The association of the change in renin with cardiovascular and inflammatory markers in a bi-ethnic population: The SABPA study

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Affirmation by authors

The following researchers contributed in making this study possible:

Ms. R Swart

She was responsible for the planning, writing and composition of the manuscript. This included literature research about the topic, writing and submitting an ethics application as well as statistical analyses. She was involved in clinical measurements such as pulse wave velocity (PWV) in research projects.

Prof. JM van Rooyen (supervisor)

Prof. Van Rooyen gave recommendations for the framework, writing and composition of the manuscript as well as the methodology. He supervised the statistical analyses and helped with the formulation of the tables and figures. Prof. Van Rooyen played an important part in gaining funding for this project.

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The statements mentioned above are for the co-authors to confirm their separate roles in the study and thus giving their permission that the article may form part of this dissertation.

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Summary

Motivation

A study done in South Africa stated that black participants have lower renin levels in comparison to white participants, which may be linked to the increased prevalence of cardiovascular disease (CVD) in this population. In relation to the lower renin levels, angiotensin II (Ang II) levels may also be suppressed in this population. It was also indicated that black South Africans are prone to chronic low grade inflammation. If the renin angiotensin-system (RAS) is activated it may lead to increased inflammation due to the actions of Ang II which may indirectly increase C-reactive protein (CRP) levels. The RAS is suppressed in the black participants, thus they may have lower Ang II levels, but increased levels of inflammation and high risk of CVD. Therefore, an investigation into these mechanisms may give insight into the interaction between the RAS, inflammation and cardiovascular variables.

Aim

The aim of the study was to investigate associations of change in renin with cardiovascular and inflammatory markers in a low renin black and white population.

Methods

This study formed part of the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study that took place between 2008 and 2011. The study population consisted of 73 black and 81 white teachers between 20-65 years of age, from the Dr. Kenneth Kaunda Education District in the North-West Province of South Africa. The anthropometric variables included height and weight. Regarding cardiovascular measurements systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), total peripheral resistance (TPR), Windkessel compliance (Cwk) and carotid intima media thickness (cIMT) were determined. Biochemical variables included renin, CRP, interleukin-6 (IL-6), glucose,

von Willebrand factor (vWf), triglycerides and cholesterol levels. Means and proportions were compared using independent t-tests and Chi-square tests, respectively. Variables were compared using analyses of covariance (ANCOVA). Correlations of variables were performed using partial regression analyses. Forward stepwise multiple regression analyses were performed to determine independent associations between variables.

Results and conclusion

Percentage change in renin levels was independently and inversely associated with percentage change in systolic blood pressure (β=-0.27; p=0.011) only in black participants with low renin. Among the white participants percentage change in renin was negatively associated with percentage change in IL-6 (β=-0.24; p=0.005). In conclusion, in black participants with low renin levels at baseline, a further decrease in renin levels over three years is independently associated with an increase in SBP. These results strengthen the notion that low renin may be a causative factor in the development of hypertension in black South Africans, but that the renin- angiotensin system is not the driving force behind the increased inflammation observed in this low renin black group.

Keywords

C-reactive protein, hypertension, inflammation, interleukin-6, low renin, systolic blood pressure.

Opsomming

Motivering

Volgens 'n studie wat in Suid-Afrika gedoen is, het swart deelnemers laer renien vlakke in vergelyking met wit deelnemers, wat gekoppel kan word aan die verhoogde voorkoms van kardiovaskulêre siekte (CVD) onder hierdie bevolkingsgroep. Met betrekking tot die laer vlakke renien, kan angiotensien II (AngII) vlakke ook onderdruk word in dié bevolkingsgroep. Dit is ook beduidend dat swart Suid-Afrikaners geneig is tot chroniese lae graad van inflammasie. As die renien-angiotensien-sisteem (RAS) geaktiveer word kan dit lei tot verhoogde inflammasie as gevolg van die Ang II, wat C-reaktiewe proteïen (CRP) vlakke indirek kan verhoog. Die RAS is onderdruk in die swart deelnemers, daarom het hulle laer Ang II, maar verhoogde inflammasie en risiko vir kardiovaskulêre siekte. Vir hierdie rede kan 'n ondersoek na dié meganismes, insig in die interaksie tussen die RAS, inflammasie en kardiovaskulêre veranderlikes meebring.

Doel

Die doel van die studie was om assosiasies van verandering in renien te ondersoek met kardiovaskulêre en inflammatoriese merkers in 'n lae renien bi-etniese groep

Metodes

Hierdie studie het deel uitgemaak van die “Sympathetic Activity and Ambulatory Blood Pressure in Africans” (SABPA) studie wat plaasgevind het tussen 2008 en 2011. Die ondersoekgroep het bestaan uit 73 swart en 81 wit onderwysers, tussen die ouderom van 20 en 65 jaar, van die Dr. Kenneth Kaunda Onderwys-distrik in die Noordwes-provinsie van Suid-Afrika. Die antropometriese veranderlikes het ingesluit lengte en gewig. Rakende kardiovaskulêre metings is sistoliese bloeddruk (SBP), diastoliese bloeddruk (DBP), polsdruk (PP), totale perifere weerstand (TPR), arteriële meegewendheid (Cwk) en karotis intima media dikte (cIMT) bepaal. Biochemiese veranderlikes sluit in renien, CRP, interleukin-6 (IL-6), glukose, von

Willebrand faktor (vWf), trigliseriede en cholesterol vlakke. Gemiddeldes en proporsies is vergelyk met behulp van onafhanklike t-toetse en Chi-kwadraat toetse onderskeidelik. Veranderlikes is vergelyk met behulp van kovariansie analise (ANCOVA). Korrelasies tussen veranderlikes is gedoen met behulp van gedeeltelike regressie ontleding. Stapsgewyse meervoudige regressie ontledings is voorts uitgevoer om onafhanklike assosiasies tussen veranderlikes te bepaal.

Resultate en gevolgtrekking

Die persentasie verandering in renien vlakke is onafhanklik en omgekeerd geassosieer met die persentasie verandering in sistoliese bloeddruk (β=-0.27; p=0.011), slegs in swart deelnemers met lae renien vlakke. Onder die wit deelnemers het die persentasie verandering in renien ‘n negatiewe verband gehou met persentasie verandering in IL-6 (β=-0.24; p=0.005). Die gevolgtrekking wat gemaak kan word, is dat die afname in vlakke van renien oor 3 jaar kon gelei het tot verdere verhogings in SBP onder die swart deelnemers. Hierdie resultate versterk die idee dat lae renien ‘n oorsaak kan wees van die ontwikkeling van hipertensie onder swart Suid-Afrikaners, maar dat die renin-angiotensien sisteem nie die dryfkrag is agter die verhoogde inflammasie in die lae renin swart groep nie.

Sleutelwoorde

C-reaktiewe proteïen, hipertensie, inflammasie, interleukin-6, lae renien, sistoliese bloeddruk.

Preface

This dissertation is submitted in fulfilment of the requirements for the degree Master of Science in Physiology. The article format of *Journal of renin-angiotensin-aldosterone system (JRAAS)* was used throughout this dissertation and the dissertation is presented in English. The references are included at the end of each chapter in the style which *JRAAS* prescribed.

The structure of the dissertation consists of Chapter 1 which describes the background, motivation, objectives and hypotheses of this study. Chapter 2 includes the methodology consisting of a detailed description of the RAS, inflammation and CVD. Chapter 3 includes the article which consists of a title page, instructions for authors, abstract, an introduction, material and methods, results, conclusion and acknowledgements. Chapter 4 is the summary of the results and is also composed of concluding remarks and recommendations for future studies.

List of tables and figures

List of abbreviations

ACE:	angiotensin converting enzyme
Ang:	angiotensin
AT-R:	angiotensin type receptor
cIMT:	carotid intima media thickness
CRP:	C-reactive protein
CVD:	cardiovascular diseases
Cwk:	Windkessel compliance
DBP:	diastolic blood pressure
GGT:	gamma glutamyltransferase
IL-6:	interleukin-6
NO	nitric oxide
PP:	pulse pressure
RAS:	renin-angiotensin-system
ROS:	reactive oxygen species
SABPA:	Sympathetic activity and Ambulatory Blood Pressure in Africans
SBP:	systolic blood pressure
sPRR:	soluble plasma renin receptor
TNF-α:	tumor necrosis factor alpha

TPR: total peripheral resistance

vWf: von Willebrand factor

Chapter 1: Introduction

1.1 Background

Globally non-communicable diseases such as cardiovascular diseases (CVD) are a concern which may have detrimental effects on especially poor countries because of their vulnerability including socio-economic factors.[1] From 1999-2004 blood pressure was well managed in only 29% of African Americans and 35% in the white population.[2] According to statistics in 2011 more or less 8.5 million South Africans had high blood pressure of which three million were black men.[3] It was estimated that by the year 2020 non-communicable diseases and especially CVD may be the main cause of mortality globally.[4] CVD may be one of the main causes and one of four disorders that may contribute to almost 50% of mortality worldwide.[5] Hypertension which is considered as an important risk factor for the development of CVD, especially in black Africans, is also a major cause of mortality worldwide, even more than obesity and smoking.[6,7] The reason for the higher morbidity was suggested by a few studies which compared African and Caucasian populations. Black South Africans' lifestyle underwent drastic changes due to the increasing urbanization from poor communities to more westernized communities which lead to increased stress and diseases such as CVD, diabetes, coronary heart disease, atherosclerosis and hypertension.[7,8,9] These changes resulted in increased blood pressure with as much as 37 mmHg for systolic and 23 mmHg for diastolic blood pressure.[10]

CVD and cardiovascular events can be caused by the abnormal activity of the renin- angiotensin-system (RAS).[11] Previously it was indicated that black populations have a lower responsive RAS compared to white populations.[12] This implies that black participants have lower renin levels compared to white participants and are more likely to develop a phenomenon known as low renin hypertension. There are different forms of low renin hypertension including low renin/low aldosterone, low renin/normal aldosterone and low renin/high aldosterone hypertension.[13] Patients with essential hypertension tend to have lower renin levels as the result of the increased perfusion pressure at the juxtaglomerular cells which suppresses renin secretion.[14] Various mechanisms

may be involved in the low renin phenotype, such as lifestyle changes in association with urbanization,[15,16] high renal sodium reabsorption,[15,17] reduced renin secretion rate[18] which may be due to lower soluble plasma renin receptor (sPRR) in blacks and polymorphisms in genes encoding for RAS proteins.[15] All of these factors are described in detail in the literature review.

The levels of inflammatory markers also differ between blacks and whites.[19-21] In several studies done on white participants it has been shown that increases in plasma inflammatory markers lead to increased risk for the development of hypertension in whites.[22-24] Increased inflammation is a process which may lead to decreased nitric oxide (NO) bio-availability[25] and consequent endothelial dysfunction.[26,27] A decrease in NO bio-availability will lead to increased vascular tone and eventually hypertension.[25,28] The RAS have an important role in the inflammatory process.[29] Angiotensin II (Ang II) stimulates the inflammatory process by releasing inflammatory markers including tumor necrosis factor (TNF-α) and interleukin-6 (IL-6)[30] in macrophages and smooth muscle cells.[31]

1.2 Motivation

Cardiovascular diseases are more prevalent in black populations when compared to white populations.[7,8,9] Various factors may contribute to this and previous studies in South Africa suggested a role for the RAS.[15] Black participants are more likely to have low renin levels which may lead to volume expansion hypertension.[32] On the other hand, an activated RAS is linked to increased inflammation, since it has been demonstrated that Ang II may play an important role in the inflammatory process.[11] In contradiction with lower renin (and consequently lower Ang II, because renin is the rate limiting step in the synthesis of Ang II) in black populations, it has also been indicated that black participants are more prone to increased inflammation and endothelial dysfunction.[19] Therefore, since the RAS, inflammation and eventually endothelial dysfunction are interlinked, an investigation into all of these factors may contribute to clarify the mechanisms involved to explain the high prevalence of CVD in black South Africans.

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Chapter 2: Literature study

2.1 Cardiovascular diseases worldwide

In 1999 cardiovascular disease (CVD) was responsible for up to 50% of mortality worldwide.[1] In 2007 it was estimated that by the year 2020 increased CVD may be the main contributor to the mortality rate worldwide.[2] This was confirmed in 2011 when it was indicated that CVD is the number one cause of mortality where hypertension is the number one risk factor for CVD development worldwide.[3]

2.2 Cardiovascular diseases in black South Africans

In South Africa hypertension and CVD are also increasing health problems, especially among the black South Africans.[4,5] Various factors may contribute to the increasing CVD prevalence in Africans including lifestyle, environmental factors, genetic predisposition, as well as socioeconomic status.[6-11] In addition to these factors, studies have also shown that black Africans have low renin levels with a concomitant higher prevalence of hypertension and CVD in comparison with white Africans.[12]

2.3 The Renin-Angiotensin-System (RAS)

The use of plasma renin levels to diagnose and determine the prognosis of cardiovascular disease started more than 40 years ago.[13] In Figure 1 the normal renin-angiotensin system is schematically represented. The first enzymatic reaction in the RAS is executed by the enzyme renin after activation by the sympathetic nervous system. This is also the rate limiting step in this pathway. Renin is produced in the kidneys by the juxtaglomerular apparatus which was elicited through the decreased transport of sodium chloride to macula densa cells in the distal nephron.[14] Receptors for renin can be found in the liver, placenta, kidney, heart and also in subcutaneous and visceral adipose tissue.[15] Renin then acts on angiotensinogen which is converted to angiotensin I (Ang I). In turn, Ang I is then converted to Ang II by the action of the angiotensin converting enzyme (ACE).[16] There are other enzymes which have the same functions as ACE including, chymase and catepsin.[17] Ang II can bind to two different receptors including angiotensin type 1 receptor (AT_1)

and angiotensin type 2 receptor (AT_2). AT_1 receptors are found in the cardiovascular, endocrine, renal and nervous systems and most physiological effects are mediated through AT_1 receptors.[18,19] Whereas AT_2 receptors can be found in the heart, kidney, vasculature, adrenals, pancreas and in the brain.[20] Binding of Ang II to the AT_1 receptor elicits effects such as secretion of inflammatory cytokines, production of reactive oxygen species (ROS), vasoconstriction, secretion of aldosterone, hypertrophy, vascular permeability and sodium and water retention. Binding of Ang II to AT_2 may elicit two different responses. The first will result in generation of phospholipase A2 which increases cyclo-oxygenases and prostaglandin I2 leading to vasodilation. The second response will result in bradykinin (BK) release which will increase nitric oxide (NO) and cyclic guanosine 3'-5'-monophosphate (cGMP) and subsequently stimulate vasodilation.[21] Binding of Ang II to the AT_2 receptor also include, natriuresis, anti-inflammatory responses, anti-proliferative actions and arterial wall remodeling.[22]

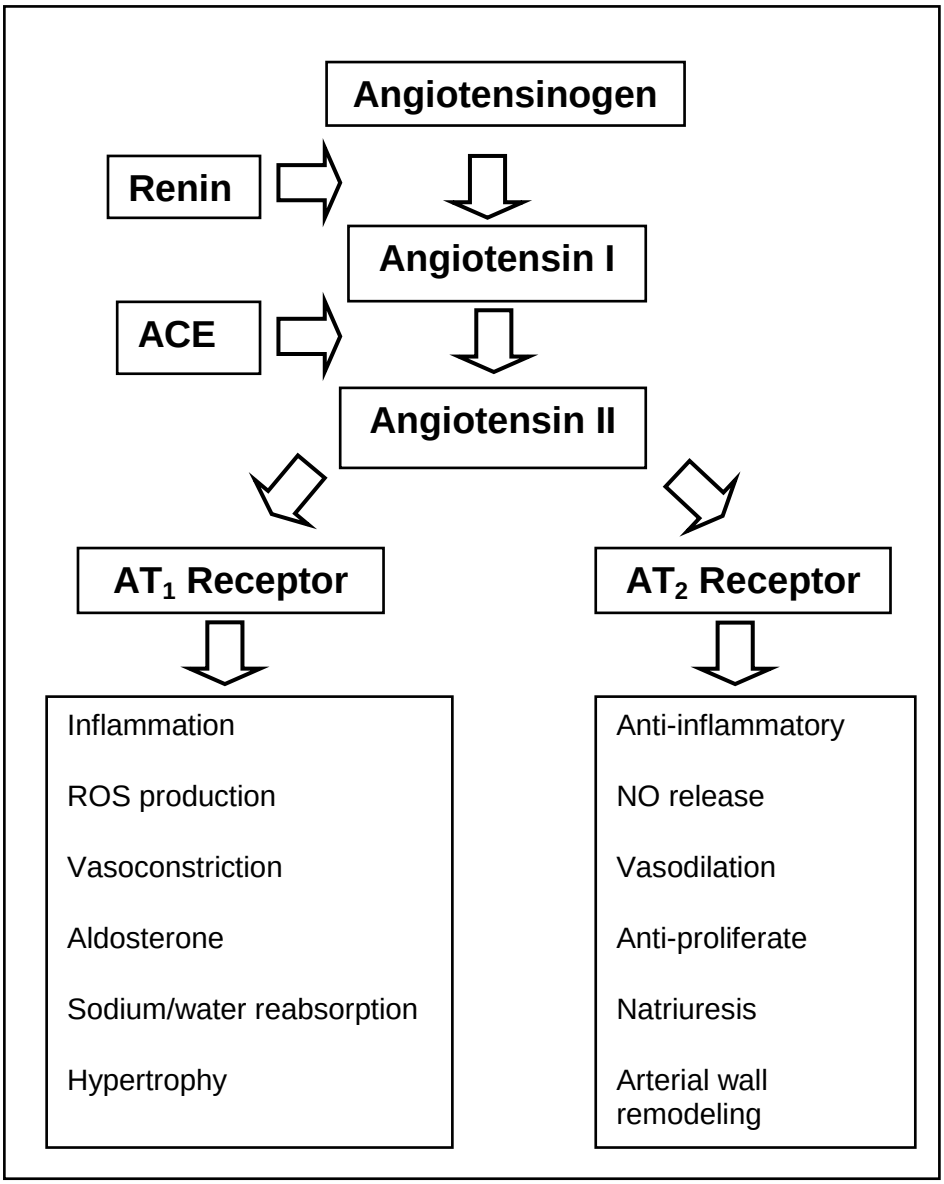

Figure 1 The biochemical pathway for the RAS and its effects of binding to AT_1- and/or AT_2 receptor.[22]

ACE: Angiotensin Converting Enzyme, AT-R: Angiotensin type receptor, ROS: reactive oxygen species, NO: nitric oxide.

2.4 Optimal renin levels and the consequences of a disturbance in the renin balance

The RAS plays an important role in the functioning of the renal system, the

cardiovascular system and the adrenal glands.[22] This is done via the regulation of the fluid volume, sodium and potassium balance as well as regulation of arterial blood pressure.[22]

In order to obtain these balances, renin secretion levels should be within the normal reference values of 0.20 to 2.80 ng/mL/h in the supine position and 1.50 to 5.70 ng/mL/h in the upright position.[23] Too low or too high renin levels may lead to a worse prognosis for patients with hypertension resulting in end-organ damage.[24,25]

2.4.1 High and low renin levels

High levels of renin, but also low renin levels may be harmful to the vasculature and may lead to stroke, myocardial infarction and eventually heart failure and also have a worse prognosis in comparison with normal renin levels. Black participants have lower renin levels which may be the cause of volume expansion hypertension.[26] In Figure 1 if renin levels are suppressed which is more prevalent in black participants, it may have an effect on Ang I, Ang II and aldosterone levels, leading to reduced excretion of sodium.[22] If there is excess salt in the body due to the low excretion of sodium through the kidneys, it may lead to volume hypertension because of the increasing extracellular fluid.[22] The renal renin secretion may in turn be suppressed by decreased aldosterone levels.[22] It was confirmed that Afro-Americans had lower renin levels in comparison with white subjects.[26] In essence, low renin levels are seen due to a higher perfusion pressure at the juxtaglomerular cells which suppresses renin release. Results also indicated that people with low renin essential hypertension may be less prone to the development of CVD than patients with hypertension and normal or high renin levels.[27] A previous cohort study stated that high plasma renin concentration may be linked to higher risks of cardiovascular events which is in contrast with blacks.[28,29] Medium renin levels (0.66-4.50 ng/mL/h) to high renin levels (>4.50 ng/mL/h)[30] may lead to the increasing development of CVD.

2.5 Inflammation

Previous studies done on Afro-Americans stated that inflammation and left

ventricular hypertrophy was positively associated,[31,32] which suggests that inflammation may lead to an increase in blood pressure.[33,34] In turn if blood pressure increases it may lead to increasing inflammatory responses.[34]

2.5.1 Interleukin-6 (IL-6)

Some studies illustrate that IL-6 is an important circulating biomarker with an important function in the inflammatory process.[35] IL-6 is synthesized by several cells including macrophages, B cells and endothelial cells.[36] The actions of IL-6 do not take place where this cytokine is synthesized.[37] IL-6 is involved in the promotion of smooth muscle cell proliferation, modulation of vascular reactivity and vascular remodeling.[22] The secretion of IL-6 is stimulated by Ang II through the AT_1 receptor and results in various biological actions including synthesis of acute- phase reactants by the liver.[38-42] The rest of the cytokines also have an important role in the inflammatory response that leads to pathology; these cytokines include CRP and TNF-α.[43-46]

2.5.2 C-reactive protein (CRP)

CRP is produced by the liver in response to other inflammatory markers such as IL-6 and intercellular adhesion molecule-1.[47] CRP is a marker of the acute phase response, such as infection and damaging of tissues. CRP increases tissue factors, leukocytes and monocyte recruitment,[22] and is furthermore associated with smooth muscle cell migration, endothelial cell dysfunction, atherosclerosis, and cell proliferation.[48] It has also been shown that CRP is associated with endothelial dysfunction and can predict cardiovascular events.[45,49]

2.5.3 Tumor Necrosis Factor-α (TNF-α)

TNF-α is known to be the mediator of inflammation and it increases other inflammatory markers including IL-6, cellular adhesion molecules and monocyte chemotactic protein-1.[50] Both TNF-α[51] and CRP[52] up-regulates the recruitment of inflammatory cytokines and vascular cell adhesion molecules. Additionally it is also involved in cell migration and matrix activation.[22] TNF-α stimulates nicotinamide adenine dinucleotide phosphate oxidase which will result in the

formation of superoxide.[53] Increased superoxide levels may be scavenged by NO, which will lead to the inactivation of NO and therefore promote vasoconstriction.[53] The promotion of vasoconstriction will impair the endothelial function due to inhibiting vasodilatation and may eventually lead to endothelial dysfunction.[54,55]

2.6 RAS and lifestyle

In addition to the link between an activated RAS on the inflammatory process, lifestyle factors such as obesity, stress and smoking can also have an activated effect on RAS.[56,57] Increased alcohol consumption may stimulate the RAS and eventually lead to the development of hypertension and CVD.[58-60] Increased alcohol consumption may lead to an increase in SBP as a result of increased sodium and water retention.[61,62] The possible mechanism which is responsible for further decreasing renin levels due to alcohol consumption, is volume hypertension which is associated with increasing sodium volume. If there is increased sodium concentration, the kidneys retain more water. This may lead to expansion of extracellular fluid volume which causes an increase in blood pressure and lower renin secretion through suppressing of renal renin secretion.[63]

2.7 Renin-angiotensin system, inflammation and cardiovascular disease

When the RAS is activated through hypertension, heart and kidney diseases, the increased availability of Ang II may stimulate increased secretion of inflammatory cytokines, which in turn may also increase oxidative stress.[35,64] Increased inflammation may then contribute to the development of CVD and cardiac events.[35,64] In this regard, previous results linked the inflammatory process with atherosclerosis, cardiomyopathy, hypertension[64] and also endothelial dysfunction.[65] Additionally, increased levels of Ang II itself is also associated with heart failure, hypertension, cardiac fibrosis and vascular fibrosis.[66,67]

There is a positive feedback system between inflammation and the RAS, which causes the inflammatory process to be persistent.[68] When arteries are injured, Ang II shifts the inflammatory cells to this injured site, thus showing the important role of Ang II in the inflammatory process.[68] In contrast inflammatory

cells can also produce Ang II.[68] Ang II can possibly generate inflammation in atherogenesis due to the fact that Ang II has the effect of releasing TNF-α and IL-6.[69,70]

Indicating the importance of inflammatory markers, people with elevated inflammation levels may be at risk for future stroke, vascular mortality and peripheral vascular disease.[37,44,45,71] In a cohort study of healthy men who had high baseline CRP levels in the highest quartiles, there was a twofold increase in risks of peripheral vascular disease and strokes.[44,45] Inflammation increases with the severity of CVD and atherosclerosis.[45,49]

If the pathway of the RAS is activated in Figure 1, it may lead to an increase in renin, Ang I and Ang II levels which lead to increases in inflammation and various other effects. A study which investigated the role of the RAS and inflammation found that if the RAS is activated through Ang II infusion it may lead to an increase in inflammatory effects of IL-6.[72]

Human studies investigating the link between an inflammatory marker IL-6, renin (Ang II) and hypertension[72] are limited. Most of the studies investigated the link between inflammation and hypertension.[73,74] One study investigated the association between IL-6, RAS activity and hypertension.[72] The study investigated 385 hypertensive and 196 normotensive subjects aged between 18-65 years in a mixed population consisting of African-Americans and Hispanics.[72] Ang II was infused and the results showed that in hypertensive and normotensive people, IL-6 levels increase with increasing activity of the RAS.[72] In hypertensive and normotensive people there was an association between renin and CRP levels.[72] This study indicates that there is a link between RAS activity and inflammation and also between hypertension and inflammation.[72]

2.8 Inflammation and endothelial dysfunction

If the RAS is activated it can lead to endothelial dysfunction and vascular remodeling,[75-77] which takes place due to the migration of smooth muscle cells, smooth muscle cell proliferation and changes in the extracellular matrix composition.[78,79] When remodeling of the small vessels takes place, it can

complicate hypertension by increasing the peripheral resistance.[80] Endothelial cells cannot undergo regeneration because Ang II inhibits this process leading to endothelial damage and dysfunction.[80] The process of endothelial damage is an on-going process. Elevated Ang II levels lead to increasing inflammatory cells and eventually lead to sustained vascular inflammation.[22] Endothelial function is furthermore also detrimentally affected by increased production of ROS, as a result of nicotinamide adenine dinucleotide phosphate oxidase stimulation by Ang II.[81] The endothelium regulates the release of NO, inflammatory cells can move into the vascular wall and the vascular function can be reduced when the tissues are damaged.[80]

Previous studies stated that inflammation and endothelial dysfunction is present in patients with coronary heart diseases.[82,83] Von Willebrand factor (vWf), a glycoprotein, is considered to be a useful marker for endothelial dysfunction and injury since it is involved in the activation of platelets to form thrombi and to increase adhesion of endothelial cell to the vessel wall.[84]

2.9 Decreased renin but increased inflammation

Black participants have lower renin levels compared to white participants,[85] however, they also have higher blood pressure and inflammation.[86] There are pathways of the formation of Ang II that do not include renin or ACE such as Ang-(1–12).[87]

The activity of chymase which acts on the novel substrate, Angiotensin-1-12 (Ang-1- 12) to form Ang II, was investigated in various studies including metabolic studies.[88-96] The Ang-(1-12) is important in the heart,[97] kidney[89,98] and brain[99-104] for the formation of Ang II. This new pathway, which does not include renin or ACE, may help to explain this phenomenon in blacks. Due to this different pathway, Ang II may be synthesized differently in blacks which may explain the decreased renin but increase inflammation.

Low renin hypertension in black participants is more prevalent than in white participants and black participants are also more likely to develop salt sensitivity, which is interlinked with the low plasma renin profile.[105,106] In the

following paragraphs mechanisms are investigated which may also explain this phenomenon.

2.10 Mechanisms to explain differences in the Renin-Angiotensin System (RAS) in blacks and whites

There were contradictions found in various studies which investigated mechanisms to explain differences in the RAS in black and white participants.[12,107] Previous results indicated that low renin levels have been found in urbanized black populations but not in rural black populations, implicating that urbanization plays a major role in the development of low renin hypertension.[108] In black participants lower renin levels may be the result of lifestyle changes such as increased stress levels due to rapid urbanization,[12] high renal sodium reabsorption,[12,10] reduced renin secretion rate,[109] which may be due to a decreased conversion of pro-renin to renin due to lower sPRR found in blacks, and different genetic expression of RAS proteins.[12]

2.10.1 Reduced renin secretion rate

There are three mechanisms by which renin are released through the juxtaglomerular cells. It includes a renal vascular baroreceptor, which will be effected by renal perfusion pressure changes in the afferent arteriole, a tubular macula densa-mediated process, which may be effected by distal tubular delivery rate and the renal sympathetic nerves.[110] Previous studies have shown that pro-renin is 20% higher in Africans than Caucasian subjects.[111,112] This result may be explained by a recent study done in 2014 which states that blacks may have a decreased conversion of pro-renin to renin due to lower sPRR.[113]

2.10.2 Polymorphisms in the genes encoding for RAS proteins

The low renin levels may also be due to genetic abnormalities caused in the RAS. A study showed that the polymorphism of the promoter region of the angiotensinogen gene (-20A→C) caused increases in SBP in black participants.[114] In another study the polymorphism of the aldosterone synthase gene, CYP11B2, was also linked to increases in SBP in black participants.[115] A

recent study done in 2015 on a West African population concluded that the ACE gene DD genotype predisposes this African population to the incidence of hypertension.[116]

2.10.3 High renal sodium reabsorption

Abnormal handling of sodium may be one of the mechanisms which lead to lower renin, thus the black's may have an abnormal ability to handle excessive sodium renal reabsorption in comparison with the white participants. Previous studies showed that blacks have lower renin levels [117], lower aldosterone levels [117] which lead to high sodium reabsorption and a lower sodium excretion. [118] This may lead to increased hypertension in comparison with whites. This might be because of the differences in the renal sodium transport mechanisms between blacks and whites. It is speculated that sodium reabsorption is increased in other sites of the nephron when comparing blacks and whites, which may lead to sodium retention, lower aldosterone secretion and lower renin in blacks.[107]

2.11 Aims, objectives and hypotheses

2.11.1 General aim

The aim of the study is to investigate associations of change in renin with cardiovascular and inflammatory markers in a low renin group of a black and white population: The SABPA-study

2.11.2 Objectives

- The first objective of this study is to investigate the association of percentage change in renin with cardiovascular markers (SBP, DBP and PP) in black and white participants with low renin levels.

- The second is to investigate the association of percentage change in renin with inflammation (IL-6 and CRP) in black and white participants with low renin levels.

2.11.3 Hypotheses

- Firstly, there is an inverse association between percentage change in renin and cardiovascular markers (SBP, DBP and PP) in black and white participants with low renin levels.

- Secondly, percentage change in renin is negatively associated with inflammation in black and white participants with low renin levels.

2.12 Procedure and methodology of the SABPA study

The SABPA study commenced in February, 2008 for 10 weeks (Phase I) on 409 black and white teachers with the same socio-economic status. To minimize seasonal effects the same protocol was be repeated in February 2009. Apparently healthy African and white secondary school teachers, recruited from governmental organizations in the North-West Province, were included as the human participants. The 3-year follow-up of the study (Phase II) had an 87.8 % successful return of original participants in 2011 and 2012, with 359 participants which consisted of 173 Africans (84 female, 89 male) and 186 white urban Africans (95 female 91 males) respectively. Exclusion criteria included the use of α- and β-blockers, an ear temperature > 37.5°C, participants who were vaccinated or who donated blood three months prior to the commencement of the study, and pregnant or lactating women. For this sub-study we also excluded participants who did not participate in both the baseline and follow-up phases (n=50), participants with no renin values at baseline (n=35) and follow-up (n=68). Additionally, in accordance with our aims participants with a renin value of ≥6.18 pg/ml at baseline (n=88) and follow-up (n= 17) was excluded in this study (table 1). The cut-off value of 6.18 pg/ml was selected based on the mean renin value in supine healthy subjects between 40-60 years of age (supine range:1.1-20.2 pg/ml) (Renin III Generation, CIS Biointernational, Cedex, France) (14). HIV infected participants (n=13) and participants on medication (n=1) that could interfere with the RAS was also excluded. After applying these exclusion criteria our baseline study population consisted of 73 black and 81 white participants while data from 66 black and 71 white participants were used to investigate change over a 3 year period. The following is a summarised protocol of the data collection activities SABPA I and II studies.

Participants spent the night at the Metabolic Unit Research Facility of the North-West University which consists of 10 bedrooms, 2 bathrooms, a kitchen, a dining room and a television room. When the participants arrived at the Metabolic Unit, they were introduced to the experimental setup to decrease anticipation stress[119]. They received a standardized dinner and had their last beverages (tea/coffee) and two biscuits at 20:30 h. The following day after the last ABPM was taken at 6:00 h,

various measurements were taken including anthropometric measurements followed by cardiovascular and biochemical measurements. Weight and height of the subjects were taken in triplicate with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, UK) and the body mass index was calculated[120]. Cardiovascular variables were continuously recorded for five minutes with the Finometer including systolic blood pressure (SBP), diastolic blood pressure (DBP), total peripheral resistance and arterial Windkessel compliance (Finapres Medical Systems, Amsterdam, The Netherlands). Carotid intima media thickness was measured with the Sonosite Micromaxx ultrasound system (SonoSite Inc., Bothell, WA) and a 6-13 MHz linear array transducer was used to measure carotid intima media thickness (cIMT), by obtaining a high resolution image from two angles from the left and right carotid artery[121]. A 10 millimetre segment with good image quality was chosen after the images were imported into the Artery Measurement System (AMS) II v1.139 (Gothenburg, Sweden)[122,123,124]. Hereafter, blood samples were taken with a sterile winged infusion set by a registered nurse, and serum and plasma were stored at -80°C for analysis. After completion of all procedures, HIV-positive participants received HIV post-counselling. Serum, ethylenediaminetetraacetic acid (EDTA) and sodium fluoride plasma were prepared according to standard procedures and stored at -80°C until biochemical analyses were performed. Active renin was determined with a radio-immunometric assay (Renin III Generation, CIS biointernational, Cedex, France) in EDTA plasma. Serum IL-6 levels were measured using a high sensitivity Quantikine enzyme linked immunosorbent assay (ELISA), (R&D Systems, Minneapolis, MN USA). High sensitivity CRP, gamma glutamyl transferase (GGT) as measure of alcohol abuse,[125] triglycerides and total cholesterol levels were determined in serum while glucose levels were determined in sodium fluoride plasma with three multiple analysers (Cobas Integra 400 plus, Roche, Basel, Switzerland; Konelab 20i; Thermo Scientific, Vantaa, Finland; Unicel DXC 800, Beckman and Coulter, Germany). The intra- and inter-coefficients of variation for all assays were below 10%. Von Willebrand Factor (vWf) was determined with an ELISA assay in which a polyclonal rabbit anti-vWf antibody and rabbit anti-vWf-horse radish peroxidase (HRP) antibody (DAKO, South Africa) were used. Serum cotinine levels were measured using a homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland).

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Chapter 3: Research article

Instructions for authors

Journal of Renin-Angiotensin-Aldosterone system (JRAAS)

Manuscript structure

An original article must consist of the following sections: Title page, Abstract (150-200 words see below) plus keywords, Introduction, Materials and methods, Results, Discussion, Conclusions, References, Tables, Figures and Acknowledgements.

Abstract:

This article should consist of an abstract of 150-200 words which must consist of a hypothesis/introduction, materials and methods, results, conclusions.

Main text:

The main text can include up to 5000 words

References

The reference style should be Vancouver reference style. The reference numbers should have full points in the reference list. The publications must be listed in the correct order as in the text. The Journal titles should be abbreviated using Index Medicus.

Tables and Figures

All the figures must have a key line. The figures do not have to be in full column width or page width. It must be numbered and cited in the correct order. Captions must be below the figures and left aligned

The association of the change in renin with cardiovascular and inflammatory markers in a bi-ethnic population: The SABPA study

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Abstract

Objective: To investigate if percentage change (%Δ) in renin is associated with %Δ in cardiovascular and inflammatory markers in a low renin bi-ethnic group.
Methods: Blood pressure, active renin, C-reactive protein and interleukin-6 levels of 73 black and 81 white teachers were measured at baseline and after three years.
Results: In the black group, %Δ renin was inversely associated with %Δ systolic blood pressure (β=-0.27; p=0.011). In the white group %Δ renin was inversely associated with %Δ interleukin-6 (β=-0.24; p=0.005).
Conclusions: Our results suggest that decreasing renin may be a causative factor in the development of hypertension in black South Africans.

Keywords: Blacks, C-reactive protein, hypertension, inflammation, interleukin-6, low renin, systolic blood pressure.

Introduction

The prevalence of hypertension in South Africa is a major concern. A health survey done in 2001 stated that 8.2 million people above the age of 15 years had hypertension.[1] Furthermore, only 2.7 million people with hypertension receive anti-hypertensive treatment.[1] More recently it was indicated that 78% of South Africans above 50 years of age have hypertension, underlining the increasing burden of cardiovascular disease in South Africa.[2]

Increased renin, the rate limiting step in the renin-angiotensin system (RAS), is associated with increased synthesis of angiotensin II (Ang II) from angiotensinogen.[3] In turn, increased Ang II levels are associated with increased inflammation[4] and may lead to the development of hypertension.[5] On the other hand, suppressed RAS activity, as a consequence of low renin levels, may lead to the development of volume loading hypertension (low-renin hypertension).[6]

Lower renin levels in black participants when compared to their white counterparts were previously reported.[7,8] Additionally, we also indicated inverse associations of renin levels with ambulatory systolic and diastolic blood pressure in black men.[9] In low-renin black men and women, renin was also associated with the albumin-to-creatinine ratio, suggesting a link between low renin levels and subclinical end-organ damage.[10]

In contrast with the lower renin activity found in black participants, levels of inflammation are higher in black participants when compared to white participants.[11] We previously linked increased blood pressure with increased inflammation in a high C-reactive protein (CRP) (>3 mg/L) group of black men.[12] In the present sub-study we therefore aimed to investigate if change (over a three-year period) in renin levels is associated with change in cardiovascular and inflammatory markers in a low renin black and white cohort.

Materials and methods

Study population and protocol

This study formed part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study that took place between 2008/9 (baseline) and 2011/12 (follow-up). A detailed description of the study population and protocol has been published.[13] At baseline 409 black and white teachers with a similar sex distribution were included and 359 teachers returned for the follow-up study (87.3 % compliance to follow-up). Exclusion criteria included the use of α- and β-blockers, an ear temperature > 37.5°C, participants who were vaccinated or who donated blood three months prior to the commencement of the study, and pregnant or lactating women. For this sub-study we also excluded participants who did not participate in both the baseline and follow-up phases (n=50) and participants with no renin values at baseline (n=35) and follow-up (n=68). Additionally, in accordance with our aims participants with a renin value of ≥6.18 pg/ml at baseline (n=88) and follow-up (n= 17) was excluded in this study. The cut-off value of 6.18 pg/ml was selected based on the mean renin value in healthy subjects between 40-60 years of age (supine range:1.1-20.2 pg/ml) (Renin III Generation, CIS Biointernational, Cedex, France).[10] HIV infected participants (n=13) and participants on medication (n=1) that could interfere with the RAS was also excluded. After applying these exclusion criteria our baseline study population consisted of 73 black and 81 white participants while data from 66 black and 71 white participants were used to investigate change over a 3 year period.

Ethical considerations

All the subjects were informed about the protocol of the study as well as the benefits and the risks involved in taking part, after which each participant signed an informed consent form. The study fulfilled all the requirements as stated in the Helsinki Declaration of 1975 (revised in 2004) for investigation on human subjects. The SABPA study was approved by the Ethics Review Board of the North-West

University and this sub-study was also approved by the Health Research Ethics Committeeof the North-West University.

Anthropometric measurements

Weight and height of the subjects were taken in triplicate with calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, UK) and body mass index was calculated.[14]

Questionnaires

All the subjects completed a general health questionnaire containing questions on their lifestyle habits such as smoking and medication usage.

Cardiovascular measurements

The Finometer was used to measure systolic blood pressure (SBP) and diastolic blood pressure (DBP) after five minutes resting period (Finapres Medical Systems, Amsterdam, The Netherlands). Total peripheral resistance (TPR) and Windkessel compliance (Cwk) were calculated using the Fast Modelflo computer software program from the Finometer data. The Sonosite Micromaxx ultrasound system (SonoSite Inc., Bothell, WA) and a 6-13 MHz linear array transducer was used to measure carotid intima media thickness (cIMT), by obtaining a high resolution image from two angles from the left and right carotid artery. A 10 millimetre segment with good image quality was chosen after the images were imported into the Artery Measurement System (AMS) II v1.139 (Gothenburg, Sweden). The mean cIMT were calculated through determining the borders of the far and near wall and the inner diameter of the vessel from 100 discrete measurements through the 10 mm segment for each participant. The analysis of the cIMT was done by one observer and the intra-observer variability was 0.04 millimeter between two measurements made four weeks apart (n=10).

Biochemical analyses

Blood samples from fasting participants were obtained from their right arm brachial vein branches with a sterile winged infusion set by a registered nurse after a 5 min rest in the semi-Fowler's position. Serum, ethylenediaminetetraacetic acid (EDTA) and sodium fluoride plasma were prepared according to standard procedures and stored at -80°C until biochemical analyses were performed. Active renin was determined with a radio-immunometric assay (Renin III Generation, CIS biointernational, Cedex, France) in EDTA plasma. Serum IL-6 levels were measured using a high sensitivity Quantikine enzyme linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN USA). High sensitivity CRP, gamma glutamyl transferase (GGT) as measure of alcohol abuse,[15] triglycerides and total cholesterol levels were determined in serum while glucose levels were determined in sodium fluoride plasma with three multiple analysers (Cobas Integra 400 plus, Roche, Basel, Switzerland; Konelab 20i; Thermo Scientific, Vantaa, Finland and Unicel DXC 800, Beckman and Coulter, Germany). The intra- and inter-coefficients of variation for all assays were below 10%. Von Willebrand Factor (vWf) was determined with an ELISA assay in which a polyclonal rabbit anti-vWf antibody and rabbit anti-vWf-horse radish peroxidase (HRP) antibody (DAKO, South Africa) were used. Serum cotinine levels were measured using a homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland).

Statistical analyses

Statistical analyses were performed with Statistica 12 (Statsoft Inc., Tulsa, OK, USA). Values are regarded as significant when $p \leq 0.05$. Data were expressed as arithmetic mean and standard deviation for normally distributed variables. Variables with a non-Gaussian distribution (renin, IL-6, CRP, vWf, cotinine, GGT) were logarithmically transformed and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions were compared between black and white groups using independent t-tests and Chi-square tests, respectively. Percentage change for all variables were calculated and compared between black and white groups while adjusting for age, sex and baseline values using analyses of covariance (ANCOVA). Correlations of percentage change

in renin with percentage change in biochemical and cardiovascular variables were performed using partial regression analyses (adjusted for sex and age at baseline). Forward stepwise multiple regression analyses were performed to determine independent associations of percentage change in renin with percentage change in inflammatory and cardiovascular variables. Two models were compiled with percentage change in renin as the dependent variable in both models. In model 1 the main independent variable was percentage change in SBP. The covariates that were entered in model 1 were age, baseline SBP, baseline renin, percentage change in IL-6, baseline IL-6, baseline GGT and percentage change in GGT. In model 2 the main independent variable was percentage change in PP. The covariates entered for model 2 were age, baseline PP, baseline renin, percentage change in IL-6, baseline IL-6, baseline GGT and percentage change in GGT. In sensitivity analyses, we substituted percentage change in IL-6 with percentage change in CRP and age with BMI in separate analyses. Since anti-inflammatory medication usage may influence the inflammatory response and the results, we also added anti-inflammatory medication usage as covariate and repeated the multiple regression analyses.

Results

The characteristics of black (n=73) and white (n=81) participants with low renin at baseline are compared in Table 1. The cardiovascular profile of black and white participants with low renin was comparable, except for the higher DBP (p=0.02) noted in the black group compared to the white group. The black group had lower renin (3.87 pg/ml vs. 4.59 pg/ml; p=0.002), glucose (p=0.001) and total cholesterol (p<0.001) levels. The inflammatory marker, CRP (p=0.006) as well as vWf levels (p<0.001) were higher in the black participants when compared to their white counterparts. Both cotinine and self-reported smoking were similar between the black and white groups, but the black participants had higher GGT levels (p<0.001) compared to the white participants.

Table 1. Characteristics of low renin black and white participants at baseline.

	Low renin		
	Black (n=73)	White (n=81)	p-value
Age (years)	41.2±6.55	46.9±8.72	<0.001
Sex (n, male)	36	31	0.167
Body mass index (kg/m²)	28.1±5.86	27.0±5.44	0.232
Cardiovascular measurements			
Systolic blood pressure (mmHg)	136±16	135±14	0.793
Diastolic blood pressure (mmHg)	79±9	76±7	0.021
Pulse pressure (mmHg)	56.4±10.4	58.9±10.7	0.141
Total peripheral resistance (mmHg/ml/s)	1.01±0.25	1.05±0.3	0.399
Windkessel compliance (ml/mmHg)	1.91±0.40	2.02±0.54	0.148
Carotid intima media thickness (mm)	0.65±0.10	0.66±0.11	0.557
Biochemical analyses			
Renin (pg/ml)	3.87 (2.21;6.66)	4.59 (2.43;6.98)	0.002
Interleukin-6 (pg/ml)	2.16 (2.06;2.22)	2.15 (2.08;2.22)	0.286
C-reactive protein (mg/l)	5.07 (2.27;26.6)	4.12 (2.99;11.0)	0.006
Glucose (mmol/l)	5.15±0.92	5.59±0.71	0.001
Von Willebrand factor (%)	86.2 (54.9;135)	60.1 (40.9;87.1)	<0.001
Triglycerides (mmol/l)	1.18±1.03	1.16±0.69	0.882
Total cholesterol (mmol/l)	4.48±1.17	5.84±1.19	<0.001
Lifestyle			
γ-Glutamyltransferase (U/l)	36.7 (16.6;180)	16.4 (7.00;76.0)	<0.001
Cotinine (ng/ml)	2.58 (2.00;114)	2.58 (2.0;245)	0.669
Smoking (n)	8	14	0.249

Data expressed as mean ± standard deviation, geometric mean (5^{th} and 95^{th} percentiles) and n.

Table 2. Percentage change in anthropometric, cardiovascular, lifestyle and biochemical variables.

	Low renin		
	Blacks (n=66)	Whites (n=71)	p-value
Body mass index (%)	4.29 (2.48;6.10)	3.35 (1.60;5.12)	0.484
Cardiovascular measurements			
Systolic blood pressure (%)	1.91 (-0.21;4.04)	-1.77(-3.84;0.30)	0.020
Diastolic blood pressure (%)	2.53 (0.29;4.77)	-0.62 (-2.80;1.56)	0.059
Pulse pressure (%)	2.43 (-1.09;5.96)	-2.52 (-5.95;0.91)	0.057
Total peripheral resistance (%)	13.0 (6.06;19.99)	2.23 (-4.55;9.01)	0.036
Windkessel compliance (%)	-3.46 (-7.68;0.76)	0.97 (-3.14;5.09)	0.169
Carotid intima media thickness (%)	1.69 (-0.73;4.12)	7.96 (5.67;10.24)	<0.001
Biochemical analyses			
Renin (%)	-17.5 (-24.3;-10.7)	2.17 (-4.39;8.74)	<0.001
Interleukin-6 (%)	59.4 (52.0;66.9)	35.5 (28.3;42.7)	<0.001
C-reactive protein (%)	0.34 (-6.80;7.48)	-9.36 (-16.3;-2.45)	0.069
Glucose (%)	0.57 (-4.73;5.87)	-22.7 (-27.9;-17.6)	<0.001
Von Willebrand factor (%)	3.11 (1.45;4.77)	5.76 (4.14;7.38)	0.048
Lifestyle			
γ-Glutamyltransferase (%)	-1.62 (-6.73;3.49)	-4.17 (-9.10;0.75)	0.512
Cotinine (%)	-1.80 (-17.2;13.6)	6.86 (-8.01;21.7)	0.443

Data expressed as adjusted mean (least square mean) percentage change with 95% confidence intervals. Adjusted for baseline age, sex and baseline values.

In Table 2, the percentage change in the anthropometric, cardiovascular, lifestyle and biochemical variables are shown. Both SBP and DBP increased in the black group, while it decreased in the white group, with the difference between the groups being significant (SBP: p=0.02 and DBP: p=0.059). Although TPR increased in both groups, the increase in the black group was significantly higher (p=0.036). The cIMT also increased in both racial groups, but to a greater extent in the whites (p<0.001).

Renin decreased in the black group and increased in the white group (-17.47% vs. 2.17%; p<0.001). Regarding the inflammatory markers, IL-6 increased in both groups but more in the black group (59.44% vs. 32.51%; p<0.001) while CRP increased slightly in the black group and decreased in the white group, with the difference between the groups being borderline significant (p=0.069).

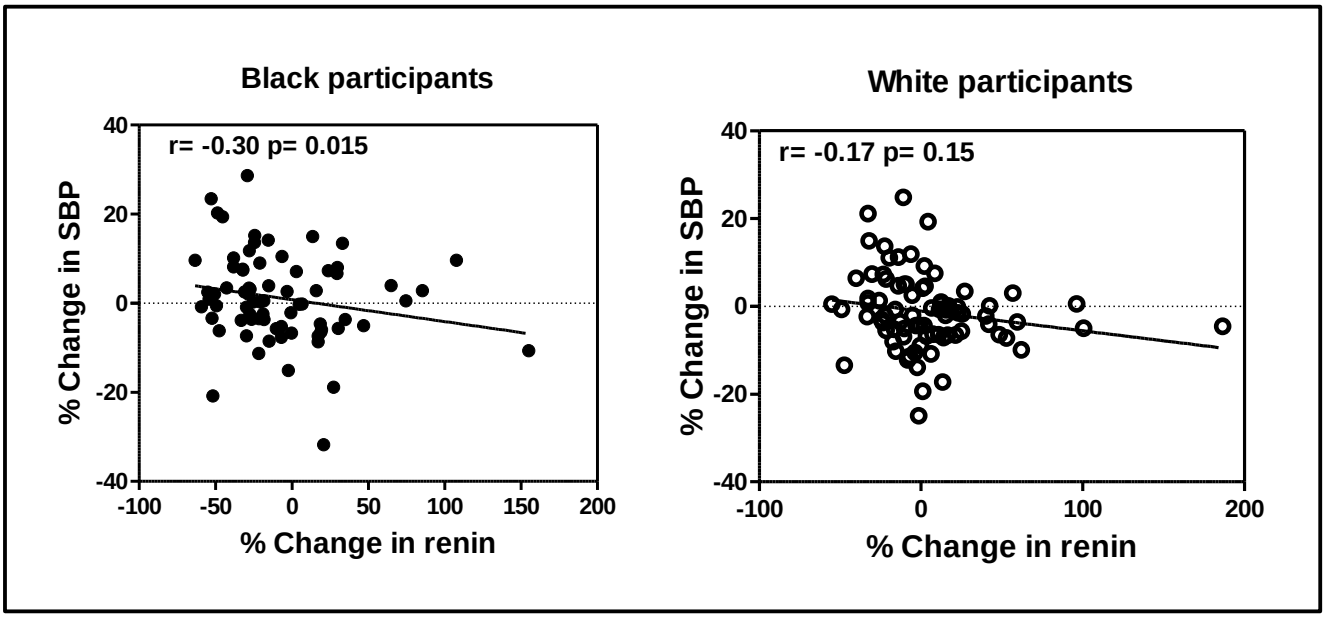

Figure 2. Single regression analyses of percentage change in renin and percentage change in SBP in low renin black and white groups

In single regression (Figure 2) and partial regression analyses (Table S1) percentage change in renin were inversely correlated with percentage change in SBP (r= -0.30; p=0.015) (Figure 2) and with percentage change in PP (r= -0.36; p=0.003) (Table S1) in the low renin black group only. These associations were absent in the low renin white group, however percentage change in renin were inversely correlated with percentage change in IL-6 (r=-0.25, p=0.037), in the low renin white group.

In multiple regression analyses, the inverse association of percentage change in renin with percentage change in SBP in the low renin black group was confirmed to be independent of various covariates (β=-0.27; p=0.011; Table 3). Additionally, percentage change in GGT (β=0.26; p=0.017) was also positively associated with percentage change in renin in the low renin black participants (model 1). In the same group, when we investigated whether the association of percentage change in renin with percentage change in PP is independent, percentage change in PP no longer entered the model (model 2). In the low renin white group the association of percentage change in renin with percentage change in IL-6 (β= -0.24; p=0.005; Table 3) was also confirmed to be independent of various covariates.

In sensitivity analyses, we substituted percentage change in IL-6 with percentage change in CRP, the results remained the same in the low renin black group, but in the low renin white group only baseline renin (β=-0.70; p<0.001) remained independently associated with percentage change in renin. When we substituted age with BMI in model 1, the results remained the same in both groups. After the addition of anti-inflammatory medication usage as covariate to the models the results also remained largely the same.

Table 3. Forward stepwise regression analysis with percentage change in renin as dependent variable in black and white participants.

	Percentage change in renin			
	Blacks (n=76)		Whites (n=99)	
Adjusted R^2	0.34		0.54	
	β (95% CI)	p-value	β (95% CI)	p-value
Age (baseline)	0.21 (0.01; 0.41)	0.046	-	-
Systolic blood pressure (% change)	-0.27 (-0.48; -0.07)	0.011	-	-
Gamma glutamyltransferase (% change)	0.26 (0.05; 0.46)	0.017	-	-
Renin (baseline)	-0.46 (-0.66; -0.25)	<0.001	-0.71 (-0.87; -0.55)	<0.001
Interleukin-6 (% change)	-	-	-0.24 (-0.40; -0.08)	0.005

Independent variables included in the model: percentage change in SBP, baseline SBP, age, percentage change in GGT, baseline GGT, baseline renin, percentage change in IL-6 and baseline IL-6. -, Covariate did not enter model

Supplementary table S1. Partial correlations of percentage change in renin with biochemical and cardiovascular variables.

	Low renin			
	Blacks (n=66)		Whites (n=71)	
	r-value	p-value	r-value	p-value
Cardiovascular measurements				
Systolic blood pressure (%)	-0.319	0.010	-0.175	0.153
Diastolic blood pressure (%)	-0.114	0.368	-0.020	0.869
Pulse pressure (%)	-0.360	0.003	-0.186	0.129
Total peripheral resistance (%)	-0.079	0.531	-0.012	0.926
Windkessel compliance (%)	0.105	0.409	0.047	0.704
Carotid intima media thickness (%)	0.112	0.400	0.011	0.929
Biochemical analyses				
Interleukin-6 (%)	0.034	0.791	-0.252	0.037
C-reactive protein (%)	0.078	0.539	-0.065	0.596
Glucose (%)	0.088	0.492	-0.214	0.077
Von Willebrand factor (%)	-0.055	0.667	0.022	0.861

Adjusted for baseline age and sex

Discussion

The aim of the study was to investigate associations of change in renin with cardiovascular and inflammatory markers in a low renin bi-ethnic South African population. The most prominent result was an independent association between a decrease in renin levels and increased SBP in black participants with low renin levels. These findings are not only in line with previous findings indicating an association between low renin and blood pressure[16] but also strengthen the suggestion that low renin may be a causative factor in the development of hypertension in black South Africans.[17]

There are conflicting results in the literature about the different mechanisms leading to low renin levels in black participants when compared to white participants. These factors include high renal sodium reabsorption,[17,18] reduced renin secretion rate[19] through decreased conversion of pro-renin to renin as a result of lower soluble plasma renin receptor found in blacks.[20] Other factors include polymorphisms in genes encoding for RAS proteins[17] and lifestyle factors such as rapid urbanization.[17]

Although the exact mechanism linking decreased renin levels with increased blood pressure is not clear, it may be a result of volume loading. Volume loading is associated with increased sodium retention, which may result in the retention of water by the kidneys. When water is retained it may lead to expansion of the extracellular fluid volume and thereby increasing blood pressure.[6] In turn, this may lead to an even further suppression of renin secretion.[6] Our results, furthermore, suggest that the development of volume loading hypertension may be aggravated in the black population through excessive alcohol consumption[21] as indicated by the association of percentage change in renin with GGT levels, although not a focus of this article.

In the low renin white group, percentage change in renin was negatively associated with percentage change in IL-6 (inflammation). This is difficult to explain since activation of the RAS, through an increase in the rate limiting step, (renin) may lead to increased inflammation, since increased Ang II may stimulate the production of

inflammatory cytokines in vascular smooth muscle and endothelial cells.[22] Although there was a slight increase (2%) in renin levels in the white group renin levels were still in the low range and may therefore not have been sufficient to elicit an increased inflammatory response.

Since there was no association between percentage change in renin levels and inflammatory markers in the low renin black group, despite the increase in inflammatory markers at follow-up. It is, therefore, expected in the black individuals that with decreased RAS activation, decreased secretion of inflammatory cytokines will ensue. However in our study, mean IL-6 levels at baseline in the low renin black participants indicated high inflammatory levels. Our results suggest that other mechanisms may be involved in the stimulation of inflammatory responses, in this group. These mechanisms may include other enzymes such as chymase and catepsin which are involved in the convertion of Ang I to Ang II.[23]

The results of this study have to be interpreted within the context of its strengths and limitations. This is a well-controlled study which included urbanized black and white participants, allowing comparison between these groups. This is, to the best of our knowledge, the first study investigating how changes in renin over a period of three years are associated with change in cardiovascular variables and inflammation in both black and white individuals. The limitation of this study is that our sample was relatively small and was recruited from urban areas in the Potchefstroom District of the North-West Province and cannot be seen as representative of the entire South African population.

In conclusion, in black participants with low renin levels at baseline, a further decrease in renin levels over three years is independently associated with an increase in SBP. These results strengthen the notion that low renin may be a causative factor in the development of hypertension in black South Africans, but that the renin- angiotensin system is not the driving force behind the increased inflammation observed in this low renin black group.

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Declaration of interest

The authors declare no conflict of interests

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Chapter 4: Concluding chapter

4.1 Summary of main findings

The aim of the study was to investigate associations of change in renin with cardiovascular and inflammatory markers in a low renin group of a black and white population.

The hypotheses will be reviewed and compared to the results of this study.

- Hypothesis 1: There is an inverse association between percentage change in renin and cardiovascular markers (SBP, DBP and PP) in black and white participants with low renin levels.

The results obtained in this study showed that there was an inverse association of percentage change in renin with percentage change in SBP in the low renin black group. With regards to DBP, we found no association between percentage change in renin and percentage change in DBP in either of the groups. When investigating whether percentage change in renin is associated with percentage change in PP, we also found an inverse relationship in partial regression analyses in the low renin black group, but in multivariate analyses, percentage change in PP no longer entered the model. We were unable to establish any of these associations in the low renin white group. Therefore, the first hypothesis is only partially accepted since we were unable to establish any relationship between change in renin and cardiovascular markers in the low renin white group and only between change in renin and change in SBP in the low renin black group.

- Hypothesis 2: The percentage change in renin is negatively associated with inflammation in black and white participants with low renin levels.

The results of this study showed that there was a negative association between percentage change in renin and percentage change in inflammation in the white low renin participants only. Although there was a slight increase (2%) in renin levels in the white group renin levels were still in the low range and may therefore not have

been sufficient to elicit an increased inflammatory response. In the low renin black group there was no association between percentage change in renin levels and inflammatory markers, but rather an increase in inflammatory markers over the three-year follow-up time. These results suggest that there may be other mechanisms involved in the stimulation of inflammatory responses in the black participant group.

Therefore, the second hypothesis is also partially accepted since we only found a negative association between percentage change in renin and percentage change in inflammation in the white low renin group only.

4.2 Strengths and limitations

4.2.1 Strengths

The SABPA study is a well-controlled and organized study. This study took place in the same environment for all the participants and they were all teachers with the same socioeconomic status at baseline and from the same province. Golden standard methods were used to measure the anthropometric, cardiovascular and biochemical measurements.

4.2.2 Limitations

The limitation of this study is that our participant group was relatively small and recruited from urban areas in the Potchefstroom District of the North-West-Province and cannot be seen as representative of the entire South African population. Although there were adjustments made for certain covariates such as age, BMI, sex and baseline values, there may be other unknown variables which could interfere with the associations found in this study.

4.2.3 Recommendations

- Future studies should make use of a larger low renin population group.
- There must be a longer period of duration between the baseline and follow-up of participants.

- Future studies should include aldosterone and the renin/aldosterone ratio should also be used in the analysis.
- Other inflammatory markers, which were not included in this study such as TNF-α should also be investigated further.

Appendix A

NORTH-WEST UNIVERSITY ®
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22 September 2014

Prof JM van Rooyen
Physiology

Dear Prof van Rooyen

HREC APPROVAL OF YOUR APPLICATION

Ethics number: NWU-00036-07-A6 (Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study)

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

Project title: The association of renin with inflammatory markers in a bi-ethnic population: The SABPA study

Project leader/supervisor: Prof JM van Rooyen

Student: R Swart

Application type: Sub-study

Risk level descriptor: Minimal

You are kindly informed that at the meeting held on 14/05/2015 of the HREC, Faculty of Health Sciences, the aforementioned was approved.

The period of approval for this project is from 22/09/2015 to 31/12/2016.

After ethical review:

Translation of the informed consent document to the language's applicable to the study participants should be submitted to the HREC (if applicable).

The HREC requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the protocol or other associated documentation must be submitted to the HREC 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.

A progress report should be submitted within one year of approval of this study 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 must be notified if the study is temporarily suspended

or terminated. The progress report template is obtainable from Carolien van Zyl at Carolien.VanZyl@nwu.ac.za.. Annually a number of projects may be randomly selected for an external audit.

Please note that the HREC 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. Ethics approval is required BEFORE approval can be obtained from these authorities.

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

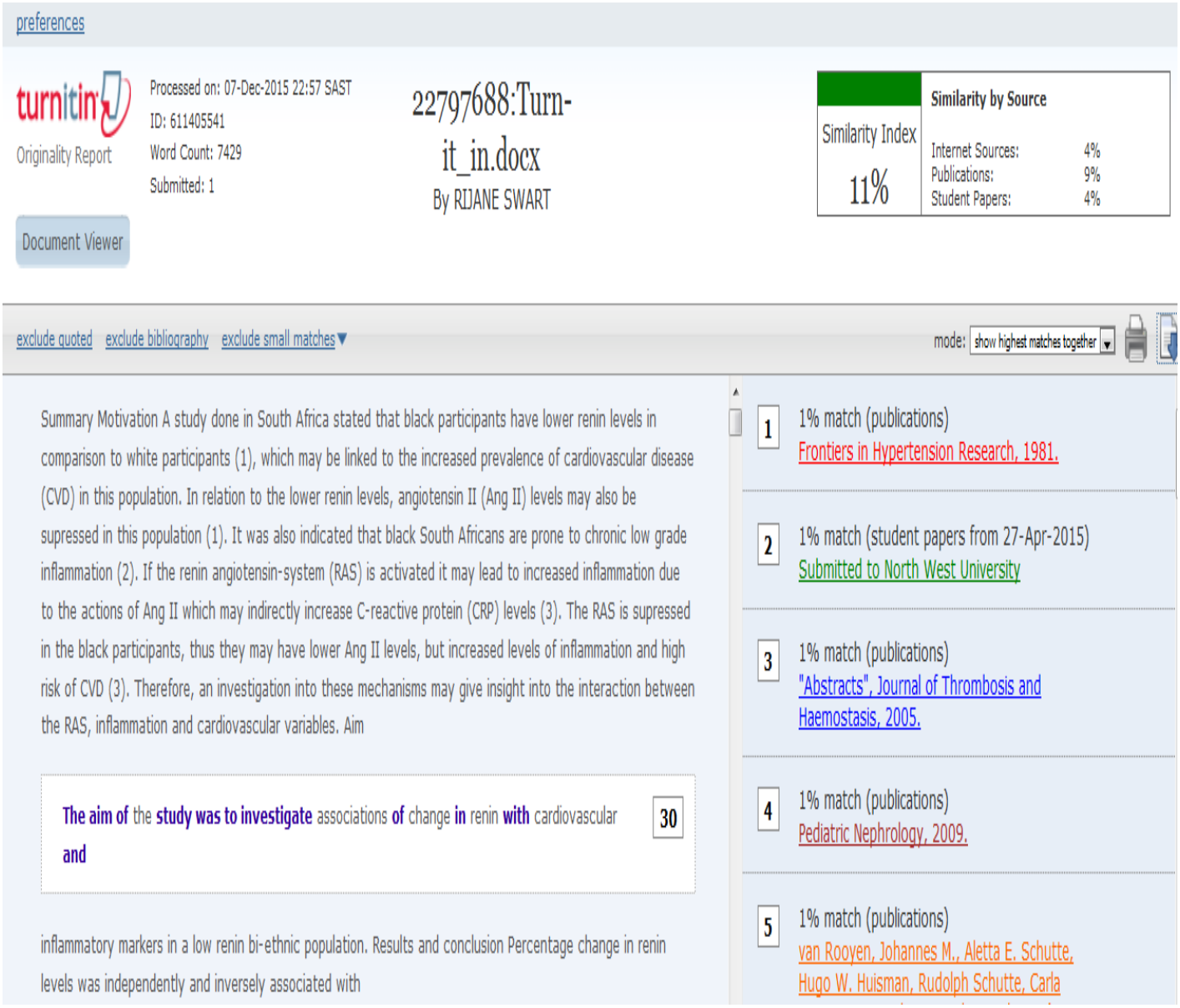
We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Ethics Office at Carolien.VanZy@nwu.ac.za or 018 299 2089.

Yours sincerely

Prof Minrie Greeff
HREC Chairperson

File reference: 9.1.5.3

Appendix B



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Summary Motivation A study done in South Africa stated that black participants have lower renin levels in comparison to white participants (1), which may be linked to the increased prevalence of cardiovascular disease (CVD) in this population. In relation to the lower renin levels, angiotensin II (Ang II) levels may also be supressed in this population (1). It was also indicated that black South Africans are prone to chronic low grade inflammation (2). If the renin angiotensin-system (RAS) is activated it may lead to increased inflammation due to the actions of Ang II which may indirectly increase C-reactive protein (CRP) levels (3). The RAS is supressed in the black participants, thus they may have lower Ang II levels, but increased levels of inflammation and high risk of CVD (3). Therefore, an investigation into these mechanisms may give insight into the interaction between the RAS, inflammation and cardiovascular variables. Aim

The aim of the study was to investigate associations of change in renin with cardiovascular and 30

inflammatory markers in a low renin bi-ethnic population. Results and conclusion Percentage change in renin levels was independently and inversely associated with

1 1% match (publications)
Frontiers in Hypertension Research, 1981.

2 1% match (student papers from 27-Apr-2015)
Submitted to North West University

3 1% match (publications)
"Abstracts", Journal of Thrombosis and Haemostasis, 2005.

4 1% match (publications)
Pediatric Nephrology, 2009.

5 1% match (publications)
van Rooyen, Johannes M., Aletta E. Schutte, Hugo W. Huisman, Rudolph Schutte, Carla

Appendix C

DECLARATION

I, Clarina Vorster (ID: 710924 0034 084), Language editor and Translator, and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of the dissertation of ms R Swart (student nr 22797688) from the Northwest-University.

Title of the article: ***Change in renin, cardiovascular and inflammatory markers over three years in a bi-ethnic population: The SABPA-study***

C Vorste _____ 25 Nov 2015

C Vorster *Date*

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1

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