
Antihypertensive drugs	0.47 (0.19-1.18)	0.11	0.77 (0.23-2.59)	0.67
Log gamma-glutamyl transferase	**1.50 (1.15-1.95)**	**0.003**	**1.90 (1.25-2.88)**	**0.003**
Tobacco use	0.87 (0.53-1.44)	0.60	0.99 (0.44-2.26)	0.99
Physical activity index	0.81 (0.56-1.18)	0.28	0.71 (0.40-1.25)	0.24
Log glucose	0.82 (0.58-1.17)	0.27	0.70 (0.42-1.16)	0.17
Total cholesterol	**0.69 (0.49-0.97)**	**0.032**	0.61 (0.32-1.13)	0.12
Log interleukin-6	1.35 (0.99-1.83)	0.052	1.49 (0.84-2.64)	0.16
Log creatinine clearance	0.95 (0.63-1.44)	0.82	**0.42 (0.20-0.90)**	**0.026**
Diabetes status	1.10 (0.24-5.04)	0.90	2.65 (0.48-14.6)	0.26
SBP	**1.48 (1.12-1.95)**	**0.006**	**2.04 (1.26-3.29)**	**0.004**
Log renin	1.57 (0.91-2.71)	0.10	2.20 (0.86-5.60)	0.099
Model 2: SBP, main independent variable				
Age	1.25 (0.87-1.78)	0.23	1.13 (0.62-2.06)	0.70
Sex	1.26 (0.60-2.65)	0.55	2.46 (0.63-9.71)	0.20
Locality	0.88 (0.43-1.82)	0.73	1.32 (0.42-4.11)	0.63
Body mass index	0.93 (0.59-1.48)	0.76	**2.13 (1.02-4.44)**	**0.043**
Antihypertensive drugs	0.49 (0.20-1.21)	0.12	0.80 (0.24-2367)	0.72
Log gamma-glutamyl transferase	**1.51 (1.17-1.96)**	**0.002**	**1.90 (1.26-2.88)**	**0.002**
Tobacco use	0.91 (0.55-1.50)	0.70	1.07 (0.47-2.44)	0.88
Physical activity index	0.80 (0.55-1.17)	0.25	0.69 (0.38-1.26)	0.23
Log glucose	0.82 (0.58-1.17	0.28	0.72 (0.44-1.19)	0.20
Total cholesterol	**0.71 (0.50-0.99)**	**0.045**	0.68 (0.37-1.24)	0.21
Log interleukin-6	**1.37 (1.01-1.85)**	**0.041**	1.49 (0.85-2.61)	0.17
Log creatinine clearance	0.99 (0.66-1.51)	0.99	**0.47 (0.22-0.99)**	**0.047**
Diabetes status	1.09 (0.24-4.94)	0.91	2.62 (0.49-14.1)	0.26
SBP	**1.42 (1.07-1.87)**	**0.014**	**1.87 (1.16-3.01**	**0.010**
Model 3: Renin*SBP, main independent variable				
Age	**1.41 (1.00-1.20)**	**0.049**	1.36 (0.76-2.42)	0.30
Sex	1.28 (0.62-2.67)	0.51	2.49 (0.66-9.30)	0.18
Locality	0.81 (0.40-1.65)	0.57	0.99 (0.32-2.20)	0.57
Body mass index	1.00 (0.63-1.59)	0.99	**2.43 (1.17-5.06)**	**0.017**
Antihypertensive drugs	0.53 (0.21-1.34)	0.17	0.92 (0.26-3.21)	0.90
Log gamma-glutamyl transferase	**1.49 (1.14-1.94)**	**0.003**	**1.85 (1.21-2.81)**	**0.004**
Tobacco use	0.86 (0.52-1.42)	0.55	0.94 (0.41-2.16)	0.89
Physical activity index	0.82 (0.57-1.19)	0.30	0.70 (0.40-1.23)	0.21
Log glucose	0.83 (0.58-1.18)	0.30	0.72 (0.44-1.19)	0.20
Total cholesterol	**0.70 (0.50-0.98)**	**0.039**	0.61 (0.33-1.14)	0.12
Log interleukin-6	**1.36 (1.01-1.83)**	**0.042**	1.46 (0.84-2.54)	0.18
Log creatinine clearance	0.97 (0.64-1.49)	0.92	**0.44 (0.20-0.96)**	**0.040**
Diabetes status	1.02 (0.22-4.70)	0.98	2.34 (0.42-12.9)	0.33
Log renin*SBP	**1.72 (1.05-2.83)**	**0.031**	**2.40 (1.06-5.46)**	**0.037**

Supplementary Table 4: Cox proportional hazard ratios with all-cause and cardiovascular mortality in the high group (N=500)

	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Model 1: Renin, main independent variable				
Age	1.29 (0.93-1.81)	0.13	1.30 (0.75-2.29)	0.35
Sex	1.04 (0.49-2.19)	0.93	0.62 (0.16-2.31)	0.47
Locality	1.12 (0.51-2.47)	0.77	1.56 (0.39-6.32)	0.53
Body mass index	0.76 (0.43-1.36)	0.36	1.01 (0.45-2.23)	0.99
Antihypertensive drugs	2.03 (0.98-4.22)	0.057	2.40 (0.63-9.19)	0.20
Log gamma-glutamyl transferase	1.28 (0.96-1.70)	0.096	1.15 (0.65-2.04)	0.63
Tobacco use	1.58 (0.81-3.07)	0.18	1.29 (0.39-4.26)	0.97
Physical activity index	**0.51 (0.31-0.84)**	**0.008**	**0.28 (0.10-0.72)**	**0.010**
Log glucose	0.77 (0.51-1.17)	0.22	1.11 0.62-1.98)	0.73
Total cholesterol	**0.61 (0.41-0.90)**	**0.013**	0.55 (0.27-1.15)	0.11
Log interleukin-6	**1.38 (1.04-1.82)**	**0.026**	1.55 (0.92-2.62)	0.10
Log creatinine clearance	0.73 (0.48-1.12)	0.15	0.63 (0.30-1.32)	0.22
Diabetes status	0.80 (014-4.40)	0.80	0.58 (0.04-7.89)	0.68
SBP	1.06 (0.73-1.55)	0.80	0.68 (0.32-1.47)	0.33
Log renin	1.10 (0.66-1.82)	0.72	1.10 (0.44-2.78)	0.83
Model 2: SBP, main independent variable				
Age	1.29 (0.92-1.80)	0.13	1.30 (0.74-2.27)	0.36
Sex	1.04 (0.50-2.20)	0.91	0.62 (0.16-2.30)	0.47
Locality	1.11 (0.51-2.42)	0.80	1.52 (0.39-5.94)	0.54
Body mass index	0.77 (0.43-1.37)	0.37	1.01 (0.46-2.23)	0.98
Antihypertensive drugs	2.04 (0.98-4.23)	0.056	2.44 (0.64-9.26)	0.19
Log gamma-glutamyl transferase	1.27 (0.95-1.68)	0.10	1.14 (0.65-1.20)	0.65
Tobacco use	1.55 (0.80-2.99)	0.19	1.26 (0.39-4.06)	0.69
Physical activity index	**0.52 (0.32-0.84)**	**0.008**	**0.28 (0.11-0.71)**	**0.007**
Log glucose	0.77 (0.51-1.17)	0.22	1.12 (0.62-1.20)	0.71
Total cholesterol	**0.61 (0.41-0.91)**	**0.015**	0.56 (0.26-1.16)	0.12
Log interleukin-6	**1.39 (1.05-1.83)**	**0.022**	1.56 (0.93-2.62)	0.089
Log creatinine clearance	0.72 (0.47-1.11)	0.14	0.63 (0.30-1.30)	0.21
Diabetes status	0.82 (0.15-4.51)	0.82	0.58 (0.04-7.98)	0.68
SBP	1.05 (0.72-1.53)	0.80	0.68 (0.32-1.44)	0.31
Model 3: Renin*SBP, main independent variable				
Age	1.30 (0.93-1.80)	0.12	1.25 (0.72-2.18)	0.42
Sex	1.04 (0.49-2.19)	0.92	0.58 (0.15-2.20)	0.42
Locality	1.11 (0.52-2.38)	0.79	1.80 (0.47-6.99)	0.39
Body mass index	0.76 (0.43-1.36)	0.36	0.97 (0.43-2.19)	0.93
Antihypertensive drugs	2.04 (0.98-4.23)	0.056	2.25 (0.59-8.56)	0.23
Log gamma-glutamyl transferase	1.28 (0.97-1.68)	0.082	1.07 (0.62-1.84)	0.80
Tobacco use	1.58 (0.81-3.06)	0.18	1.21 (0.36-4.04)	0.76
Physical activity index	**0.52 (0.31-0.84)**	**0.008**	**0.27 (0.11-0.68)**	**0.006**
Log glucose	0.77 (0.52-1.16)	0.21	1.09 (0.58-2.04)	0.79
Total cholesterol	**0.61 (0.41-0.90)**	**0.013**	0.58 (0.27-1.21)	0.14
Log interleukin-6	**1.38 (1.04-1.82)**	**0.024**	1.64 (0.99-2.71)	0.055
Log creatinine clearance	0.73 (0.48-1.11)	0.14	0.62 (0.30-1.30)	0.21
Diabetes status	0.80 (0.15-4.36)	0.79	0.52 (0.03-7.81)	0.64
Log renin*SBP	1.09 (0.69-1.71)	0.70	0.89 (0.38-2.10)	0.79

Condensed abstract

We investigated whether renin and its interactions with blood pressure are predictive of 5-year all-cause and cardiovascular mortality in a cohort of African ancestry, commonly affected by low-renin hypertension. We found that SBP, particularly the interaction between SBP and renin predicted both all-cause (HR, 1.72, *P*=0.031) and cardiovascular mortality (HR, 2.40, *P*=0.037) only in Africans with low plasma renin. In neither the total nor high renin group did SBP or the SBP*renin interaction predict cardiovascular mortality. Our findings suggest that volume-overload that elevates blood pressure, consequently suppressing renin, may increase the cardiovascular risk in this population.

Word count: 96

List of abbreviations:

CCB:	Calcium channel blocker
CrCl:	Creatinine clearance
CRP:	C-reactive protein
ENaC:	Epithelial sodium channel
GGT:	Gamma glutamyl transferase
HbA1c:	Glycated haemoglobin
HR:	Hazard ratio
HR:	Heart rate
IL-6:	Interleukin-6
MAP:	Mean arterial pressure
PP:	Pulse pressure
PURE:	Prospective Urban and Rural Epidemiology
RAAS:	Renin-angiotensin-aldosterone system
SABPA:	Sympathetic activity and Ambulatory Blood Pressure in Africans

CHAPTER 6

Summary of the main findings, conclusions and recommendations

1. INTRODUCTION

This is a summative chapter that presents an interpretation of the main findings of this thesis. A comparison of original hypotheses as detailed in *Chapter 1* is made with the results, as well as with existing literature. Conclusions are then drawn and recommendations for future research on the low renin phenotype in Africans are given.

2. INTERPRETATION OF THE MAIN FINDINGS AND A COMPARISON WITH THE RELEVANT LITERATURE

This study aimed to characterise the cardiovascular profile of Africans with predominantly low renin levels and to investigate if this profile, presented by the low renin phenotype, predisposes the population to increased cardiovascular risk.

Hypothesis 1: Low renin status is more prevalent in black compared to white adults.

The first two manuscripts were from the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, with participants aged between 20 and 65 years. The black individuals had significantly lower plasma renin than whites (4.19±2.62 vs 6.32±3.07 pg/mL; $p<0.001$), also reflected by a higher frequency of low renin cases in blacks (80.9%) compared to their white counterparts (57.8%). This was apparent at rest and when an acute stressor was applied. The stressor used, known as the cold pressor test (CPT), was performed by immersing the participant's right foot up to the ankle in 4°C water for one minute. In addition, 67% of the black adults participating in the Prospective Urban and Rural Epidemiology (PURE) study (age range, 35-94 years) had low renin levels.

It has been shown previously that black populations tend to have low plasma renin activity compared to other ethnic groups, including both normotensives and hypertensives [1-3]. However, a comparison by Rayner *et al*. between black, white and coloured hypertensives in

South Africa showed no significant difference in renin levels [1]. By making use of active plasma renin (and not renin activity), the present study confirmed the predominance of suppressed renin in black populations. After secretion from the juxtaglomerular apparatus, renin cleaves the substrate angiotensinogen to form angiotensin I [4]. Plasma renin activity is expressed as the quantity of angiotensin I formed per unit of time, while active renin measurement refers to the concentration of the cleaved form of renin [5, 6]. Low renin levels are usually a result of high blood pressure and/or volume-overload [6]. In this study, it was found that lower renin levels were accompanied by higher blood pressure in blacks compared to whites (Table 1). The sodium-volume component (calculated according to Laragh's volume equation (V=BP/R) also increased with decreasing renin levels (Table 1). These observations support the low renin volume-loading hypertension phenotype, possibly due to salt-sensitivity and enhanced sodium retention [7].

The first hypothesis is therefore accepted because low renin levels were found to be more common in blacks compared to whites.

Table 1: Renin and blood pressure profiles of the study populations

	Black (N= 153)	**White (N=188)**	***P***
Manuscript 1 (SABPA)			
Age (years)	43.1 ± 7.71	43.9 ± 10.6	0.38
Office SBP (mmHg)	140 ± 17.6	130 ± 13.3	<0.001
Office DBP (mmHg)	81.1 ± 9.83	76.3 ± 7.82	<0.001
Hypertensive n (%)	73 (47.7)	48 (25.5)	<0.001
Renin (pg/ml)	4.19 ± 2.62	6.32 ± 3.07	<0.001
Manuscript 2 (SABPA)			
	Black (N= 162)	**White (N=206)**	***P***
Age (years)	42.6 ± 7.56	44.8 ± 10.5	0.055
24 hour SBP (mmHg)	132 ± 16.8	125 ± 12.9	<0.001
24 hour DBP (mmHg)	83.2 ± 11.2	77.0 ± 8.20	<0.001
*Sodium-volume	55.4 ± 40.8	35.3 ± 21.5	<0.001
Hypertensive n (%)	80 (61.1)	46 (38.7)	<0.001
Renin (pg/ml)	3.51 (1.10; 9.24)	5.86 (2.07; 14.6)	<0.001
Manuscript 3 (PURE), black participants only			
	Low renin (N=1002)	**High renin (N=500)**	***P***
Age (years)	51.2 ± 10.7	50.8 ± 9.71	0.49
Office SBP (mmHg)	138 ± 24.8	130 ± 22.1	<0.001
Office DBP (mmHg)	89.4 ± 14.6	86.2 ± 13.5	<0.001
*Sodium-volume	49.9 (20.3; 130)	9.90 (2.73; 21.9)	<0.001
Hypertension, n (%)	568 (56.7)	246 (49.2)	0.006
Renin (pg/ml)	2.71 (1.11; 5.94)	13.0 (6.54; 44.7)	<0.001

*Sodium-volume component calculated according to Laragh's volume equation (V=BP/R). Values are arithmetic mean ± standard deviation; geometric mean (5^{th} and 95^{th} percentile interval) for logarithmically transformed variables. Abbreviations: BP, blood pressure. Bold text indicate p<0.05. SBP, systolic blood pressure; DBP, diastolic blood pressure,

Hypothesis 2: The low renin group will exhibit higher cardiovascular reactivity to stress and there will be adverse associations between active plasma renin and cardiovascular responses to stress.

This hypothesis is based on data from the SABPA study and was tested in *Chapter 3*. Despite suppressed renin levels at rest and during stress, renin reactivity was positively associated with total peripheral resistance (TPR) reactivity. In addition, diastolic blood pressure (DBP) reactivity was higher in blacks compared to whites. However, it was only in whites whereby DBP was positively associated with renin reactivity.

The cold pressor test elicits mainly a peripheral vascular (α-adrenergic) effect characterised by higher peripheral vascular resistance and DBP [8]. In blacks, resting TPR associated negatively with renin, while during stress TPR increased, but renin did not. Nevertheless, TPR reactivity was positively associated with renin reactivity. This association may be due to α-adrenergic receptor-mediated vasoconstriction during sympathetic activation [9, 10]. Vasoconstriction of the afferent arteriole may also result in renin secretion by reducing intrarenal blood pressure [11]. The direct effects of renin on the vasculature independent of angiotensin II are mediated by the (pro)renin receptor. The receptor may be activated by both renin and prorenin, which is supposedly 20% higher in blacks [12-14]. Recently, Van Rooyen *et al*. showed that angiotensin I and II were lower in black hypertensive men compared to their white counterparts, and suggested that the increased peripheral vascular resistance due to sympathetic output may be the predominant mechanism of maintaining blood pressure in this population, since the RAAS is suppressed [10, 15].

In the white group, there was a significant increase in renin secretion during application of a laboratory stressor and this renin reactivity was associated with DBP reactivity. The increase in renin secretion during stress could be explained by β-adrenergic receptor mediated renin

secretion at the juxtaglomerular apparatus [11, 16], leading to an increase in angiotensin II formation, vasoconstriction and ultimately increased afterload and DBP [8, 17].

The second hypothesis is therefore partially accepted because blacks, with predominantly low renin levels showed higher DBP reactivity and a positive association between renin- and TPR reactivity. In whites, there was higher renin reactivity that associated adversely with DBP.

Hypothesis 3: The low renin group will present with adverse associations of active plasma renin, aldosterone and ARR, with ambulatory blood pressure as well as surrogate measures of sympathetic activity.

This hypothesis is also based on data from the SABPA study and was tested in *Chapter 4,* where I only focused on black and white individuals with low renin levels. Associations suggested that aldosterone and its ratio to renin (ARR) may reduce dipping in nocturnal heart rate. Furthermore, the interaction of aldosterone and noradrenaline supports a possible synergy between the RAAS and sympathetic drive in influencing the dipping of nocturnal heart rate. Additionally, increased renin associated with an increased mean ambulatory heart rate.

Blockade of aldosterone reduces heart rate and its variability by promoting parasympathetic dominance in the early morning hours when sympathetic activity is usually dominant [18]. Recent evidence indicates that higher heart rate and high salt intake may lead to salt-sensitive hypertension [19]. The observed adverse association between aldosterone and surrogate measures of sympathetic activity in this study suggest that aldosterone and higher sympathetic activity may synergistically contribute to the development of low-renin hypertension in Africans. Aldosterone may contribute by its direct fibrotic effects on the vasculature and the heart as well as via increased sodium retention, leading to volume expansion [20-22], while sympathetic activity contributes to increased blood pressure via elevated vascular resistance [10]. We also found a negative association of both diastolic and systolic blood pressure with renin, while ARR showed a borderline significant positive association with ambulatory blood

pressure, suggesting a potential role of aldosterone on blood pressure even when renin is suppressed. The ratio of aldosterone to renin associates adversely with central and peripheral blood pressure, while in black populations ARR was shown to influence the relationship between salt intake and blood pressure [23-25]. In addition to sodium retaining effects, aldosterone has been linked to cardiac remodelling via the mineralocorticoid receptors found in the heart [21].

I therefore partially accept the third hypothesis because aldosterone and ARR were negatively associated with dipping in night-time heart rate, while renin was positively associated with ambulatory heart rate.

Hypothesis 4: Black South Africans with the low renin phenotype are at an increased risk for mortality compared to those with normal/high renin.

The last objective of this study was to follow-up 1502 black South Africans over five years. This made possible a comparison of mortality in blacks with low versus high renin. It was found only in the low renin group (N=1005) that SBP and its interaction with renin, predicted all-cause and cardiovascular mortality. Since black populations tend to retain more sodium [3, 26], the suggestion is that the observed relationship may be partly due to the state of increased sodium and water retention, leading to volume-overload. The comparisons made across quartiles of plasma renin shows the suppression of renin with increasing blood pressure and the sodium-volume component. A state of excessive volume expansion may exert strain on the heart, augmenting left ventricular mass and subsequently left ventricular hypertrophy [27, 28]. In addition, salt-sensitivity, a component of the low renin, volume-loading hypertension, has been linked to target organ damage as reflected by left ventricular hypertrophy and microalbuminuria, particularly in blacks and the elderly [29, 30].

Regarding the association between renin and mortality in the high renin group, it was observed that although all-cause and cardiovascular mortality rates increased with increasing renin, no

significant associations were found between either SBP, renin or their interaction, and all-cause or cardiovascular mortality. In the total group, renin independently predicted all-cause mortality, which is in agreement with previous studies that found an independent relationship between renin and all-cause mortality [31], but not with cardiovascular events [32].

The fourth hypothesis is therefore accepted because systolic blood pressure (that also suppresses renin) and its interaction with renin were associated with a two-fold increase in cardiovascular mortality in the low renin group.

3. STRENGTHS

The SABPA study consisted of a homogenous sample, providing a valuable comparison of black and white school teachers from the same province. This study confirmed the high occurrence of low renin in blacks (using the proposed cut-offs from the Renin III CISBIO kit (Cedex, France) from two independent studies including men and women from both urban and rural areas and across a wide age range. This is important in a South African context, because the majority of studies reporting on renin were not performed in sub-Saharan Africa. In addition to the stimulatory effect of sympathetic activation on β-adrenergic-mediated renin secretion, this study showed a possible interaction of aldosterone and its ratio to renin with the sympathetic nervous system, which is not clear in black populations and probes further investigations into the relationship between sympathetic activity and the RAAS in these individuals. To the best of my knowledge the PURE study is also the first longitudinal study in Africa where the prognostic value of renin could be studied. Another strength of this study is the relatively large sample size of the PURE study and the randomized selection of households for inclusion in the study.

4. LIMITATIONS, CHANCE AND CONFOUNDING

The main limitation of the present study was the lack of all the components of the RAAS for the SABPA and PURE studies. This study did not make use of gold standard methods to assess sympathetic activity, such as microneurography and regional noradrenaline spillover [33] in the SABPA study. There was no collection 24-hour urine samples, which could have provided additional valuable information on salt intake and catecholamines. Measurement of renin while the participants were on antihypertensive drugs may have influenced the results. Therefore individual adjustments for the different classes of drugs were performed and the results remained significant in *Chapter 4*, while in *Chapter 5*, removing those on antihypertensive treatment did not affect the results in the low renin group.

In all three manuscripts, there was not an equal distribution in the number of participants in the low and high renin groups. The cut-off used to split the study populations into low and high renin groups may not have been suitable for the South African population, but there is also no evidence indicating the contrary, namely that these cut-offs are not suitable for black populations. The CISBIO kit age-specific cut-off values, 8.11 pg/ml for age range of 20-40 years and 6.18 pg/ml for 40-60 years were used for this study. Furthermore, mean plasma renin value of the study population was used as cut-off and confirmed significant results in the low renin group.

This study population was from the North West province, and is possibly not representative sample of the general population of South Africa. The cross-sectional observations made do not infer any causal relationship between components of the RAAS and applicable measures of cardiovascular structure and function. The possibility of chance should also be considered. Performing multiple correlations and adjusting for a number of covariates could have under- or overestimated the associations observed.

5. SUMMARY OF MAIN FINDINGS

The findings from this thesis confirm a high prevalence of a low renin status in blacks. In a black population with suppressed renin, renin reactivity related to peripheral vascular resistance reactivity, indicative of a blood pressure elevating effect of the sympathetic nervous system, even at low renin levels. In addition, renin associated with an increase in ambulatory heart rate, while aldosterone and its ratio to renin was associated with attenuated dipping in night-time heart rate (Figure 1).

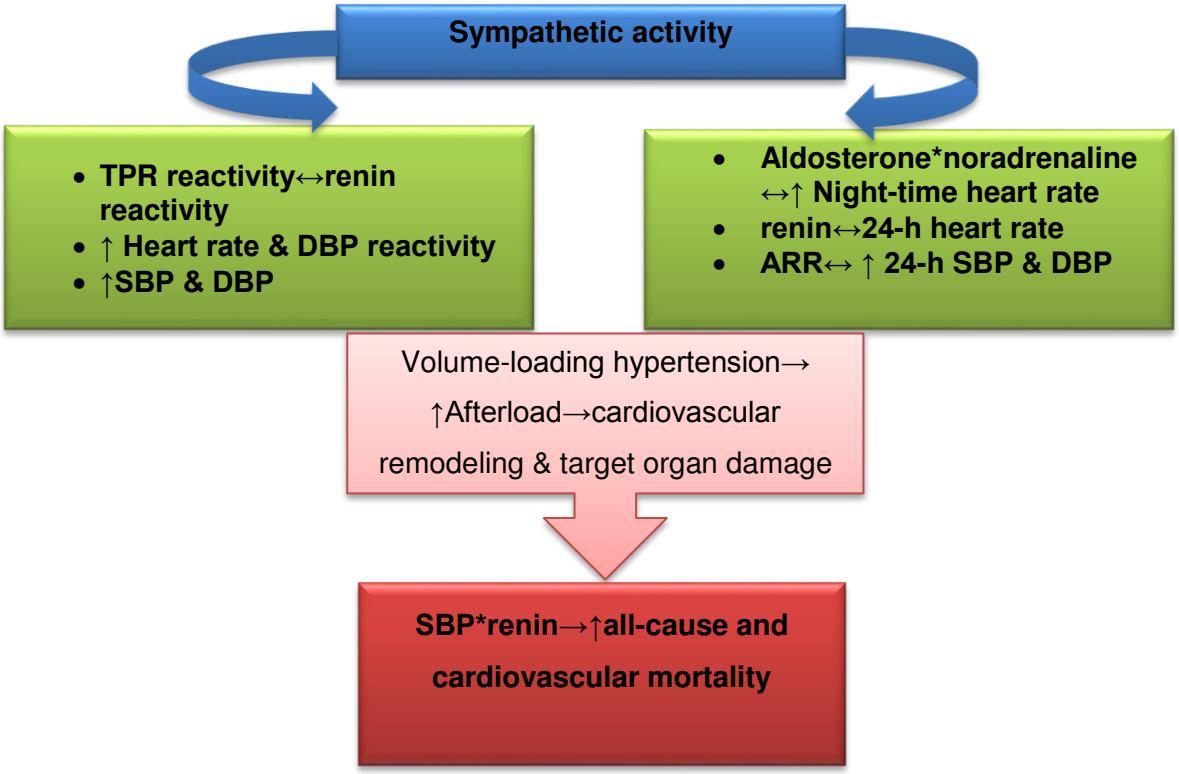

Figure 1: The low renin phenotype in Africans: the possible role of sympathetic activity, aldosterone and the implications for cardiovascular mortality. ↔, association; TPR, total peripheral resistance; DBP, diastolic blood pressure; SBP, systolic blood pressure; ARR, aldosterone-to-renin ratio.

The findings from the first two manuscripts suggest that when the RAAS is supressed, the sympathetic nervous system may increase blood pressure via vasoconstriction, and that the

effects of aldosterone on the heart and sympathetic drive may be aligned in low-renin hypertension common in black populations. ARR also tended to increase with increasing ambulatory blood pressure. In this population, despite suppressed renin, aldosterone levels were similar between blacks and whites, and therefore higher ARR in blacks. The higher levels of aldosterone in the state of low renin may be an indication of possible mineralocorticoid excess or aldosterone secretion that is independent of angiotensin II [34, 35]. As previously shown in populations of African ancestry, a higher ARR modifies the relationship between blood pressure and salt intake, indicative of a role in salt-sensitive hypertension [25].

Sodium handling defects in the kidneys seem to be the basis of low-renin hypertension in blacks. The epithelial sodium channel (EnaC) is the rate limiting barrier in the control of sodium reabsorption in the mineralocorticoid sensitive distal collecting tubule [36]. Under normal circumstances, when there is excess salt in the body, the activity of EnaC is downregulated to reduce sodium reabsorption. Any alteration in this regulatory process will result in excessive sodium retention and volume expansion [34]. Mutations of the EnaC results in enhanced function such as in Liddle syndrome [37]. A variant of the EnaC (R563Q) was found by Rayner and colleagues in individuals of African ancestry. This mutation has been associated with low renin and low aldosterone hypertension in black South Africans [38, 39]. Furthermore, in patients presenting with the R563Q and resistant hypertension, amiloride, an inhibitor of EnaC activity, significantly reduced mean blood pressure [40]. On the other hand, Bochud *et al*. showed that blacks tend to reabsorb more sodium than whites, particularly in the proximal tubule and less in the aldosterone-sensitive distal tubule [41]. The poor response to amiloride in African-American adolescents suggest that sodium reabsorption may be augmented in other segments of the tubules except the distal segment, thereby suppressing aldosterone secretion and EnaC activity [42]. The effects of amiloride on sodium channels extend beyond the kidney. Amiloride can improve vascular function by reducing stiffness and alleviating

endothelial swelling caused by insertion of sodium sensitive channel and may be mediated by aldosterone [43-45].

Among other genes that regulate sodium balance in the kidney, it was recently shown that dopamine and G protein-coupled receptor kinase 4 (GRK4) may be involved in the development of low-renin, salt-sensitive hypertension. G protein-coupled receptor kinase 4 variants are associated with low aldosterone and altered natriuretic response in blacks [46, 47]. The distribution of the GRK4 variant, p.Ala142Val is different between blacks and whites. This variant reduced sodium excretion in rats, and may therefore contribute to the low renin/ low aldosterone phenotype in normotensive blacks [48].

Finally, it was found that the low renin phenotype in this study is characterised by high blood pressure and a higher prevalence of hypertension (Table 1), and that the interactions between renin and SBP predicted cardiovascular and all-cause mortality. This relationship may be mediated by volume-loading that increases the afterload and results in cardiovascular remodelling and damage leading to mortality. These results suggest that the widespread low renin states that are likely to be accompanied by sympathetic dominance, higher aldosterone relative to renin and salt-sensitivity in black populations increase their risk for cardiovascular mortality.

6. CONCLUSION

This study aimed to profile and analyse the low renin phenotype and how it relates to cardiovascular haemodynamics, as well as cardiovascular and all-cause mortality. It was found that suppressed renin accompanied by high blood pressure was prominent in blacks compared to whites. Additionally, renin, aldosterone and their ratio associated adversely with vascular resistance and surrogate measures of sympathetic activity. This suggests that augmented sympathetic activity and aldosterone may be determinants of the cardiovascular

profile of the low renin phenotype. Finally, the interaction of renin and systolic blood pressure predicted both all-cause and cardiovascular mortality in blacks with low renin levels. Collectively, the results of this thesis suggest that the low renin phenotype and the adverse associations with cardiovascular function it presents may increases the risk for cardiovascular mortality.

7. RECOMMENDATIONS

To further our understanding of the mechanisms involved in the pathophysiology of hypertension in black populations, particularly the low renin phenotype, it would be useful to profile the entire RAAS by measuring prorenin, angiotensinogen, angiotensin-converting enzymes (ACEs), angiotensins, aldosterone (preferably in 24-hour urine) and assess salt-sensitivity in a highly controlled salt-loading study.

The roles of the recently discovered components of the RAAS, including the two counter-regulatory pathways ACE-2-(Angiotensin (1-7)-*Mas* receptor cascade and angiotensin II type 2 receptor pathway that opposes the effects of angiotensin II should also be studied. Evidence regarding the effect of intra-renal RAAS on the circulating RAAS and blood pressure regulation may shed light on the mechanisms that lead to increased blood pressure when the circulating RAAS is suppressed.

Individuals taking antihypertensive medications that cause a reactive renin secretion such as diuretics, ACEs and angiotensin receptors blockers (ARBs) and those that supress renin such as beta blockers should be excluded from future investigations [49]. Measurement of renin in the presence of ACEs and ARBs may overestimate the true activity of renin up to ten times *in*

vivo, while in the absence of beta blockers, renin levels in the lower ranges could fall in the normal or high renin ranges [49, 50].

Future longitudinal studies should also target young and healthy normotensive adults followed up over time to observe any adverse cardiovascular changes associated with components of the RAAS and if such changes will lead to hypertension. Since females may present with high renin levels during the luteal phase of the menstrual cycle [51], plasma renin activity should also be measured, importantly, for calculating ARR.

In South Africa, volume-loading, inadequate diuretic therapy and incorrect drug combinations are among the causes of resistant hypertension [52], which is more common in blacks compared to whites [53]. Mechanisms seem multifactorial and common characteristics include low renin states associated with volume-overload. It may be necessary to define exactly what constitutes low-renin hypertension (cut-offs for components of the RAAS and corresponding blood pressures for the specific cut-off, responses to antihypertensive drugs, particularly mineralocorticoid receptor antagonists, which are underused as well as amiloride) in populations of African descent. This may be useful in identifying the factors that must be targeted for therapeutic intervention in order to achieve optimal blood pressure and reduce the severe cardiovascular outcomes due to hypertension.

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ANNEXURES

ANNEXURE A

Ethics approval

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8 September 2014

Dear Prof Schutte

Ethics Application: NWU-00001-12-A1 "African Predict Study"

Thank you for the amendments made to your application. All ethical concerns have now been addressed and your application to include the sub-study, entitled "The cardiovascular profile of the low renin phenotype in a black South African population" under the umbrella project has been approved until 31/12/2017.

Yours sincerely

Prof Minrie Greeff
Health Research Ethics Committee Chairperson

Original details: Prof Minrie Greeff(10187308) C:\Users\13210572\Documents\ETIEK\2014 ETHICS\NWU-00001-14-A1 (AE Schutte-LF Gafane) - Approval letter.docm
8 September 2014

File reference: 9.1.5.3

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Ethics Committee
Tel: 018 2994237

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To whom it may concern

31 August 2012

Dear Prof./Dr./Mr./Me.

Ethics application: NWU-00036-07-S6 (L. Malan)

"SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans)" study

The additional request for continuation of the SABPA studie till 2017 has been approved.

Kind regards

Prof. H.H. Vorster
Chair person

YUNIBESITI YA BOKONE-BOPHIRIMA
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2 September 2004

Dr A Kruger
Bussie 594
Noordwes-Universiteit
(Potchefstroomkampus)

Geagte dr Kruger

GOEDKEURING VIR EKSPERIMENTERING MET MENSE

Hiermee wens ek u in kennis te stel dat u projek getiteld *"PURE study (Prospective Urban and Rural Epidemiology study)"* deur die Etiekkomitee goedgekeur is met nommer 04M10.

Gebruik asseblief die nommer genoem in paragraaf 1 in alle korrespondensie rakende bogenoemde projek en let daarop dat daar van projekleiers verwag word om jaarliks in Junie aan die Etiekkomitee verslag te doen insake etiese aspekte van hulle projekte asook van publikasies wat daaruit voortgespruit het. U sal in Mei 2005 die dokumentasie hieroor ontvang.

Goedkeuring van die Etiekkomitee is vir 'n termyn van hoogstens 5 jaar geldig (volgens Senaatsbesluit van 4 November 1992, art 9.13.2). Vir die voortsetting van projekte na verstryking van hierdie tydperk moet opnuut goedkeuring verkry word.

Die Etiekkomitee wens u alle voorspoed met u werk toe.

Vriendelike groete

ESTELLE LE ROUX
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2010-02-23

ETHICS APPROVAL OF PROJECT

This is to certify that the next project was approved by the NWU Ethics Committee:

Project title : *PURE study (Prospective Urban and Rural Epidemiology study)*

Project leader / Student : Prof Annamarie Kruger

Ethics number: NWU-00016-10-A1

Expiry date: 20/01/2015

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

The formal Ethics approval certificate will be sent to you as soon as possible.

Yours sincerely

Me.Marietjie Halgryn
NWU Ethics Secretariate

ANNEXURE B

Declaration of language editing

cumlaude
languagepractitioners

Director: CME Terblanche - BA (Pol Sc), BA Hons (Eng), MA (Eng), TEFL

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Baillie Park, 2531

Tel 082 821 3083
cumlaudelanguage@gmail.com

DECLARATION OF LANGUAGE EDITING

I, Christina Maria Etrecia Terblanche, hereby declare that I edited the following research study:

The cardiovascular profile of the low renin phenotype in a black South African population

for **Lebo F Gafane** for the purpose of submission as a thesis for examination. Changes were suggested in track changes and implementation was left up to the author.

Regards,

CME Terblanche

Cum Laude Language Practitioners (CC)

SATI accreditation nr: 1001066

Registered with PEG

ANNEXURE C

Publications

Journal of Human Hypertension (2015), 1–6

www.nature.com/jhh

ORIGINAL ARTICLE

Plasma renin and cardiovascular responses to the cold pressor test differ in black and white populations: The SABPA study

LF Gafane[1], R Schutte[1,2,3], JM Van Rooyen[1] and AE Schutte[1,2]

Low plasma renin levels and augmented cardiovascular reactivity to stress are common in blacks and have been linked to the development of hypertension in this population. We (i) compared cardiovascular and plasma renin reactivity to a cold pressor test between a black and white population; and (ii) investigated the associations between cardiovascular and plasma renin reactivity within the black and white populations. Our population consisted of 153 black and 188 white men and women (age range, 20–65 years). We measured blood pressure (BP), heart rate (HR), stroke volume (SV), total peripheral resistance (TPR), Windkessel arterial compliance, and determined plasma renin levels at rest and during the cold pressor test. Reactivity was calculated for each participant as the percentage change from the resting value. We found lower renin and elevated BP in blacks compared with whites at rest and during stress (both, $P < 0.001$). During stress, HR increased more in blacks ($P < 0.001$), whereas SV ($P < 0.001$) and arterial compliance ($P = 0.013$) decreased more in blacks compared with whites. TPR reactivity was positively associated with renin reactivity in blacks only ($\beta = 0.17$; $P = 0.041$), while in whites diastolic BP reactivity was positively associated with renin reactivity ($\beta = 0.21$; $P = 0.005$). Although blacks had suppressed renin levels at rest and during acute stress, vascular resistance reactivity associated positively with renin reactivity only in the black population. These results suggest that low renin levels in blacks during rest and stress are linked to increased peripheral vascular responses to stress, which may contribute to elevated BP in blacks.

Journal of Human Hypertension advance online publication, 27 August 2015; doi:10.1038/jhh.2015.88

INTRODUCTION

The incidence of cardiovascular morbidity and mortality continues to increase in black South Africans, with hypertension being the most common cardiovascular risk factor.[1,2] It is known that blacks tend to have a suppressed renin-angiotensin-aldosterone system (RAAS) activity, including low renin status, and accordingly, low renin hypertension is common.[3] Furthermore, black populations have higher sympathetic nerve activity[4,5] and peripheral resistance at rest and when the cardiovascular system is challenged[6,7] when compared with whites.

The cold pressor test is a method used to study cardiovascular stress reactivity by immersing an individual's foot or hand in ice water for 1 min. This results mostly in a peripheral vascular effect by stimulating the α-adrenergic receptors that cause vasoconstriction and a subsequent elevation in total peripheral resistance (TPR), ventricular afterload and blood pressure (BP), thereby interfering with the heart's ability to increase stroke volume (SV) during stress.[8–10] In addition, the secretion of renin during sympathetic activation promotes vasoconstriction, further contributing to systolic (SBP) and diastolic BP (DBP) elevation via the actions of angiotensin II.[3] Renin can also result in BP elevation by activation of the prorenin receptors in the vasculature, independent of other components of the RAAS.[11]

High plasma renin has detrimental effects on the vasculature such as activation of profibrotic and proinflammatory pathways through the prorenin receptors,[12,13] however, in black populations, low renin is positively associated with increased BP and target organ damage.[3,14–16] Despite the known low renin phenotype and increased cardiovascular reactivity in black populations, to the best of our knowledge, no previous studies have investigated the specific haemodynamic and renin responses during a stressor in blacks. The aims of this study were (i) to compare cardiovascular and plasma renin reactivity with a cold pressor test between a black and white population; and (ii) to investigate the associations between cardiovascular and plasma renin reactivity within the black and white populations.

MATERIALS AND METHODS

Study design and population

The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study was conducted between February 2008 and May 2009. The study included 200 black (101 women and 99 men) and 209 white (108 women and 101 men) urban teachers in the North West Province of South Africa. The black group was mostly Setswana speaking. The reason for this selection was to have a homogeneous sample from a similar socioeconomic class. We invited eligible participants in the age range between 20 and 65 years. Exclusion was based on the following criteria: ear temperature > 37.5 °C; vaccinated or donated blood in the previous 3 months before the study commenced; pregnancy, lactation, HIV infection, diabetes, any acute/chronic medication and psychotropic substance abuse or dependence. From 409 participants, we had renin data available for 153 black and 188 white participants.

Participants were fully informed about the objectives and procedures of the study before enrolment. Assistance was given to any participant who requested conveyance of information in their home language. All participants signed an informed consent form. The study complied with all applicable requirements of the international regulations, in particular,

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the Helsinki declaration of 1975 (as revised in 2008) for investigation of human participants. The Health Research Ethics Committee of North-West University (Potchefstroom campus) approved this study (NWU-00036-07-S6). Participants were transported at 16h30 to the Metabolic Unit Research Facility of the North-West University, and they were familiarised with the experimental setup. After receiving a standardised dinner, participants were encouraged to go to bed at around 22h00. The participants woke up at 05h45 and the measurements commenced.

Questionnaires

We administered validated general health and sociodemographic questionnaires.

Anthropometric measurements

Weight, height, waist and hip circumferences were measured in triplicate by anthropometrists according to standardised methods with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, London, UK; Holtain non-stretchable metal flexible measuring tape). Body mass index (BMI) was calculated and expressed as $kg\,m^{-2}$.[17]

Cardiovascular measurements

The validated[18,19] Finometer device (FMS, Finapres Measurement Systems, Amsterdam, the Netherlands) was connected, and after a 10-min resting period, a 2-min calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial arterial pressure.[19] The highest precision in cardiovascular measurement can be achieved only after this calibration,[19] and BP measurements complied with the requirements of the Association for the Advancement of Medical Instrumentation (AAMI).[19,20] Continuous measurement of resting cardiovascular variables was performed for a 5-min period, after which the participant was exposed to the cold pressor test for 1 min. It was performed by immersing the participant's right foot up to the ankle in ice water with a temperature of 4 °C for 1 min. The Beatscope software was used to calculate SBP and DBP, heart rate (HR) and computed SV, TPR, and 'Windkessel' compliance of the arterial system (Cwk).[21] Cwk is the 'Windkessel' compliance of the arterial system also referred to as Windkessel compliance, or buffer compliance. As part of the nonlinear three-element model (aortic characteristic impedance, arterial compliance and systemic vascular resistance), it is computed from an age-dependent, aortic pressure–area relationship and represents the lumped compliance of the entire arterial system.[21] We used the average of 1 min of the resting recording and the average of the last 20 s of the stressor recordings. Cardiovascular reactivity was calculated for each participant as the percentage change from the resting value to the stressor value. All measurements were performed at room temperature.

Biological sampling and biochemical analyses

Participants were requested to be in a fasted state by not eating or drinking anything except water for approximately 8–10 h prior to sample collection in the mornings. An 8-h morning spot urine sample was collected to measure norepinephrine using the 3-Cat Urine ELISA Fast Track kit (LDN, Nordhorn, Germany). A registered nurse obtained the first blood sample with a sterile winged infusion set from the antebrachial vein branches whilst the participant was in a supine position for a period of 30 min. Samples were prepared according to appropriate methods and stored at −80 °C in the laboratory. Sequential multiple analysers (Konelab 20i, ThermoScientific, Vantaa, Finland; and Cobas Integra 400 plus, Roche, Basel, Switzerland) were used to analyse total and high density lipoprotein cholesterol, fasting glucose, high sensitivity C-reactive protein, gamma-glutamyltransferase and glycosylated haemoglobin (HbA1c). Serum creatinine was analysed using an enzymatic colorimetric test (Cobas Integra 400 plus, Roche). The Modification of Diet in Renal Disease formula was used to estimate the glomerular filtration rate. Active plasma renin was analysed in duplicate using the high sensitivity radio-immunometric assay and cross-reaction with prorenin was 0.4% (Renin III Generation, CIS Biointernational, Codolet, France). The source of reagents was mouse anti-human-active renin monoclonal antibody (IBL Lab, 38T501, Minneapolis, MN, USA). Another blood sample to measure renin was collected 5–10 min after exposure to the cold pressor test, and renin reactivity was calculated for each participant as the percentage change from the resting value to the stressor value.

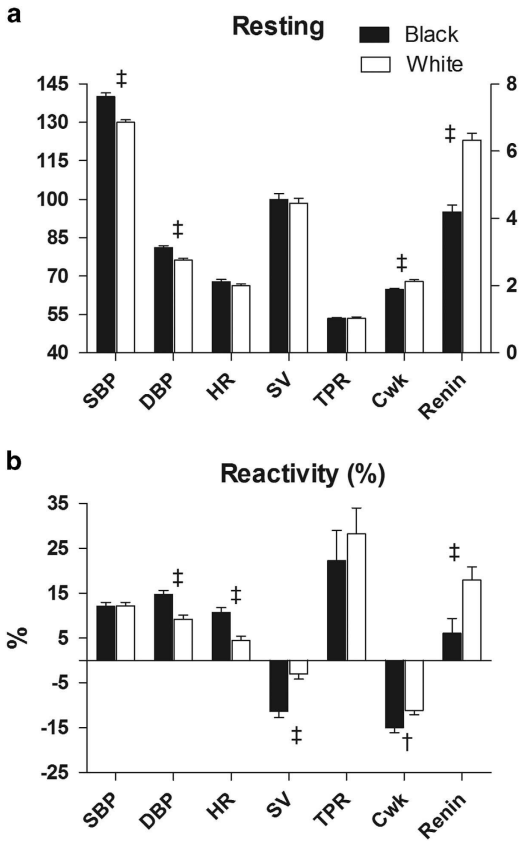

Figure 1. Comparison of haemodynamic variables and plasma renin between blacks and whites. (**a**) Resting. (**b**) Reactivity (%) from baseline during the cold pressor test. $^{\dagger}P < 0.05$; $^{\ddagger}P \leqslant 0.001$. Reactivity values are adjusted for baseline haemodynamic and plasma renin measurements. Plasma renin ($pg\,ml^{-1}$).

Statistical analyses

We used Statistica Version 12 for all statistical analyses (Statsoft Inc., Tulsa, OK, USA). Power analyses were performed for the SABPA study to obtain relevant effect sizes based on differences in biological profiles and genotyping hypothalamic-pituitary adrenal axis variation. Resulting sample sizes of 50–416 would enable explanation of biological differences and detection of single nucleotide polymorphisms with a statistical power of 0.8, and level of significance of 0.05. We tested for interaction of sex on the associations between TPR and renin, SBP and renin as well as between DBP and renin, but none were significant (all $P \geqslant 0.06$). The distribution of HbA1c, gamma-glutamyltransferase, total cholesterol, high density lipoprotein cholesterol and C-reactive protein were normalised by logarithmic transformation. The central tendency and spread of these variables were represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions were compared by using two-sided independent *t*-tests and Chi-square tests. In Figure 1, error bars represent the standard error of mean. We performed single, partial and multiple regression analyses to investigate associations between relevant cardiovascular variables and renin. In partial regression analyses, we adjusted for age, BMI and sex. After considering several variables for inclusion in the multiple regression model, we finally included age, BMI, sex, gamma-glutamyltransferase, total cholesterol to high density lipoprotein cholesterol ratio, antihypertensive medication, HbA1c, C-reactive protein and estimated glomerular filtration rate as covariates.

RESULTS

Characteristics of the population

Table 1 lists the characteristics of the population stratified by ethnicity. Age was similar between the two groups, but the black group had a higher mean BMI ($P = 0.005$) and a larger proportion (47.7%) was hypertensive ($P < 0.001$) compared with the whites

Table 1. Characteristics of the population

	Black	*White*	P-*value*
N	153	188	
Age (years)	43.1 ± 7.71	43.9 ± 10.6	0.38
Women, *n* (%)	73 (47.7)	99 (52.7)	0.36
Anthropometric measurements			
Weight (kg)	80.2 ± 18.7	84.1 ± 21.7	0.077
Body mass index (kg m^{-2})	29.7 ± 7.13	27.6 ± 6.09	0.005
Waist circumference (cm)	92.2 ± 15.7	92.7 ± 16.5	0.75
Resting cardiovascular measurements			
Systolic BP (mm Hg)	140 ± 17.6	130 ± 13.3	< 0.001
Diastolic BP (mm Hg)	81.1 ± 9.83	76.3 ± 7.82	< 0.001
Biochemical measurements			
Resting plasma renin (pg ml^{-1})	4.19 ± 2.62	6.32 ± 3.07	< 0.001
Glycosylated haemoglobin (%)	5.89 (5.10; 7.50)	5.48 (5.00; 6.29)	< 0.001
Gamma-glutamyltransferase (U l^{-1})	44.4 (19.9; 177)	19.1 (7.00; 75.9)	< 0.001
TC: HDL	4.11 (0.32; 7.46)	4.71 (2.85; 7.86)	< 0.001
C-reactive protein (mg l^{-1})	3.80 (0.45; 26.33)	2.05 (0.99; 8.99)	< 0.001
Serum creatinine (μmol l^{-1})	75.1 ± 0.08	72.3 ± 0.08	< 0.001
eGFR (ml min^{-1} 1.73 m^{-2})	113 ± 27.6	95 ± 16.9	< 0.001
Norepinephrine (ng ml^{-1})	39.6 ± 40.8	45.6 ± 32.1	0.002
Lifestyle factors			
Smoking, *n* (%)	28 (18.3)	28 (15.0)	0.41
Hypertensive, *n* (%)	73 (47.7)	48 (25.5)	< 0.001
Alcohol use, *n* (%)	38 (24.8)	95 (50.8)	< 0.001
Antihypertensive medication, *n* (%)	27 (17.6)	11 (5.9)	< 0.001

Abbreviations: BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; TC, total cholesterol. Values are arithmetic mean ± standard deviation or geometric mean (5th and 95th percentile interval) for logarithmically transformed variables. Reactivity values are adjusted for baseline haemodynamic and plasma renin measurements.

(25.5%). Under resting conditions, SBP and DBP were higher in blacks compared with whites ($P < 0.001$) (Table 1), whereas HR ($P = 0.16$), SV ($P = 0.59$) and TPR ($P = 0.97$) were not different (Figure 1). Cwk and renin were lower in the black compared with the white group (both $P < 0.001$) (Figure 1).

In both groups during the cold pressor test, SBP, DBP, HR and TPR increased, whereas SV and Cwk decreased (all $P < 0.001$). Renin did not increase ($P = 0.67$) in blacks during stress (Table 2). DBP and HR increased more in blacks (both $P < 0.001$), whereas SV ($P < 0.001$) and Cwk ($P = 0.013$) decreased more in blacks compared with whites (Figure 1).

Adjusted regression analyses

We determined the correlations between haemodynamic variables and renin under resting conditions and during the cold pressor test while adjusting for age, BMI and sex (Table 3). At rest, SBP and DBP associated negatively with renin in both groups (both $P < 0.05$), while TPR associated negatively with renin in blacks only ($P = 0.017$). Cwk associated positively with renin in both blacks ($P < 0.001$) and whites ($P = 0.018$). When assessing similar correlations, but for the cold pressor test, DBP reactivity associated positively with renin reactivity in whites ($P = 0.010$), while TPR reactivity associated positively with renin reactivity in blacks ($P = 0.041$) only.

Table 2. Changes from baseline within the black and white groups

Variables	*Resting*	*Stressor*	P-*value*
Black			
SBP (mm Hg)	140 ± 17.6	155 ± 22.6	< 0.001
DBP (mm Hg)	81.1 ± 9.83	91.8 ± 12.4	< 0.001
HR (b.p.m.)	67.4 ± 10.4	73.9 ± 11.3	< 0.001
SV (ml)	101 ± 26.1	88.3 ± 26.3	< 0.001
TPR (mm Hg ml^{-1} s^{-1})	1.01 ± 0.29	1.22 ± 0.41	< 0.001
Cwk (ml mm Hg^{-1})	1.89 ± 0.42	1.61 ± 0.48	< 0.001
Renin (pg ml^{-1})	4.17 ± 2.62	4.21 ± 2.29	0.67
White			
SBP (mm Hg)	130 ± 13.3	147 ± 15.9	< 0.001
DBP (mm Hg)	76.3 ± 7.82	83.9 ± 10.3	< 0.001
HR (b.p.m.)	66.2 ± 10.7	69.1 ± 12.5	< 0.001
SV (ml)	98.5 ± 24.7	94.7 ± 24.7	< 0.001
TPR (mm Hg ml^{-1} s^{-1})	1.02 ± 0.54	1.26 ± 1.06	< 0.001
Cwk (ml mm Hg^{-1})	2.13 ± 0.52	1.89 ± 0.51	< 0.001
Renin (pg ml^{-1})	6.34 ± 3.07	6.72 ± 3.15	0.001

Abbreviations: Cwk, 'Windkessel' compliance of the arterial system; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; SV, stroke volume; TPR, total peripheral resistance.

Table 3. Partial regression analysis between haemodynamic variables and plasma renin, adjusted for age, body mass index and sex

Resting variables	*Resting plasma renin (pg ml^{-1})*			
	Black		*White*	
	r	P-*value*	*r*	P-*value*
SBP (mm Hg)	**−0.33**	**< 0.001**	**−0.29**	**< 0.001**
DBP (mm Hg)	**−0.22**	**0.01**	**−0.25**	**0.001**
HR (b.p.m.)	0.15	0.07	−0.002	0.98
SV (ml)	0.05	0.56	0.03	0.73
TPR (mm Hg ml^{-1} s^{-1})	**−0.19**	**0.017**	−0.12	0.09
Cwk (ml mm Hg^{-1})	**0.36**	**< 0.001**	**0.17**	**0.018**
Reactivity variables	*Plasma renin reactivity (%)*			
	Black		*White*	
	r	P-*value*	*r*	P-*value*
SBP (%)	0.09	0.32	0.06	0.40
DBP (%)	0.08	0.35	**0.19**	**0.010**
HR (%)	−0.08	0.35	−0.03	0.70
SV (%)	−0.09	0.25	−0.02	0.84
TPR (%)	**0.18**	**0.041**	0.02	0.84
Cwk (%)	−0.07	0.45	−0.14	0.063

Abbreviations: Cwk, 'Windkessel' compliance of the arterial system; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; SV, stroke volume; TPR, total peripheral resistance. Reactivity values are adjusted for baseline haemodynamic and plasma renin measurements. Bold values indicate statistical significance ($P < 0.05$).

We performed forward stepwise multiple regression analyses (Table 4) to determine independent associations between haemodynamic variables and renin under resting conditions and during application of the cold pressor test. All of the above associations were confirmed. In addition, it is noteworthy to mention the borderline significant association between DBP reactivity and renin reactivity in blacks ($P = 0.060$).

Table 4. Forward stepwise multiple regression analyses between haemodynamic variables and plasma renin

Resting variables	*Resting plasma renin (pg ml^{-1})*					
	Black			*White*		
	R^{2a}	*β (95% CI)*	P-*value*	R^2	*β (95% CI)*	P-*value*
SBP (mm Hg)	**0.30**	**−0.29 (−0.48; −0.16)**	**< 0.001**	**0.27**	**−0.27 (−0.40;00.16)**	**< 0.001**
DBP (mm Hg)	**0.24**	**−0.19 (−0.34; −0.04)**	**0.012**	**0.29**	**−0.23 (−0.35; −0.10)**	**< 0.001**
HR (b.p.m.)	—	—	—	—	—	—
SV (ml)	—	—	—	—	—	—
TPR (mm Hg ml^{-1} s^{-1})	**0.16**	**−0.18 (−0.34; −0.04)**	**0.016**	0.09	−0.12 (0.26; 0.03)	0.11
Cwk (ml mm Hg^{-1})	**0.59**	**0.24 (0.14; 0.35)**	**< 0.001**	**0.69**	**0.11 (0.02; 0.19)**	**0.014**
Reactivity variables	*Plasma renin reactivity (%)*					
	Black			*White*		
	R^{2a}	*β (95% CI)*	P-*value*	R^2	*β (95% CI)*	P-*value*
SBP (%)	0.05	0.10 (−0.06; 0.27)	0.22	—	—	—
DBP (%)	0.17	0.15 (−0.01; 0.30)	0.060	**0.07**	**0.21 (0.07; 0.37)**	**0.005**
HR (%)	—	—	—	—	—	—
SV (%)	0.02	−0.09 (−0.26; 0.07)	0.25	—	—	—
TPR (%)	**0.07**	**0.17 (0.01; 0.34)**	**0.041**	—	—	—
Cwk (%)	0.03	−0.09 (−0.26; 0.07)	0.28	0.01	−0.13 (−0.28; 0.02)	0.09

Abbreviations: Cwk, 'Windkessel' compliance of the arterial system; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; SV, stroke volume; TPR, total peripheral resistance. —, did not enter the model. Independent variables included in the model: age, body mass index, sex, gamma-glutamyltransferase, total cholesterol to high-density lipoprotein cholesterol ratio, antihypertensive medication, glycosylated haemoglobin, C-reactive protein and estimated glomerular filtration rate. Reactivity values are adjusted for baseline haemodynamic and plasma renin measurements. Bold values indicate statistical significance ($P < 0.05$). [a]Adjusted R^2.

Sensitivity analysis

We investigated whether the association found in blacks between TPR reactivity and renin reactivity was confounded by norepinephrine, and thus included norepinephrine in our multiple regression model. In doing so, norepinephrine did not enter the model, but renin remained significant ($R^2 = 0.07$; $\beta = 0.17$; $P = 0.049$).

DISCUSSION

We compared renin and cardiovascular responses with the cold pressor test between blacks and whites, and investigated whether renin and cardiovascular reactivity are associated. The main finding of our study is that despite suppressed levels at rest and during stress, renin was positively associated with TPR in blacks at rest and when the cardiovascular system was challenged. In whites, an increase in renin was associated with an increase in DBP. These results suggest a possible difference in the role of renin in mechanisms controlling BP between blacks and whites, which may be influenced by the characteristics of our population such as hypertension, obesity and lifestyle factors.

The cardiovascular stress challenge results in activation of the sympathetic nervous system,[4,9] as reflected by the increased vascular resistance, BP, HR and a decrease in SV and arterial compliance in our study. Changes in DBP, HR, SV and arterial compliance were more pronounced in blacks compared with whites as previously shown in the same population.[5,22] Vascular resistance reactivity was similar between the groups, however, previous findings showed that TPR tends to be higher in blacks compared with whites.[6] It is noteworthy that the changes in basic haemodynamics were accompanied by an increase in renin only in the white group ($P = 0.001$ versus $P = 0.67$ in blacks).

Our results on suppressed renin during the cold pressor test are in contradiction with previous findings from the same study population that reported an increase in renin during a laboratory mental stressor in blacks which was associated with increased carotid intima-media thickness.[16] In the previous study, Hamer *et al.*[16] focused on recovery values and used the STROOP Colour Word Conflict Test, which is known to elicit a mixed α-adrenergic and β-adrenergic response. The resultant effects include norepinephrine-induced myocardial responses via central mechanisms and renin secretion from the juxtaglomerular apparatus by stimulation of β-adrenergic receptors.[23,24] On the other hand, the cold pressor test has a predominant α-adrenergic response with a peripheral vasoconstrictive effect.[23,24] Sympathetic nerve stimulation can also increase renin secretion by stimulating α-adrenergic receptors that cause constriction of the afferent arteriole, a fall in intrarenal BP and eventually elevated renin secretion.[25] Blacks seem to have a higher density of α-adrenergic receptors responsible for higher peripheral vasoconstriction and BP reactivity.[9] Increased sodium retention, which is common in black hypertensives[26] may have had a role in the suppression of renin secretion during a stressor[27] in the black group. Sensitivity analysis indicated that norepinephrine did not have a role in this association.

Although blacks tend to have low renin levels, their prorenin levels are reportedly higher than that of renin and also 20% higher in blacks compared with whites.[28,29] The prorenin receptor is located in the vasculature,[30] and it has been linked to elevated SBP and DBP in Japanese men.[12] Binding of renin and/or prorenin to this receptor can activate tissue renin angiotensin system, resulting in pressure overload as well as end-organ damage in the heart and kidneys independent of the circulating RAAS.[28,31,32] It was recently indicated that urinary angiotensinogen is positively associated with BP in a salt-sensitive, low renin group of blacks, thus showing that intrarenal renin angiotensin system can contribute to BP control independent of the circulatory RAAS.[33] Also, in salt-sensitive individuals, the low renin phenotype is characterised by impairment of the compensatory mechanism for

renal sodium reabsorption, renin production, formation of angiotensin II and constriction of the renal efferent arteriole.[26,34] This suggests that because renin is suppressed in our black and mostly hypertensive population, other mechanisms such as vascular hyper-reactivity to stress,[5,6,22] prorenin receptor activation[30] and local tissue renin angiotensin system[33] may be responsible for the associations found between vascular resistance reactivity and renin reactivity.

Our study should be interpreted within the context of its limitations and strengths. We did not measure baseline and stressor reactivity for norepinephrine, prorenin, angiotensin II and aldosterone. By the time of the writing of this manuscript, plasma aldosterone was not available, but it is planned for analysis in the near future. We did not assess salt sensitivity. The higher vascular reactivity to a cold stimulus in blacks may also be due to an exaggerated perception of cold pain.[5] Some of the participants used antihypertensive medication such as beta blockers, diuretics and angiotensin receptor blockers that influence the RAAS and therefore could have affected our results. The medication was not taken on the day of the measurement and this was authorised by a medical doctor. This was a cross-sectional study, therefore causality cannot be inferred, and our study population cannot be regarded as representative of the multi-ethnic South African population. However, to our knowledge, this is the first study to compare cardiovascular and plasma renin responses with a laboratory stressor between black and white South Africans. Our study was performed under well-controlled experimental conditions.

In conclusion, despite suppression of renin secretion during application of a stressor, TPR reactivity was positively associated with renin reactivity only in the black population. These results suggest that although renin is suppressed in blacks during rest and stress, it may have a role in the underlying mechanisms that act on the peripheral vasculature to elevate BP.

What is known about this topic?

- Blacks tend to have a low renin status; consequently, low renin hypertension is common in this group.
- Blacks have increased cardiovascular reactivity to acute stressors as compared with whites.
- Low renin levels are associated with target organ damage and increased blood pressure in blacks.

What this study adds?

- To the best of our knowledge, this is the first study to indicate that plasma renin is suppressed during acute stress in blacks compared with whites.
- Low renin is positively associated with total peripheral resistance in blacks only.
- Although renin is suppressed in blacks during rest and stress, its ability to elicit responses in the peripheral vasculature remains active.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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duced not only the level of phosphorylated Akt and e-NOS but also oxidative stress. In experiment 2, body weight and rotarod test showed significant increasing in RD group compared to sham group.

Conclusions: Our results suggest that acute blood pressure control by RD is promising therapeutic strategy to improve prognosis after ischemic stroke in hypertensive patients.

OS 32-05 EFFECTS OF SGLT2 INHIBITORS ON CIRCADIAN RHYTHM OF BLOOD PRESSURE IN RATS

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Objective: Disrupted circadian rhythm of blood pressure is associated with cardiovascular events in metabolic syndrome and obesity. Experiments were conducted to examine the effects of sodium-glucose co-transporter 2 (SGLT2) inhibitors on circadian rhythm of blood pressure in a genetic model of obese metabolic syndrome (SHR/NDmcr-cp (+/+) (SHRcp)) and salt-treated obese Otsuka Long Evans Tokushima Fatty (OLETF) rats.

Design and method: Luseogliflozin (10 mg/kg/day, p.o.) and empagliflozin (10 mg/kg/day, p.o.) were administered for 5 weeks in metabolic syndrome SHRcp rats and 1% NaCl (in drinking water)-treated obese OLETF rats, respectively. Blood pressure was measured continuously by telemetry system. Glucose metabolism and insulin resistance were evaluated by oral glucose tolerance test.

Results: Both SHRcp and salt-treated OLETF rats developed non-dipper type of hypertension with altered glucose metabolism and insulin resistance. Administration of luseogliflozin and empagliflozin resulted in a remarkable increase in urinary glucose excretion and improved glucose metabolism and insulin resistance in SHRcp and salt-treated OLETF rats, respectively. Furthermore, luseogliflozin and empagliflozin significantly attenuated the development of hypertension with normalization of circadian rhythm of blood pressure, which was associated with an increase in urinary sodium excretion.

Conclusions: These data suggest that SGLT2 inhibitors elicit beneficial effects on circadian rhythm of blood pressure during the development of hypertension in subjects with metabolic syndrome and obese.

OS 32-06 MOUSE GLOMERULAR INJURY REDUCED BY BLOCKING N-TYPE CALCIUM CHANNEL

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Objective: Previous studies have indicated the beneficial effects of N-type calcium channel blocker, cilnidipine, on protecting renal podocyte injury in metabolic syndrome model rats. In the present study, we examined the effects of cilnidipine (L/N-type calcium channel blocker) and nifedipine (L-type calcium channel blocker) on the glomerular filtration barrier damage in the doxorubicin-induced nephropathy mice model.

Design and method: Balb/c mice (male, 5 week-old) were treated with vehicle (n = 5), doxorubicin (20 mg/kg, i.v., n = 6) + carboxymethyl cellulose (vehicle for calcium channel blockers), doxorubicin + cilnidipine (30 mg/kg, twice a day, p.o., n = 6), doxorubicin + nifedipine (30 mg/kg, twice a day, p.o., n = 6) for 8 weeks.

Results: Doxorubicin injection induced the development of albuminuria in 8 weeks. Cilnidipine significantly suppressed the development of albuminuria. The effect was accompanied by an increase in glomerular desmin staining, a marker of glomerular podocyte damage. On the other hand, nifedipine failed to prevent the development of albuminuria or the increase in glomerular desmin staining.

Conclusions: These data suggest that cilnidipine suppressed the development of doxorubicin-induced nephropathy more efficiently than nifedipine does, possibly through inhibiting N-type calcium channel in mice.

OS 32-07 LEVOROTATORY-AMLODIPINE PLUS FLUVASTATIN CONFER ADDITIVE EFFECTS ON RETARDING ENDOTHELIUM-MESENCHYMAL TRANSITION THROUGH SUPPRESSING ROCK1 EXPRESSION

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Objective: To investigate the effects and mechanisms of levorotatory (L)-amlodipine combined fluvastatin treatment on endothelium-mesenchymal transition (EndMT), a process related to hypertension development.

Design and method: Human umbilical vein endothelial cells (HUVECs) were used and divided to six groups based on different therapeutic strategies as follows: Blank Control, angiotensin-II (Ang-II), Ang-II+ rho-associated kinase (ROCK) inhibitor, Ang-II+ L-amlodipine, Ang-II+ fluvastatin, and Ang-II +L-amlodipine+ fluvastatin groups. Twenty-four hours later, HUVECs were collected for immunofluorescence staining and western blot analyses to evaluate EndMT, and for flow cytometry to analyze cells apoptosis.

Results: Compared to Blank Control group, ROCK1 expression was significantly increased and phosphorylated endothelial nitric oxide synthase (p-eNOS) and eNOS expressions were profoundly reduced in Ang-II group. Immunofluorescence staining revealed that CD31 expression was down-regulated and a-SMA expression was up-regulated in HUVECs in Ang-II group. Western blot showed that endothelium-specific markers (CD31 and VE-cadherin) were significantly diminished and fibroblasts-specific markers (a-SMA and FSP-1) were significantly increased by Ang-II stimulation. Both L-amlodipine and fluvastatin were beneficial to improve these adverse changes, and these favorable effects were further enhanced by L-amlodipine combined fluvastatin treatment. HUVECs apoptosis induced by Ang-II were mitigated by both L-amlodipine and fluvastatin, and this benefit was further promoted by L-amlodipine combined fluvastatin treatment. Notably, all these improvements achieved by L-amlodipine combined fluvastatin treatment were comparable to those of ROCK1 inhibitor.

Conclusions: Ang-II is a significant contributor to EndMT via increasing ROCK1 expression and L-amlodipine combined fluvastatin treatment confers additive effects on improving EndMT and endothelium apoptosis, which may be beneficial for preventing hypertension development.

OS 32-08 SYMPATHETIC NERVE ACTIVITY AND THE LOW RENIN PHENOTYPE: THE SABPA STUDY

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Objective: Hypertension, particularly in black populations is often accompanied by low renin levels, indicative of possible blood pressure (BP) dysregulation by the renin-angiotensin-system (RAS). The potential role of aldosterone in sympathetic nerve activation in the context of low renin warrants clarification. We therefore explored whether measures of sympathetic nerve activity (noradrenaline, 24-hour heart rate (HR) and % dipping in night-time HR) relate to renin, aldosterone and aldosterone-to-renin ratio (ARR) in low renin blacks and whites.

Design and method: We included black (N = 162) and white (N = 206) participants, stratified by low and high renin status, and focused on the low renin groups. We measured 24-hour BP, HR and calculated night-time dipping. We determined renin and aldosterone in plasma and calculated ARR. Noradrenaline and creatinine were determined in urine and noradrenaline: creatinine ratio calculated.

Results: More blacks had low renin (80.9%) compared to whites (57.8%) ($P < 0.001$). In univariate and after multivariate analysis the following significant associations were evident only in low-renin blacks: noradrenaline: creatinine ratio associated positively with aldosterone ($\beta = 0.32$, $P = 0.001$), 24-hour HR associated positively with renin ($\beta = 0.17$, $P = 0.041$), while HR dipping associated negatively with aldosterone ($\beta = –0.30$, $P = 0.001$) and ARR ($\beta = –0.23$, $P = 0.010$). No significant findings were obtained in low-renin whites.

Conclusions: In a black low-renin population, the observed associations of sympathetic nerve activity indices and components of the RAS suggest that higher aldosterone levels relative to renin may have detrimental effects on the cardiovascular system and that the effects of aldosterone may be coupled to sympathetic nerve activity.

OS 32-09 THE BENEFICIAL EFFECT OF LOSARTAN ON PLATELET AGGREGATION RESULTS FROM THE REDUCTION OF ENDOGENOUS ASYMMETRIC DIMETHYL ARGININE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Objective: Previous studies show that platelet aggregation and asymmetric dimethyl arginine (ADMA) are increased in hypertensive patients and animals.

ANNEXURE D

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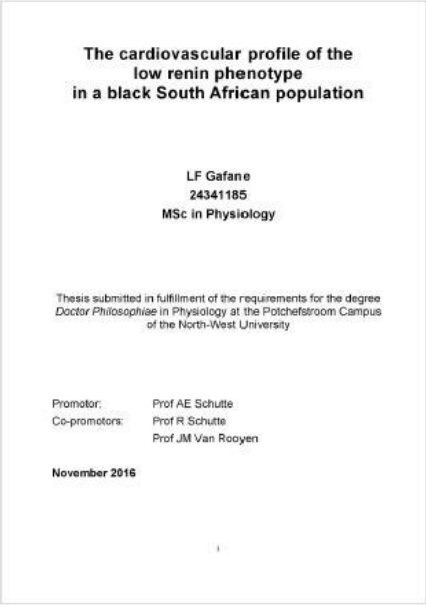

The cardiovascular profile of the low renin phenotype in a black South African population

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Thesis submitted in fulfillment of the requirements for the degree *Doctor Philosophiae* in Physiology at the Potchefstroom Campus of the North-West University

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