

Chronic depression symptoms, hypertension and renal impairment in a bi-ethnic sex cohort: the SABPA study

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Dissertation submitted in fulfillment of the requirements for the
degree *Magister Scientiae* in Physiology at the Potchefstroom
Campus of the North-West University

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November 2016

Dedicated to my parents, for their unwavering love and support.

Everything I have done has only been done through the grace of God.

I would also like to thank the following:

Prof L Malan, my supervisor and mentor, who believed in me even when I did not. Thank you for reminding me of the importance of tempering science with humanity.

Prof NT Malan, who reminded me that statistical results are meaningless without critical thought. Thank you for the serenity and kindness you added to my chaos. I am a better researcher for it.

Mrs M Cockeran, thank you for guiding me through the theory behind statistics and for taking the time to explain the hidden mechanics when it seemed that statistics blurred into the realm of magic.

Everyone at Physiology and HART for all their kind support, wisdom and advice. In particular, Prof A Schutte, for her kindness and generosity in my hour of need.

Family and friends, for all their support and understanding in my long absences. Thank you for humoring my countless renditions of physiological mechanics. I'd like to especially thank everyone who contributed advice with such sincerity as if this project was as dear to your hearts as it is to mine. Willem van der Merwe, Monica Young, Kobus Scheepers, Hermoine Venter, Robyn Hyslop and Annemarie Wentzel, thank you for not making me do this alone. Cindy Smith, thank you for carrying me that last bit, you made my world a better place.

It doesn't stop being magic just because you know how it works. – Terry Pratchett

Summary

Title: Chronic depression symptoms, hypertension and renal impairment in a bi-ethnic sex cohort: the SABPA study.

Motivation: Although elevated renin levels are associated with volume-loading hypertension, African ethnicities are prone to develop low-renin hypertension. Chronic depressive symptoms were associated with vascular dysfunction and may disrupt the renin-angiotensin-aldosterone-system (RAAS). This may underscore a plausible mechanism for the association between depressive symptoms, sensitization of the RAAS in facilitating hypertension and renal dysfunction.

Objectives: The aim of this study was to investigate prospective changes and independent associations between depression symptoms, RAAS mediators (renin, aldosterone), blood pressure and estimated glomerular filtration rate (eGFR) in a bi-ethnic sex cohort from South Africa.

Methodology: This sub study forms part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, conducted in 2008 and 2009. The study sample consisted of 359 participants with comparable socio-economic status, who participated in both legs of sampling. All users of hypertensive medication were excluded, as were participants diagnosed with diabetes or HIV infection. The final study population thus consisted of 195 participants (33 black men, 54 white men, 35 black women, and 73 white women). The study was approved by the Ethics Review Board of the North-West University and adhered to the requirements set in the Declaration of Helsinki.

Clinical measurements included ambulatory blood pressure (ABPM) and electrocardiogram (ECG) measures using the Cardiotens CE120® and interpreted with the Cardiovisions 1.19 software. A psychological battery of questionnaires was administered under supervision of

registered psychologists. The battery included the Patient Health Questionnaire-9 (PHQ-9) used to determine the number of depression symptoms in participants. Physical activity recorded total energy expenditure (kcal/24h), considering resting metabolic rate and body surface area (m²) measurements were also obtained.

An 8-hour overnight midstream urine sample was collected to determine estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease Study equation. Fasting blood samples were collected by a registered nurse. All blood and urine samples were dealt with and stored according to secure standardized methods. Serum samples were analyzed for creatinine, glucose, and cholesterol, serum C-reactive protein (CRP), and gamma glutamyl transferase (γ -GT), serum cotinine levels, and glycosylated A_{1C} (HbA_{1C}). Plasma renin and plasma aldosterone were also measured.

Statistica 13.0 and IBM SPSS v 23 were used for all statistical analyses of the data. *A priori* covariates included age, body surface area, physical activity γ -GT and cotinine. *A priori* hypotheses were tested for all cardiovascular risk markers independent of baseline *a priori* and respective baseline risk markers. Normal distribution of data was tested, and box-cox logarithmic transformations were done for physical activity, cotinine, γ -GT, CRP, estradiol, renin, aldosterone, eGFR, PHQ, SBP, DBP and pulse pressure (PP) data.

Independent *t*-tests and Chi-square tests were used to describe the study population. Chi-square tables determined prevalence, while dependent sample *t*-tests determined differences over time. McNemar chi-square equations were used to calculate Odds Ratios and obtain p-values.

Multiple linear regression analyses were computed to determine the adjusted associations of changes over time in depressive symptoms, BP and eGFR in the bi-ethnic sex cohort, independent of baseline *a priori* covariates, estradiol, and baseline values of the respective cardiovascular risk markers. Statistical significance level was set at $p < 0.05$ (two-tailed).

Optimal cut points 1) of depression symptoms associated with chronic diastolic hypertension (DBP-HTN) and 2) of renin values associated with moderately severe depression were computed from the maximum of the Youden index (J) (sensitivity + specificity – 1) using non-parametric receiver operating characteristic (ROC) curves. The statistical significance level was set at $p \leq 0.05$ (two-tailed).

Results: Africans (Blacks) had more depressive symptoms and a mean hypertensive state, as well as lower plasma renin levels than Caucasians (Whites). Ethnic differences adjusted for *a priori* covariates were apparent for depressive symptoms ($F_{1, 198} = 24.529$), eGFR ($F_{1, 198} = 14.360$), SBP ($F_{1, 198} = 13.929$), DBP ($F_{1, 198} = 13.998$), and renin ($F_{1, 196} = 11.286$) ($p < 0.001$).

Forward stepwise multiple regression analyses and receiver operating characteristics were used to assess associations. An inverse association between depression and renin levels [Adj R^2 0.38; β -0.27 (-0.5, -0.1), $p = 0.009$] with [AUC=0.61 (0.47-0.74); sensitivity/specificity 63.6/61.0%] was found in Blacks only. Chronic depression symptoms were associated with chronic DBP-HTN [AUC =0.58 (0.45-0.72); sensitivity/specificity 72.4/46.2%]. Similar findings were not traced for the White cohort.

Conclusions: Chronic depression symptoms may desensitize the RAAS by maintained activation of central neural control centers. Neural control may compensate by lowering renin and improving renal function to protect against volume-loading hypertension in Blacks. These findings emphasize the impact of depression on low renin and hypertension in Blacks in terms of prevention, diagnosis and treatment.

Chronic depression symptoms predicted chronic 24-hour DBP-HTN and may suggest a desensitization of the RAAS over time. Protection by central neural control centers may lower renin levels to protect against volume-loading hypertension and renal impairment.

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Nomenclature

24h	24 hour
ACE	angiotensin converting enzyme
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
ABPM	ambulatory blood pressure measurement
ANCOVA	analysis of co-variance
ARR	aldosterone-renin ratio
BP	blood pressure
BSA	body surface area
CI	confidence interval
CRP	C-reactive protein
CVD	cardiovascular disease
DBP	diastolic blood pressure

DBP-HTN	diastolic hypertension
DSM-IV-R	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
ECG	Electrocardiogram
ECLA	electrochemiluminescence immunoassay
eGFR	estimated glomerular filtration rate
GFR	glomerular filtration rate
HART	Hypertension in Africa Research Team
HPAA	hypothalamic-pituitary-adrenal axis
HPLC-MS	high-performance liquid chromatography mass spectrometry
IQR	interquartile range
IRMA	immunoradiometric assay
LVH	left ventricular hypertrophy
MDRD	modification of diet in renal disease

MUSA	Medicine Usage in South Africa
NO	nitric oxide
NRF	National Research Foundation
OR	odds ratio
PHQ-9	Patient Health Questionnaire-9
PP	Pulse pressure
PRA	plasma renin activity
RAAS	renin-angiotensin-aldosterone system
RMR	resting metabolic rate
ROS	reactive oxygen species
SABPA	Sympathetic activity and Ambulatory Blood Pressure in Africans
SARChI	National Research Foundation South African Research Chair Initiative
SBP	systolic blood pressure

SD	standard deviation
SI	Système International
SNS	sympathetic nervous system
TEE	total energy expenditure
γ -GT	γ -glutamyl transferase

Chapter 1: Preface

1.1 Foreword

This dissertation is submitted in fulfillment of the requirements for the degree *Magister Scientiae* in *Physiology* at the Potchefstroom Campus of the North-West University. The peer-reviewed journal: *Stress: The International Journal on the Biology of Stress* has been considered for publication and the article and supporting dissertation is thus presented in the prescribed format of the journal. Relevant citations and references appear at the end of each chapter as applicable, and are set out in the bibliographic style of the aforementioned journal as stipulated in Chapter 3 under the title *'Instructions for Authors'*.

1.2 Outline of the Dissertation

This document is divided into four chapters, as follows:

Chapter 1 consists of the preface, the outline of the dissertation, the authors' contributions and the skills acquired by the student during study.

Chapter 2 consists of the introduction, the literature study to supply brief and relevant background, as well as research motivation, aims, objectives, and hypotheses.

Chapter 3 denotes the relevant author instructions as prescribed by the journal: *Stress: The International Journal on the Biology of Stress*, and further comprises of the manuscript for publication: "*Chronic depression symptoms, 24h diastolic hypertension and low renin levels in a Black African sex cohort: the SABPA study.*"


Chapter 4 consists of the conclusions and limitations of the study, implications of the study and recommendations for future research.

1.3 Authors' contributions

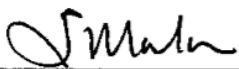
The researchers involved in this study contributed in the following ways:

- ❖ Miss AC De Vos (Hons BSc) was responsible for the writing of this dissertation, which included the literature study, statistical analyses and the interpretation of the results.
- ❖ Prof L Malan (RN, HED, PhD) as supervisor and head of the Sympathetic and Ambulatory Blood Pressure in Africans (SABPA) study, contributed to the study design and data collection, and also provided supervision and guidance for the dissertation.
- ❖ Prof NT Malan (DSc), as co-supervisor, contributed to data collection and assisted in the planning, writing, and review processes of this dissertation.
- ❖ Mrs M Cockeran (MSc), as co-supervisor provided statistical consultation and supervision for all statistical analyses, and assisted in reviewing this dissertation.


Herewith I, Arnoldeen Christien de Vos, student number 20673914, declare the aforementioned an accurate reflection of my contribution and hereby consent to the inclusion of this manuscript in this dissertation for the degree *Magister Scientiae* in *Physiology*.


Miss AC De Vos (Hons BSc)

The co-authors of the manuscript in Chapter 3, we the undersigned, hereby give permission that the manuscript '*Chronic depression symptoms, 24h diastolic hypertension and low renin levels in a Black African sex cohort: the SABPA study*' may form part of this dissertation for the degree *Magister Scientiae* in *Physiology* by Arnoldeen Christien de Vos.


Prof L Malan (RN, PhD)


Mrs M Cockeran (MSc)


Prof NT Malan (DSc)

Chapter 2: Introduction, Literature study, Background, and Research Motivation

2.1 General introduction

Blacks have been noted to be markedly susceptible to vascular adrenergic responses and low-renin hypertension [1]. This can possibly be due to a variety of contributing factors such as environmental factors, increased sodium reabsorption, genetics, and attenuated renin secretion responses [2], although the exact mechanisms and causes are still widely disputed [2-4].

To date, no comprehensive national registry exists to report the prevalence of non-communicable diseases of the South African population. Epidemiological studies in South Africa lack the data to sufficiently portray exact, in-depth representative patterns in different population groups. This scarcity of research available may be attributed to population distribution dynamics, as well as under-equipped health care and research facilities [5].

Global mortality and morbidity trends have shown a notable shift in prevalence from communicable diseases to non-communicable diseases in recent years [6]. These non-communicable diseases such as cardiovascular dysfunction [7], hypertension, as well as type 2 diabetes, are often considered 'lifestyle diseases'. Lifestyle diseases may also be the result of an unbalanced diet, excessive alcohol consumption [8], smoking, sedentary lifestyle and exposure to chronic stress [9], in addition to *a priori* confounder such as age and gender [10]. Behavioral alterations and psychosocial stress often originate from globalization and urbanization. However, increased emotional demands on individuals could contribute to the rise in prevalence of lifestyle diseases [6].

In a meta-analysis involving under-developed and developing countries, South Africa was rated as one of the countries with the highest hypertension prevalence rates [5], with alcohol abuse often used as a coping mechanism for emotional stress, being one of the most significant predictors of hypertension in Blacks [11]. Emotional stress as experienced in an urban-dwelling

environment induces sympathetic hyperactive responses [12, 13] and might reflect in sensitization of the renin-angiotensin-aldosterone system (RAAS).

2.2 Chronic depression

Chronic exposure to a stressful lifestyle, as is often the case in an over-demanding urban environment, has been linked to the development of depression [14]. Indeed, an urban-dwelling environment was associated with enhanced vascular reactivity, a decrease in plasma renin activity, and perception of poorer health values in a Black male cohort [15]. Depression, a common affective disorder, has also been associated with cardiovascular morbidity and mortality in the western world [16]. Due to the difficulties presented in finding a clear, quantitative, empirical marker, depression is often dismissed in clinical studies. However, both clinical and sub-clinical depression has been shown to have many physiological manifestations, including several mechanistic and even structural system disruptions [17]. Coping with chronic psychosocial stress causes higher metabolic demands and induces sympathetic nervous system dysfunction, neural and adrenal fatigue [18], as well as depression [19, 20], thereby explaining the link between mental health and sympathetic nervous system function.

Depression symptoms score can be quantified via the Patient Health Questionnaire-9 (PHQ-9) of Kroenke and Spitzer [21]. This questionnaire is ideal for the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study as it was validated in African cohorts for use in primary health care settings [22, 23]. Each item evaluates the presence of one of the nine Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-R) criteria for major depression. The recommended and established PHQ-9 cut-off point of ≥ 10 may indicate the presence of moderately severe depression symptoms [21].

2.2.1 Depression and sympathetic stimulation

Recently associations between mental stress responses and renin secretion were indicated [24], as well as the direct effect of sympathetic stimulation on renal tubular function [25]. These associations may underscore a plausible mechanism for the interaction between chronic depression, renin activity, vasoconstrictive agents of the renin-angiotensin-aldosterone system (RAAS), such as anti-diuretic hormone (ADH), angiotensin, and aldosterone via angiotensin II [26-29], and volume-loading hypertension [5, 13, 30]. Chronic depression symptoms may promote endothelial dysfunction [31, 32], and may change RAAS mediator sensitivity. Indeed, depression may impact key components in the maintenance of blood pressure and may well be a critical component to many mechanisms of hypertension [33]. Acute mental stress responses reflective of everyday life stress [34] may thus guide renin secretion [8, 24].

2.3. Renin-Angiotensin-Aldosterone System (RAAS)

2.3.1 The RAAS Pathway

The macula densa cells of the distal convoluted tubules orchestrate the release of renin from the juxtaglomerular apparatus in the nephron in response to several stimuli. These stimuli include activation of mechanoreceptors by reductions in blood pressure, stimulation from tunica externa sympathetic nerves, and a low sodium ion concentration in the distal convoluted tubules [35]. Renin catalyzing the cleaving of angiotensinogen to form angiotensin I serves as the rate-limiting step in the resulting RAAS cascade, an intricate enzyme-catalyzed cascade that predominantly mediates long-term fluid-volume retention by altering the rate of filtration and reabsorption of sodium and water in the kidney [35, 36]. Renin travels through the circulatory system and cleaves angiotensinogen as released by the liver (Figure 2-1). This produces angiotensin I, which is then converted by angiotensin converting enzyme (ACE), primarily in the lungs, to the vasoactive peptide, angiotensin II.

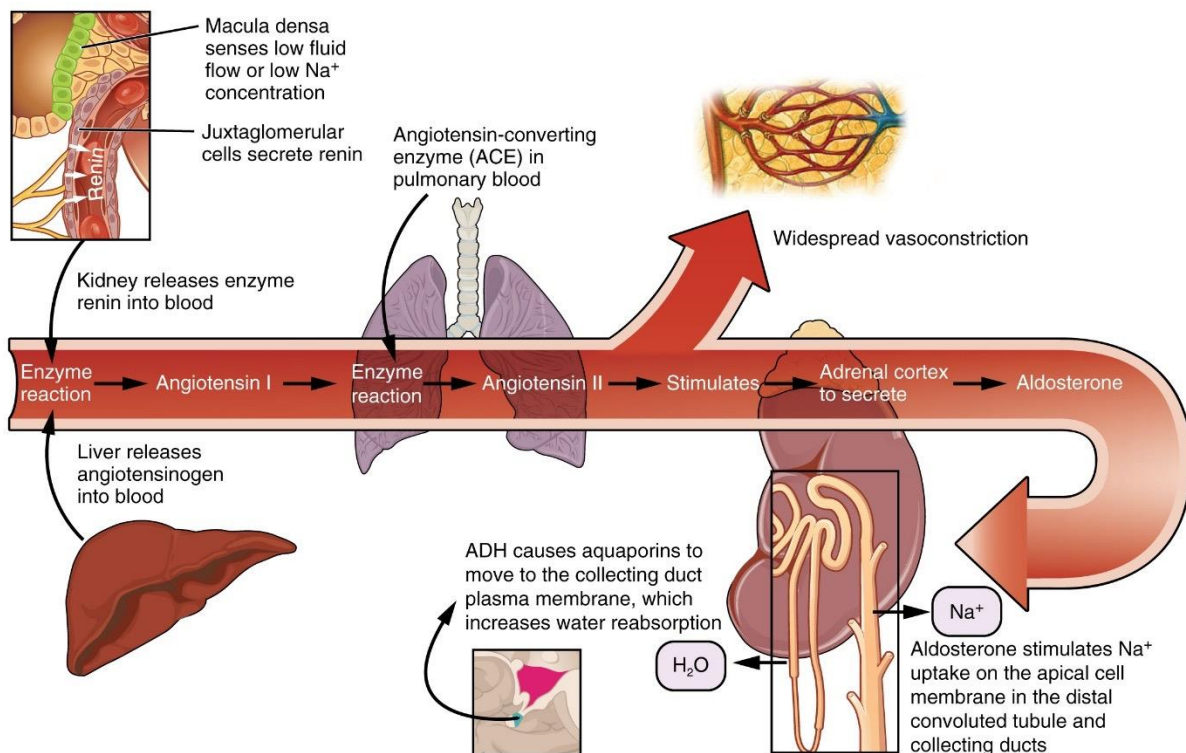


Figure 2.3.1-01: The enzyme renin converts the pro-enzyme angiotensin I; the lung-derived enzyme Angiotensin-converting enzyme (ACE) converts angiotensin I into active angiotensin II. Image courtesy of OpenStax College, Rice University [1]

Aldosterone promotes conservation of sodium in exchange for potassium in the nephron's collecting ducts and distal tubules, which leads to increased water retention. The resultant fluid-volume increase in conjunction with activation of the above-mentioned vasoconstrictive mechanisms leads to an increase in blood pressure [37, 38].

2.3.2 Losing the path – the ethnic discrepancy paradox

Occlusion of the afferent renal artery by cholesterol or calcification will lead to a decrease in blood pressure in the glomerulus, where the reduction in mechanical pressure would stimulate an increase of renin released into the blood. This increase in renin triggers the RAAS cascade which activates mechanisms to increase blood pressure throughout affected systems already mentioned. Hypertension stemming from high renin levels is relatively commonly managed by

the prescription of renin suppressant medication [39]. However, it proves ineffective for the almost paradoxical hypertension in African and African-American Blacks with low renin states, as renin activity is already suppressed [40, 41]. If it is assumed that renin drives increases in blood pressure, then low renin levels should naturally lead to low blood pressure. While there is still much speculation as to the mechanism of low-renin hypertension, there is general consensus that the well-documented and disproportionate increase in aldosterone levels that accompany low-renin hypertension is a crucial part of the mechanism [42, 43]. Suppression of renin secretion during a state of elevated blood pressure is a natural defense mechanism that protects the body from the adverse effects of sustained high blood pressure [4]. Thus leading to the postulation that chronically elevated blood pressure is at least in part responsible for the suppression of renin if renin no longer mediates blood pressure (BP), and may maintain this cycle once renin regulation of BP has been compromised. However, the rising rate of hypertension prevalence indicates that there also might be additional factors responsible for the hyper- and/or hypo-secretion of renin [8].

The dimorphic disparity may be augmented by a variety of contributing factors such as environmental factors, increased sodium reabsorption, genetics and attenuated renin secretion responses [2]. Regardless, this physiological occurrence is largely attributed to a result of downstream mediators of the RAAS such as angiotensin II and aldosterone, although the exact mechanism remains unclear [44]. To maintain homeostasis, renin secretion will be suppressed [2, 44], resulting in low-renin hypertension [35]. Low renin values are however related to greater vasoconstriction, and resulting vascular load, despite enhanced renin responses. Indeed, low PRA levels have been associated with a suboptimal intake of Ca^{2+} that suppresses Ca^{2+} -ATPase mediated Ca^{2+} efflux with resulting increases in intracellular Ca^{2+} (Ca_i), vascular resistance and hypertension [40].

2.3.3 Aldosterone-to-Renin-Ratio

The aldosterone-to-renin ratio (ARR), as mediator of the RAAS system, may be a way to evaluate dysfunction in the RAAS system [45-47]. Renin acts as the rate-limiting step of the RAAS cascade, suggesting aldosterone, one of the more potent effectors of the RAAS cascade, to be proportionally mediated by renin activity. However, aldosterone activity may be enhanced independently of renin activity, through the influence of factors such as sympathetic nervous system stimulation, baroreceptor activity, plasma sodium and potassium concentrations and adrenocorticotrophic hormone (ACTH) [48, 49]. Abnormal ARR may thus be indicative of various dysfunctions associated with renal modulation. As such, ARR is often used as a diagnostic tool in testing for primary aldosteronism, as well as for essential or primary hypertension, often associated with renal dysfunction [5, 43, 45, 47, 50-52]. ARR is currently an accepted method of measurement for RAAS dysfunction, especially in the detection of primary aldosteronism. It should, however, be noted that measurements may be compromised by certain factors such as pregnancy, or use of hypertensive medications, diuretics, or other interfering agents [53].

2.4 Renal impairment

2.4.1 Regulation by the RAAS (hormonal)

Exhaustion of psychophysiological reserves by continued experience of a chronic depressive state-induced increase in sympathetic activity [54, 55] and subsequent stimulation of innervated tissues such as the hypothalamic-pituitary-adrenal axis (HPAA) [33, 34, 56, 57]. HPAA stimulation in turn increases secretion of corticotrophin releasing hormone [58, 59], antidiuretic hormone (ADH) [60] and ACTH [61, 62] as well as downstream aldosterone secretion in the adrenal cortex [63, 64]. Collectively, this activation of downstream hormonal RAAS mediators functions to increase mean arterial pressure [35, 36, 44]. Elevated blood

pressure in the kidney serves to increase the glomerular filtration rate (GFR), thereby suppressing the production of renin by the juxtaglomerular cells, while the downstream mediators of the RAAS system remain active in stimulating fluid retention and maintaining “elevated” blood pressure levels [24, 35, 65].

2.4.2 Glomerular filtration rate and endothelial dysfunction (nephron)

Various studies have found associations between depressive symptoms and endothelial dysfunction [13, 19, 57, 66, 67], suggesting that a depressed emotional state has direct and indirect effects on endothelial dysfunction. Endothelial dysfunction negatively affects renal function estimated glomerular filtration rate (eGFR) on both systemic and glomerular level. By compromising renal function, blood pressure regulatory mechanisms are disrupted, and the resultant chronic elevation in mean arterial pressure further degrades endothelial function and, consequently, vascular health [68-71]. Endothelial structure and function differ depending on location in the body, as the endothelial layer is specialized to offer varying levels of protection, permeability, structural support, vasomotor reactivity and endocrine function to meet the requirements of each role. While the endothelium may functionally adapt to alterations in its environment, sustained strain may lead to structural changes and, thereby, compromising function [72, 73]. Systemic endothelial dysfunction caused by chronically elevated blood pressure entails functional and structural changes that reinforce the vasculature to withstand pressure at the cost of distensibility and elasticity, which again increases blood pressure [74]. The glomerular vascular wall consists of an endothelial layer permeated by fenestrae, a basal layer and a podocyte layer. These structures act synergistically to comprise the functional barrier-mediating permeation, where damage or loss of the integrity of any layer results in dysfunction of the glomerular capillary wall [68]. The kidney has the largest total endothelial surface area of any organ, and endothelial dysfunction at the renal level promotes a positive

feedback loop that increases renal endothelial dysfunction, while also affecting blood pressure and thus promoting cardiovascular dysfunction [74]. Linking emotional distress to renal function still remains to be investigated in Africans. Recent findings of HPAA hypo-activity associated with increased albumin-creatinine ratio and decreased eGFR in an African male cohort were enhanced by coping disability [56]. These findings underscore the need to show the link between depression, RAAS and renal function.

2.4.3 Fluid retention

The mechanism of fluid volume-regulation as discussed in the text can be summarized as presented in figure 2-1.

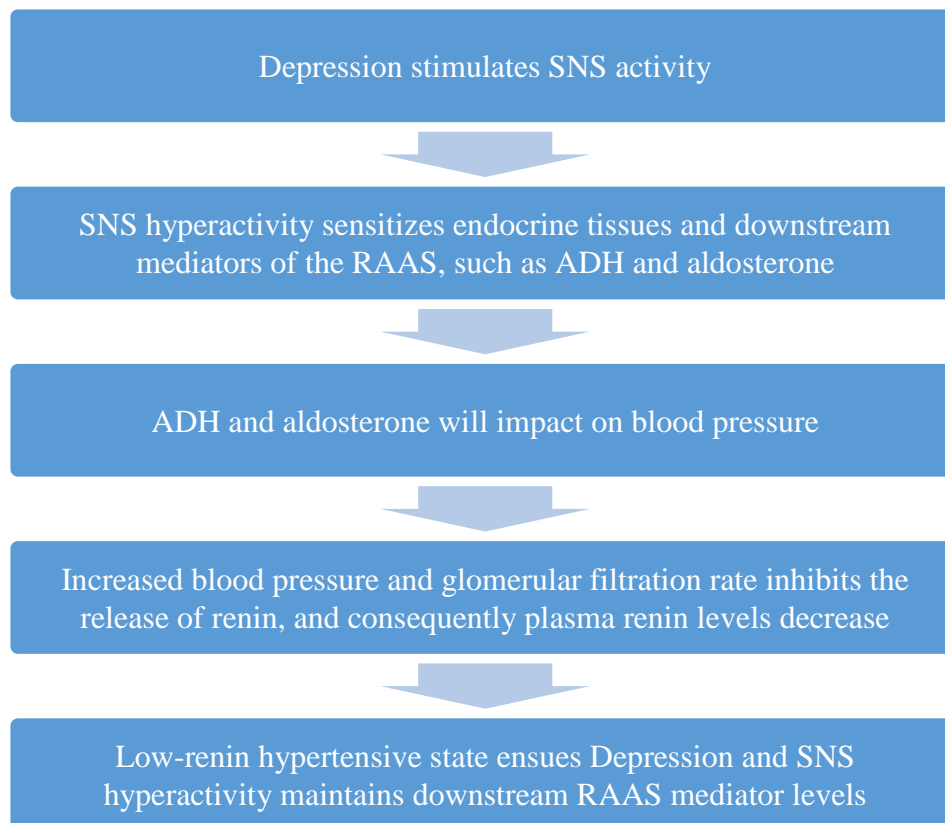


Figure 2-1: Integrated flow diagram outlining fluid volume-regulation. Where SNS, sympathetic nervous system; RAAS, renin-angiotensin-aldosterone system; ADH, anti-diuretic hormone.

2.5 Cardiovascular impact

2.5.1 Depression and cardiovascular disease

Both clinical and sub-clinical depression has been linked to a higher incidence of cardiac events in individuals with cardiovascular disease (CVD) and in healthy populations [57, 75]. In Africans, symptoms of depression have been associated with an increase in cardiovascular risk and structural wall remodeling [2, 19, 76]. Several mechanisms have been proposed as possible mediators in the depression-CVD association in Africans, including sympathetic hyperactivity and metabolic factors / changes [18, 76]. Additionally, hypertension has been linked to detrimental health such as end-organ damage [77]; specifically left ventricular hypertrophy [78], renal failure [79], aneurysm, heart failure, stroke [80], and decline of cognitive function [81].

2.5.2 Vasculature and endothelial dysfunction

The mediators for the RAAS further suppress vasodilation by affecting the synthesis of nitric oxide (NO), a potent vasodilator, as follows: Angiotensin II binds to AT-1 receptors expressed on vascular endothelial cell surfaces, down-regulating the synthesis of NO. ACE also has the additional function of degrading bradykinin, required for nitric oxide synthesis [82, 83]. Thus the reduced NO bioavailability, coupled with stimulation of AT-1 receptors on vascular smooth muscle cells, results in an overall increase in vasoconstriction by impeding the vasodilatory mediator, NO [83, 84]. Furthermore, angiotensin II activation of AT-1 receptors simultaneously stimulates increased aldosterone synthesis and secretion in the adrenal glands [84, 85].

Sustained activation of the sympathetic nervous system will have additional consequences mediated by AT-1 stimulation including reactive oxygen species (ROS) formation, fibrosis [36], increased ADH secretion [86], vascular smooth muscle cell proliferation, and

inflammatory responses [87]. Recent findings by Hamer et al. in 2011 showed that exposure to acute rather than chronic mental stress [88] elicited an elevated release of renin which was associated with a marker of sub-clinical atherosclerosis [24]. The study further proposed that mental stress exposure and subsequent sympathetic stimulation may act as a potential mechanism of the increased burden of CVD in urbanized Africans [24].

2.5.3 End-organ damage

Given the complex nature of factors contributing to hypertension, the distinction is not always drawn between low-renin hypertension and other variants of hypertension by studies assessing the damage resulting from hypertension. Augmented plasma renin and aldosterone levels have been speculated to be a causative factor in primary hypertension [5]. Conversely, low renin levels (hypo-renin) in Africans and African-Americans [40] have been linked to end-organ damage [4]. It has been speculated that black African men are more prominently at risk for hypertension [89], hypertension-related target organ damage [90, 91] and cardiovascular disease [30]. Hypertension-related target organ damage includes renal dysfunction [4, 37], left ventricular hypertrophy (LVH) [19], cardiac wall remodeling [92], as well as silent ischemic events [66].

Black men, compared to White men, in addition to presenting a higher prevalence of low-renin hypertension [93, 94], have also been found to be more susceptible to hypertension-related target organ damage, including renal dysfunction (eGFR) and silent ischemic events [4, 19, 37, 91]. Susceptibility increases as various studies found that the Black men from the SABPA study are more vulnerable to cardiometabolic diseases and hypertension compared to Black women or Whites [5, 15, 18, 19, 24, 40, 44, 56, 95].

2.6 Research Motivation

Cardiovascular disease is becoming increasingly more prevalent in the black population of South Africa [96]. While this phenomenon is currently attributed to the population dynamics of a large-scale transition of Black people from rural to urban environments, further research is required to confirm the current findings and discern the specific causes that lead to this increase [97]. Despite a significant correlation demonstrated between plasma renin levels and systolic blood pressure [24], little is known about the effect of depression symptoms on renin levels, blood pressure and renal function in South African populations. A need exists to examine whether chronic depression symptoms could predict hypertension or renal impairment in South African populations. To date, no studies have been conducted on the South African population to investigate the possible correlation between chronic depressive symptoms, blood pressure and renal impairment. This sub-study will therefore endeavour to contribute to the knowledge regarding increased prevalence of hypertension in an urban-dwelling bi-ethnic sex cohort by investigating renin levels when more depressive symptoms are apparent and the consequences thereof on renal impairment.

2.7 Questions arising from literature

2.7.1 Will changes in depression symptoms be associated with renin-, blood pressure- and renal function levels over a time period of three years amongst a bi-ethnic sex cohort from the SABPA study?

2.7.2 Will chronic depression symptoms predict hypertension and renal impairment amongst a bi-ethnic sex cohort from the SABPA study?

2.8 Aim and objectives of the study

2.8.1 Aim

The aim of this sub-study was to investigate prospective associations between depression, blood pressure and renal function over a time period of three years in a bi-ethnic sex cohort from South Africa.

2.8.2 Objectives

2.8.2.1 To determine depression symptoms, blood pressure, RAAS mediators (renin and aldosterone) levels and renal function differences between Blacks and Whites over a time period of three years.

2.8.2.2 To determine the possible associations between depression symptoms, RAAS mediators (renin and aldosterone) levels, blood pressure and renal function in Blacks and Whites.

2.9 Hypotheses

2.9.1 Depression symptoms will be positively associated with blood pressure in the Black participants of the SABPA study.

2.9.2 Depression symptoms will be positively associated with ARR and inversely associated with renal function among the black participants of the SABPA study.

2.9.3 Chronic depression symptoms will predict hypertension and renal impairment in the black participants of the SABPA study.

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Chapter 3: Manuscript for publication

Chronic depression symptoms, 24h diastolic hypertension and low renin levels in a Black African sex cohort: the SABPA study.

Instructions for Authors: *Stress: The International Journal on the Biology of Stress*

The complete Guide for Authors for the journal: *Stress: The International Journal on the Biology of Stress* may be accessed at the URL: <http://www.tandfonline.com/action/authorSubmission?journalCode=ists20&page=instructions>

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Stress: The International Journal on the Biology of Stress requires that studies involving humans be approved by an institutional review board, in accordance with approved published guidelines, prior to actually performing the research and publishing the data. This approval should be explicitly stated in the methods section

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Your paper should be compiled in the following order: title page with author details; abstract (not more than 250 words); 6 keywords; main text; acknowledgments; declaration of interest (disclosure) statement; references (listed alphabetically by first author surname); appendices/supplemental material (as appropriate).

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Abstract: A non-structured abstract of no more than 250 words. This should contain the rationale for the study, the hypothesis that was tested, and give some detail about what was done; state the sex of the subjects and numbers per groups; state how effects were measured. Abbreviations should be defined as first used in the Abstract, and on first use in the main text. Single words should not be abbreviated. The impact of the results for the field should also be summarized.

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Please choose American spelling style consistently throughout your manuscript.

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- **Two authors:** Prokai-Tatrai K, Prokai L. Modifying peptide properties by prodrug design for enhanced transport into the CNS. *Prog Drug Res*. 2003;61:155–188.
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- **No author:** Pelvic floor exercise can reduce stress incontinence. *Health News*. 2005;11(4):11.
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Funding details. Please supply all details required by your funding and grant-awarding bodies as follows:

For single agency grants: This work was supported by the [Funding Agency] under Grant [number xxxx].

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Chronic depression symptoms, 24h diastolic hypertension and low renin levels in a Black African sex cohort: the SABPA study.

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Abstract

Although elevated renin levels in Western populations are associated with volume-loading hypertension, African ethnicities are prone to develop low-renin hypertension. Chronic depressive symptoms, associated with vascular dysregulation, may reflect a disturbed renin-angiotensin-aldosterone-system (RAAS). We aimed to investigate prospective changes in depression symptoms, RAAS (renin, aldosterone), blood pressure and estimated glomerular filtration rate in a bi-ethnic sex cohort. Socio-economically similar participants (43.7 ± 9 years) participated in both legs of sampling of this study. Participant exclusion criteria comprised the use of hypertension medication, as well as a diagnosis of diabetes or human immune deficient virus (HIV) infection. [N=195: 68 Blacks (33 males, 35 females); 127 Whites (54 males, 73 females)] Depression symptoms (PHQ-9), 24h blood pressure measurements, fasting blood samples were obtained. Blacks displayed higher prevalence of diastolic hypertension (DBP-HTN). Linear regression analyses and receiver operating characteristics showed an inverse association between chronic depression and renin levels [Adj R^2 0.38; β -0.27 (-0.5, -0.1), $p = 0.009$] with [AUC=0.61 (0.47-0.74); sensitivity/specificity 63.6/61.0%] in Blacks only. Chronic depression symptoms were associated with chronic DBP-HTN [AUC =0.58 (0.45-0.72); sensitivity/specificity 72.4/46.2%]. Similar findings were not found for the White cohort. Chronic depression symptoms desensitized the RAAS by activating central neural control centers by lowering renin and improving renal function to protect against volume-loading hypertension in Blacks. These findings emphasize the impact of depression on low renin and hypertension in Blacks in terms of prevention, diagnosis and treatment.

Keywords: *Depression symptoms; renin; renal impairment; diastolic hypertension; urban men; ethnicity.*

Introduction

Taxing emotional demands may exhaust the psychophysiological resources in an effort to cope with these demands, leading to sympathetic nervous system dysfunction, neural and adrenal fatigue, [1] and depression [2, 3]. Both clinical and sub-clinical depressive states have been linked to a higher incidence of cardiac events in individuals with existing cardiovascular disease (CVD) as well as in healthy populations [4]. In Africans, symptoms of depression were shown to be associated with an increase in cardiovascular risk and structural wall remodeling [2]. Consequently, several mechanisms have been proposed as possible mediators in the depression-CVD association in Africans, including sympathetic hyperactivity and metabolic changes [1, 5].

Sympathetic hyperactivity and the afflicted functionality of the renin-angiotensin-aldosterone system (RAAS) may underscore a plausible mechanism for the association between chronic depression, renin activity as trigger enzyme, vasoconstrictive agents, and volume-loading hypertension [6-8]. Black men are at higher risk for hypertension and cardiovascular disease (CVD) compared to Caucasian men, due to seeking social support in an urban environment which is not forthcoming to enable effective coping [5]. Indeed, the demands of an urban-dwelling environment were associated with enhanced vascular reactivity, decreased plasma renin activity and perception of own poorer well-being in a Black male cohort [9].

Augmented plasma renin and aldosterone levels may contribute to essential or primary hypertension [6], while low renin levels in Africans and African-Americans have been linked to target-organ damage [10]. Blacks are more susceptible to hypertension-related target organ damage including renal dysfunction, left ventricular hypertrophy (LVH), as well as silent ischemic events, than are Whites [2, 10-14]. This is largely a result of downstream signaling in

the RAAS, such as angiotensin II and aldosterone, although the exact mechanism is still unclear [15]. Estradiol has however been implicated RAAS regulation [16].

Another suggestion is that stress activates sympathetic activity and subsequent stimulation of the hypothalamic-pituitary-adrenal axis (HPAA), increasing secretion of corticotrophin releasing hormone, antidiuretic hormone (ADH) and adrenocorticotrophic hormone (ACTH) from the HPAA [5, 7, 13]. Physiologically there is evidence to suggest that sympathetic activity may increase ADH and downstream aldosterone secretion in the adrenal cortex and ultimately increasing mean arterial pressure [17-19]. Subsequently, higher blood pressure will increase the estimated glomerular filtration rate (eGFR) in the kidneys, thereby decreasing the production of renin by the juxtaglomerular cells. Despite the decrease in renin levels, the levels of downstream mediators of the RAAS system remain active in stimulating fluid retention [13].

Whether depressive symptoms sensitize the RAAS system and facilitate volume-loading hypertension, needs to be investigated. We, therefore, aimed at determining whether associations exist between depression symptoms, RAAS markers, blood pressure and eGFR in a bi-ethnic sex cohort.

Methods

Study design

The Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study is a prospective cohort study. Teachers from 43 schools in the Dr Kenneth Kaunda Education District (Klerksdorp and Potchefstroom) in the North-West Province of South Africa, were invited to participate in the study (N=2170). The SABPA study exclusion criteria comprised pregnancy, lactation, users of alpha and beta blockers, psychotropic substance abuse, participants whose tympanum temperatures exceeded 37.5°C, or had been vaccinated or had donated blood three months prior to participation. Preliminary screening identified eligible

participants (N=409) from the respondents according to the SABPA protocol. Only participants who also completed the 3 year follow-up phase were considered for inclusion in the sub-study (N=359).

Use of hypertension medication warranted exclusion (N=127) as per contra-indication for renin analyses [20]. Participants with clinical diagnoses of diabetes and human immune deficient virus (HIV) infection were also excluded (N=23). The final study sample comprised 195 participants for prospective analyses, including Black males (N=33), White males (N=54), Black females (35), and White females (N=73). The participants in the first phase of the study were between the ages of 21 and 62 years with comparable socio-economic status. A description of the protocol is described in detail elsewhere [5].

All participants provided voluntary written consent prior to participation, were ensured complete anonymity and cannot be identified via this paper. The SABPA study has been approved by the Ethics Review Board of the North-West University (Potchefstroom Campus: NWU-00036-07-S6) and adheres to the requirements outlined in the Declaration of Helsinki [21].

General procedure

Clinical assessments were obtained over two days. On a working day (Monday through Thursday), between 07:00–08:00, 24h ambulatory blood pressure (BP) and a 2-lead electrocardiogram (ECG) device, as well as an accelerometer were attached to the participants after which they resumed normal activities. A 24h standardized diet commenced.

At 16:00 the participants were relocated to the Metabolic Unit Research Facility of the North-West University, where they were introduced to the experimental set-up and assigned a private bedroom. A psychological battery was administered under supervision of registered psychologists. Hereafter participants engaged in passive, leisurely recreational activities of

their choice and were requested to remain fasting and go to sleep by 22:00. The ambulatory blood pressure device was removed at 07:30 on the second day after the last BP measurement had been obtained, where after a urine sample was collected, followed by anthropometric assessments. From here on participants were in a semi-recumbent position for at least 45-60 minutes prior to blood sampling obtained from the dominant arm of the subject by a registered nurse using a sterile winged infusion set. All biological samples were securely handled and stored according to standardized procedures.

Lifestyle risk factors

Anthropometric measures included weight, height and circumferences and were obtained in triplicate by qualified personnel. Inter- and intra-observer variability was less than 10% at baseline. Body surface area in (m²) was calculated according to the Mosteller formula [22], total energy expenditure (TEE in kcal/24h), considered Resting Metabolic Rate (RMR), was measured by the Actical® activity monitor (Mini Mitter Co., Inc., Bend). Serum γ -glutamyl transferase (γ -GT) was measured as a marker for alcohol consumption [23], and serum cotinine measurement was taken as a measure of smoking status [24].

Cardiovascular measures

Mean 24-hour systolic and diastolic blood pressure and 2-lead ECG measurements were recorded using the Cardiotens (CE120®, Meditech, Hungary), and programmed to take measurements at 30-minute intervals between 08:00 and 22:00 and in 60-minute intervals between 22:00 and 06:00 [25]. A mean 24-hour systolic blood pressure (SBP) of ≥ 130 mm Hg and/or diastolic blood pressure (DBP) of ≥ 80 mm Hg was classified as hypertension [26]. The mean 24h pulse pressure was defined as 24h SBP – 24h DBP as a measure of arterial stiffness [27] and might impact on arterial tone and wall permeability.

Psychometric questionnaires

The Patient Health Questionnaire-9 (PHQ-9) [28], with an α -Cronbach reliability index: 0.81 in the SABPA study sample was used to determine depression symptoms in participants. The PHQ-9 has been validated in various ethnic groups for use in primary health care settings [29]. Each item evaluates the presence of one of the nine criteria for major depression according to the DSM-IV criteria [30]. The recommended and established PHQ-9 cut-off point of ≥ 10 indicates the presence of moderately severe depression symptoms.

Biochemical measures

The Sequential Multiple Analyzer Computer was used to calculate fasting glucose from sodium fluoride samples. Triglycerides, cholesterol, gamma glutamyl transferase (γ -GT), Cotinine, and HbA_{1C} % levels were obtained from the serum blood samples using the KonelabTM 20i (ThermoScientific, Vantaa, Finland). The Integra 400, Roche from Switzerland was used to perform Turbidometric inhibition immunoassays.

Serum creatinine (for the calculation of eGFR), glucose, and cholesterol were measured using the timed-end-point method, and serum high sensitive C-reactive protein (CRP) was measured using a highly sensitive turbidimetric method (Particle enhanced turbidimetric assay, Cobas Integra 400 plus, Roche, Basel, Switzerland) and γ -GT was measured using an enzyme rate method (Unicel DXC 800, Beckman Coulter, Germany). Serum cotinine levels were determined by means of homogenous immunoassays (Modular ROCHE Automated, Switzerland). Glycosylated hemoglobin A_{1C} (HbA_{1C}), a reflection of average capillary glucose for the preceding three months was measured using a turbidimetric inhibition immunoassay (Cobas Integra 400plus, Roche, Switzerland). Serum estradiol (pmol/l) was measured by electrochemiluminescence immunoassay (ECLA, Roche, Switzerland). Plasma renin was measured using plasma renin III generation kits (August 2008, Model 13) via

Immunoradiometric assay (IRMA), high sensitivity radioimmunoassay for quantitative measurement of active resting renin (IBL Lab, 38T501, MN, USA). Plasma aldosterone was measured with competitive radioimmunoassay (Beckman Coulter) on a Wizard2 Automatic Gamma Counter (PerkinElmer, Waltham, MA). Inter-assay reproducibility was 0.9% - 3.6% for renin, while aldosterone had an intra- and inter-assay variation of 3.14% - 6.05% respectively. eGFR was calculated using the modification of diet in renal disease (MDRD) formula: $186 * [\text{serum creatinine } (\mu\text{mol/l})/88.4]^{-1.154} * [\text{age}]^{-0.203}$. Consider: * 1.210 (if Black) and/or * 0.742 (if female) [31].

Statistical analyses

Statistica 13.0 (Statsoft, Inc., Tulsa, USA) and IBM SPSS v 23 (SPSS, Inc, Chicago, IL) were used for all statistical analyses of the data. Main effects (sex x ethnicity) a priori hypotheses at follow-up were tested for cardiovascular risk variables independent of baseline a priori and respective baseline risk markers. Normal distribution of data was tested, and box-cox logarithmic transformations were done for physical activity, cotinine, γ -GT, CRP, estradiol, renin, aldosterone, eGFR, PHQ-9, SBP, DBP and pulse pressure (PP) data. *A priori* covariates included age, body surface area, physical activity γ -GT [23] and cotinine [24].

Independent *t*-tests and Chi-square tests were used to describe the study population. Chi-square tables determined proportions while dependent sample *T*-tests determined differences over time. McNemar chi-square equations were used to calculate change in prevalence risk over time.

Multiple linear regression analyses determined associations between changes over time in depressive symptoms, BP and eGFR in the bi-ethnic sex cohort, independent of baseline *a priori* covariates (age, body surface area (BSA), total energy expenditure (TEE), cotinine, γ -GT), estradiol [16], and baseline values of the respective cardiovascular risk markers. Several

models were computed and dependent variables included changes (Δ) in Depression, SBP, DBP and eGFR. The independent variables included a priori confounders at baseline, delta (Δ) change in the risk marker and respective baseline value. Independents included *a priori* confounders at baseline, change in depression, renin and aldosterone and baseline values of the respective risk markers. Sensitivity analysis included repetition of linear regression analyses by 1) adjusting eGFR for baseline DBP and depression, individually as well as concurrently 2) additional adjustment for CRP as independent covariate and 3) testing associations between mediators of RAAS – renin and aldosterone, depression, eGFR and pulse pressure at baseline and follow-up using the same a priori confounders at baseline. Statistical significance level was set at $p < 0.05$ (two-tailed).

Optimal cut points of 1) depression symptoms predicting chronic DPB hypertension; and of 2) renin values predicting moderately severe depression were computed from the maximum of the Youden index (J) ($\text{sensitivity} + \text{specificity} - 1$) using non-parametric receiver operating characteristic (ROC) curves. The statistical significance level was set at $p \leq 0.05$ (two-tailed).

Results

Ethnic differences adjusted for a priori covariates were apparent for depressive symptoms ($F_{1, 198} = 24.529$), eGFR ($F_{1, 198} = 14.360$), SBP ($F_{1, 198} = 13.929$), DBP ($F_{1, 198} = 13.998$), and renin ($F_{1, 196} = 11.286$) ($p < 0.001$).

Hereafter, ethnic groups were compared with baseline characteristics in Table 1 by ethnicity (N = 68 Black and N = 127 Whites, with $p \leq 0.001$ unless otherwise indicated). Median values and interquartile ranges were reported to portray a more accurate representation of distributions. Blacks had higher levels of γ -GT and CRP in comparison to Whites. Black populations also showed higher average scores than Whites on the depression symptom questionnaire and had a higher tendency of the score exceeding the clinical cut point of ≥ 10

as indicated in table 1 [28]. Over all, Blacks had lower renin levels ($p=0.002$) than did Whites, with no differences in aldosterone levels ($p=0.735$) between ethnicities. Blacks also showed higher average BP in comparison to Whites.

Table 1: Baseline characteristics by ethnic status.

Variables	Blacks (N =68) Median (IQR)	Whites (N = 127) Median (IQR)	p values
<i>Lifestyle risk markers</i>			
Age, (years) †	40.5 (8)	46 (11)	0.005
Body surface area, (m ²) †	1.84 (0.37)	1.95 (0.41)	0.021
Physical activity, (kcal/day) †	2,550.3 (878.1)	2,845.1 (1,099.5)	0.004
Cotinine, (ng/ml) †	0.01 (8.5)	0.01 (0)	0.498
cGGT, (u/l) †	37.74 (34.11)	16 (16)	< 0.001
Glucose (mmol/l) †	4.96 (0.84)	5.5 (0.7)	< 0.001
C-Reactive protein, (mg/l) †	3.45 (6.51)	1.4 (2.01)	< 0.001
HbA _{1c} % †	5.7 (0.6)	5.4 (0.4)	< 0.001
Estradiol (pmol/L) in men †	77.02 (53.33)	68.93 (32.24)	0.008
Estradiol (pmol/L) in women †	227.9 (569.74)	131.8 (321.32)	0.082
<i>Depressive symptoms</i>			
PHQ-9 Depression symptom score †	9 (8)	4 (6)	< 0.001
PHQ-9 Depression ≥ 10, N (%) ‡	27 (30.71%)	17 (13.39%)	< 0.001
<i>Renin angiotensin system</i>			
Renin (pg/ml) †	4.22 (3.4)	5.81 (4.13)	0.002
Aldosterone (pg/ml) †	39.29 (66.57)	46.68 (49.83)	0.735
Aldosterone-Renin Ratio †	11.79 (16.89)	9.58 (10.91)	0.003
eGFR †	112.31 (28.61)	92.93 (22.76)	< 0.001
eGFR, cut point, N (%) ‡	12 (17.65%)	55 (43.31%)	< 0.001

Cardiovascular measures

24h SBP, (mmHg) †	124 (18.5)	121 (13)	0.001
24h DBP, (mmHg) †	79 (13)	75 (10)	< 0.001
Pulse Pressure †	46 (9.5)	46 (8)	0.310
DBP Hypertension, N (%) ‡	29 (42.65%)	32 (25.20%)	0.012
SBP and DBP Hypertension, N (%) ‡	31 (45.59%)	40 (31.50%)	0.052

Abbreviations: IQR, interquartile range; GGT, gamma glutamyl transferase; HbA_{1C}, glycated hemoglobin; PHQ-9, Patient health questionnaire - depression symptom score; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure. †Values presented as median (interquartile range); ‡Values are presented as N, number of observations (percentage of the group %). p-values were obtained from T-tests, independent by sample.

Table 2 shows the comparative differences by ethnicity over a three-year period. Blacks have higher depression symptom scores in comparison to Whites, although there is a slight decrease in depressive symptoms over time in Blacks (p=0.048). Renin levels remain lower in blacks than in Whites, but decreases over time in both groups, whereas Aldosterone levels decreased less in Blacks (p=0.043) than in comparison to Whites. While Whites showed no significant change in eGFR, eGFR increased to almost double its value at baseline in Blacks. Blacks showed consistently higher levels of BP compared to their White counterparts, additionally displaying an 8.82% increase in DBP hypertension prevalence over time.

Table 2: Comparing differences over a three-year period by ethnic status

Variable	Blacks (N=68)			Whites (N=127)		
	Baseline/follow-up	Difference (95% CI)	p	Baseline/follow-up	Difference (95% CI)	p
<i>Lifestyle risk markers</i>						
Age, (years) †	41/44	3 years	0.001	45/48	3 years	0.001
Body surface area, (m ²) †	1.87/1.93	0.06 (0.04, 0.08)	0.001	1.96/1.99	0.03 (0.01, 0.04)	0.001
Physical activity, (kcal/day) †	2,571.6/3,438.3	866.8 (469.5, 1,264.0)	0.001	3,057.4/3,411.6	354.2 (-44.13, 752.4)	0.081
Cotinine, (ng/ml) †	19.17/30.68	11.51 (-2.36, 25.37)	0.102	28.17/25.53	-2.64 (-9.14, 3.87)	0.424
cGGT, (u/l) †	48.14/46.09	-2.05 (-9.92, 5.82)	0.604	25.65/24.9	-0.74 (-6.04, 4.56)	0.782
Glucose (mmol/l) †	4.99/4.99	0 (-0.13, 0.14)	0.945	5.55/4.28	-1.27 (-1.47, -1.07)	0.001
C-Reactive protein, (mg/l) †	6.77/4.32	-2.46 (-4.77, -0.14)	0.038	3.06/2.59	-0.47 (-1.97, 1.03)	0.540
HbA _{1c} % †	5.71/5.77	0.06 (-0.01, 0.14)	0.106	5.46/5.5	0.04 (-0.02, 0.1)	0.146
Estradiol (pmol/L) in men †	87.55/138.7	51.16 (40.51, 61.81)	0.001	69.27/50.93	-18.33 (-26.63, -10.03)	0.001
Estradiol (pmol/L) in women †	437.4/376.4	-60.99 (-199.8, 77.8)	0.378	283.8/193.4	-90.33 (-187.4, 6.73)	0.068
<i>Depression symptoms</i>						
PHQ-9 Depression symptom score †	10/8	-1.44 (-2.87, 0.02)	0.048	6/6	0.35 (-0.35, 1.04)	0.329
PHQ-9 Depression ≥ 10, N ‡	27/25	-2.95 [0.14 (0.55, 2.42)]	0.705	17/26	7.08 [4.26 (0.13, 0.99)]	0.039
<i>Renin angiotensin system</i>						
Renin (pg/ml) †	4.76/3.3	-1.46 (-2.24, -0.69)	0.001	6.35/5.04	-1.32 (-1.92, -0.71)	0.001
Aldosterone (pg/ml) †	66.55/50.43	-16.12 (-31.71, -0.53)	0.043	64.84/29.37	-35.47 (-45.24, -25.7)	0.001
Aldosterone-Renin Ratio †	16.98/22.52	-5.54 (-10.81, -0.28)	0.039	12.01/6.84	5.17 (3.68, 6.66)	0.001

Est GFR †	112.9/222.3	109.4 (88.14, 130.6)	0.001	93.83/94.41	0.59 (-2.06, 3.23)	0.662
eGFR, cut point, N ‡	12/1	-16.18 [OR not possible]	0.001	55/58	2.36 [0.27 (0.42, 1.65)]	0.602
Cardiovascular measures						
24h SBP, (mmHg) †	127 /131	3.26 (0.71, 5.82)	0.013	122/122	0.35 (-0.85, 1.54)	0.566
24h DBP, (mmHg) †	80/81	0.87 (-0.75, 2.48)	0.287	75/75	-0.76 (-1.62, 0.11)	0.085
24h Pulse Pressure †	47/50	2.38 (1.03, 3.73)	0.001	46/47	1.09 (0.38, 1.81)	0.003
DBP Hypertension, N ‡	29/35	8.82 [2.57 (0.13, 1.28)]	0.109	(32/31)	-0.79 [0.03 (0.52, 2.22)]	0.853

Abbreviations: GGT, gamma glutamyl transferase; HbA_{1c}, glycated hemoglobin; PHQ-9, Patient health questionnaire - depression symptom score; eGFR, estimated glomerular filtration rate; DBP, diastolic blood pressure. †Values presented as arithmetic mean at baseline/follow up as well as the difference over three years' time (95% CI); p-values were obtained from dependent t-tests. ‡Values are presented as N, number of observations at baseline/follow up as well as the percentage difference over three years' time followed by the Odds Ratio (95% Confidence Interval); p-values were obtained by McNemar chi-square equations.

In Table 3, linear regression analyses showed an inverse association between change over time in depression and chronic renin levels [Adj R² 0.35; β -0.24 (-0.44, -0.03), p = 0.027] in Blacks only. In the Black group, change in DBP was inversely associated with chronic depressive symptoms [Adj R² 0.23; β -0.28 (-0.50, -0.06), p = 0.016].

Table 3: Multiple linear regression associations between changes in depression symptoms, diastolic blood pressure, estimated glomerular filtration rate and the RAAS in a bi-ethnic cohort.

Associations presented as regression coefficients (95% confidence interval) p-value. Co-variates included: age, body surface area, physical activity, gamma

Blacks			
β (95% CI)	Δ Depression	Δ DBP	Δ eGFR
<i>Adjusted R²</i>	0.35	0.23	0.17
Chronic Depression	-	-0.28 (-0.50, -0.06) p=0.016*	-
Chronic Renin, ng/l	-0.24 (-0.44, -0.03) p=0.027*	-	-
Chronic Aldosterone	-	-	-0.21 (-0.43, 0.02) p=0.076†

*glutamyl transferase, cotinine, estradiol, and pulse pressure. Where F to enter = 2.5 and F to remove = 0.5. Sensitivity analysis adjusting eGFR for baseline DBP and depression, individually as well as concurrently did not change the results obtained. No associations were found for the change in independents, and are subsequently not shown. *, $p \leq 0.05$; **, $p \leq 0.01$; †, $p < 0.10$.*

In table 4, further analyses on these associations in linear regression analyses and receiver-operating characteristics (ROC) analyses showed an inverse association between follow-up depression and baseline renin levels [Adj R² 0.38; β -0.27 (-0.5, -0.1), $p = 0.009$] as well as an association between baseline depression and follow-up renin levels with an AUC=0.61 (0.47-0.74); sensitivity/specificity 63.6/61.0% in Blacks only. In this group, renin was positively associated with aldosterone at follow-up in Blacks ($p=0.005$) and Whites ($p=0.001$), independent of baseline and a

priori covariates. Baseline depression symptoms were associated with baseline DBP hypertension [AUC =0.58 (0.45-0.72); sensitivity/specificity 72.4/46.2%]. Pulse pressure was positively associated with eGFR [Adj R² 0.16; β -0.36 (-0.6, -0.1), p = 0.004.] in Blacks only.

Table 4: Associations between depression symptoms, renin and aldosterone at follow-up in a bi-ethnic cohort.

<i>β</i> (95% CI)	Blacks		Whites	
	Follow-up Depression symptoms	Follow-up Renin	Follow-up Depression symptoms	Follow-up Renin
Adjusted R²	0.38	0.35	0.47	0.32
Baseline Renin, pg/ml	-0.27 (-0.5, -0.1) p=0.009	-	-	-
Follow-up Renin, pg/ml	-	-	-	-
Baseline Aldosterone, pg/ml	-	-0.46 (-0.7, -0.2) p=0.001	-	-
Follow-up Aldosterone, pg/ml	-	0.30 (0.1, 0.5) p=0.005	-	0.28 (0.1, 0.4) p=0.001

Values presented as associations (95% confidence interval) p-value. Co-variates included: age, body surface area, physical activity, gamma glutamyl transferase, cotinine, estradiol, and pulse pressure. Where F to enter = 3.0 and F to remove = 0.5.

All findings remained unchanged after sensitivity analyses were computed.

Discussion

We aimed at investigating whether depressive symptoms might facilitate volume-overloading hypertension and low renin in a bi-ethnic cohort from South Africa. The main findings revealed Blacks as the more vulnerable group in terms of cardiovascular risk, consistently being found to record higher BP levels than their White counterparts. Overall, chronic depression levels were inversely associated with follow-up renin levels and associated with chronic 24h DBP hypertension in the Black cohort. Chronic depression symptoms desensitized the RAAS by activating central neural control centers in lowering renin and improving renal function to protect against volume-loading diastolic hypertension in Blacks. These findings emphasize the impact of depression on low renin and hypertension in Blacks in terms of prevention, diagnosis and treatment.

An apparent desensitization in the RAAS system may have occurred due to chronic depressive symptoms in the Black cohort to prevent volume-overload by lowering renin levels to maintain homeostasis. As for renal impairment, there seems to be an observable alteration in renin to aldosterone ratio, where the Black groups maintain relatively higher aldosterone levels despite a similar decrease in renin levels. Furthermore, despite the decrease in renin and aldosterone levels, the eGFR increases dramatically, which enforces improved renal function. Similar findings were not evident in the Whites.

Renin functions as the rate limiting factor in mediating the volume-loading effects of the RAAS by triggering a cascade of responses, including the release of angiotensin and aldosterone, amongst other mediators. Renin thus activates mediators of the RAAS, responsible for stimulating retention of ions and fluid in the kidney to increase blood volume and by proxy, blood pressure [32, 33]. Our findings showed higher depression symptoms (PHQ-9), attenuated renin levels with no differences in aldosterone levels, suggesting that depression may indirectly

desensitize the RAAS (as evidenced by aldosterone-renin ratio (ARR)) in mediating low-renin hypertension in the Black men. Various studies supported the influence of depression on increased sympathetic activity [34, 35], and the subsequent stimulation thereof of the HPAA [4, 29, 36, 37]. Increased HPAA activity increases the secretion of corticotrophin-releasing hormones, antidiuretic hormone and adrenocorticotrophic hormone from the HPAA. ADH will increase aldosterone secretion in the adrenal cortex while aldosterone increases the mean arterial pressure [15, 32, 33].

Our findings of low renin levels in the Black cohort seem to contradict functionality of renin-release by exhibiting hypertension levels while presenting lower plasma renin levels. Opie and Seedat, as well as Hamer et al., have demonstrated an inverse association between baseline renin levels and 24h BP in a Black cohort from South Africa [6, 13]. In support, normal aldosterone ranges between 55-138 pmol/l; and Blacks were within the lower normal ranges (55-65 pmol/l). Despite no differences in the aldosterone levels, the BP and ARR levels were increased in the Blacks compared to their ethnic counterparts. This indicates that chronic depression symptoms augmented ARR in Blacks can be attributed due to maintenance of low to normal levels of aldosterone, despite decreasing renin levels over time. A detrimental cycle may develop where higher BP might, if continuously present and uncontrolled, further desensitize the RAAS system and suppress renin secretion in an attempt to maintain normal BP and fluid balance. In other studies, low-renin hypertension was explained through a disturbed ARR that functionally result in elevated or compensatory blood pressure increases to mediate functionality in the RAAS. In turn, elevated blood pressure can possibly down-regulate the release of renin [15, 38] (Figure 3-1).

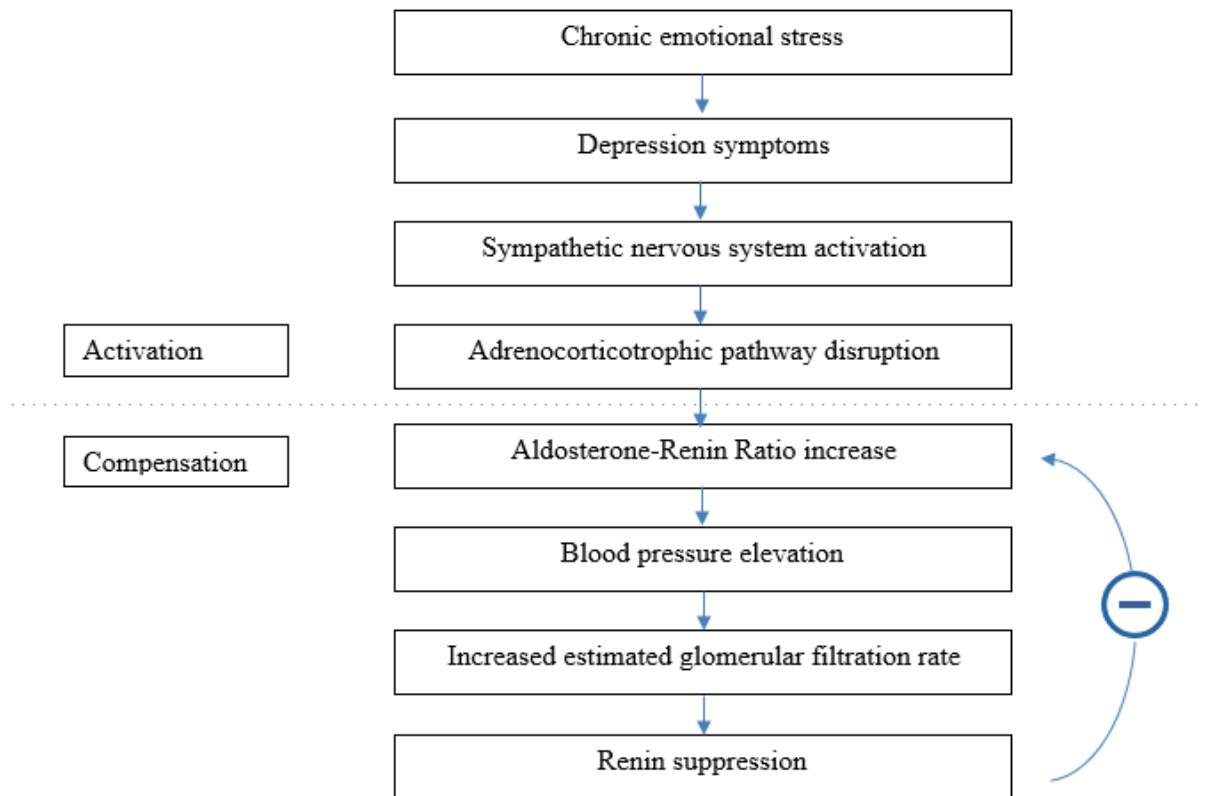


Figure 3-1: Depression symptoms may indirectly disrupt the RAAS through activation of the adrenocorticotrophic pathway and facilitate blood pressure elevation and renal dysfunction. To maintain homeostasis, possible RAAS desensitization will suppress renin secretion and result in low-renin hypertension.

Augmented plasma renin and aldosterone levels have shown to be possible contributory factors or determinants of essential or primary hypertension in literature [6] which was not reflected in the Black cohort. However, Blacks demonstrated a higher risk profile for cardiometabolic diseases and hypertension in this and other studies [1, 2, 6, 9-13, 15, 37]. Furthermore, in our study Black men showed hypertension-related target organ damage including renal dysfunction (eGFR). The negative association of renal function (eGFR) with aldosterone accompanied by lower renin levels suggests that low renin-lower aldosterone levels in Black South Africans may be indirectly indicative of renal damage, as a protective physiological response [10].

Chronic depression may additionally exhaust psychophysiological resources, leading to sympathetic nervous system dysfunction, neural and adrenal fatigue, [1] and depression [2, 3]. Both clinical and sub-clinical depression has been linked to higher incidence cardiac events in individuals with CVD and in healthy populations [4]. In Blacks from the SABPA study, symptoms of depression were associated with an increase in cardiovascular risk and a stroke risk marker [2, 39, 40]. Several mechanisms have been proposed as possible mediators in the depression-CVD association in Africans, including sympathetic hyperactivity and metabolic changes [1, 5]. By affecting endothelial dysfunction and indirectly altering RAAS mediator sensitivity, depression symptoms affect key components in the maintenance of blood pressure and may well be a critical component of a mechanism for explaining hypertension prevalence. Our data may indirectly support the proposed molecular mechanism to explain low-renin hypertension [41]. Low renin values were related to greater vasoconstriction and resulting vascular load. Indeed, low plasma renin levels have been associated with a suboptimal intake of Ca^{2+} that suppresses Ca^{2+} -ATPases mediated Ca^{2+} efflux with resulting increases in intracellular Ca^{2+} (Ca_i), vascular resistance and hypertension [41].

Chronic stress stimulates the HPA axis and may lead to increased activity of the adrenal glands [42, 43]. Increased adrenal activation of vasoconstrictors and anti-diuretics such as angiotensin II and aldosterone will increase blood volume, and arterial tone (24h PP) potentially explaining an overload mechanism. Downstream signaling will desensitize the RAAS to lower renin activity and to maintain normal volume loading.

Our study therefore suggests a desensitized RAAS system as a plausible mechanism for the association between chronic depression symptoms, low-renin activity and aldosterone, vasoconstrictive agents of the RAAS via angiotensin II [44-47]; and volume-loading DBP hypertension [6-8]. Depression symptoms may thus indirectly sensitize the RAAS system

through activation of the adrenocorticotrophic pathway and facilitate volume-loading hypertension. To maintain homeostasis, renin secretion will be suppressed (desensitization of RAAS) and may possibly result in low-renin hypertension.

Another concerning factor is that despite none of the participants taking hypertension medication, Blacks still showed a 8.82 % increase in DBP hypertension prevalence compared to -0.79% in Whites. Therefore it is imperative to advocate awareness of depression symptoms in a conflict-ridden environment.

Depression and alcohol consumption

On average, Whites showed no clinical level of moderate to severe depression symptoms opposed to the Blacks manifesting chronic clinical depression. To elucidate findings, the rate of alcohol consumption should be acknowledged, as levels at baseline and follow-up exceeded the value of chronic alcohol abuse which is in line with previous findings [23]. This trend may suggest hyper-filtration due to endothelial dysfunction, which was not evident in Whites. This poses the question of whether Blacks are depressed and consume more alcohol as coping mechanism to alleviate depression [48], or whether depression stems from alcohol intake. Alcohol is a central nervous system depressant as well as a diuretic, and therefore may induce sodium retention to increase blood volume and would be more likely to trigger renin activity than reduce renin levels [49]. Therefore we can only suggest that chronic emotional distress is more likely to be causative of lower renin levels. In agreement, chronic depression levels in this cohort were associated with vascular dysregulation and -tone, as well as perfusion deficits in the microvasculature [29, 50].

Limitations

A larger study population would improve the validity of statistical analyses as well as provide an improved representation of the population as a whole. Furthermore, classification of race as

Black or White may not represent accurately the nuances of physiological differences between the various ethnicities those classifications represent.

Conclusion

In conclusion, chronic depression symptoms seem to desensitize the RAAS system by inducing low renin and aldosterone levels among the Black participants of the SABPA study. Chronic depression symptoms may have facilitated sympathetic stimulation contributing to the development of volume-loading (DBP) hypertension, possible hyper-filtration and associated renal impairment.

Acknowledgements

The research was funded by the Metabolic Syndrome Institute, France; South African Medical Research Council; National Research Foundation (NRF), North-West University, North-West Department of Education and ROCHE Diagnostics, South Africa.

This work was supported by Professor A Schutte, of the National Research Foundation South African Research Chair Initiative (SARChI) under Grant 86895.

Disclosure of interest

The authors report no conflicts of interest.

The funding organizations played no role in designing or conducting the study; collecting, managing, analyzing and interpreting the data; preparing, reviewing, or approving the manuscript. The authors declare no conflict of interest with the content of this article. Opinions and conclusions expressed in this article exclusively are those of the authors.

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Chapter 4: Conclusion, Implication of Study and Recommendations.

4.1 Introduction

Chapter 4 recounts a brief summary of the findings in the manuscript of the article in Chapter 3, focused on the aims, results and conclusions. The deductions made are compared with literature available from other studies on the same subject. Also in this chapter, the recognized limitations and weaknesses of the study are evaluated and recommendations are made for future research regarding depressive symptoms and renal dysfunction.

4.2 Summary and conclusions based on main findings

The dissertation title and main research focus of this study is: “*Chronic depression symptoms, hypertension and renal impairment in a bi-ethnic sex cohort: the SABPA study*”. Specifically, the article title and main variables investigated are: “*Chronic depression symptoms, 24h diastolic hypertension and low renin levels in a Black African sex cohort: the SABPA study*”.

The main purpose of this sub-study was to investigate depression, renin-angiotensin-aldosterone system (RAAS) markers, blood pressure and renal dysfunction in a prospective bi-ethnic sex cohort from South Africa. The objectives of the study were to determine changes over time, as well as possible associations between depression symptoms, RAAS markers, blood pressure, renin levels and renal function in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study bi-ethnic cohort. Three hypotheses were proposed: Firstly, that depression symptoms will be positively associated with blood pressure in the Black participants. Secondly, Depression symptoms will be positively associated with aldosterone-renin ratio (ARR) and inversely associated with renal function among the black participants. The third hypothesis stated that chronic depression symptoms will predict hypertension and renal impairment in the black participants of the SABPA study. All three hypotheses were accepted. Notably the ARR increased in the Black population group, indicating disproportionate aldosterone activity despite the low renin levels. The conclusion therefore

proposed was that chronic depression symptoms and associated diastolic blood pressure (DBP) hypertension seem to desensitize the RAAS system by inducing low renin levels among the Black participants of the SABPA study. Chronic depression symptoms may have facilitated sympathetic stimulation contributing to the development of volume-loading hypertension, possible hyper-filtration and associated renal impairment.

4.3 Comparison of findings with literature

Comparison of these findings with those of similar studies allows the evaluation of whether the findings reflect, contradict or extend previous research. Such scrutiny decreases bias and assists in putting the findings of the study into perspective.

4.3.1 In accordance with literature

Various studies have supported our findings, confirming our findings that Blacks present as the more vulnerable group in terms of cardiovascular risk, consistently having higher blood pressure (BP) levels [1] and lower renin levels [2] than their White counterparts.

4.3.2 Contradictory to literature

Blood pressure and symptoms of depression and anxiety: a prospective study found no significant relationship between depression symptoms and alterations in blood pressure during a four-year follow-up [3]. Furthermore, in a collaborative meta-analysis, Matsushita, et al. linked estimated glomerular filtration rate (eGFR) decreases to cardiovascular mortality [4], while the eGFR in Black populations with weaker cardiovascular profiles had shown a marked increase in the study. This increase in eGFR may however be attributed to hyper-filtration as a result of endothelial damage and increased alcohol consumption as found by Wong et al. [5]. However, the role of depressive symptoms was not considered in the two last-mentioned studies.

4.3.3 Findings in literature that are not evident in this study

Depressive symptoms were associated with reduced baroreflex in coronary artery disease [6] which may explain, in part, the potential maintenance of an elevated BP set point. Various studies supported the influence of depression on increased sympathetic activity [7, 8], and the subsequent stimulation thereof of the hypothalamic-pituitary-adrenal axis (HPAA) [9-12].

4.3.4 Findings not yet documented by current literature

Overall, chronic depression levels were inversely associated with follow-up renin levels and directly associated with chronic 24h diastolic blood pressure (DBP) hypertension in the Black cohort. Depressive symptoms may therefore partly explain low-renin hypertension through more than molecular mechanisms [13]. An apparent desensitization in the RAAS system may have occurred due to chronic depressive symptoms in the Black cohort to prevent volume-overload by lowering renin levels to maintain homeostasis.

4.4 Confounders and chance

Objective evaluation of the integrity of data collected is of critical importance to ensure reliable results and reasonable conclusions. Hence it is imperative to assess any shortcomings in the methods or analyses that may influence findings.

- ❖ We included objective markers as confounders. The *a priori* confounders in the study, age, body surface area, the alcohol metabolite γ -glutamyl transferase (γ -GT), cotinine as an indicator for smoking status, and physical activity were independent predictors in all our models to avoid bias.
- ❖ The use of γ -GT as marker for alcohol consumption [14] is widely implemented and accepted, though various other conditions may result in false positives [15]. Other than alcohol consumption, obesity, diabetes, hypertension, and hypertriglyceridemia may also increase γ -GT levels [16, 17].

- ❖ The validity of estradiol levels obtained is compromised by the unknown stage of menstruation or menopause in the female participants [18]. Furthermore, the method of electrochemiluminescence immunoassay used to obtain estradiol values could be improved by rather using high-performance liquid chromatography mass spectrometry (HPLC-MS).
- ❖ Renin levels are subject to fluctuation with the use of certain drugs, phases of menstrual cycle and dietary salt consumption. Dietary salt consumption is believed to be elevated in South African populations [19]. Despite the 24 h diet at follow-up in participants, the individual dietary salt consumption is unknown, which may have affected renin levels.
- ❖ Participants were all teachers from the Kenneth Kaunda district in the North West Province of South-Africa and may not accurately represent the whole of the South African population

While there is always the possibility of an element of chance in any study, the collection protocol was strictly adhered to, maintaining high integrity of the data set. Furthermore, various exclusion criteria ensured minimal possible compromise of data point. Finally, all statistical analyses were conducted following detailed protocol and reviewed by a qualified statistician. Other co-authors are experts in psychophysiological research and contributed to an improved understanding of sympathetic activation, depressive symptoms and the RAAS.

4.5 Strengths of study

- ❖ The study featured an award-winning design (Metabolic Syndrome Institute, France), and all measurements and conditions were rigorously controlled.
- ❖ The study focused on participants from homogenous socio-economic backgrounds, minimizing social and economic influences on study parameters. The regional focus of

the study also allows for relative similarity in environmental factors which could affect results.

- ❖ The study was conducted in one season, to avoid seasonal variations in physiological processes, thereby providing more reliable and consistent results.
- ❖ The prospective nature allows the observation of development of various dysfunctions, reactions and developments. This allows a distinct advantage over a cross-sectional study, which has the distinct disadvantage of only working with once-off measurements, thereby not being as accurate or comprehensive when compared with a longitudinal study.

4.6 Limitations of study

- ❖ While the SABPA study adheres to stringent exclusion criteria and manages to exclude much of the socio-economical variation that is believed to affect results, it may not be representative of the South African population as a whole. Ideally, a larger study population would improve the validity of statistical analyses as well as provide a better representation of the population as a whole. Furthermore, classification of race as Black or White may not accurately represent cultural differences and nuances of physiological differences between the various ethnicities.
- ❖ The intricate mechanisms of the RAAS, autonomic nervous system and psychological depression cannot be separated from physiological interactions of the body as a whole. As such, it cannot be discounted that all possible variables that may factor in their regulation have been accounted for. Ideally, data on angiotensinogen, angiotensin I, angiotensin II, bradykinin, angiotensin-converting-enzyme, RAAS fingerprinting, dietary salt intake, or markers of other pathways that may undergo similar changes such as cortisol, adrenaline and nor-adrenaline would provide a more in-depth scrutiny of this proposed mechanism.

- ❖ Depression symptoms are a useful measurement tool in quantifying emotional distress. However, still much social and cultural stigma surrounds depression, and severity of depression is often under diagnosed. While every precaution was taken to ensure that the questionnaires were correctly understood by each participant, the answers are still subject to personal perception.

4.7 Recommendations for future research

- ❖ Similar studies in other cohorts would aid in confirming our hypotheses or, if ulterior results are obtained, conflicting results may prove valuable in identifying additional unknown variables.
- ❖ Larger study population groups would provide greater statistical power and allow for more representative results. An increase in population sample size would more easily accommodate analyses not only between race groups, but also concurrently between genders.
- ❖ Investigating coping mechanisms along with depression symptoms may further elucidate the possible disruptive effects of chronic psychosocial reserve depletion and facilitate better insights and understanding of the neurological impact on renal impairment and associated hypertensive states.
- ❖ Additionally, recording dietary salt intake and menstrual or menopausal state at the time of sampling would provide a more accurate measure of estradiol and renin levels. Another possible method of compensating for the level of reliability of these hormonal measurements could be to repeat sampling at set intervals during each phase, which would provide more accurate and representative data.
- ❖ Additionally, alcohol consumption is known to be a prominent feature in the urban South African lifestyle. Alcohol consumption has been implicated as coping mechanism, sympathetic nervous system depressant, diuretic effect and risk factor for

endothelial dysfunction and renal dysfunction and risk factor for cardiovascular diseases. As such, effects of chronic excessive alcohol consumption should further be examined to ascertain its role in facilitating or aggravating the effect of depressive symptoms on renal dysfunction.

4.8 Conclusions

In conclusion, all three the hypotheses stated in section 2.9 were accepted based on results obtained. Chronic depression symptoms seem to desensitize the RAAS system by inducing low renin and higher ARR levels among the Black participants of the SABPA study. Chronic depression symptoms may have facilitated sympathetic stimulation contributing to the development of volume-loading hypertension, possible hyper-filtration and associated renal impairment.

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Appendices

Appendix A: Patient Health Questionnaire (PHQ-9)

DSM-IV criteria (Kroenke, Spitzer & Williams, 2001)

Instructions:

Please indicate how often over the last 2 weeks you have been bothered by any of the following problems by ticking the appropriate box.

		not at all	several days	more than half the days	nearly every day
1.	Little interest/pleasure in doing things	0	1	2	3
2.	Feeling down/depressed/hopeless.	0	1	2	3
3.	Trouble falling or staying asleep/ <i>OR</i> sleeping too much	0	1	2	3
4.	Feeling tired/ having little energy	0	1	2	3
5.	Poor appetite <i>OR</i> overeating	0	1	2	3
6.	Feeling bad about yourself <i>OR</i> that you are a failure/ that you have let yourself or your family down	0	1	2	3
7.	Trouble concentrating on things, such as reading the newspaper/ watching television	0	1	2	3
8.	Moving or speaking so slowly that other people could have noticed <i>OR</i> being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9.	Thoughts that you would be better off dead/ of hurting yourself in some way	0	1	2	3
If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home or get along with other people?		not difficult at all	somewhat difficult	very difficult	extremely difficult

Appendix B: Ethics approval for SABPA study



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Dear Dr Malan

6 February 2008

ETHICS APPROVAL OF PROJECT

The North-West University Ethics Committee (NWU-EC) hereby approves your project as indicated below. This implies that the NWU-EC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans)

Ethics number:

N	W	U	-	0	0	0	3	6	-	0	7	-	S	6
Institution			Project Number					Year		Status				

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

Approval date: 12 November 2007

Expiry date: 11 November 2012

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principle investigator) must report in the prescribed format to the NWU-EC:
 - annually (or as otherwise requested) on the progress of the project,
 - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-EC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-EC and new approval received before or on the expiry date.
- In the interest of ethical responsibility the NWU-EC retains the right to:
 - request access to any information or data at any time during the course or after completion of the project;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the project are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the NWU-EC or that information has been false or misrepresented,
 - the required annual report and reporting of adverse events was not done timely and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.

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.

Yours sincerely

Prof M M J Lowes
(chair NWU Ethics Committee)

Appendix C: Extension of ethics approval for SABPA study



Private Bag X8001, Potchefstroom
South Africa, 2520

Tel: 018 299-1111/2222
Web: <http://www.nwu.ac.za>

To whom it may concern

Ethics Committee
Tel: 018 2994237
E-mail: 10055355@nwu.ac.za

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

H.H. Vorster

Prof. H.H. Vorster
Chair person

Appendix D: Ethics approval for sub-study



Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: <http://www.nwu.ac.za>

Faculty of Health Sciences
Health Sciences Ethics Office for Research,
Training and Support
Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

1 August 2016

Prof L Malan
Physiology

Dear Prof Malan

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-020053-16-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: Chronic depression symptoms, hypertension and renal impairment in a bi-ethnic sex cohort: the SABPA study

Study leader/supervisor: Prof L Malan

Student: AC de Vos

Application type: Single study

Risk level: Minimal

You are kindly informed that your application was reviewed at the meeting held on 08/06/2016 of the HREC, Faculty of Health Sciences, and was approved on 01/08/2016.

The commencement date for this study is 01/08/2016 dependent on fulfilling the conditions indicated below. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form at Ethics-HRECIncident-SAE@nwu.ac.za.

A monitoring report should be submitted within one year of approval of this study (or as otherwise stipulated) and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-Monitoring@nwu.ac.za. Annually a number of studies may be randomly selected for an external audit.

Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC, Faculty of Health Sciences. Ethics approval is required BEFORE approval can be obtained from these authorities.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely



Dr Wayne Towers
HREC Chairperson



Prof Minrie Greeff
Ethics Office Head

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2016 Applications\Applications 05 - 8 June 2016\NWU-00053-16-S1 (L. Malan-AC de Vos)\NWU-00053-16-S1(L. Malan-AC de Vos)-AL.docm
01 August 2016

File reference: 9.1.5.3

Appendix E: Originality report

Turnitin Originality Report for Chronic depression symptoms, hypertension and renal impairment in a bi-ethnic sex cohort: the SABPA study.

Student: AC De Vos (20673914)

Final_Dissertation_ACDV.docx

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Jacobus De Wet Scheepers

20765274

Appendix F: Language editing certificate



11 November 2016

I, **Ms Cecilia van der Walt**, hereby confirm that I took care of the **editing** of the **dissertation** of **Ms Arnoldeen de Vos** titled ***Chronic depression symptoms, hypertension and renal impairment in a bi-ethnic sex cohort: the SABPA study.***

MS CECILIA VAN DER WALT

BA (*Cum Laude*)

HED (*Cum Laude*),

Plus Language editing and translation at Honours level (*Cum Laude*),

Plus Accreditation with SATI for Afrikaans and translation

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