N-terminal prohormone B-type natriuretic peptide, inflammation and the vasculature: exploring the links in a bi-ethnic South African population

Ruan Kruger

20035632

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Promoter: Prof. R Schutte

Co-promoters: Prof. AE Schutte

Prof. HW Huisman

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"Seek a valuable lesson in every difficulty, because you become what you think about most of the time." - ANON

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SUMMARY

Motivation

Cardiovascular disease states including hypertension and vascular stiffness are precursors of cardiac damage such as heart failure. The prevalence of cardiovascular disease among the African population in South Africa is also increasing dramatically. The N-terminal prohormone B-type natriuretic peptide (NT-proBNP) is a reliable biomarker and predictor of cardiovascular risk and heart failure. During the onset and development of heart failure, the heart undergoes structural and functional changes including hypertrophy and vascular remodelling. NT-proBNP levels are normally lower in men compared to women, but less is known about ethnic differences and also the associations between NT-proBNP and measures of cardiovascular function. Information on factors affecting vascular function and therefore the synergy between blood vessels and the heart leading to cardiac damage in a bi-ethnic South African population is also scant. Therefore, this study included markers of both atherosclerosis (C-reactive protein, soluble urokinase plasminogen activator receptor, fibulin-1) and arteriosclerosis (arterial compliance and alkaline phosphatase) to address the underlying vascular changes that augment cardiac load and damage. The lack of information in this regard, especially in South Africans, serves as motivation for this study.

Aim

The purpose of this study was to explore the possible associations of NT-proBNP with cardiovascular function and also biochemical components that may contribute to the development of cardiovascular disease in both African and Caucasian men and women.

Methodology

Data from the South African study regarding the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC) were used, and presented in the manuscript Chapters 3, 4, 5 and 6. This study included 756 Africans and Caucasians in total. Groups were stratified by

ethnicity or gender, or both ethnicity and gender as specified by statistical interaction terms. Cardiovascular measurements were performed and NT-proBNP, fibulin-1, high sensitivity C-reactive protein (CRP), soluble urokinase plasminogen activator receptor (suPAR) and also alkaline phosphatase (ALP) levels were determined. Means were compared with either T-tests or analysis of variance (ANOVA). Significant differences between groups were also tested with analysis of covariance (ANCOVA) with adjustments applied for age, body mass index (BMI) and systolic blood pressure (SBP). Partial correlations were performed to investigate associations between various variables with adjustments applied for age, BMI and SBP. Multiple regression analyses were performed to investigate independent associations between variables in the different groups.

Results and conclusions of each manuscript

- The first paper in this thesis (Chapter 3) aimed to compare NT-proBNP as a marker of cardiac load and its possible associations with markers of cardiovascular function in Africans and Caucasians. The results indicated that the African population revealed higher NT-proBNP levels compared to Caucasians, however, these were partially confounded by SBP and completely by arterial compliance. NT-proBNP was positively associated with both SBP and pulse pressure in Africans, but not in Caucasians. Also, after adjustments were applied for significant covariates and confounders, the positive significant association remained in Africans only. These associations may suggest early vascular changes contributing to cardiac alterations in Africans.

- The aim in Chapter 4 was to explore the relationship between NT-proBNP and fibulin-1 (an extracellular matrix component and also expressed in atherosclerotic lesions) in African and Caucasian men and women. NT-proBNP was positively associated with fibulin-1 in African men only after adjustments were applied for age, BMI, SBP, heart rate and estimated creatinine clearance. No significant link existed between NT-proBNP and measures of arterial stiffness in any of the groups. However, after full adjustment, the positive significant association between

NT-proBNP and fibulin-1 was confirmed in African men and also in younger African men and women after excluding participants older than 55 years. These associations were not present in the Caucasians. This suggests that vascular alterations also occur in young African men and women and that they may be prone to develop cardiovascular disease much earlier as opposed to Caucasian men and women.

- Due to earlier vascular changes present in the African population, we aimed to investigate the link between NT-proBNP and inflammatory markers (both CRP and suPAR) in African men and women (Chapter 5), independent of a hypertensive state. Although the levels of NT-proBNP and inflammatory markers were lower in men compared to women, the results showed that NT-proBNP is positively and significantly associated with both CRP and suPAR in the normotensive African men only. No significant association was observed in normotensive African women. After full adjustments in multiple regression analyses, the positive significant association between NT-proBNP, CRP and suPAR was confirmed in African men. This suggests that in a low-grade inflammatory state, normotensive African men are more susceptible to developing vascular alterations that may result in cardiac overload and damage.

- In Chapter 6 we explored the possible association of NT-proBNP with a marker of osteoblast-activity, alkaline phosphatase (ALP). This sub-study was performed in a bi-ethnic male population. The results revealed a positive association between NT-proBNP and ALP in African men, but not in Caucasian men. African men also had higher NT-proBNP and ALP levels as opposed to Caucasian men. After adjusting for significant covariates, the link between cardiac strain and osteoblastic activity, and possible vascular calcification was confirmed in African men. This population seems to have a higher susceptibility to develop sclerosis in either the media or intima, which could contribute to cardiovascular damage due to a possible increased cardiac afterload.

General conclusion

NT-proBNP, a reliable marker of cardiac overload and damage, was positively associated with systolic blood pressure, pulse pressure, fibulin-1, C-reactive protein, soluble urokinase plasminogen activator receptor and alkaline phosphatase. Throughout this study, our findings were persistent in the black South African population, especially African men. These results indicate that the earlier burden of cardiovascular disease in young Africans may result from early vascular changes due to inflammation, extracellular matrix alterations and calcification which could cascade into cardiac strain and damage.

Key words: NT-proBNP, cardiovascular function, fibulin-1, inflammation, Africans

AFRIKAANSE TITEL: N-terminaal prohormoon B-tipe natriuretiese peptied, inflammasie en die vaskulatuur: ‘n ondersoek na die verbande in 'n bi-etniese Suid-Afrikaanse populasie.

OPSOMMING

Motivering

Kardiovaskulêre siekte toestande, insluitende hipertensie en vaskulêre styfheid, is voorlopers van kardiale skade soos hartversaking. Die voorkoms van kardiovaskulêre siektes onder die swart bevolking van Suid-Afrika is ook besig om dramaties toe te neem. Die N-terminaal prohormoon B-tipe natriuretiese peptied (NT-proBNP) is ’n betroubare biomerker en voorspeller van kardiovaskulêre risiko en hartversaking. Tydens die aanvang en ontwikkeling van hartversaking, ondergaan die hart strukturele en funksionele veranderinge wat hipertrofie en vaskulêre hermodellering insluit. NT-proBNP vlakke is normaalweg laer in mans as in vrouens en weinig is bekend oor die rasverskille asook die assosiasies tussen NT-proBNP en kardiovaskulêre metings. Inligting oor veranderinge en komponente wat bydra tot die aanvang van kardiale skade in ’n bi-etniese Suid-Afrikaanse bevolking is ook skaars. Hierdie studie sluit dus merkers van beide aterosklerose (C-reaktiewe proteïen, oplosbare urokinase plasminogeen aktiveerder reseptor, alkaliese fosfatase) en arteriosklerose (arteriële meegewendheid en fibulien-1) in, om sodoende die onderliggende vaskulêre veranderinge wat kardiale lading en skade ondersteun, aan te spreek. Die tekort aan inligting in hierdie verband, veral in Suid-Afrikaners, dien as motivering vir hierdie studie.

Doelstelling

Die doel van hierdie studie is om die moontlike assosiasies van NT-proBNP met kardiovaskulêre funksie en ook biochemiese komponente wat kan bydra tot die ontwikkeling van kardiovaskulêre siekte in beide Afrikaan en Kaukasiër mans en vrouens te ondersoek.

Metodologie

Die data van die Suid-Afrikaanse studie rondom die rol van geslag, ouderdom en etnisiteit op insuliensensitiwiteit en kardiovaskulêre funksie (SAfrEIC) is gebruik, wat in die manuskrip Hoofstukke 3, 4, 5 en 6 voorgestel is. Die studie sluit ’n totaal van 756 Afrikane en Kaukasiërs in. Die groepe is verdeel in Afrikane en Kaukasiërs óf mans en vrouens, óf beide Afrikaan en Kaukasiër mans en vrouens, wat deur statistiese interaktiewe terme bepaal is. Kardiovaskulêre metings is geneem en NT-proBNP, fibulien-1, hoë sensitiewe C-reaktiewe proteïen (CRP), oplosbare urokinase plasminogeen aktiveerder reseptor (suPAR) vlakke en ook alkaliese fosfatase (ALP) is bepaal. Gemiddelde waardes is vergelyk met óf t-toetse óf die variansie analises (ANOVA). Betekenisvolle verskille tussen groepe is ook met behulp van kovariansie analises (ANKOVA) getoets, terwyl daar vir ouderdom, liggaamsmassa indeks (LMI) en sistoliese bloeddruk (SBD) gekorrigeer is. Parsiële korrelasies is gebruik om assosiasies tussen verskillende veranderlikes te vergelyk, terwyl daar vir ouderdom, LMI en SBD gekorrigeer is. Meervoudige regressie analises is uitgevoer om onafhanklike assosiasies tussen veranderlikes in die verskillende groepe te bepaal.

Resultate en gevolgtrekkings van onderskeie manuskripte

- Met die eerste artikel in dié proefskrif (Hoofstuk 3) is beoog om NT-proBNP as ’n merker van kardiale lading asook die moontlike assosiasies met kardiovaskulêre komponente tussen Afrikane en Kaukasiërs te vergelyk. Die resultate dui aan dat die swart bevolking hoër NT-proBNP vlakke toon in vergelyking met Kaukasiërs, alhoewel die vlakke gedeeltelik deur SBD en heeltemal deur arteriële kompliansie beïnvloed word. NT-proBNP het positief met beide SBD en polsdruk in Afrikane geassosieer, maar nie in Kaukasiërs nie. Hierdie positiewe betekenisvolle assosiasie is bevestig nadat die nodige kovariante in ag geneem is. Hierdie assosiasies kan moontlik dui op vroeë vaskulêre veranderinge wat verder kan bydra tot kardiale veranderinge in Afrikane.

- Die doel van Hoofstuk 4 is om die verhouding tussen NT-proBNP en fibulien-1 ('n ekstrasellulêre matriks komponent wat in aterosklerotiese letsels voorkom) in Afrikaan en Kaukasiër mans en vrouens te ondersoek. NT-proBNP het positief en betekenisvol met fibulin-1 alleenlik in Afrikane mans geassosieer, nadat daar vir ouderdom, LMI, SBD, harttempo en voorspelde kreatinien opruiming gekorrigeer is. Geen betekenisvolle verbande is tussen NT-proBNP en merkers van arteriële styfheid in enige groep gevind nie. Die positiewe en betekenisvolle verband tussen NT-proBNP en fibulien-1 is in swart mans asook in jonger swart mans en vrouens bevestig, nadat daar vir betekenisvolle kovariante gekorrigeer is. Hierdie assosiasies is afwesig in die Kaukasiërs. Gevolglik kan die resultate dui op vaskulêre veranderinge wat in jong Afrikane mans en vrouens voorkom en dat hulle meer blootgestel is om vroeër kardiovaskulêre siektes te ontwikkel in vergelyking met Kaukasiërs.

- As gevolg van vroeë vaskulêre veranderinge wat in die swart populasie voorkom, het ons gepoog om die verband tussen NT-proBNP en inflammatoriese merkers (beide CRP en suPAR) in Afrikane mans en vrouens, onafhanklik van 'n hipertensiewe toestand (Hoofstuk 5), te ondersoek. Alhoewel die vlakke van NT-proBNP en inflammatoriese merkers laer was in die mans as in vrouens, het die resultate getoon dat NT-proBNP positief en betekenisvol met beide CRP en suPAR geassosieer word in die normotensiewe swart mans alleenlik. Geen betekenisvolle verbande het te voorskyn gekom in die swart vrouens nie. Die positiewe en betekenisvolle verbande van NT-proBNP met CRP en suPAR is in swart mans bevestig, nadat daar vir betekenisvolle kovariante gekorrigeer is. Hierdie resultate weerspieël dat normotensiewe swart mans meer onderwerp is aan die vatbaarheid om kardiovaskulêre veranderinge te ontwikkel in 'n laegraadse inflammatoriese toestand wat kan aanleiding gee tot verhoogde kardiale nabelading en skade in vergelyking met normotensiewe swart vrouens.

- In Hoofstuk 6 het ons die moontlike assosiasie van NT-proBNP met ’n merker van osteoblast-aktiwiteit, alkaliese fosfatase, ondersoek. Hierdie sub-studie is in ’n bi-etniese manlike populasie ondersoek. Die resultate toon dat ’n positiewe assosiasie tussen NT-proBNP en ALP in Afrikane mans bestaan, maar nie in Kaukasiër mans nie. Afrikane mans het ook hoër NT-proBNP en ALP vlakke getoon in vergelyking met Kaukasiër mans. Nadat daar vir betekenisvolle kovariante gekorrigeer is, is die onafhanklike verband tussen kardiale belading en moontlike vaskulêre kalsifisering bevestig in swart mans. Hierdie populasie dui dus ’n baie hoër waarskynlikheid om sklerose in óf die media óf die intima te ontwikkel wat kan bydra tot verhoogde kardiale nabelading.

Algemene gevolgtrekking

NT-proBNP, ’n betroubare merker van kardiale oorbelading en skade, is positief geassosieer met sistoliese bloeddruk, polsdruk, fibulin-1, C-reaktiewe proteïen, oplosbare urokinase plasminogeen aktiveerder reseptor en alkaliese fosfatase. Deurlopend in hierdie studie is ons volgehoue bevindinge slegs gevind in die swart bevolking, veral mans. Hierdie resultate dui op die voorkoms van die kardiovaskulêre belading in die jong Afrikane wat onderwerp is aan vroeë vaskulêre veranderinge wat gedryf word deur inflammasie, ekstrasellulêre matriks veranderinge en kalsifisering wat kan aanleiding gee tot kardiale oorbelading en skade.

Sleutelwoorde: NT-proBNP, kardiovaskulêre funksie, fibulien-1, inflammasie, Afrikane

PREFACE

This thesis is presented in the article-format, consisting of peer-reviewed published or submitted articles. This format is approved, supported and defined by the North-West University guidelines for post graduate PhD level studies. The first chapter of this thesis is a detailed literature review, aside from the appropriate literature backgrounds discussed in each of the manuscripts. Chapter 2 is an overview of the study protocol together with all appropriate information on the materials and methods used to obtain the data. Chapters 3, 4, 5 and 6 comprise the articles in the form of original contributions. All the articles were submitted for publication in peer reviewed journals. The promoter and co-promoters were included as co-authors in each paper, together with international collaborators, where applicable. The first author was responsible for the initiation and all parts of this thesis, including literature searches, data mining and statistical analyses, the interpretation of results as well as writing the research papers. All co-authors gave their consent that the research articles may form part of this thesis.

The first article was submitted to the *Heart, Lung and Circulation* journal (published), the second to *Atherosclerosis* (published), the third to *Inflammation Research* (submitted) and the fourth and final paper to *Ethnicity & Disease* (submitted). The relevant references are provided at the end of each chapter according to the instructions for authors of the specific journal in which the papers were published or submitted for publication.

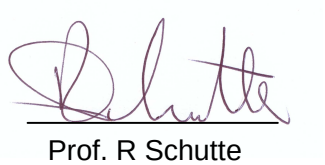
STATEMENT BY THE AUTHORS

The contribution of each researcher in this study is provided in the following table:

NAME	ROLE IN THE STUDY
Mr. R Kruger	Responsible for initial proposal of this study along with all extensive literature searches, critical evaluation of study protocol and methodology, statistical analyses, design and planning of research articles and thesis, interpretation of results and writing of all sections of this thesis.
Prof. R Schutte	**Promoter.** Guidance, intellectual input, data collection and critical evaluation of statistical analyses and also the final product.
Prof. AE Schutte	**Co-promoter.** Intellectual contributions, critical appraisal of the final product, project leader of the SAfrEIC study, data collection and connection with international collaborators for research manuscripts.
Prof. HW Huisman	**Co-promoter.** Intellectual input, data collection and assessment of physiological quality of the final product.
Prof. MH Olsen	**Mentor.** Professional, logistic and academic input to all research manuscripts.
Prof. WS Argraves	**Co-author.** Valued academic and expert input pertaining to fibulin-1 in the paper published in *Atherosclerosis*.
Prof. L Rasmussen	**Co-author.** Intellectual and well-grounded input regarding fibulin-1 in the paper published in *Atherosclerosis*.
Prof. P Hindersson	**Co-author.** Supportive and intellectual input of the manuscripts of Chapters 3 (published in *Heart, Lung and Circulation*) and 5 (submitted to *Inflammation*).
Prof. J Eugen-Olsen	**Co-author.** Expert academic commentary regarding suPAR in the paper submitted for publication in *Inflammation*.

The following is a statement of the co-authors verifying their individual contribution and involvement in this study and granting their permission that the relevant research articles may form part of this thesis:

Hereby, I declare that I approved the aforementioned manuscripts and that my role in this study, as stated above, is representative of my actual contribution. I also give my consent that these manuscripts may be published as part of the Ph.D. thesis of Ruan Kruger.



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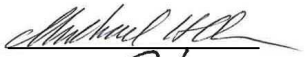

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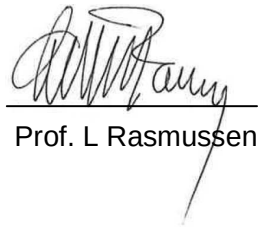
Prof. WS Argraves



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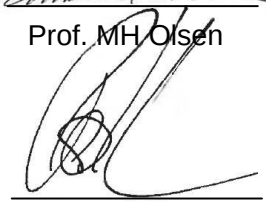

Prof. P Hindersson

Prof. J Eugen-Olsen

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LIST OF ABBREVIATIONS

χ^2 – Chi-square

ALP – Alkaline phosphatase

ANCOVA – Analysis of covariance

ANOVA – Analysis of variance

ANP – Atrial natriuretic peptide

ATP – Adenosine triphosphate

AUUUAA – (A): adenine and (U): uracil bases

BMI – Body mass index

BNP – B-type natriuretic peptide

bpm – Beats per minute

cGMP – Cyclic guanosine monophosphate

cm – Centimetre

CO – Cardiac output

CNP – C-type natriuretic peptide

CRP – C-reactive protein

CVD – Cardiovascular disease

Cωk – Windkessel arterial compliance

DBP – Diastolic blood pressure

DNP – D-type natriuretic peptide

ECM – Extracellular matrix

EDTA – Ethylenediaminetetraacetic acid

EGF – Epidermal growth factor

ELISA – Enzyme-linked immunosorbent assay

Et al. – Et alia “and others”

HIV – Human immunodeficiency virus

HOMA (IR) – Homeostasis model assessment (insulin resistance score)

HT – Hypertension

i.e. – That is

kg – Kilogram

kg/m^2 – Kilograms per metre squared

L – Liter

Log – Logarithm

LVH – Left ventricular hypertrophy

m/s – Meters per second

mg/L – Milligrams per litre

ml/min – Milliliters per minute

mmHg – Millimeters Mercury

mmol/L – Millimole per litre

mRNA – Messenger ribonucleic acid

n – Number of

NEP – Neutral endopeptidase

ng/mL – Nanograms per millilitre

NO – Nitric oxide

NPR-A/B – Natriuretic peptide receptor type A or B

NS – Not significant

NT-proBNP – Amino (N)-terminal prohormone B-type natriuretic peptide

p – Probability

PDE – Phosphodiesterase

pg/mL – Picograms per millilitre

PP – Pulse pressure

PWV – Pulse wave velocity

r – Regression coefficient

R^2 – Relative predictive power of a model

RAAS – Renin-angiotensin-aldosterone system

RNA – Ribonucleic acid

SAfrEIC – South African study regarding the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function

SBP – Systolic blood pressure

SD – Standard deviation

Std β – Standard Beta

suPAR – Soluble urokinase plasminogen activator receptor

TC:HDLC – Total cholesterol and high density lipoprotein cholesterol ratio

TPR – Total peripheral resistance

UK – United Kingdom

uPA – Urokinase plasminogen activator

uPAR – Urokinase plasminogen activator receptor

USA – United States of America

USD – United States Dollar

vs. – Versus

VSMCs – Vascular smooth muscle cells

WHO – World Health Organization

yrs. – Years

β – Beta

γ – Gamma

µmol/L – Micro mole per liter

µU/mL – Micro-units per milliliter

CHAPTER 1

INTRODUCTION AND LITERATURE STUDY

1. GENERAL INTRODUCTION

Sub-Saharan Africa is a collection of 47 countries comprising approximately 12% of the world's population.[1, 2] According to The World Bank database, 49.3 million people of the 12% are South Africans. South Africa is a country which extends from highly developed cities to remote rural areas where citizens follow a westernized or traditional lifestyle, respectively. Recently Twagirumukiza et al. reported that 23% of cardiovascular disease (CVD) in South Africa is attributed to hypertension.[3] Additionally, it seems that most CVD arise in developing regions.[1] The current knowledge on the prevalence of CVD is growing. However, the prevention of CVD is mainly derived from studies done in populations of European or American origin.[4] This creates a concern regarding the lack of information that exists in African populations of South Africa, since it is not known whether the information derived from such studies conducted in North America or Western Europe is applicable to South African blacks.[5]

For the purpose of this thesis, data will be used from the South African study regarding the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC), which included African and Caucasian men and women from urban areas of the North-West province. This study will focus on the comparison between these groups regarding the possible links that exist between a reliable marker of cardiac strain and cardiovascular components as well as possible factors that may alter vascular integrity and in turn result in cardiac overload.

This chapter entails the applicable literature to provide the necessary background and is additional to the appropriate and relevant literature overviews given in each separate article. In the literature study, the prevalence of CVD in Africans and Caucasians, especially from South Africa, will be addressed. The involvement of the natriuretic peptide system in cardiovascular function will be the focus, as well as the contribution of vascular remodelling and inflammation to cardiac alterations. In addition, this chapter includes a short motivation for each research article as well as a motivation for the subdivision of the target population. The aims and hypotheses of each manuscript, as well as the structure of the thesis will be provided in this chapter.

2. LITERATURE OVERVIEW

2.1 DEMOGRAPHICS

An estimated 17.3 million people died from CVD in 2008 of which over 80% of CVD deaths were from low- and middle-income countries. This makes CVD the leading non-communicable disease.[6] In the developing world (e.g. Africa and Asia) the prevalence of CVD is twice as high compared with those in the developed parts of the world (e.g. USA, UK and Australia) and the relatively young age of CVD-related deaths are becoming an even greater concern.[7] CVD is not limited to geographical areas, gender or socioeconomic classes and is ever increasing.[8] The World Health Organization projected that by 2030 almost 23.6 million people will die from CVD.[6] It is also noteworthy to mention that the current global life expectancy is postulated at 69 years, whereas The World Bank estimates the life expectancy of South Africans to be 51 years.

In South Africa, between 1997 and 2004, 195 people died per day because of some form of CVD.[9] The Heart and Stroke Foundation of South Africa estimated that about 33 people die per day because of a heart attack, while almost 60 die daily because of stroke and 37 of heart failure.[9] Although the white and black African people have similar mortality rates for these diseases, their patterns differ considerably.[10] Caucasians mainly reflect a pattern of death caused by heart attacks, while Africans reflect that of death caused by stroke, diseases of the cardiac muscle and also hypertension.[10] Although infectious disease is a well-known problem in African countries and also receives interest from government and international bodies, it is important to stress the immediate consideration for non-communicable diseases, which includes CVD.[11] Non-communicable diseases are becoming the main cause of morbidity and mortality, since it is postulated that they will overtake communicable diseases by 2030.[12]

CVD in South Africa, especially among the black Africans, develops chronically with underlying components which are attributed to unhealthy lifestyle choices along with recently described transition from rural to urban regions.[13] These components include unhealthy dietary intake,[14] physical inactivity,[15,16] alcohol abuse,[17,18] cigarette smoking[19] and other modifiable risk factors

which contribute to elevating blood pressure and cardiovascular burdens resulting in cardiac stress load and dysfunction. All these contributing factors initiate systemic alterations including extracellular remodelling and adverse inflammatory states, consequently cascading into the damaging effects such as heart failure and death.[20, 21]

2.2 CONTRIBUTING FACTORS OF CARDIOVASCULAR DISEASE

2.2.1 Environment and lifestyle

As black South Africans are subjected to urbanisation, they tend to change their dietary patterns and time management, and inherently develop a physiologically stressful state. Hence, coping strategies (smoking, use of alcohol and pharmacological substances) are initiated to manage stress subsiding with urbanisation.[22] Therefore, lifestyle contributes significantly to the onset and/or maintenance of increasing blood pressure.[23] Hypertension is not the only disease of lifestyle during this transition, but also diabetes, coronary heart and cerebrovascular disease.[4]

The importance of environmental components associated with urbanisation and its demands on blood pressure status has been previously described.[24] These environmental factors which include harsh geographic environments, unhealthy dietary intake, physical inactivity, psychosocial stress, poor socioeconomic conditions, excessive alcohol intake and cigarette smoking,[4,6] adversely contribute to their risk of cardiovascular disease. Multiple factors contributing to the high susceptibility of developing hypertension include: altered plasma renin levels, sodium abnormalities, epithelial sodium channel alterations,[25] altered genes regulating the renin-angiotensin-aldosterone system,[26] increased peripheral vascular resistance,[27] increasing obesity,[28] and low socioeconomic status.[29,30]

2.2.2 Hypertension

Normal arterial blood pressure is determined by cardiac output (CO) and total peripheral resistance (TPR).[31,32] Heart rate determines CO via beta-1 and cholinergic receptors controlled by sympathetic and parasympathetic stimulation.[33] The CO is further determined by the intravascular fluid volume and the venous capacitance influencing the filling pressure of the heart and the force of contraction that determines the stroke volume.[33] Therefore, the relationship between CO and TPR has to be critically controlled. The components determining the stroke volume, heart rate and intravascular volume is further and more complexly influenced by multiple vasoactive mechanisms which are controlled by local and systemic neural, hormonal and renal factors.[34] It is clear that the control of normal blood pressure is a complex multifactorial harmonisation of different mechanisms. However, once imbalances occur, a disease state called hypertension develops. Although hypertension has received much attention over the past few decades, the primary cause remains inconclusive.

High blood pressure is now a major public health concern in South Africa.[29,35,36] The study by Twagirumukiza et al. projected that the number of hypertensives (in 2008) is almost four times higher than the estimated 2005 number from the World Health Organization's Africa regional Office.[3] The number is a staggering 74.7 million people from the sub-Saharan countries, which is predicted to increase by 68.0% in 2025.[3] Hypertension is also described as one of the most common risk factors for cardiovascular morbidity and mortality,[37] and even slightly elevated blood pressure is associated with an increased risk of myocardial infarction, heart failure, stroke and renal failure.[38] In addition, a study revealed that the prevalence of hypertension with inadequate blood pressure treatment is high among the African population of South Africa.[39]

Arterial hypertension is the result of interactions of interrelated mechanisms, which ultimately lead to an increased cardiovascular risk and other disease states.[40,41] Interactions of demographics, lifestyle and genetic factors are some of the key components that are commonly associated with elevated blood pressure.[4,39] Additionally, obesity, insulin resistance, elevated activity of the sympathetic nervous system,[42] over stimulation of the renin-angiotensin-

aldosterone systems, abnormal renal sodium handling, endothelial dysfunction, and alterations of the central vessels contribute largely to hypertension.[40] With the onset of hypertension, CO increases, but the TPR may remain normal. However, in time this ratio becomes inverted.[43] Increasing vasoconstriction, endothelial dysfunction, structural remodelling and vascular inflammation are some of the factors contributing to elevated TPR.[44]

Bakris et al. eloquently summarised the pathophysiologic state of chronically elevated blood pressure.[34] Chronic elevations in arterial blood pressure result from combinations of inappropriate balance between CO and TPR. Table 1 illustrates the adverse combinations of CO and TPR.

Table 1 – Relationships between cardiac output and total peripheral resistance and disease.

Relationship	**↑ CO and normal/↓ TPR**	**↑ TPR and normal/↓ CO**	**↑ CO and ↑ TPR**
Conditions	• early diabetes mellitus, • dialysis patients, • hyperdynamic or -adrenergic HT (seen in youth)	• accelerated or malignant HT • HT in the elderly	• reno-vascular HT

Key and abbreviations: ↑ - high; ↓ - low; HT – hypertension; CO – cardiac output; TPR – total peripheral resistance

CO and TPR are two major components directly influenced by the integrity of the blood vessels. Therefore, if and when the vasculature is chronically compromised, either via environmental or lifestyle factors, or perhaps due to genetic susceptibility, blood pressure will be altered. Structural and functional changes of blood vessels result in stiffness and further contribute to chronically elevated blood pressure.[45,46]

2.2.3 Vascular stiffness

Blood pressure contributes significantly in determining the blood vessel wall structure by initiating remodelling mechanisms to compensate for changes during wall stress.[47] Therefore the regulation of the variability in blood pressure is very important to maintain normal cardiovascular function. Degradation of elastin fibres, deposition of collagen, increased calcium content and also hypertrophy of the arterial media of large blood vessels are the accepted causes of age-related arterial stiffening.[47,48] These functional and structural changes are more distinct in central arteries (aorta, carotid) than in the peripheral vasculature (femoral or radial).[49] When the interaction between the mechanics and the structure of the blood vessel wall components is altered, it affects the cardiovascular system by initiating the development of athero- or arteriosclerosis.[50] The ultimate result of these arterial wall alterations is the elevation of systolic blood pressure along with pulse pressure due to a change in the diameter of the blood vessels.[34,48] This creates a vicious cycle since the increasing blood pressure accelerates further arterial damage.

There exist numerous endogenous mechanisms that influence normal vascular function. The bioavailability of nitric oxide (NO) is one of the key contributors in healthy blood vessel endothelium.[51] NO is an effective vasodilator, which inhibits platelet aggregation and adhesion, limits vascular smooth muscle proliferation, inhibits the formation of neo-intima, prevents monocyte chemotaxis and inhibits leukocyte adhesion to the endothelium.[52] Natriuretic peptides, bradykinin and prostacyclin inhibit the growth of vascular smooth muscle cells (VSMCs) and contribute to healthy vasculature.[31] Opposed to NO, endothelin-1 and angiotensin II enhance vasoconstriction which contribute to elevated intravascular pressure and consequently augment blood pressure.[53] The balance between vasoconstriction and vasodilatation is therefore important to preserve normal vascular function. In states of decreased NO-bioavailability, the relatively unopposed effects of endothelin-1 cascades into vasoconstriction and contributes to endothelial dysfunction.[54] In addition, angiotensin II mediates vascular remodelling by directly stimulating protein synthesis in VSMCs, inducing growth factor synthesis and altering the extracellular matrix (ECM).[55,56]

Another mechanism, in which enzymes modulate ECM proteins, is the matrix metalloproteinases.[57] In the event of elevated blood pressure due to increasing angiotensin II, matrix metalloproteinase 9 activity increases and results in enhanced collagen degradation in order to improve the intrinsic distensibility of elastic arteries and in turn reduces blood pressure.[57] Although the identification of molecules that contributes to arterial stiffness are still largely unknown, components of the ECM, the structure of the matrix, and the cell–matrix interactions are considered to be the major determinants of arterial stiffness.[58] With all these functional and structural alterations there is no doubt that increased arterial stiffness contributes to progressive CVD.[58] Increased arterial stiffness increases cardiovascular morbidity and mortality as a result of elevated systolic blood pressure and a decrease in diastolic blood pressure, which in turn increases left ventricular afterload and alters coronary perfusion.[59] As mentioned previously, arterial stiffness is also a major determinant of pulse pressure, which also predicts coronary heart disease and stroke as well as cardiovascular events,[60] and is described as an independent predictor of cardiovascular mortality.[61]

The relationship between arterial stiffness and the ECM is largely explained in the literature on the interaction between structural collagen and elastin. However, many other adhesion ECM components such as integrins, fibronectin, vitronectin, urokinase plasminogen activator receptor, fibulins, focal contact proteins etc. are important in the normal morphology to regulate extracellular-matrix assembly, cell proliferation, differentiation, and cell death.[62] Ultimately when the ECM is compromised, vascular stiffness results and contributes to adverse cardiac effects.

2.2.4 Cardiac volume load and hypertrophy

In the event of arterial stiffness due to arterial damage, the amplitude and velocity of the pulse waves increase.[63,64] This causes an early return of reflected waves from the peripheral vasculature to the aorta.[64] In turn, the aortic and left ventricular pressures increase during systole whereas the mean diastolic pressure decreases. Synchronisation between the ejected and reflected wave is now disturbed, which reaches the aorta during systole in older individuals,

increasing left afterload and leading to hypertrophy of the left ventricle.[65] Normally, cardiac hypertrophy acts as an adaptive response of the heart due to elevated volume overload or afterload and subsides once the balance is restored. Cardiomyocytes increase in size without undergoing mitosis during the normal hypertrophic response.[66] Although the hypertrophic response maintains cardiac function to some extent and only for a certain period, progressive hypertrophy becomes harmful and results in cardiac dysfunction and eventual congestive heart failure.[67]

The main event leading to heart failure is the loss of a critical amount of functioning myocytes after an injury to the heart or prolonged contributing factors of cell death.[21] This injury may be a result of acute myocardial infarction, toxins such as alcohol, or prolonged cardiovascular stress, such as hypertension,[21] and also adverse regulation of the renin-angiotensin system in the heart, systolic calcium toxicity, elevated endothelin and electrolyte imbalance.[68] The ventricle therefore has a decreased ability to eject blood during systole and in turn increases the tension on the non-injured parts of the heart. Subsequently, the response of the ventricle to the increase in diastolic preload is inadequate with a low ejection fraction.[20] As a result, the sympathetic nervous system activates and stimulates β-adrenergic receptors in the non-injured myocardium to increase both the force and frequency of the contraction.[69] Heart failure chronically progresses as ventricular dilatation augments (cardiomyopathy) due to compensatory physiological processes.[70-72]

As a result of chronic cardiac myocyte stretch, as seen in heart failure, there is an upregulation of ventricular natriuretic peptide production that acts in a counteractive manner to curb overload.[73-75] However, after damage of cardiac myocytes (as a result of haemodynamic stress load), natriuretic peptides are even more profoundly expressed. Elevated natriuretic peptides are a consistent independent predictor of mortality and other cardiac composite endpoints for populations at risk for CVD.[76]

2.3 THE NATRIURETIC PEPTIDE SYSTEM

It was established in the early 1980's that the heart exhibits endocrine functions in the form of peptides released by its myocytes.[77] These peptides are involved in the long-term regulation of sodium and water balance, blood volume and arterial pressure. Two of the major pathways of these peptides include the vasodilator effects to lower blood pressure and also the renal effects that lead to natriuresis and diuresis.[78]

About 29 years ago, the first of four types of natriuretic peptides was discovered; today it is known as atrial natriuretic peptide (ANP) and was formerly known as atrial natriuretic factor.[79] The ANP is a 28-amino acid peptide that is synthesized, stored, and released by atrial myocytes in response to atrial distension, angiotensin II stimulation, endothelin secretion, and sympathetic stimulation. Elevated ANP levels occur during hypervolemic states (elevated blood volume) and congestive heart failure.[79] In the late 1980s, Sudoh et al. revealed the second natriuretic peptide, similar to ANP, from the porcine brain, named B-type natriuretic peptide (BNP). BNP is also produced in cardiac myocytes and shares peripheral receptors with ANP.[80]

A third homologous natriuretic peptide produced by the brain and endothelium, but not in cardiac myocytes, is called CNP or C-type natriuretic peptide. CNP is a 22-amino acid peptide that was initially identified in the central nervous system. It plays a role together with other local systems in the control of vascular tone, due to the novel endothelial site of production of CNP and the proximal situation of its receptor in vascular smooth muscle.[81] The last type is called dendroaspis natriuretic peptide or DNP, which is a 38 amino acid peptide, isolated from the venom of the Green Mamba (*Dendroaspis angusticeps*) and has structural similarities to the three known human natriuretic peptides.[82] The human chromosomal location, clearance mechanism as well as other physiological properties of the DNP gene is unknown. However, a DNP-like peptide has been isolated in human plasma and human atria, but no conclusive evidence with regard to the presence of DNP in humans has been reported yet.[83]

Recent studies reported that BNP and more likely the cleaved amino (N)-terminal of the peptide derived from the BNP prohormone called N-terminal prohormone B-type natriuretic peptide (NT-

proBNP), are used as strong predictors and biomarkers of cardiovascular risk.[84-86] Therefore, this literature overview will focus on NT-proBNP with regard to its biochemical synthesis, physiology and clinical relevance.

2.3.1 Biochemistry of the N-terminal prohormone B-type natriuretic peptide

The human BNP is encoded by a single copy gene consisting of three exons and two introns (Figure 1) that is transcribed into precursor mRNA and then cut out of the message by RNA-splicing in the nucleus, leaving a mature mRNA that is then translated in the cytoplasm.[87] The BNP mRNA has a distinctive feature of the presence of four AUUUAA repeated sequences within the carboxylterminal-untranslated region that is considered to produce mRNA stability.[87] The post-translational processing of BNP regulation takes place during gene expression.[88]

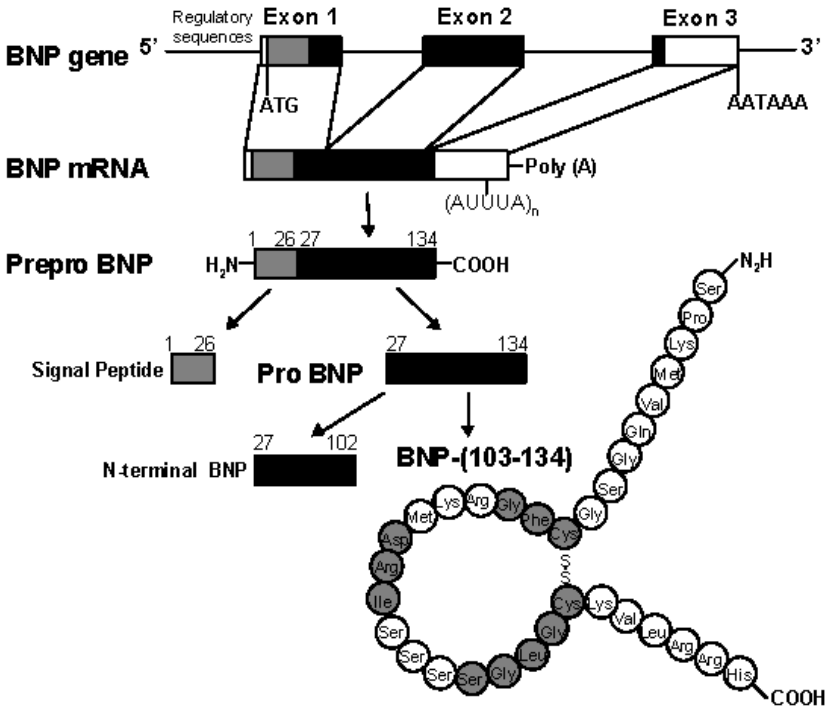

FIGURE 1 – Schematic illustration of BNP gene expression and cleavage of BNP and NT-proBNP showing encoding (exon) and non-coding (intron) sequences.[89]

The human BNP gene is located on the first chromosome and expresses a cardiac hormone which consists of 108 amino acids called the prohormone proBNP.[90] Furin (a proteolytic enzyme) catalyzes the split of proBNP into two parts (Figure 2). The first end-product of this enzymatic cleavage is the 32 amino acid BNP hormone, which is biologically active in the circulation and is separate from the N-terminal of proBNP. The remaining 76 amino acid subsection is NT-proBNP.[90,91]

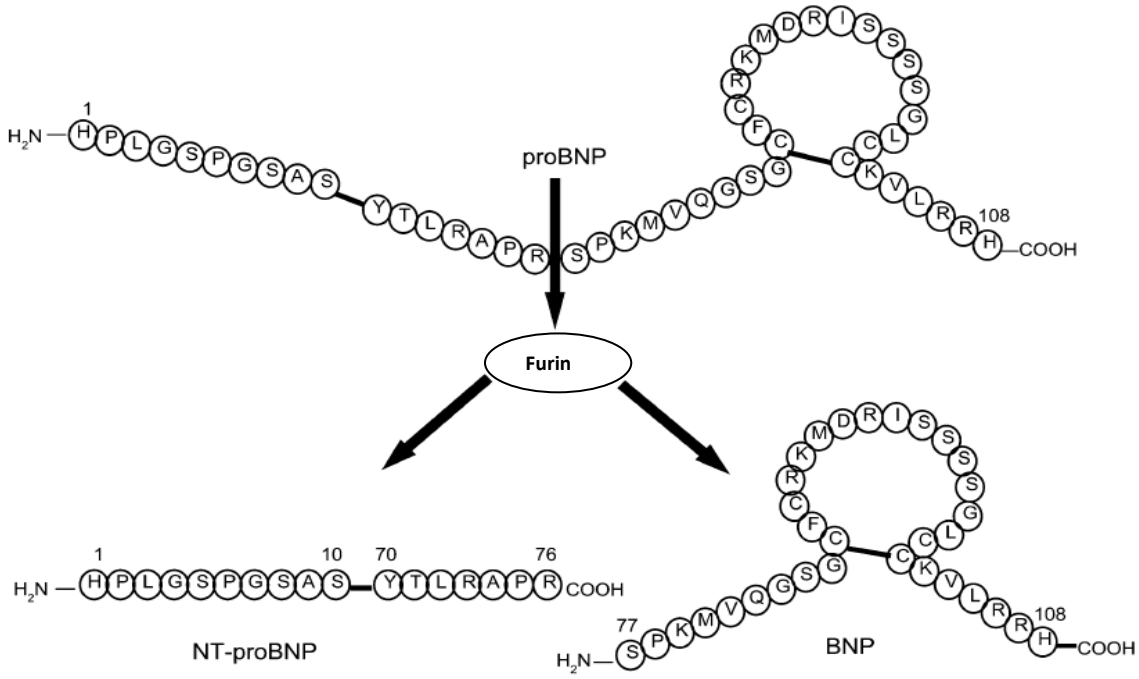

FIGURE 2 – Enzymatic cleavage of proBNP into NT-proBNP and BNP.[81]

2.3.2 Physiology of NT-proBNP

In normal physiological conditions BNP and NT-proBNP (primarily secreted by cardiomyocytes in the atria) concentrations increase in response to elevated blood pressure and plasma volume.[92] By means of exposing cells or organs to increased (NT-pro)BNP concentrations, or a mouse model over-expressing (NT-pro)BNP or (NT-pro)BNP gene knockout, the physiological effects of NT-proBNP have been studied in the intact organism.[81] This has shown that NT-proBNP binds to natriuretic peptide receptor type A (NPR-A) or B (NRP-B), causing an increased production of cyclic guanosine monophosphate (cGMP).[93] The cGMP exerts its

biologic effects indirectly either through cGMP-dependent protein kinase G, phosphodiesterases (PDEs), or by direct action on effectors such as amiloride-sensitive sodium channels in the kidney (Figure 3). The biological effects of NT-proBNP therefore include natriuresis, diuresis, vasodilatation, inhibition of renin and aldosterone production and inhibition of cardiac and vascular myocyte growth.[92,94]

NT-proBNP also binds to natriuretic peptide receptor C, which is thought to function as a clearance receptor, after which it is internalised and degraded.[95] NT-proBNP may be degraded by the extracellular neutral endopeptidases (NEPs) in the kidney and vasculature. In short, the release of these peptides compensates for the volume and pressure overload by lowering salt and water reabsorption and inhibits sympathetic outflow via the long reflex of the central nervous system, respectively. In addition, these elevated natriuretic peptides inhibit the renin-aldosterone activity to further lower blood pressure and plasma levels.[96]

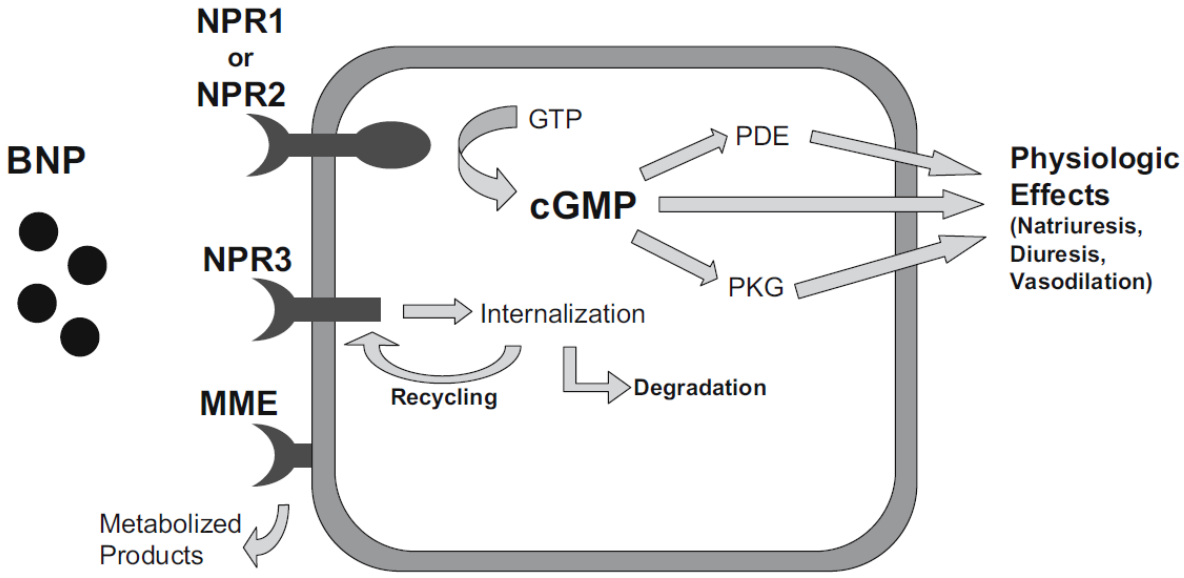

FIGURE 3 – Mechanistic diagram of the physiological pathway of NT-proBNP secretion.[97]

Although the synthesis of BNP-related peptides is still undergoing investigation, it is known that cardiac myocytes comprise the major source of these peptides in the circulation,[81] but is also produced by cardiac fibroblasts.[98] In 1994, Magga et al. reported that the main stimulus for both

ANP and BNP peptide synthesis and secretion is cardiac wall stress. The tension in the cardiac wall is a common cause for many diseases such as hypertensive heart disease and heart failure and natriuretic peptides may serve as good clinical biochemical markers of these states.

2.3.3 Clinical relevance of NT-proBNP

The primary site of NT-proBNP production is the myocytes in the atria, however once afterload augments due to arterial damage (arterial stiffness), the ventricle becomes the distinct site of secretion. With chronic myocyte stretch as seen in hypertension, left ventricular hypertrophy (LVH), myocardial infarction and cardiac heart failure, there is an upregulation of ventricular natriuretic peptide production possibly due to secondary local stretch mechanisms.[73-75,99] Therefore, NT-proBNP is often called the ventricular hormone with higher production especially in heart failure, since its natural regulatory effects are superseded. In normal subjects the plasma concentrations of BNP and NT-proBNP are relatively similar, as they are both continuously secreted from the atria of the heart. It has been confirmed that the half-life of NT-proBNP is 5.45 times (120 vs. 22 minutes) longer than that of BNP.[87,100,101]

2.3.4 NT-proBNP and cardiovascular disease

A major complication of hypertension is LVH. Hypertrophy of cardiac muscle is also a known risk factor for all CVDs independent of elevated blood pressure.[102] It has been reported that in patients with left ventricular dysfunction the NT-proBNP levels rise 2-10 times higher than the plasma concentration of BNP. Therefore, the greater rise in NT-proBNP during or prior to heart failure, may make it a better marker compared to BNP along with its longer half-life.[94]

NT-proBNP levels have been reported to be higher in hypertensive subjects, especially with LVH or left ventricular dysfunction, compared to normotensives.[103] NT-proBNP strongly predicts cardiovascular events in patients with hypertension and LVH without diabetes or clinically overt cardiovascular disease.[92] Those with diastolic dysfunction with preserved left ventricular ejection fraction are also subjected to clinically adverse cardiovascular outcome.[104] In response to

cardiac overload, one has to consider its effects on the structure of the tissue. Therefore, cardiac remodelling may occur in order to sustain the ever on-going, damaging load on the heart.

Sustained ventricular hypertrophy leads to thinning, necrosis and fibrosis of the ventricular wall, which compromises the hypertrophic response and limits the capacity of the heart to counteract wall stress.[105] Prolonged distension in the atria also leads to the depletion of natriuretic peptide expression.[106] To compensate for this loss, natriuretic peptides are synthesized by the ventricles, but with the development of hypertrophy. The scale of this response is insufficient. Subsequently, the ability of the circulation to limit ventricular wall stress and the release of vasoconstrictor hormones decreases.[107,108] The failing ventricle then loses its capacity to enhance its function to overcome increases in volume afterload and as ventricular dilatation enhances systolic ejection, an enlargement in chamber size depresses cardiac function in heart failure.[108] The resulting constriction of systemic arteries and veins markedly increases the pressure and volume in the heart and aggravates the load on the ventricle. Hence, the same endogenous mechanisms that exerted favourable effects in the normal heart, by increasing an inotropic state, produce damaging effects in the weakening heart by increasing wall stress.[109,110] In addition, systolic function cannot be sustained and cardiac output decreases.

Both haemodynamic stress and neurohormonal activation increase cardiac wall stress, and this can cause irreversible structural remodelling of the heart because of slippage and elongation of myocardial fibres and its extracellular components.[20] The hypertrophic response to stress may further increase circulating concentrations of potentially cardiotoxic cytokines.[70] At this point it is important to highlight interweaved connections between the heart and the blood vessels. They are interdependent in both normal and adverse conditions. In the next and final section of this literature overview, emphasis will be on NT-proBNP in relation with cardiovascular components which adds to the reasoning and motivation of this study.

2.3.5 NT-proBNP and the vasculature

2.3.5.1 Blood vessel walls and extracellular matrix

The adventitia, media and intima of blood vessel walls comprise many ECM components which include collagens, thrombospondin, osteopontin, fibrilin, elastins, fibulins, laminins, proteoglycans, fibrinogen, fibronectin, nidogen-1, endostatin, aggrecan and versican.[111,112] In this study fibulin-1 was measured. The fibulins are a family of six proteins that are associated with basement membranes and elastic ECM fibres.[113] The fibulins are minimally defined as having a series of epidermal growth factor (EGF)-like modules, followed by a carboxy-terminal fibulin-type module. Fibulins are hypothesized to function as intramolecular bridges that stabilize the organization of supramolecular structures, such as elastic fibres and basement membranes of the ECM,[114] which could be in close association with integrins. All fibulins except fibulin-6 and -7 are found in elastic tissues.[115] Fibulin-2 and -4 are at the border between the central elastin core and its surrounding microfibrils. Fibulin-1 is located within the elastin core and fibulin-5 is associated with the microfibrils.[112] Fibulin-1 is produced by migratory cardiac mesenchymal cells that have trans-differentiated from endocardial cells.[116] Fibulins are also prominently expressed in blood vessel walls; however, during development fibulin-1 is expressed by the primordial VSMCs which are associated with the ventral endothelium of the dorsal aorta.[117] In adult blood vessels, pronounced fibulin-1 deposition occurs in the matrix that surrounds VSMCs and in the elastic laminae of arteries.[118]

The significance of fibulin-1 in the development of cardiovascular physiology and pathology is inconclusive, but Argraves et al. speculated that plasma fibulin-1 could be important as a risk factor for cardiovascular diseases and atherosclerosis progression.[119] However, one study found reduced levels of plasma fibulin-1 in patients with unstable angina pectoris and acute myocardial infarction.[120] Another study by Cangemi et al. found fibulin-1 to be upregulated in non-atherosclerotic arterial tissue in Type II diabetic patients.[121] The same study reported associations between elevated circulating fibulin-1 levels and glycemic status, cardiovascular variables, and mortality.[121]

The finding that plasma levels of fibulin-1 are reduced in coronary heart disease patients[119] raises questions as to the molecular basis for this and whether this protein is a useful diagnostic marker. No information is available on the interactions or possible relationship between cardiac strain and fibulin-1.

2.3.5.2 Inflammation

Inflammation is a process that stretches beyond its classic involvement of lipid accumulation and infection caused by pathogens. It is known that inflammatory cytokines including C-reactive protein (CRP), interleukin-6, tumour necrosis factor-α, monocyte chemoattractant protein-1 and interleukin-8 is associated with CVD.[122,123] Elevated wall stress can promote the production of proteoglycans that binds and retains lipoproteins. This escalates into oxidative alteration and initiates an inflammatory response, which in turn cascades into lesion formation in arterial smooth muscle cells.[124] The developing lesion is normally the work of leukocytes penetrating the intima by means of the monocyte chemoattractant protein-1.[125] A local inflammatory response follows, which includes macrophage foam cells and also T-lymphocytes that signal γ-interferon and tumour necrosis factor-α.[126] As this process progresses, fibrogenic mediators are released. These mediators include peptide growth factors that further add to the advanced atherosclerosis lesion.[127]

Inflammation participates in the development of hypertension, which makes hypertension one of the classical risk factors for atherosclerosis.[126,128] This is based on the principle that vasoconstrictor agents such as angiotensin II contribute to increased oxidative stress in smooth muscle cells of arterial walls.[129] Augmented reactive oxygen species will in turn increase the expression of interleukin-6 and monocyte chemoattractant protein-1. This will activate adhesion molecules such as VCAM-1 on the vascular endothelium and stimulate the abovementioned process to enhance lesion formation.[130,131] Although these are important and basic principles of inflammation in atherosclerosis, one should stress that there are some inflammatory markers that prove more reliable and specific than others.

CRP is synthesized by the liver in response to interleukin-6 and is probably the well-known studied inflammatory marker.[132-134] In the literature there is controversy regarding CRP being a risk marker rather than a causal factor in the atherosclerotic process.[135,136] In 2003 Vasan et al. reported that interleukin-6 has a stronger role for prediction of congestive heart failure among elderly subjects without previous myocardial infarction than for CRP or tumour necrosis factor-α.[137] However, CRP remains the accepted marker for cardiovascular risk. Moreover, NT-proBNP in combination with CRP is associated with an increased risk of CVD with high predictive mortality.[133] CRP stimulates the onset phagocytosis by binding to receptors on monocytes, macrophages and neutrophils and further activates the classic complement pathway.[138] Ishikawa et al. reported that CRP levels predict mortality in patients with dilated cardiomyopathy[139] and correlate negatively with left ventricular function in patients with and without heart failure.[139,140] CRP is considered as an acute-phase reactant rather than an initiator of inflammation, since it functions near the end of the inflammatory cascade.

Another inflammatory marker, suPAR (soluble urokinase plasminogen activator receptor), has recently been shown to be a significant predictor of cardiovascular events independent of subclinical organ damage.[141,142] The urokinase plasminogen activator (uPA) along with its receptor (uPAR) is not only present on monocytes and activated T-cells, but also in smooth muscle and endothelial cells.[143] The cell surface receptor (uPAR) sheds a subunit, producing a soluble form of uPAR.[143,144] The uPAR is involved in numerous immune functions which include adhesion, migration, angiogenesis, fibrinolysis, and cell proliferation.[143,145] The soluble bioactive form (suPAR) is cleaved from the cell surface and released in plasma and urine.[134] Elevated suPAR levels indicates adverse clinical outcome in patients suffering from CVD, such as atherosclerotic plaque.[146]

2.3.5.3 Calcification of blood vessels

Osteoblasts have a distinct purpose as they share important connections with mineralisation mechanisms and gene expression which include alkaline phosphatase (ALP), core binding factor-1 and osteopontin.[147] Evidence indicates that phosphates regulate and coordinate cell signalling as well as gene expression.[148] Jono et al. suggested that extracellular phosphate directly regulates the ability of VSMCs to initiate matrix mineralization.[149] Calcification of blood vessels is an active regulated process in which VSMCs gain osteoblast-like functions.[150,151] Therefore, vascular calcification refers to the ectopic deposition of phosphate minerals in arteries, heart valves, and cardiac muscle.[149] Vascular calcification is found in atherosclerotic lesions of the intima and is associated with increasing age.[152] Vascular calcification is also regarded as a clinical marker for atherosclerosis and perhaps also arteriosclerosis.[147] Since atherosclerosis develops under chronic inflammatory conditions, VSMCs are subjected to modified lipids, lipoproteins and inflammatory cytokines that may regulate osteoblastic differentiation and mineralisation that could cascade into metastatic calcification.[153-155]

Calcified coronary arteries is positively correlated with atherosclerotic plaque,[156,157] myocardial infarction[158] and plaque instability.[159] Calcification in the media of large arteries leads to augmented stiffness and therefore decreased arterial compliance. The consequent loss of elasticity is associated with increased arterial pulse wave velocity and pulse pressure.[48,160] In turn, this will result in increased afterload contributing to left ventricular hypertrophy and impaired coronary perfusion as explained previously. Medial arterial calcification predicts future cardiovascular events in patients with diabetes mellitus[161] and is a prognostic marker of CVD mortality in patients with kidney disease.[162]

Currently three different types of calcification in the vasculature are known (Figure 4), which include (1) the onset of chronic inflammation at soft tissue levels due to pathogen invasion, (2) infiltration of leukocytes in the arterial intima contributing to atherosclerotic plaque development and finally (3) direct alterations in medial integrity due to modifiable risk factors of lifestyle. All

these processes adversely alter osteogenic gene expression and augments ectopic calcification.[147]

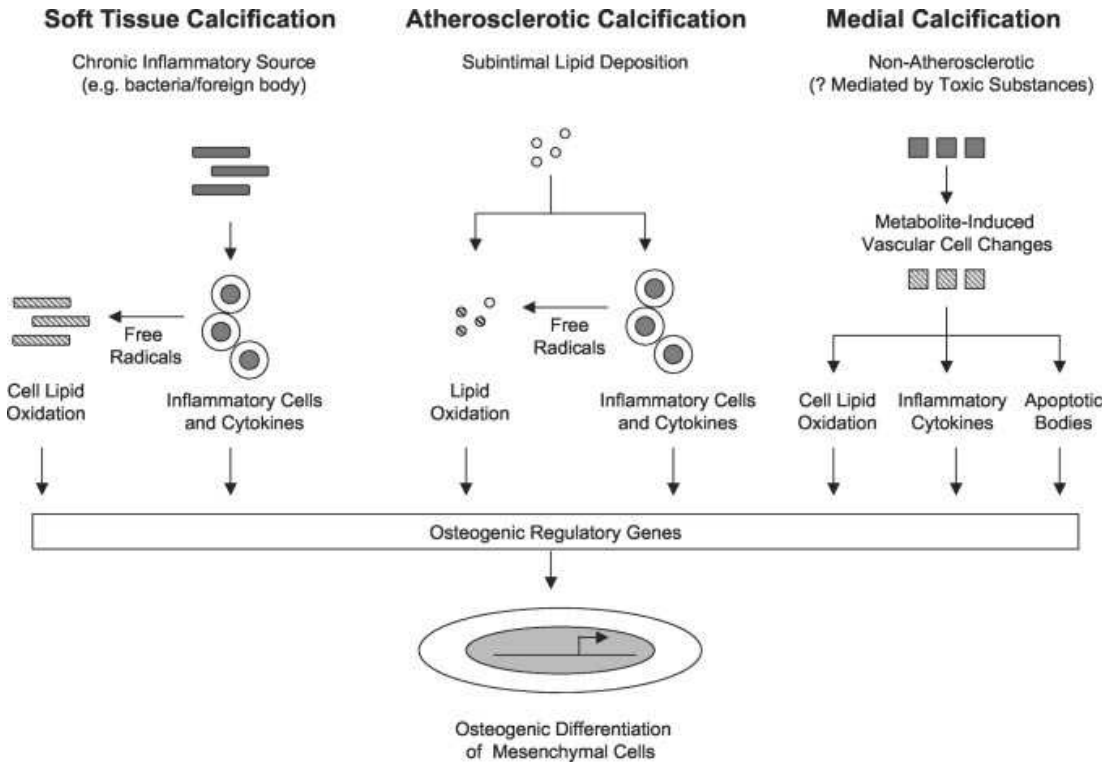

FIGURE 4 – Parallel mechanisms in soft tissue versus atherosclerotic and non-atherosclerotic vascular calcification.[147]

Four exclusive mechanisms have also been described by Giachelli.[160] The first is the loss of inhibition of pyrophosphates and matrix gla proteins. These are the normally expressed inhibitors of mineralization in blood vessel walls. In turn, this leads to spontaneous vascular calcification and increased mortality.[163] The second osteogenic mechanisms where bone proteins including osteopontin,[160] osteocalcin,[164] matrix vesicles[165] and cartilage formation is expressed in calcified vascular lesions.[166] Thirdly, osteoporosis in postmenopausal women can result in bone turnover which may lead to release of circulating nucleation complexes contributing to vascular calcification.[167-169] The last mechanism is apoptosis or cell death. This is normally membranous debris rich in phospholipids and apoptotic bodies that contribute to nucleate apatite. This is typically seen in atherosclerosis.[165,170]

2.4 INTEGRATION OF CONCEPTS

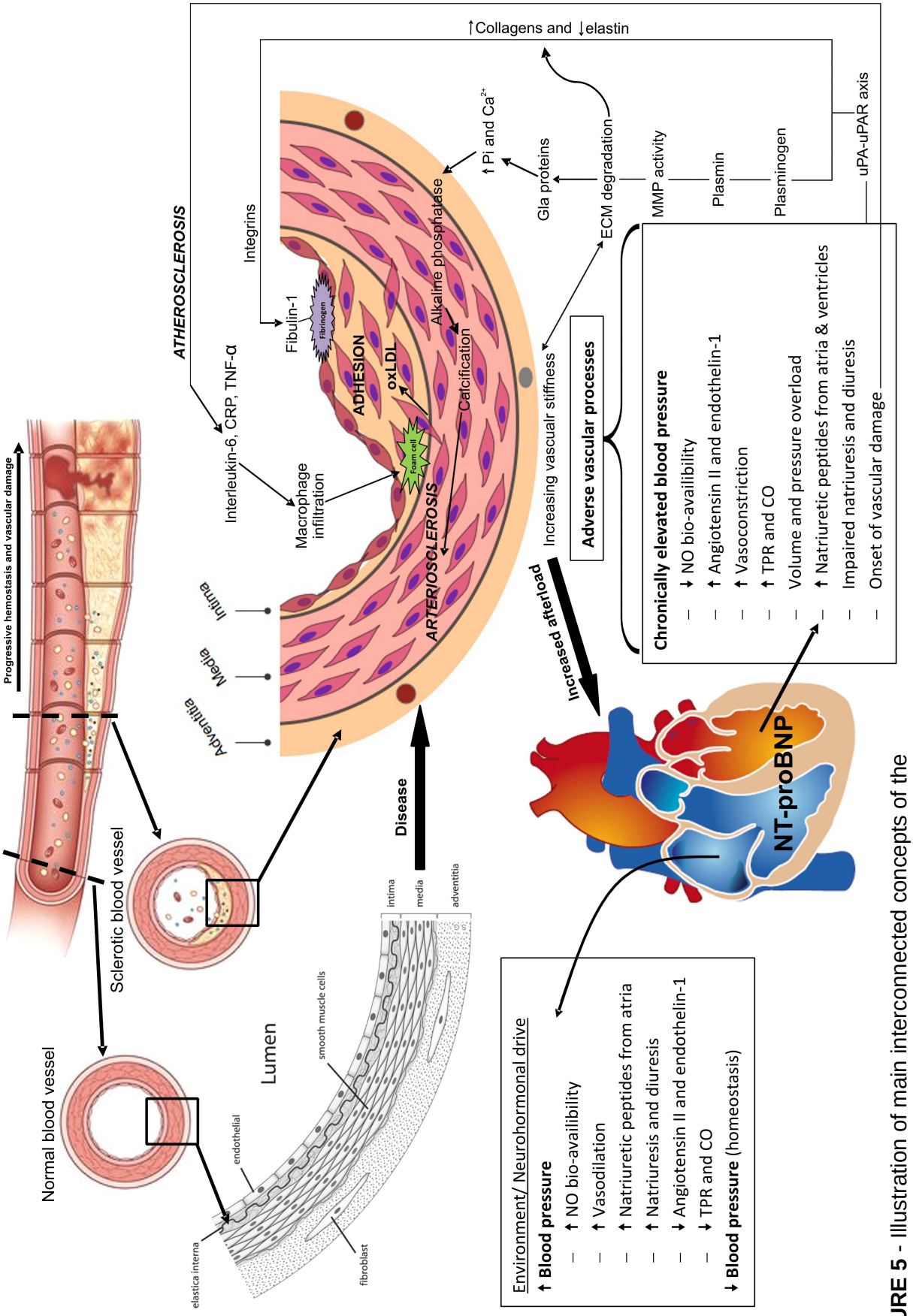

FIGURE 5 - Illustration of main interconnected concepts of the thesis

3. MOTIVATIONS

This thesis consists of four research articles submitted for peer reviewed publication. Since the relevant literature background for each manuscript is discussed and embedded in those chapters as well as in the broad literature overview, only a concise motivation for each chosen topic will be provided in this section.

3.1 NT-proBNP and cardiovascular function in Africans and Caucasians

In a study of coronary atherosclerosis, plasma NT-proBNP levels were significantly lower in African Americans compared to Caucasians, and likewise, lower plasma BNP levels in African Americans than Caucasians with heart failure.[171,172] It is also evident in the literature that NT-proBNP levels are normally higher in women compared to men.[173] However, less is known about the differences between Africans and Caucasians regarding NT-proBNP levels in South Africa and also the associations thereof with cardiovascular function.

3.2 NT-proBNP and fibulin-1 in African and Caucasian men and women

Numerous studies investigated the ECM with regards to structural and functional changes in organs and also blood vessels.[112] However, most of these studies focused on the structural scaffold of collagen and elastin with less reference to the smaller regulatory components of the ECM.[174,175] Fibulin-1 is regarded as an extracellular matrix component expressed in blood vessels.[176] It has also been shown that fibulin-1 may contribute to vascular stiffness, due to possible remodelling of the scaffolding matrix proteins in close association with fibrinogen and integrins.[177] However, to our knowledge, no studies investigated the associations between NT-proBNP, fibulin-1 and arterial function.

3.3 NT-proBNP and inflammatory markers in normotensive African men and women

It is known that inflammation contributes largely to cardiovascular disease such as coronary artery disease, hypertension, left ventricular hypertrophy and chronic heart failure.[70,178-180] CRP is the leading marker of inflammation with high predictive value in cardiovascular risk. However, it seems as if a multimarker approach is becoming a trend in clinical screening. Although it is known that CRP in combination with interleukin-6 and tumour necrosis factor-α reliably estimates cardiovascular risk, one should also consider novel markers that recently showed promising results.[141,142,181] suPAR has shown to be an independent predictor of subclinical organ damage as well as a reliable marker of both low-grade and systemic inflammation.[143] This novel marker is receiving increasing attention, while information on this inflammatory marker lacks in South African populations. It is evident that NT-proBNP, CRP and suPAR are reliable biomarkers in risk stratification. However, one should consider whether results from European countries are applicable in the African context.

3.4 NT-proBNP and alkaline phosphatase in African and Caucasian men

Calcification of blood vessels (especially in the coronary arteries and aorta) is concomitant with ageing.[147] It is also shown to contribute to adverse ectopic lesions in the arterial tree.[152,161,182] There are different forms of vascular calcification mediated by separate mechanism. However, phosphatases are mediators of osteoblast activity and contribute to calcification.[149] Alkaline phosphatase (ALP), is one such mediator. ALP indicates calcification of blood vessels by means of overlapping mineralization where VSMCs develop osteogenic properties.[183] Therefore, the interaction between cardiac and vascular function is inevitable. Although this connection seems apparent, no evidence is available on the relationship between NT-proBNP as marker of cardiac load and alkaline phosphatase.

4. MOTIVATION FOR THE POPULATION SUBDIVISION

In each research article the particular population has been divided into specific groups after introducing the proper interaction terms, which is also discussed as part of the statistical analyses in the result section of each manuscript. If and when a certain population group was excluded, the appropriate reasons were given due to a previous insignificant finding or to fit the research question.

5. AIMS

General aim

The overall aim of this study was to explore associations between NT-proBNP and vascular function as well as biochemical components affecting the vascular wall leading to cardiac alterations as reflected by NT-proBNP levels in both African and Caucasian men and women.

The more detailed aims are:

5.1 CHAPTER 3 – N-terminal prohormone B-type natriuretic peptide and cardiovascular function in Africans and Caucasians: the SAfrEIC study

- To compare NT-proBNP levels between Africans and Caucasians.
- To explore the associations between NT-proBNP and markers of cardiovascular function in Africans and Caucasians.

5.2 CHAPTER 4 – NT-proBNP is associated with fibulin-1 in Africans: the SAfrEIC study

- To investigate the associations between NT-proBNP, fibulin-1 and measures of arterial function in African and Caucasian men and women.

5.3 CHAPTER 5 – NT-proBNP and inflammatory markers in normotensive Africans: the SAfrEIC study

- To investigate the associations of NT-proBNP with known and novel markers of inflammation in normotensive African men and women.

5.4 CHAPTER 6 – NT-proBNP and alkaline phosphatase in African and Caucasian men: the SAfrEIC study

- To explore whether a relationship exists between NT-proBNP and alkaline phosphatase as a marker of vascular calcification in African and Caucasian men.

6. HYPOTHESES

The hypotheses of the individual research articles were:

6.1 CHAPTER 3 – N-terminal prohormone B-type natriuretic peptide and cardiovascular function in Africans and Caucasians: the SAfrEIC study

- NT-proBNP levels are higher in Caucasians compared to Africans.
- NT-proBNP is adversely associated with measures of cardiovascular function in both Africans and Caucasians.

6.2 CHAPTER 4 – NT-proBNP is associated with fibulin-1 in Africans: the SAfrEIC study

- NT-proBNP is positively associated with fibulin-1 as well as measures of arterial function including pulse wave velocity and arterial compliance in African and Caucasian men and women.

6.3 CHAPTER 5 – NT-proBNP and inflammatory markers in normotensive Africans: the SAfrEIC study

- NT-proBNP is positively associated with both CRP and soluble uPAR in both African men and women, independent of a hypertensive state.

6.4 CHAPTER 6 – NT-proBNP and alkaline phosphatase in African and Caucasian men: the SAfrEIC study

- An independent positive association exists between NT-proBNP and alkaline phosphatase in African and Caucasian men.

7. STRUCTURE OF THE THESIS

This thesis consists of four research manuscripts submitted for publication in peer-reviewed international journals. After this first chapter, Chapter 2 will discuss and describe the study protocol along with the materials and methods used to obtain the data. Chapter 3 compares the NT-proBNP levels in Africans and Caucasians as well as the associations between NT-proBNP and measures of cardiovascular function in the same groups. Chapter 4 explores the associations between NT-proBNP levels and arterial stiffness as well as fibulin-1 as marker of the extracellular matrix in African and Caucasian men and women. Chapter 5 investigated the associations between NT-proBNP and inflammatory markers in African men and women who were apparently healthy, young and normotensive. The final research article (Chapter 6) investigated the possible relationship of NT-proBNP with a marker of vascular calcification in African and Caucasian men. The final chapter (Chapter 7) provides a summary of the main findings as well as a discussion of all the results presented in the thesis, and followed by conclusions and recommendations.

The relevant references are provided at the end of each chapter according to the instructions for authors of the particular journal in which the articles were published or submitted for publication. It was decided to use the American Medical Association (9^{th} edition) reference style in all unpublished chapters.

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CHAPTER 2

STUDY PROTOCOL AND PROCEDURES

A. STUDY DESIGN

The South African study regarding the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC) was a cross-sectional study that was conducted from March to July 2007. This study was designed from a multi-disciplinary angle in order to address many research questions regarding the health of South Africans. Trained field workers recruited a total of 756 volunteers from urban areas of the North West Province of South Africa, which included African and Caucasian men and women (aged 20 to 70 years). A transitory break-down is displayed below:

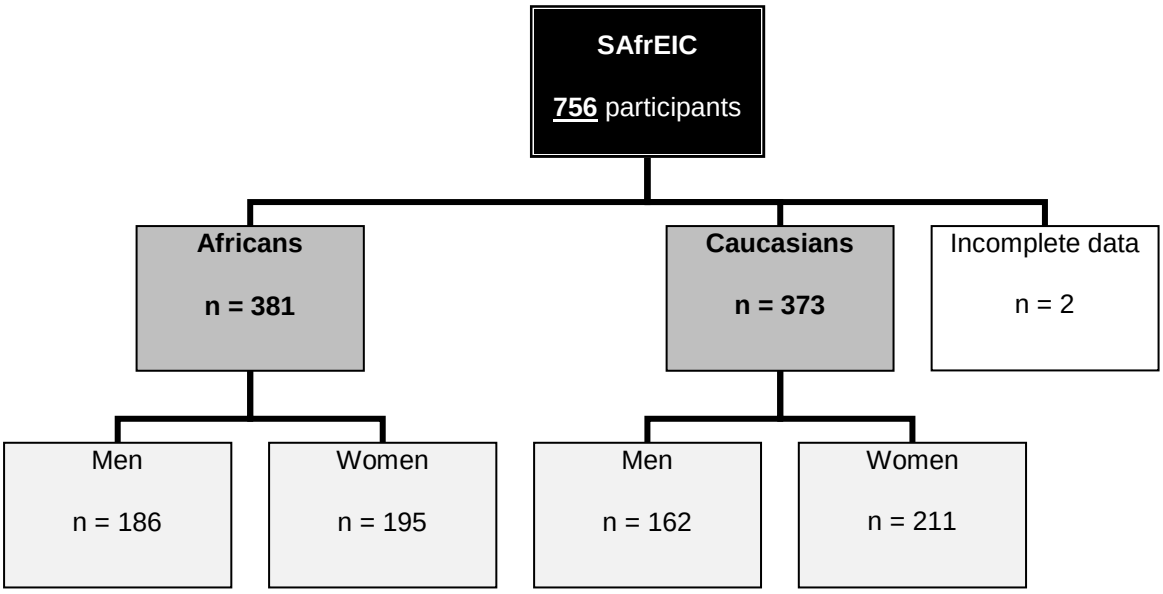

Figure 1: Number of participants that took part in the SAfrEIC study.

Prior to this study all the participants that were apparently ill, pregnant or lactating were excluded. The reason for this was to exclude any known major conditions that may alter or influence the homogenous nature of this population. The four subgroups of African and Caucasian men and women were more than adequate for statistical power. The Ethics Committee of the North-West University (Potchefstroom campus) approved this study and all participants gave written informed consent after the procedures were thoroughly explained to them. African field workers were available to relay information to the African participants in their home language.

B. MATERIALS AND METHODS

Organisational procedures

The participants were invited for a non-recurring clinical examination at a Metabolic Unit facility on the campus of the North-West University. They arrived at 07h00 and were introduced to the research environment after which all the organisational procedures were explained to them and informed consent forms were signed. During the course of the morning, basic health and demographic questionnaires were completed. A fasting blood sample was collected from each participant by a registered nurse from the antebrachial vein using a sterile winged infusion set and syringe. Anthropometric measurements were subsequently taken in a private bedroom. Afterwards cardiovascular measurements were obtained. Each participant received breakfast as well as financial compensation for travel expenses to our facility. In the event of a participant being identified with any abnormalities (such as hypertension or diabetes), the participant was referred to their local clinic, hospital or physician. Each participant received a feedback report containing their basic health information.

Biochemical analyses

The participants were required to fast for at least 8 hours before the fasting blood glucose level could be determined. Capillary blood glucose was directly measured in the Metabolic Unit by a nurse by means of an enzymatic method in order to screen for diabetes mellitus (LifeScan SureStep® Blood Glucose Monitoring System, LifeScan Inc., Milputas, CA 9535). Serum and plasma samples were also taken and stored at -80℃ until further biochemical analyses. Serum blood glucose, lipids (high density lipoprotein, low density lipoprotein, triglycerides and total cholesterol), creatinine, albumin, high-sensitivity C-reactive protein and liver enzymes (aspartate aminotranspeptidase, γ-glutamyltransferase, alanine aminotransferase and alkaline phosphatase) were determined later in the laboratory with the Konelab™ 20i auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). Creatinine clearance was estimated using the Cockcroft-Gault method.[1]

Insulin was determined using the ST AIA-PACK IRI kit (Cat. No 025260) using two-site immunoenzymometric assay. The homoeostasis model assessment (HOMA) index was used to estimate and quantify insulin resistance, which was calculated as the product of fasting glucose and insulin divided by 22.5. Serum cotinine was determined with the IMMULITE 2000 nicotine metabolite assay (Siemens Medical Solutions Diagnostics Ltd., Los Angeles, CA); this metabolite was measured in order to support self-reported smoking data obtained from basic health questionnaires.

Serum NT-proBNP was determined using the Elecsys proBNP sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). To assess the stability of NT-proBNP in the frozen samples, serum NT-proBNP was plotted as a function of the time the samples had been frozen, which varied from 8.5 to 10.5 years, and no association existed between the NT-proBNP level and the time from which the sample was obtained.[2] This ruled out a systemic change in NT-proBNP over this time interval, suggesting that the peptide is likely to be stable when preserved from 2007 as described in the present protocol. The analytical range extended from 5.1 to 34 927 pg/mL. Between-assay coefficients of variation in low and high ranges of NT-proBNP were 4.8% and 2.7%, respectively.

Fibulin-1 was determined by using a sandwich immunoassay.[3] Plasma (EDTA) suPAR levels were measured using the suPARnostic® ELISA kit (ViroGates, Copenhagen, Denmark). The human immunodeficiency virus (HIV) status was determined immediately after blood sampling with a rapid test according to the protocol of the National Department of Health of South Africa. Serum was used for testing with the First Response Test and was repeated with the Pareeshak test for confirmation.

Anthropometric measurements

Qualified anthropometrists measured the body height, body mass as well as waist and hip circumferences of each participant according to standard procedures.[4] The circumferences

were measured in triplicate with a flexible metallic measuring tape (Holtain unstretchable metal tape). Maximum height was measured to the nearest 0.1 cm using the Invicta Stadiometer (Invicta Plastics Ltd. 1465, U.K.). Weight was measured to the nearest 0.1 kg using a digital scale (Precision Health Scale, A & D Company, Japan).

Cardiovascular measurements

Blood pressure measurements were acquired by means of two devices. Firstly, after an initial 10-minute rest in the sitting position, blood pressure (systolic and diastolic) and heart rate were measured using the OMRON HEM-757 (Omron Healthcare, Kyoto, Japan) apparatus, with the blood pressure cuff on the left upper arm. Appropriate cuff sizes were used for obese participants. The participants were seated comfortably during these measurements. Two measurements were taken, with a five-minute rest interval. Secondly, we monitored blood pressure and other cardiovascular parameters with the Finometer™ device (FMS, Finapres Medical Systems, Amsterdam, Netherlands), based on the vascular unloading technique of Peñáz together with the three element model of Wesseling.[5] This entailed a five-minute continuous recording of each participant's cardiovascular parameters under resting, yet awake, conditions. The cardiovascular/ hemodynamic parameters included systolic and diastolic blood pressure, mean arterial pressure, heart rate, inter-beat interval, cardiac output, stroke volume, pulse rate variability and total peripheral resistance. Data files were analysed with the Beatscope 1.1 software to obtain Windkessel arterial compliance.

Pulse wave velocity (PWV) was measured using non-invasive accessible superficial pulses and the Complior SP device (Artech-Medical, Pantin, France, over the carotid radial segment and in an elastic-muscular mixed arterial segment over the carotid dorsalis pedis segment. All measurements were taken by the same two observers for all participants. The PWV was measured on the left side of each participant, while the participant was in a supine position.

Statistical analyses

All statistical analyses were performed using Statistica version 10 software. Variables with a non-Gaussian distribution were logarithmically transformed and the central tendency and spread were represented by the geometric mean and the 5^{th} and 95^{th} percentile intervals. Independent sample T-tests was performed to compare the means of the two ethnic/gender groups. Chi-square tests were performed to compare proportions between groups. Unadjusted and adjusted correlations between NT-proBNP and variables associated with cardiovascular function and vascular components were performed. Multivariable linear regression models with forward stepwise selection for each gender and ethnic group were performed with NT-proBNP as the main dependent variable.

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CHAPTER 3

RESEARCH ARTICLE 1

ORIGINAL ARTICLE

Original Article

N-terminal Prohormone B-type Natriuretic Peptide and Cardiovascular Function in Africans and Caucasians: The SAfrEIC Study

Ruan Kruger[a], Rudolph Schutte[a,*], Hugo W. Huisman[a], Peter Hindersson[b], Michael H. Olsen[c] and Aletta E. Schutte[a]

[a] *Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa*
[b] *Department of Biochemistry, Hospital Vendsyssel, Hjørring, Denmark*
[c] *Cardiovascular Research Unit, Division of Cardiology, Department of Internal Medicine, Glostrup Hospital, University of Copenhagen, Denmark*

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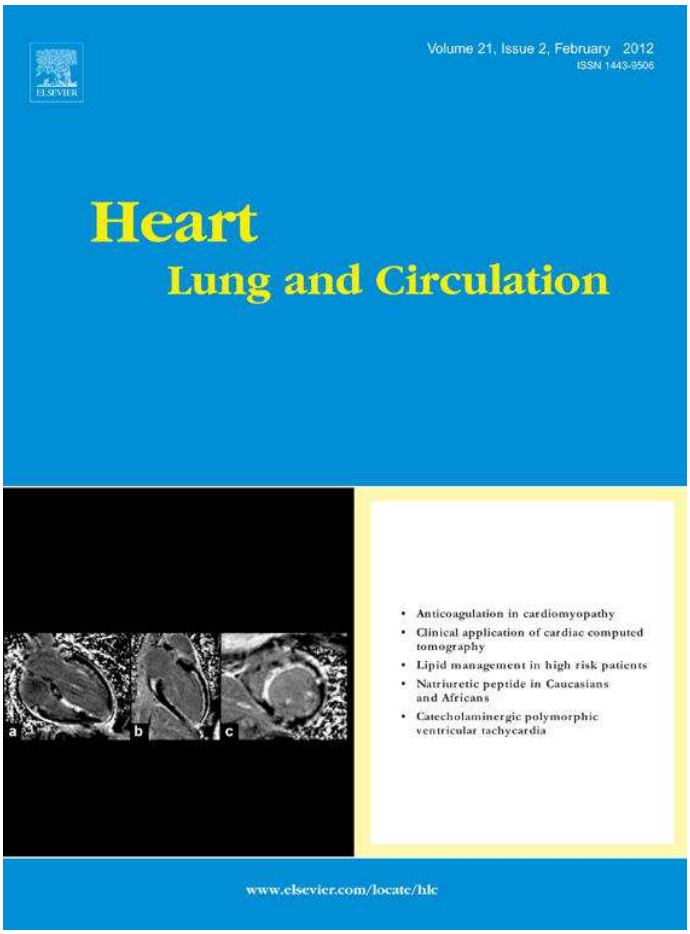

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Abstract

Background: This study compared NT-proBNP levels and the association with cardiovascular markers between Africans and Caucasians from South Africa.

Methods: This cross-sectional study involved 201 Africans and 255 Caucasians from the North West province, South Africa. Serum NT-proBNP concentrations, blood pressure, pulse wave velocity and arterial compliance were measured.

Results: NT-proBNP levels were significantly higher (P<0.001) in Africans than Caucasians, also after adjusting for gender, body mass index (BMI) and pulse wave velocity (P=0.008). This significant difference became borderline significant after adjusting for systolic blood pressure (SBP) (P=0.060), and non-significant after adjusting for arterial compliance (P=0.35). In single regression, a significant positive correlation of NT-proBNP with SBP (r=0.26; P<0.001) and pulse pressure (PP) (r=0.28; P<0.001) were shown for Africans only. After multiple adjustments, the associations of NT-proBNP with SBP and PP remained significant in Africans (SBP: β=0.187, P<0.01; PP: β=0.234, P<0.001), with no significant associations in Caucasians.

Conclusions: NT-proBNP levels were higher in Africans than Caucasians, independently of BMI and gender. This difference was partly driven by higher SBP and lower arterial compliance in Africans. NT-proBNP was persistently associated with SBP and PP in Africans, but not in Caucasians. These associations may suggest early vascular changes contributing to cardiac alterations in Africans.

Key Words: NT-proBNP, blood pressure, ethnicity, cardiovascular function, compliance.

Introduction

The N-terminal prohormone B-type natriuretic peptide (NT-proBNP) has been underlined in the assessment and diagnosis of congestive heart failure [1]. Since NT-proBNP has a much longer half-life than BNP, it is used as a reliable biochemical predictor and marker of cardiovascular risk [2]. Patients with cardiac hypertrophy and resulting systolic and diastolic dysfunction are subjected to increasing myocardial stress load, causing a rise in plasma levels of NT-proBNP [3]. Normally, NT-proBNP is up-regulated in the atria as a response to this cardiac overload [4,5], but is also extensively secreted by the ventricular cardiomyocytes as a result of elevated cardiac wall stress [5,6]. NT-proBNP functions as a ventricular cardiac hormone and partakes in the control of myocardial structure and function [7]. It is also involved in the lowering of sodium reabsorption in the kidneys, resulting in decreased blood volume and subsequent arterial pressure [8,9], therefore counteracting left ventricular wall tension and increasing plasma volume [10,11]. In a study of coronary atherosclerosis, plasma NT-proBNP levels were significantly lower in African Americans compared to Caucasians [10,12], but less is known in the bi-ethnic populations of South Africa.

In general, black South Africans are subjected to rapid epidemiologic and socioeconomic transition as opposed to the gradual westernisation of African Americans; and this transition increases the incidence of cardiovascular related morbidity and mortality [13,14]. In a large study on hospitalised black South Africans, it became evident that most patients were presented with advanced cardiac disease, predominantly heart failure [14]. This was more prominent in patients from urban settings [15] however despite the high prevalence of cardiovascular disease amongst these black South Africans, the cause is still inconclusive. It is evident that health inequalities exist between Africans and Caucasians and could largely be due to socioeconomic transition [16]. Although some information is available on the associations between NT-proBNP and measures of

cardiovascular function in American and European Caucasians, there exists an inherent lack of information with regard to the South African population [14,17].

The aims of this comparative study were to compare NT-proBNP levels between Africans and Caucasians and also to explore the associations of NT-proBNP with markers of cardiovascular function in this target population.

Methods

Study population and procedures

The South African study regarding the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC) was a cross-sectional study that in total involved 756 Africans and Caucasians (aged 20–70 yrs.) from the North West Province of South Africa. Pregnant or lactating women were initially excluded from the study. In the present study, persons who were diagnosed positive with the human immunodeficiency virus (Africans: n=114; Caucasians: n=1) were excluded, as well as 8 participants of whom were diabetics (type 1 or 2), 91 participants that used antihypertensive or anti-inflammatory medication and participants older than 55 years (n=86). After excluding the relevant cases, a total of 456 participants (201 Africans and 255 Caucasians) were included.

Ten to twenty participants visited the Metabolic Unit facility daily at the Potchefstroom campus of the North West University from March until July in 2007. All the procedures were comprehensively explained to the participants and they all gave written informed consent to participate in the study. A participant sheet was given to each person that guided them through the different research stations where various measurements were done. Basic health and demographic questionnaires were completed during the morning. In the event where abnormalities were identified in a

participant (i.e. hypertension or diabetes), the participant was advised to visit their local clinic, hospital or physician. An informative description regarding the results of the health assessment was given to each participant at the end of the study. This study was approved by the Ethics Review Board of the North West University and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki (2008) for investigation of human participants.

Cardiovascular measurements

The OMRON HEM-757 (Omron, Kyoto, Japan) apparatus was used to determine systolic and diastolic blood pressure with the cuff on the left upper arm in the sitting position. Two blood pressure measurements were done, the first after an initial 10 minute resting period and the second reading after a 5 minute waiting period. Participants with a systolic blood pressure (SBP) ≥140mmHg and/or diastolic blood pressure (DBP) ≥90mmHg were considered hypertensive [18]. Pulse pressure was subsequently calculated by subtracting the DBP from SBP. Heart rate, cardiac output, stroke volume, arterial compliance and total peripheral resistance were determined with the Finometer apparatus (FMS, Finapres Measurement Systems, Amsterdam, The Netherlands). The carotid dorsalis-pedis pulse wave velocity (PWV) was measured with the Complior SP Acquisition system (Artech-Medical, Pantin, France).

Anthropometric measurements

Applying standard procedures, body height was measured to the nearest 0.1 cm by using the Invicta Stadiometer (IP 1465, London, UK) and body weight was measured to the nearest 0.1 kg (Precision Health Scale, A & D Company, Japan). Subsequently, the body mass index (BMI) was calculated for each participant as weight (kilograms) divided by height (meters) squared. The waist circumference was measured at the maximal girth with a Holtain non-stretchable, flexible metal measuring tape [19].

Biochemical analyses

All participants were requested to fast for a period of eight hours. Fasting lipids (total cholesterol, high-density lipoprotein cholesterol, and triglycerides), serum glucose, γ-glutamyl transferase, creatinine and high-sensitivity C-reactive protein were determined with the Konelab autoanalyzer (Thermo Fisher Scientific Oy, Vantaa, Finland). The Cockcroft–Gault formula [20] was used to determine estimated creatinine clearance ($ml.min^{-1}$) = (140–age) × mass (kg) × constant/serum creatinine ($\mu mol.l^{-1}$), where the constant is 1.23 for men and 1.04 for women. Serum cotinine was determined with the IMMULITE 2000 nicotine metabolite assay (Siemens Medical Solutions Diagnostics Ltd., Los Angeles, CA). Insulin was determined with the ST AIA-PACK IRI kit (TOSOH AIA, Inc., Toyama, Japan; catalog no. 025260) using a two-site immune-enzymometric assay. HOMA-IR (homeostasis model assessment) was calculated by using the following formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l) / 22.5. Serum NT-proBNP was determined using the Elecsys proBNP sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). Human immunodeficiency virus status was determined directly after blood sampling with rapid tests according to the protocol of the National Department of Health of South Africa. Serum was used for testing with the First Response Test and was repeated with the Pareeshak test to confirm results. All tests were done at the same laboratory in South Africa, except NT-proBNP analyses which were done for all participants in Denmark.

Statistical analyses

Statistica software v10.0 (StatSoft, Inc., Tulsa, OK, USA) was used for database management and statistical analyses. Normal distribution of the variables was tested prior to any further statistical analyses. Variables that did not fulfil these criteria (NT-proBNP, C-reactive protein, γ-glutamyl transferase, serum glucose and cotinine) were logarithmically transformed. NT-proBNP associations with systolic blood pressure were tested for interaction with gender or ethnicity by

introducing appropriate interaction terms in multiple regression analysis. Chi-square tests (χ^2) were used to compare proportions. Mean values of NT-proBNP were compared between Africans and Caucasians by means of ANOVA, and after adjustments were applied for gender, BMI, SBP, arterial compliance and PWV with ANCOVA analysis. GraphPad Prism v5.03 (GraphPad Software, Inc., San Diego, California, USA) was used to plot and determine unadjusted associations between NT-proBNP and cardiovascular variables. Multiple forward stepwise regression analyses were done to investigate independent associations between NT-proBNP and cardiovascular variables (SBP and PP). Covariates included in the model were age, gender, BMI, blood glucose, total cholesterol and high density lipoprotein cholesterol ratio (TC:HDLC), C-reactive protein and γ-glutamyltransferase. *P*-values of ≤0.05 were considered statistically significant.

Results

Table 1 lists the general characteristics of the Africans and Caucasians. The total group was divided in Africans and Caucasians due to the significant interaction between the associations of NT-proBNP and SBP with ethnicity ($p<0.0001$). No significant interaction was established with gender ($p=0.47$). The mean ages of the groups were similar. More Africans smoked compared to Caucasians (58.7% vs. 16.1%; $P<0.0001$), which was confirmed by the cotinine levels ($P<0.0001$). The self-reported use of alcohol was similar between Africans and Caucasians (67.8% vs. 68.6%; $P=0.85$), however the levels of γ-glutamyltransferase of Africans were significantly higher than those of Caucasians ($P<0.0001$). In total, 89.0% of this African population earned less than USD290 per month opposed to 86.0% of Caucasians earning more than USD730 per month ($P<0.0001$). The prevalence of hypertension was also noticeably higher in Africans compared to Caucasians (28.0% vs. 8.0%; $P<0.001$).

Table 1 – General characteristics of Africans and Caucasians

	Africans *n = 201*	**Caucasians** *n = 255*	***P*-value**
Age (years)	36.4 ± 9.3	35.0 ± 9.3	0.071
Gender, men/women (%)	48/52	42/58	<0.05
Body mass index (kg.m^{-2})	24.3 ± 7.4	27.2 ± 5.9	<0.0001
Waist circumference (cm)	78.0 ± 13.4	85.1 ± 14.6	<0.0001
Biochemical analyses			
NT-proBNP (pg.mL^{-1})	23.2 (19.6 – 28.2)	16.4 (14.0– 19.4)	<0.001
C-reactive protein (mg.L^{-1})	2.0 (1.5 – 2.6)	1.2 (0.94 – 1.5)	<0.01
Serum creatinine (µmol.L^{-1})	66.1 ± 11.6	72.1 ± 12.7	<0.0001
Estimated creatinine clearance (mL.min^{-1})	1.30 ± 0.38	1.55 ± 0.49	<0.0001
TC:HDLC (mmol.L^{-1})	3.11 ± 1.07	4.44 ± 1.79	<0.0001
Serum glucose (mmol.L^{-1})	5.1 (4.9 – 5.2)	5.3 (5.2 – 5.4)	0.002
Triglycerides (mmol.L^{-1})	1.14 ± 0.75	1.36 ± 0.84	0.004
Insulin (µU.mL^{-1})	8.07 ± 7.36	9.58 ± 7.64	0.039
HOMA-IR	2.01 ± 2.38	2.37 ± 2.18	0.10
Cardiovascular measurements			
Systolic blood pressure (mmHg)	122.1 ± 18.8	114.8 ± 12.7	<0.0001
Diastolic blood pressure (mmHg)	82.8 ± 13.2	76.0 ± 8.9	<0.0001
Pulse pressure (mmHg)	39.4 ± 10.0	38.8 ± 8.9	0.51
Heart rate (bpm)	68.6 ± 12.3	67.5 ± 8.9	0.27
Cardiac output (L.min^{-1})	5.15 ± 1.39	6.14 ± 1.63	<0.0001
Stroke volume (mL)	78.4 ± 19.9	90.7 ± 21.5	<0.0001
Total peripheral resistance (mmHg.L.min^{-1})	1.28 ± 0.36	1.01 ± 0.26	<0.0001
Arterial compliance (mL.mmHg^{-1})	1.76 ± 0.43	2.27 ± 0.48	<0.0001
Pulse wave velocity (m.s^{-1})	7.83 ± 1.40	7.56 ± 1.03	0.016
Lifestyle			
Income, *n* (% above USD 221)	22 (11)	219 (86)	<0.0001
Hypertension status, *n* (%)	56 (28)	21 (8)	<0.001
Cotinine (ng.mL^{-1})	79.9 (65.6 – 97.4)	15.3 (12.8 – 18.2)	<0.0001
γ-Glutamyl transferase (U.L^{-1})	58.7 (52.7 – 62.3)	28.2 (25.7 – 31.0)	<0.0001

Values are arithmetic mean ± SD, geometric mean (95% confidence interval) or number of participants.
Abbreviations: HOMA-IR – homeostasis model assessment - insulin resistance; TC:HDLC – Total cholesterol and high density lipoprotein cholesterol ratio.

Unadjusted correlations between NT-proBNP and cardiovascular variables (SBP and PP) are plotted in Figure 1. Significant positive correlations of NT-proBNP with SBP (r=0.26; p<0.001) and PP (r=0.28; p<0.001) were established in Africans, with no significance in Caucasians. Since the prevalence of smoking was higher, and the socioeconomic status significantly lower in the Africans than the Caucasians, we considered cotinine and monthly income in the multiple regression model. None of these variables entered the model. Variables that entered the multiple

regression analyses include age, gender, BMI, serum glucose, TC:HDLC, C-reactive protein and γ-glutamyl transferase. After full adjustment, the associations of NT-proBNP with SBP (Table 2) and pulse pressure (Table 3) were confirmed in Africans. Independent associations of NT-proBNP with arterial compliance and significant covariates were also tested in the total group (R^2=0.25; β=–0.089; *P*=0.21), Africans (R^2=0.19; β=–0.126; *P*=0.24) and Caucasians (R^2=0.30; β=0.123; *P*=0.18), showing no significance.

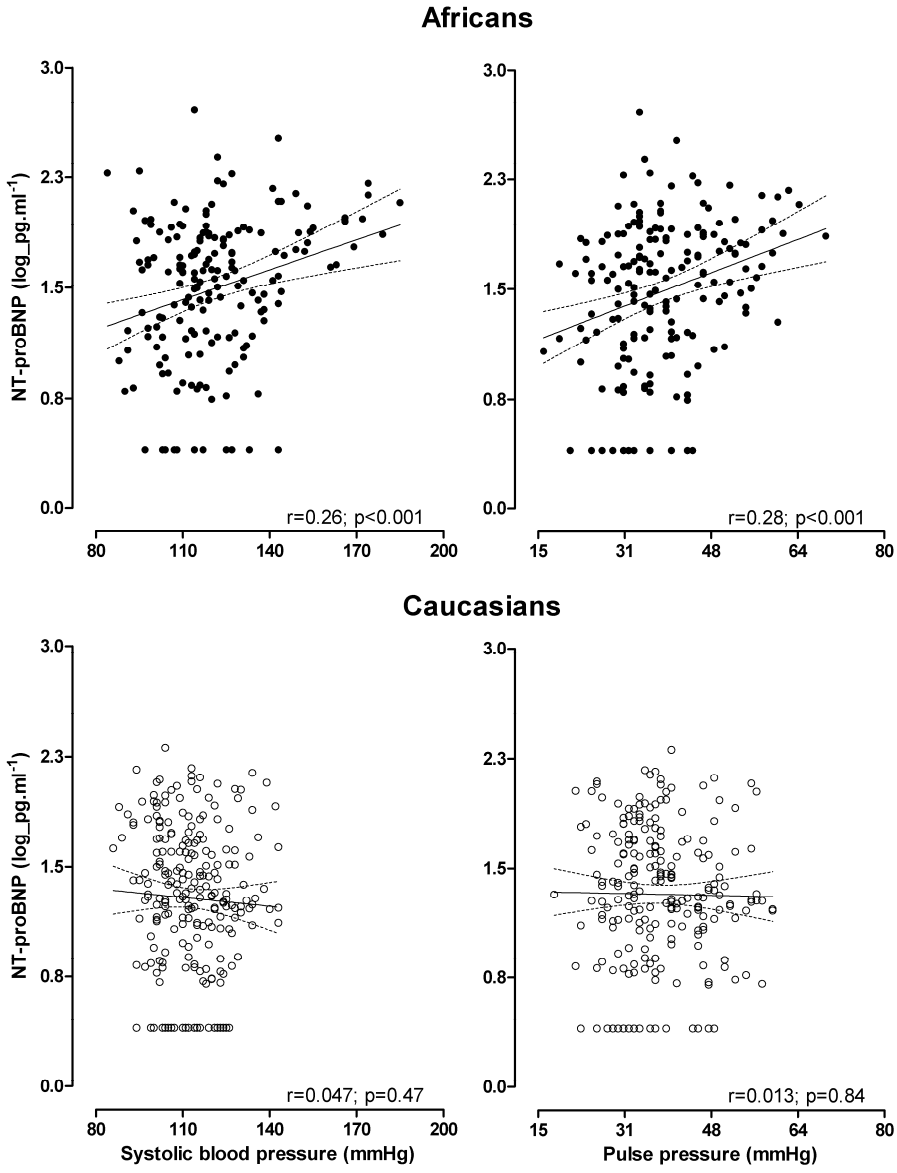

Figure 1: Single regression analyses of NT-proBNP with measures of cardiovascular function.

Table 2 – Independent forward stepwise analyses between NT-proBNP and systolic blood pressure

	Total population	**Africans**	**Caucasians**	**Ethnic interaction**
	n=456	*n=201*	*n=255*	*P value*
	NT- proBNP (pg.ml^{-1})			
R^2	0.28	0.25	0.33	
Adjusted R^2	0.27	0.22	0.31	
Std β (95% confidence interval)				
Systolic blood pressure (mmHg)	0.161 (0.069 to 0.254)†	0.187 (0.050 to 0.325)†	–	<0.01
Age (years)	0.215 (0.125 to 0.305)‡	0.313 (0.162 to 0.465)‡	0.160 (0.051 to 0.268)†	<0.001
Gender	0.417 (0.326 to 0.508)‡	0.271 (0.116 to 0.426)‡	0.458 (0.344 to 0.572)‡	<0.001
Body mass index (kg.m^{-2})	–0.217 (–0.312 to –0.123)‡	NS	–0.126 (–0.250 to –0.003)*	<0.001
Glucose (mmol.L^{-1})	NS	–0.161 (–0.300 to –0.022)*	–	0.072
TC:HDLC (mmol.L^{-1})	–0.085 (–0.170 to –0.0004)*	–	NS	0.22
γ-Glutamyl transferase (U.L^{-1})	–0.109 (–0.201 to 0.017)*	–0.186 (–0.330 to 0.041)*	NS	0.005
C-reactive protein (mg.L^{-1})	0.123 (0.034 to 0.212)†	NS	NS	0.011

Superscript symbol denotes significance for: *$P \leq 0.05$; †$P \leq 0.01$; ‡$P \leq 0.001$.

Abbreviations: TC:HDLC – Total cholesterol and high density lipoprotein cholesterol ratio; NS – not significant.

Table 3 – Independent forward stepwise analyses between NT-proBNP and pulse pressure

	Total population	**Africans**	**Caucasians**	**Ethnic interaction**
	n=456	*n=201*	*n=255*	*P value*
	NT- proBNP (pg.ml^{-1})			
R^2	0.29	0.27	0.34	
Adjusted R^2	0.27	0.24	0.32	
Std β (95% confidence interval)				
Pulse pressure (mmHg)	0.174 (0.083 to 0.264)‡	0.239 (0.100 to 0.378)‡	NS	<0.0001
Age (years)	0.230 (0.142 to 0.318)‡	0.325 (0.178 to 0.472)‡	0.154 (0.046 to 0.262)†	<0.0001
Gender	0.447 (0.352 to 0.542)‡	0.316 (0.157 to 0.475)‡	0.515 (0.387 to 0.643)‡	<0.0001
Body mass index (kg.m^{-2})	–0.181 (–0.275 to –0.087)‡	NS	NS	0.004
Glucose (mmol.L^{-1})	NS	–0.189 (–0.327 to –0.050)†	–	0.050
TC:HDLC (mmol.L^{-1})	–0.085 (–0.169 to –0.001)*	–	NS	0.32
γ-Glutamyl transferase (U.L^{-1})	NS	–0.160 (–0.302 to –0.019)*	NS	0.015
C-reactive protein (mg.L^{-1})	0.123 (0.034 to 0.212)†	NS	NS	0.015

Superscript symbol denotes significance for: *$P \leq 0.05$; †$P \leq 0.01$; ‡$P \leq 0.001$.

Abbreviations: TC:HDLC – Total cholesterol and high density lipoprotein cholesterol ratio; NS – not significant.

In exploratory analyses (Table 4), NT-proBNP levels of the African and Caucasian groups were compared before and after adjustments were applied. After adjusting for BMI and gender (*P*=0.003) and additionally for PWV (*P*=0.008), the differences observed between Africans and Caucasians remained significant. However, when additionally adjusting for SBP (*P*=0.060) the significant difference became borderline significant and became non-significant after additionally adjusted for arterial compliance (*P*=0.35).

Table 4 – NT-proBNP concentration levels before and after adjustments for confounders

	Africans	**Caucasians**	
	n=201	*n=255*	
	NT-proBNP (pg.ml^{-1})		
			***P*-value**
Before adjustments	23.2 (19.6 – 28.2)	16.4 (14.0– 19.4)	<0.001
After adjustments applied for:			
BMI and gender	22.9 (19.4 – 27.0)	16.4 (14.2 – 19.0)	0.003
BMI, gender and PWV	22.5 (19.1 – 26.4)	16.7 (14.5 – 19.3)	0.008
BMI, gender and SBP	21.5 (18.2 – 25.3)	17.3 (15.0 – 20.0)	0.060
BMI, gender and Cwk	20.4 (17.0 – 24.4)	18.0 (15.4 – 21.0)	0.35

Values are shown as the geometric mean (95% confidence interval).

Abbreviations: BMI – Body mass index; Cwk – Arterial compliance; PWV – pulse wave velocity; SBP – systolic blood pressure.

Discussion

This study aimed to compare NT-proBNP levels between Africans and Caucasians and to explore associations with markers of cardiovascular function. The key findings of this study were that NT-proBNP levels were significantly higher in Africans compared to Caucasians, however seemed driven by the higher SBP and lower arterial compliance. This was supported by the independent associations of NT-proBNP with SBP and PP. Moreover, higher levels of subclinical vascular disease are well known in the black South African population and may be due to modifiable risk factors such as smoking and alcohol use [16]. However, socioeconomic status as a social determinant of health did not enter the multiple regression analyses, suggesting that socioeconomic class does not drive the prevalence of hypertension in these populations. However, the significant interaction of NT-proBNP and SBP with ethnicity suggests that the prevalence of higher blood pressure is due to ethnic inequalities. These results in Africans versus a Caucasian group of similar ages (36.4 vs. 35.0 years), suggest that the Africans already exhibit early cardiac alterations under conditions of higher SBP and pulse pressure.

It is known that NT-proBNP levels are elevated in patients with left ventricular hypertrophy (LVH) and congestive heart failure [21], and an early increase in plasma levels of NT-proBNP is related to both cardiovascular morbidity and mortality [22]. In line with recent epidemiological evidence, NT-proBNP is a very powerful predictor of mortality in hypertensive patients, even in those without LVH [23]. This is a concern as this indicates that especially the Africans from our study are subjected to a significant increased risk of cardiovascular mortality at a very young age.

Our results are consistent with studies that have shown positive associations between NT-proBNP and SBP, but we also show that PP may be an important determinant of natriuretic peptide levels, as it reflects aortic stiffness and increased stress load on the heart [24,25].

Elevated levels of NT-proBNP, as a result of chronic cardiac overload [26], predicts cardiovascular events and mortality in the general population [27], as it reflects the degree of LVH in patients with or without hypertension [3]. LVH is an independent risk factor for all cardiovascular complications in hypertension [28]. Our results therefore indicate that early cardiac changes are already present in relatively young (~36 years) Africans with higher SBP and PP, emphasizing the importance of early counteractive measures regarding hypertension prevention in the black population of South Africa.

The original pathophysiological process for increasing NT-proBNP levels was initially suggested to be left ventricular systolic or diastolic dysfunction caused by myocardial ischemia as a result of augmented cardiac wall stress [5]. However, NT-proBNP reflects hemodynamic myocardial stress, independent of underlying pathology, for instance heart failure, and is subsequently not specific for distinct pathology, but for cardiovascular diseases in general [29,30]. The general physiological functions of NT-proBNP include peripheral vasodilatation, diuresis/natriuresis, inhibition of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system [30], and contribute to the protection against elevated blood volume and pressure [31]. In addition, the natriuretic peptides act as an important counter-regulatory mechanism, as it causes a shift in the fluid from the capillaries into the interstitium which leads to decreasing intravascular volume [32] and lowered blood pressure [33]. The natriuretic peptide system is largely activated in ventricular dysfunction (both the right and left ventricle) [34,35] and hypertension [36].

The prevalence of hypertension and hypertensive heart disease is still increasing in South Africa [37]. It is reported that Sub-Saharan Africans are subjected to salt-sensitive hypertension as a result of low renin levels, raised aldosterone concentrations and increased peripheral vascular resistance which are common characteristics observed in hypertensive Africans [38]. The RAAS

is mainly regulated by the mechanisms that stimulate renin secretion, but it is also modulated by natriuretic peptides released by the cardiac myocytes [39]. Furthermore, in this African population, the retention of excess sodium and water causes volume overload and may contribute to the development of sustained high blood pressure and elevated levels of NT-proBNP. This could perhaps be the link between the mechanisms of cardiac NT-proBNP expression and the higher blood pressure seen in our African population, explaining the persistent association of NT-proBNP with SBP and pulse pressure in African men and women, as both SBP and pulse pressure are regarded as risk factors for heart disease [17,40]. The overall health profile in the African population is a major concern and is the result of multiple contributing factors including socio-economic status, diet and lifestyle. The higher NT-proBNP levels and the associations with SBP and PP suggest that Africans are at higher risk for future cardiac events. Therefore, establishing a low cost method for the measurement of NT-proBNP could provide a stable reference in our African population to curb future cardiovascular events.

This study needs to be interpreted within the context of its limitations and strengths. This was a cross-sectional study and therefore causality cannot be inferred, however these results were consistent after multiple adjustments. Nevertheless, one cannot rule out that our findings were because of confounding variables or because of unknown factors that are associated with both NT-proBNP and cardiovascular function, especially the great socio-economic difference that was evident between our study populations. The carotid femoralis pulse wave velocity was not measured in this study, but the carotid dorsalis-pedis pulse wave velocity. NT-proBNP is a stable and sensitive marker of cardiac function including right ventricular dysfunction [41], left ventricular systolic dysfunction, and early cardiac alterations [42], but do not add further clinical information about LVH [43]. Hence, electro- and echocardiography data for LVH was not available for this study. NT-proBNP is also expensive to determine, but a good marker of cardiovascular risk.

Although there are other cost-effective markers for LVH, the strengths of NT-proBNP might encourage the development of a more affordable commercial screening method for NT-proBNP. This was a well-designed observational study implemented under controlled conditions, and was the first study to compare NT-proBNP levels and associations between NT-proBNP and cardiovascular function in Africans and Caucasians from South Africa, providing useful information in understanding this unique cohort.

In conclusion, NT-proBNP levels are higher in African compared to Caucasian men, however confounded by arterial compliance and SBP. In addition, NT-proBNP is strongly associated with SBP and PP in Africans but not in Caucasians of similar age, suggesting early onset cardiac changes under conditions of higher SBP and the association of NT-proBNP with pulse pressure in Africans. This emphasizes the importance of early intervention or counteractive measures to lower blood pressure and aortic stiffness to reduce cardiac damage.

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Disclosure

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CHAPTER 4

RESEARCH ARTICLE 2

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NT-proBNP is associated with fibulin-1 in Africans: The SAfrEIC study

R. Kruger[a,*], R. Schutte[a], H.W. Huisman[a], W.S. Argraves[b], L.M. Rasmussen[c], M.H. Olsen[d], A.E. Schutte[a]

[a] Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa
[b] Medical University of South Carolina, Department of Cell Biology, Charleston, SC, USA
[c] Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Denmark
[d] Cardiovascular Research Unit, Division of Cardiology, Department of Internal medicine, Glostrup Hospital, University of Copenhagen, Denmark

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INSTRUCTIONS FOR AUTHORS

Original articles should report original research not previously published or being considered for publication elsewhere. Manuscripts should be written in the English language (using either American or British spelling). The number of words per manuscript should not exceed 4000 (including tables and legends to figures). As a rule, research papers should be divided into sections, headed by a caption (e.g. Abstract, Introduction, Materials, Methods, Experimental Results, Discussion, etc.). Please include a short paragraph of conclusions (at the end of the text), indicating the relevance of the study with regard to the basics and/or clinical aspect of atherosclerosis. A statement concerning the source of funding, conflicts of interests and disclosures of financial support is highly recommended.

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Abstract

Objectives: The N-terminal prohormone B-type natriuretic peptide (NT-proBNP) is involved in the regulation of volume load and secreted when systemic cardiac overload occurs. Fibulin-1 on the other hand is a component of many extracellular matrix proteins including those present in atherosclerotic lesions, expressed in elastin-containing fibres of blood vessels, and also in the heart. Due to an alarming prevalence of hypertensive heart disease in black South Africans, we investigated the associations of NT-proBNP with fibulin-1 and markers of arterial stiffness in Africans and Caucasians.

Methods: We included 231 Africans and 238 Caucasians from South Africa aged 22 to 77 years. Serum NT-proBNP and fibulin-1 levels were determined, and arterial compliance and pulse wave velocity were measured.

Results: Africans had significantly higher blood pressure and NT-proBNP levels than Caucasians and African men had higher fibulin-1 levels than Caucasian men. In single regression analysis, NT-proBNP was significantly associated with fibulin-1 in African men and Caucasian women. NT-proBNP correlated negatively with arterial compliance in all groups except Caucasian women. After partial adjustments, the association between NT-proBNP and fibulin-1 strengthened in African men only. After full adjustment in multiple regression analysis, the association of NT-proBNP with fibulin-1 was confirmed in African men (R^2=0.41; β=0.26; p<0.01) and also in younger women (R^2=0.34; β=0.251; p=0.012).

Conclusions: Only Africans indicated a significant independent association between NT-proBNP and fibulin-1, suggesting that cardiovascular alterations are already present in this relatively young African population as opposed to Caucasians.

Key Words: NT-proBNP, fibulin-1, cardiovascular function, arterial stiffness, extracellular matrix remodelling.

Introduction

The N-terminal prohormone B-type natriuretic peptide (NT-proBNP) and other natriuretic peptides are endogenous counteractive mediators of the body's defence against plasma volume expansion and elevated blood pressure [1]. NT-proBNP is traditionally known to be secreted by the atria; however it is also prominently expressed by ventricular cardiomyocytes in response to cardiac wall tension or overload [2, 3]. Therefore, increasing myocyte stretch stimulates the secretion of vasodilatory NT-proBNP, which lowers blood volume and arterial pressure via its natriuretic and diuretic properties [4]. In disease states such as hypertension and heart failure, NT-proBNP levels are elevated and therefore suggested as a reliable hemodynamic indicator of cardiovascular risk [5]. Augmented natriuretic peptide gene expression and production in cardiac myocytes may result in alterations of cardiac cellular function and structure via changes in extracellular matrix components. These changes may contribute to the development of hypertension [6] and cardiac hypertrophy [1].

The extracellular matrix of the heart and vasculature is dynamically adaptive, playing a fundamental role in myocardial ventricular remodelling as it is regulated by hemodynamic stress, neuro-hormonal activation, inflammation and oxidative stress [7]. Fibulin-1 is a fibrinogen binding glycoprotein in the blood. It is a component of many extracellular matrix proteins including those present in cardiovascular tissues such as elastin-containing fibres of the arteries and also cardiac tissue [8]. Fibulin-1 was recently identified by transcriptomic screening, as upregulated in non-atherosclerotic arterial tissue from patients with type 2 diabetes mellitus and was shown to correlate with glycemic status and indices of arterial stiffness [9]. Alterations in the extracellular matrix contribute to arterial stiffness of especially the conduit arteries, which has an independent predictive value of cardiovascular mortality in hypertensive patients or patients subjected to renal failure or diabetes mellitus [10]. Black South Africans have an elevated risk for the development of hypertension and subsequent cardiovascular disease [11]. A recent study also showed that

Africans are more subjected to the progression of arterial stiffness than Caucasians [12], but no information about fibulin-1 is available in this regard. Since the associations of NT-proBNP with fibulin-1 and arterial stiffness have not yet been investigated, this study was motivated by the possible link that may exist between the natriuretic peptide system, cardiovascular extracellular remodelling and arterial stiffness.

Therefore, the aim of this study was to investigate the possible associations of the cardiovascular risk marker, NT-proBNP with fibulin-1 as well as markers of arterial function in African and Caucasian men and women.

Methods

Study Population

This cross-sectional sub-study formed part of a larger South African study regarding the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC study) and involved 231 Africans and 238 Caucasians from the North West Province of South Africa. Pregnant or lactating women were initially excluded from the study. Female participants using contraceptive medication (n=64), as well as type 1 or 2 diabetics (n=8), participants using antihypertensive or anti-inflammatory medication (n=91) and those diagnosed positive with the human immunodeficiency virus (n=115) were also excluded. This study was approved by the Ethics Review Board of the North-West University and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki (2008) for investigation of human participants.

Basic procedures

Ten to twenty participants visited the Metabolic Unit facility over a period of seven weeks at the Potchefstroom campus of the North-West University from March until July 2007. Each participant gave written informed consent to participate in the study after all the procedures were

comprehensively explained. A participant sheet guided the participants through the different research stations where various measurements were done. Basic health and demographic questionnaires were completed during the morning. An informative description regarding the results of the health assessment was given to each participant at the end of the study. In the event where abnormalities were identified in a participant (e.g. hypertension or diabetes), the participant was advised to visit their local clinic, hospital or physician.

Cardiovascular measurements

Blood pressure measurements were done in duplicate after a 10 minute resting period and a 5 minute interval between the two measurements. The OMRON HEM-757 (Omron Healthcare, Kyoto, Japan) apparatus was used to determine systolic and diastolic blood pressure with the cuff on the left upper arm in the sitting position. Pulse pressure was subsequently calculated by subtracting the diastolic blood pressure (DBP) from systolic blood pressure (SBP). Participants with a SBP ≥140mmHg and/or DBP ≥90mmHg were considered hypertensive [6]. Heart rate and arterial compliance were determined with the Finometer apparatus (FMS, Finapres Measurement Systems, Amsterdam, The Netherlands). The carotid-dorsalis pedis pulse wave velocity (PWV) was measured with the Complior SP Acquisition system (Artech-Medical, Pantin, France).

Anthropometric measurements

Body height was measured to the nearest 0.1 cm by using the Invicta Stadiometer (Invicta Plastics Ltd. 1465, London, UK) and body weight to the nearest 0.1 kg (Precision Health Scale, A & D Company, Japan), according to standard procedures. Body mass index (BMI) was calculated for each participant as weight (kilogram) divided by height (meter) squared. The waist circumference was measured at the maximal girth with a Holtain non-stretchable, flexible metal measuring tape [13].

Biochemical measurements

All participants were requested to fast for a minimum period of eight hours. Fasting lipids (total cholesterol, high-density lipoprotein cholesterol, triglycerides), serum glucose, γ-glutamyl transferase, serum creatinine and serum high-sensitivity C-reactive protein (CRP) levels were determined with the Konelab autoanalyzer (Thermo Fisher Scientific Oy, Vantaa, Finland). The Cockcroft–Gault formula was used to determine estimated creatinine clearance.[14] Serum cotinine was determined with the IMMULITE 2000 nicotine metabolite assay (Siemens Medical Solutions Diagnostics Ltd., Los Angeles, CA). The Elecsys proBNP sandwich immunoassay was used on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany) to determine the serum NT-proBNP concentration of each participant. Fibulin-1 was determined by using a sandwich immunoassay [9]. The human immunodeficiency virus status was determined directly after blood sampling with rapid tests according to the protocol of the National Department of Health of South Africa. Serum was used for testing with the First Response (PMC Medical, India) rapid HIV card test and was repeated with the Pareeshak test (BHAT Bio-tech, India) to confirm the results.

Statistical analyses

Statistica software v10.0 (StatSoft, Inc., Tulsa, OK, USA) was used for database management and statistical analyses. Normal distribution of the variables was tested prior to any statistical analyses. Variables that did not fulfil these criteria (NT-proBNP, fibulin-1, CRP, γ-glutamyl transferase, serum glucose and cotinine) were logarithmically transformed. The association of NT-proBNP with fibulin-1 was tested for interaction with gender or ethnicity by introducing appropriate interaction terms and performing univariate ANCOVA analyses. T-tests were performed to compare means and Chi-square tests to compare proportions between groups. Pearson correlations were done to investigate unadjusted associations of NT-proBNP with fibulin-1 and arterial stiffness indices. Partial correlations were performed by adjusting for age, BMI, SBP, heart rate and estimated creatinine clearance.

Independent associations were determined by performing forward stepwise regression analyses. Variables considered for entry in the model were age, BMI, SBP, heart rate, total cholesterol and high density lipoprotein cholesterol ratio (TC:HDLC), serum glucose, cotinine, γ-glutamyl transferase, estimated creatinine clearance, and CRP. Of these variables, age, BMI, SBP, heart rate, TC:HDLC, serum glucose, γ-glutamyl transferase and CRP entered the model together with fibulin-1 and arterial compliance as the main independent variables. P-values of ≤0.05 were considered statistically significant.

Results

The general characteristics of the study population are listed in Table 1. The total population were divided into African and Caucasian men and women, due to the significant interactions determined by univariate ANCOVA analyses to test for the main effects of ethnicity ($F(469)=28.4$; $p<0.0001$) and gender ($F(469)=35.5$; $p<0.0001$) on the association between NT-proBNP and fibulin-1. The mean ages of men and women differed (≈ 5 years) between ethnic groups. Cigarette smoking was significantly higher in Africans compared to Caucasians (66.1% vs. 17.1%; $p<0.0001$) and also higher in men than in women (50.0% vs. 31.7%; $p<0.0001$), which was confirmed by the cotinine levels (Table 1). The self-reported use of alcohol was similar (72.7% vs. 67.2%; $p=0.18$), however the γ-glutamyl transferase levels were significantly higher in African men and women than Caucasians ($p<0.0001$). African men had significantly higher mean BMI than Caucasian men, but the BMI was similar in the women. NT-proBNP levels along with blood pressure values were significantly higher in Africans than Caucasians. Fibulin-1 levels were higher in African compared to Caucasian men, but similar in African and Caucasian women.

Table 1 – General characteristics of African and Caucasian men and women

	Men			Women		
	Africans *n = 128*	**Caucasians** *n = 118*	***p*-value**	**Africans** *n = 103*	**Caucasians** *n = 120*	***p*-value**
Age (years)	41.1 ± 13.6	36.5 ± 11.6	<0.01	44.3 ± 11.8	38.9 ± 11.6	<0.001
Body mass index (kg.m^{-2})	20.4 ± 4.1	27.8 ± 4.9	<0.0001	27.4 ± 7.8	27.0 ± 6.6	0.68
Waist circumference (cm)	74.3 ± 10.2	92.4 ± 13.1	<0.0001	83.5 ± 15.0	81.3 ± 13.9	0.26
Biochemical analyses						
NT-proBNP (pg.ml^{-1})	24.4 (19.1 – 32.2)	8.4 (6.9 – 10.2)	<0.0001	40.4 (32.0 – 51.1)	27.2 (22.5 – 33.0)	<0.01
Fibulin-1 (µg.ml^{-1})	68.7 (65.4 – 72.2)	61.7 (59.0 – 64.6)	<0.01	71.3 (67.1 – 75.9)	71.6 (68.6 – 74.6)	0.93
C-reactive protein (mg.l^{-1})	1.55 (1.10 – 2.24)	1.03 (0.74 – 1.43)	0.102	3.63 (2.82 – 4.69)	0.96 (0.68 – 1.34)	<0.0001
Serum creatinine	64.3 ± 10.0	71.2 ± 10.5	<0.0001	66.8 ± 12.6	71.1 ± 13.1	0.015
Estimated creatinine clearance	1.25 ± 0.38	1.85 ± 0.42	<0.0001	1.17 ± 0.43	1.29 ± 0.39	0.026
TC:HDLC (mmol.l^{-1})	2.93 ± 1.08	5.24 ± 2.05	<0.0001	3.29 ± 1.04	4.00 ± 1.33	<0.0001
Serum glucose (mmol.l^{-1})	4.98 (4.85 – 5.12)	5.56 (5.43 – 5.69)	<0.0001	5.25 (5.03 – 5.49)	5.25 (5.06 – 5.44)	0.97
Triglycerides (mmol.l^{-1})	1.11 ± 0.578	1.56 ± 0.947	<0.0001	1.18 ± 0.85	1.22 ± 0.70	0.72
Cardiovascular measurements						
Systolic blood pressure (mmHg)	130.4 ± 20.6	121.8 ± 10.9	<0.0001	124.4 ± 22.7	111.1 ± 13.4	<0.0001
Diastolic blood pressure (mmHg)	84.4 ± 13.8	77.8 ± 8.1	<0.0001	85.7 ± 13.2	75.2 ± 9.8	<0.0001
Pulse pressure (mmHg)	46.0 ± 11.4	43.9 ± 8.5	0.11	38.7 ± 13.4	35.9 ± 8.3	0.053
Heart rate (bpm)	67.4 ± 14.5	66.1 ± 8.9	0.38	73.4 ± 11.9	67.5 ± 9.0	<0.0001
Arterial compliance (ml.mmHg^{-1})	1.68 ± 0.496	2.53 ± 1.01	<0.0001	1.47 ± 0.472	1.96 ± 0.420	<0.0001
Pulse wave velocity (m.s^{-1})	8.58 ± 1.52	7.94 ± 1.14	<0.001	7.94 ± 1.64	7.44 ± 0.953	0.0051
Lifestyle						
Hypertension status, n (%)	50 (39.1)	9 (7.6)	<0.001	37 (35.9)	11 (9.1)	<0.001
Cotinine (ng.ml^{-1})	126.1 (96.9 – 164.2)	19.3 (15.1 – 24.8)	<0.0001	69.6 (50.8 – 95.2)	13.4 (11.0 – 16.3)	<0.0001
γ-Glutamyl transferase (U.l^{-1})	78.7 (66.2 – 93.4)	35.1 (31.9 – 38.5)	<0.0001	59.5 (48.9 – 72.3)	24.4 (22.2 – 26.8)	<0.0001

Values are arithmetic mean ± SD, geometric mean (95% confidence interval) or number of participants.
Abbreviations: TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio.

The unadjusted and adjusted associations of NT-proBNP with fibulin-1 and markers of arterial stiffness are presented in Table 2. In single regression analysis, NT-proBNP was positively associated with fibulin-1 in African men and Caucasian women. A significant negative correlation was obtained between NT-proBNP and arterial compliance in all groups except Caucasian women. NT-proBNP correlated positively with PWV in Africans only. Fibulin-1 was not associated with either arterial compliance or PWV (data not shown) in any of the groups.

Table 2 – Unadjusted and adjusted correlations of NT-proBNP with fibulin-1 and measures of arterial stiffness

	Men		Women	
	African *n=128*	**Caucasian** *n=118*	**African** *n=103*	**Caucasian** *n=120*
	Unadjusted correlations			
Fibulin-1 (µg.ml^{-1})	r = 0.20; p = 0.023	r = 0.15; p = 0.10	r = 0.083; p = 0.40	r = 0.18; p = 0.047
Arterial compliance (ml.mmHg^{-1})	r = –0.44; p < 0.0001	r = –0.31; p = 0.001	r = –0.36; p < 0.0001	r = –0.069; p = 0.46
Pulse wave velocity (m.s^{-1})	r = 0.30; p = 0.001	r = 0.15; p = 0.097	r = 0.33; p < 0.001	r = 0.13; p = 0.15
	Adjustments: age, body mass index, systolic blood pressure, heart rate and estimated creatinine clearance			
Fibulin-1 (µg.ml^{-1})	r = 0.32; p < 0.0001	r = 0.12; p = 0.21	r = 0.16; p = 0.13	r = 0.16; p = 0.11
Arterial compliance (ml.mmHg^{-1})	r = 0.15; p = 0.11	r = 0.048; p = 0.62	r = 0.033; p = 0.75	r = 0.21; p = 0.036
Pulse wave velocity (m.s^{-1})	r = –0.14; p = 0.12	r = 0.004; p = 0.97	r = 0.026; p = 0.80	r = 0.068; p = 0.50

After adjustments were applied for age, BMI, SBP, heart rate and estimated creatinine clearance (Table 2, Figure 1), the association of NT-proBNP with fibulin-1 became stronger in African men ($r=0.32$; $p<0.0001$) with no significance in the other groups. After these adjustments, the previous significant association between NT-proBNP and arterial compliance disappeared in all groups, but a positive relationship became evident in Caucasian women. No significant correlations existed between NT-proBNP and PWV.

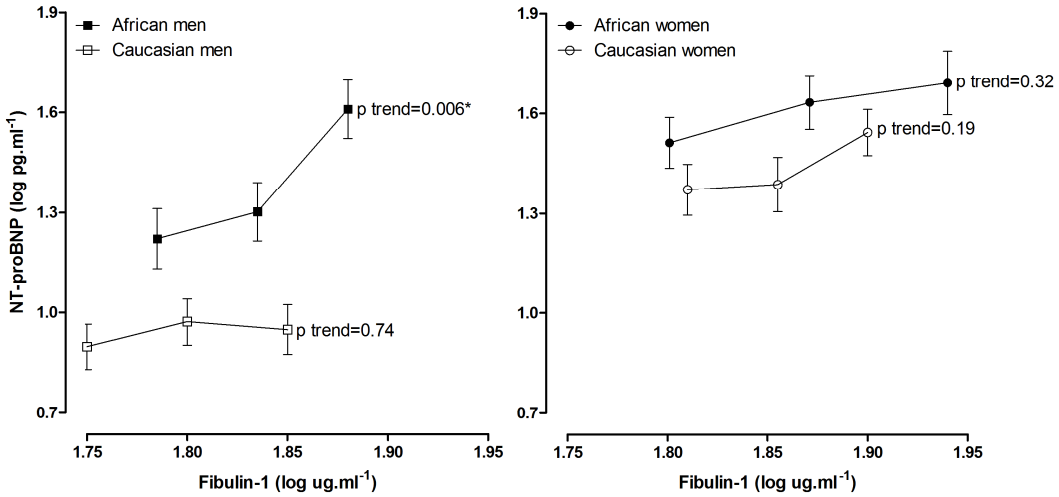

Figure 1 – NT-proBNP levels by tertiles of fibulin-1 in African and Caucasian men and women adjusted for age, body mass index, systolic blood pressure and heart rate.
P denotes significance for trend; *p<0.01 (tertile1 vs. tertile3).

In forward stepwise regression analysis (Table 3), adjusting for significant covariates (age, BMI, SBP, heart rate, TC:HDLC, serum glucose, γ-glutamyl transferase and CRP), the significant positive association between NT-proBNP and fibulin-1 was confirmed in African men only, also showing a significant relation with SBP and CRP.

Table 3 – Forward stepwise regression analyses with NT-proBNP as dependent variable

	Men		Women	
	African *n=128*	**Caucasian** *n=118*	**African** *n=103*	**Caucasian** *n=120*
	NT- proBNP (pg.ml^{-1})			
R^2	0.414	0.197	0.287	0.115
Adjusted R^2	0.383	0.175	0.257	0.089
Std β (95% confidence interval)				
Fibulin-1 (μg.ml^{-1})	0.262 (0.121 to 0.404)‡	NS	–	NS
Arterial compliance (ml.mmHg^{-1})	–	–	–	–
Age (years)	0.374 (0.208 to 0.541)‡	0.341 (0.174 to 0.507)†	0.227 (0.024 to 0.431)*	0.274 (0.087 to 0.461)*
Body mass index (kg.m^{-2})	–0.186 (–0.332 to –0.040)*	–0.270 (–0.439 to –0.101)*	–	–
Systolic blood pressure (mmHg)	0.253 (0.088 to 0.418)*	–	0.351 (0.153 to 0.549)†	–
Heart rate (bpm)	NS	–	–	–
Serum glucose (mmol.l^{-1})	–	–	–0.277 (–0.451 to –0. 104)*	–
γ-Glutamyltransferase (U.l^{-1})	–	–	NS	NS
C-reactive protein (mg.l^{-1})	0.212 (0.060 to 0.364)*	–	–	–

Superscript symbol denotes significance for: *p ≤ 0.05; †p ≤ 0.01; ‡p ≤ 0.001.

Abbreviations: NS – not significant, TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio.

In exploratory analysis, we performed similar multiple regression analyses only in participants arbitrarily younger than 55 years of age to determine whether the association between NT-proBNP and fibulin-1 remains. This analysis confirmed the significant positive relationship between NT-proBNP and fibulin-1 in younger African men (R^2=0.27; β=0.322; p=0.002; n=97), but the same association became significant in younger African women (R^2=0.34; β=0.251; p=0.012; n=78). No significance was observed in the Caucasians.

Discussion

This study aimed to investigate the possible link of NT-proBNP with fibulin-1 and markers of arterial stiffness in African and Caucasian men and women. We found a significant association between NT-proBNP and fibulin-1, which was independent of SBP, arterial compliance and other significant covariates only in African men. African men also had higher levels of NT-proBNP and fibulin-1 compared to Caucasian men. When investigating this relationship only in participants younger than

55 years, this association was confirmed in African men (mean age of 35.1 ± 9.5 years) and also became significant in African women. This link between NT-proBNP and fibulin-1 suggests early cardiovascular extracellular matrix alterations especially in African men. It is also noteworthy to mention the complete absence of this relationship in Caucasians. This result further may explain why Africans have an increased risk for future cardiovascular events [11,12].

Evidence indicates that lower natriuretic peptide levels may contribute to the development of hypertension and cardiovascular damage [15, 16]. However, elevated NT-proBNP levels also indicate cardiovascular damage due to chronic overload of the heart [17]. The association between NT-proBNP and fibulin-1 possibly signifies that elevated ventricular natriuretic peptide gene expression is a result of sustained hemodynamic overload, thereby contributing to extracellular matrix remodelling in blood vessel walls and cardiac tissue. Although fibulin-1 is not one of the conventional markers of extracellular matrix remodelling, it is a component of the extracellular matrix. Furthermore, Neiman et al. suggested that fibulin-1 could serve as a potential indicator of kidney damage [18]. However, even after adjustments were applied for estimated creatinine clearance, the relationship between NT-proBNP and fibulin-1 was confirmed in African men with significantly higher fibulin-1 levels than Caucasian men. The possibility of cardiovascular alterations in African men may subdue normal cardiovascular function even at a relatively young age.

It is also known that the Africans from South Africa are more prone to develop arterial stiffness [12]. Even though African men and women exhibited stiffer arteries as reflected by their lower arterial compliance and higher pulse wave velocity compared to Caucasians, we found no significant associations of NT-proBNP with measures of arterial stiffness or with fibulin-1 and stiffness indices. However, our result suggests that intrinsic or early developing changes may be present in the African men and women. This raises a concern of clinical importance since there may be other contributing factors that still need considerable attention. A possible reason for the lack of

association between fibulin-1 and arterial stiffness indices may be that the elevated fibulin-1 in Africans reflects changes in the heart rather than in the blood vessels with reference to associations found between fibulin-1 and left ventricular diastolic dysfunction in diabetic patients [9].

Zieman *et al*. suggested that alterations in the arterial extracellular matrix are associated with arterial stiffness, myocardial dysfunction and cardiovascular disease [19]. Although their study focused on the large scaffolding proteins (such as collagen and elastin) other extracellular matrix proteins may also take part in the long-term cascade of vascular stiffening. Fibulin-1 binds other matrix components and is present in both the arterial wall, as well as in valvular and heart tissue [20]. Recently, it was shown that fibulin-1 is increased in the arterial wall and in the blood from patients with diabetes [9]. In that same study, fibulin-1 was also positively correlated with measures of arterial stiffness and NT-proBNP. The lack of association between fibulin-1 and stiffness indices could also be explained by the young age and apparently healthy state of the cohort in which the progression of subclinical arterial damage and stiffness is not yet pathological. The significant association of NT-proBNP with fibulin-1 encourages further clinical and molecular investigations, especially since this relatively young African population is already subjected to vascular changes with possible predisposition of early vascular stiffening [12].

We postulate that the arranged elastic fibers and laminae in the media of elastic arteries may lose its structural integrity and elasticity relatively sooner in Africans than Caucasians due to pressure overload of the vasculature as a result of their higher mean blood pressure values. This may contribute to arterial stiffening and elevated ventricular afterload. Hence, elevated myocyte stretch and augmented NT-proBNP levels [21, 22] follow and reflects the risk of cardiac damage [23], especially in the Africans. Subsequently, the degeneration of elastic fibers in blood vessels and cardiac tissue contribute to the remodelling of the extracellular matrix. These alterations cause migration of vascular smooth muscle cells, infiltration of macrophages, elevated matrix

metalloproteinases and cytokines [19]. Seemingly, this cascade starts earlier in Africans compared to Caucasians and therefore the causative factors need to be identified.

Our study also underlines the different sites of expression of both NT-proBNP and fibulin-1. The fact that NT-proBNP is associated with fibulin-1, but fibulin-1 is not associated with arterial stiffness in this cohort, suggests that fibulin-1 could also reflect cardiac extracellular matrix alterations or fibrosis, perhaps more so than vascular extracellular matrix alterations. NT-proBNP is a cardiac hormone expressed by myocytes in the atria and more distinctly in the ventricles during hemodynamic myocyte stretch [2]. Fibulin-1 on the other hand is expressed in elastin containing tissues. These include large amounts in the walls of blood vessels [8] and the heart [20, 24], indicating that extracellular matrix remodelling in the vasculature depicts subsequent demands on the heart. Furthermore, it is clear that there is no specific mechanism or pathway involved in the alarming decline in cardiovascular health of the African population, but rather a cascade of adverse cardiovascular changes. Abundant contributing factors such as genetic predisposition, environmental factors or impaired intrinsic pathways may result in changes of both structural and functional components of the vasculature and cardiac tissue [25]. Although this study found a strong independent association between NT-proBNP and fibulin-1, further clinical investigation on possible mechanisms contributing to the early onset of cardiovascular alterations in the African population of South Africa is encouraged.

The findings of this study need to be interpreted within the context of its limitations and strengths. Since this study was cross-sectional, causality cannot be inferred. Our findings may have been due to confounding or unknown factors associated with NT-proBNP, fibulin-1 or arterial function. In addition, the Africans from this study were from a low socio-economic status, which could contribute to their higher risk of disease. The carotid dorsalis-pedis PWV was measured instead of the carotid-femoralis PWV, which is regarded as the golden standard of arterial stiffness

measurements. Although, NT-proBNP has been described as a stable and sensitive marker of cardiac function and a predictor of left ventricular hypertrophy [26, 27], other measures of left ventricular hypertrophy such as echocardiography and electrocardiography were not examined for this study. Nevertheless, this was a well-designed study implemented under controlled conditions. This was also the first study, to our knowledge, that investigated the associations of NT-proBNP with fibulin-1 and markers of arterial function in Africans and Caucasians from South Africa.

In conclusion, NT-proBNP is significantly associated with fibulin-1 in African men and younger African women, but not in Caucasians. This result could be of clinical importance since early-onset cardiovascular extracellular matrix alterations may be present in this African population. This, in turn, may possibly contribute to cardiac alterations. Further clinical studies are required to understand the possible higher risk of cardiovascular damage in Africans as opposed to Caucasians, and also in other ethnic groups.

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Disclosure

No conflicts of interest to declare.

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CHAPTER 5

RESEARCH ARTICLE 3

NT-proBNP and inflammatory markers in normotensive Africans: the SAfrEIC study.

R Kruger[a]; R Schutte[a]; HW Huisman[a]; P Hindersson[b]; MH Olsen[c]; J Eugen-Olsen[d]; AE Schutte[a]

[a] *Hypertension in Africa Research Team (HART); North-West University, Potchefstroom, South Africa.*
[b] *Department of Biochemistry, Hospital Vendsyssel, Hjørring, Denmark.*
[c] *Cardiovascular Prevention Clinic, Department of Endocrinology, Odense University Hospital, University of Southern Denmark.*
[d] *Hvidovre Hospital, University of Copenhagen, Denmark.*

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- Tables should be numbered in one consecutive series of Arabic numerals and referred to by number in the text. Each table should be typed on a separate sheet of paper and should have a descriptive title.

Abstract

Objective and design: This cross-sectional study aimed to investigate whether a marker of cardiac strain, the N-terminal prohormone B-type natriuretic peptide (NT-proBNP), is associated with inflammatory markers in normotensive Africans.

Methods and subjects: We measured NT-proBNP, C-reactive protein (CRP) and plasma-soluble urokinase plasminogen activator receptor (suPAR) levels along with conventional biomarkers in normotensive African men (n=78) and women (n=84).

Results: NT-proBNP, CRP and suPAR levels were lower in African men than in women. However, NT-proBNP was significantly associated with both CRP (r=0.38; *P*=0.001) and suPAR (r=0.46; *P*<0.001) in African men only. After full adjustment in multiple regression analyses, the associations of NT-proBNP with CRP (*β*=0.292; *P*=0.043) and suPAR (*β*=0.430; *P*<0.001) were confirmed in African men.

Conclusion: These results suggest that a low-grade inflammatory state may put normotensive African men at higher risk to develop subclinical cardiovascular damage than normotensive African women. We encourage population studies in other parts of South Africa to determine whether this adverse subclinical trend persists in the general population and clinical intervention and follow-up studies to address the concern of low-inflammation in these normotensive African men.

Key Words: NT-proBNP, inflammation, C-reactive protein, soluble uPAR, cardiovascular function.

Introduction

High morbidity and mortality among the African population in South Africa is a major concern due to the high prevalence of communicable and non-communicable diseases, especially in communities of low socio-economic status with limited healthcare facilities [1]. A low-grade inflammatory state, aggravated by lifestyle and environmental factors, is believed to underlie the elevating adverse health outcomes observed in this group [2]. Traditional cardiovascular risk factors provoke the biological effects of pro-inflammatory cytokines and other components of inflammation [3]. Pro-inflammatory cytokines, acute-phase reactants and cell adhesion and signaling molecules such as plasma-soluble urokinase plasminogen activator receptor (suPAR) and C-reactive protein (CRP) seem to play a role in low-grade inflammation [3].

CRP, in combination with NT-proBNP, has been proposed as reliable risk markers of cardiovascular disease as well as cardiovascular morbidity and mortality [4-6]. CRP is a hepatocyte-derived inflammatory cytokine, and is elevated in acute heart failure [7]. CRP also indicates active systemic inflammation [8], which is associated with cardiovascular events in patients with or without atherosclerotic disease and is a strong predictor of future cardiovascular events [4]. In addition, a novel marker (suPAR) of inflammation and atherosclerosis, was positively associated with the risk of developing cardiovascular disease [9], and suggested to be an independent marker of subclinical organ damage and increased cardiovascular risk [10]. It is therefore seems that these two inflammatory markers depict different aspects of inflammation regarding disease states, i.e., CRP reflects systemic inflammation whereas suPAR is more specific to the vasculature and thrombosis. These markers might be a valuable combination together with NT-proBNP to illustrate cardiovascular risk.

NT-proBNP is elevated in heart disease with or without symptoms of heart failure, and described as a marker for functional cardiac impairment, cardiovascular risk [11,12] and a reliable predictor of cardiovascular morbidity and mortality [13]. The associations of NT-proBNP with markers of

inflammation have been studied in part in Caucasian populations of American and European descent, but little is known about these associations in African populations [14,15]. The aim of this study was therefore to investigate whether subclinical cardiovascular damage assessed by NT-proBNP is associated with inflammation assessed in apparently healthy Africans.

Methods

Study population

This cross-sectional study formed part of a larger South African investigation on the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC) study that involved 382 Africans and 372 Caucasians from the North West province of South Africa. This sub-study included 162 normotensive Africans (78 men and 84 women) after excluding all those who were considered hypertensive (n=64), were over the age of 55 years (n=39), were using antihypertensive or anti-inflammatory medication (n=3), and who were HIV infected (n=114). The SAfrEIC study was approved (06M01) by the Ethics Review Board of the North-West University and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki (2008) for investigation of human participants.

Clinical procedures

Ten to 20 participants visited the Metabolic Unit facility daily on the Potchefstroom campus of the North-West University over a period of seven weeks. Each participant gave written informed consent to take part in the study after all the procedures were comprehensively explained. A participant sheet guided the participants through the different research stations where various measurements were done. Basic health and demographic questionnaires were completed during the morning. An informative description regarding the results of the health assessment was given to each participant at the end of the study. In the event where abnormalities were identified in a

participant (e.g. hypertension or diabetes), the participant was advised to visit their local clinic, hospital or physician.

Cardiovascular measurements

The OMRON HEM-757 (Omron, Kyoto, Japan) apparatus was used to determine systolic (SBP) and diastolic blood pressure (DBP) with the cuff on the left upper arm and the participant in the sitting position. The first blood pressure measurement was taken after an initial 10 minute resting period and a second measurement was taken 5 minutes after the first. Pulse pressure was subsequently calculated by subtracting the DBP from SBP. Participants with a SBP ≥140 mmHg and/or DBP ≥90 mmHg were considered hypertensive [16]. Heart rate and arterial compliance were determined with the Finometer apparatus (FMS, Finapres Measurement Systems, Amsterdam, the Netherlands) [17,18]. The carotid-dorsalis pedis pulse wave velocity (PWV) was measured with the Complior SP Acquisition system (Artech-Medical, Pantin, France).

Anthropometric measurements

Body height was measured to the nearest 0.1 cm by using the Invicta Stadiometer (IP 1465, London, UK) and body weight to the nearest 0.1 kg (Precision Health Scale, A & D Company, Japan). Subsequently, the body mass index (BMI) was calculated for each participant as weight (kg) divided by height (m) squared. The waist circumference was measured at the midpoint between the lowest rib and the top of the iliac crest with a Holtain non-stretchable, flexible metal measuring tape [19].

Biochemical measurements

Participants were requested to fast for a minimum of eight hours. Fasting lipids (total cholesterol, high-density lipoprotein cholesterol, and triglycerides), serum glucose, γ-glutamyl transferase and high sensitivity serum CRP were determined with the Konelab autoanalyzer (Thermo Fisher Scientific, Vantaa, Finland). Serum cotinine was determined with the IMMULITE 2000 nicotine

metabolite assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA). The Elecsys proBNP sandwich immunoassay was used on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany) to determine the serum NT-proBNP concentration of each participant. Plasma (EDTA) suPAR levels were measured using the suPARnostic® ELISA kit (ViroGates, Copenhagen, Denmark). Human immunodeficiency virus status was determined directly after blood sampling with rapid tests according to the protocol of the National Department of Health of South Africa. Serum was used for testing with the First Response Test (PMC Medical, India) and was repeated with the Pareeshak test (BHAT Bio-tech India) to confirm the results.

Statistical analyses

Statistica software v10.0 (StatSoft, Inc., Tulsa, OK, USA) was used for database management and statistical analyses. We tested the normal distribution of the variables prior to any further statistical analyses. Variables that deviated from normality (NT-proBNP, CRP, γ-glutamyl transferase, blood glucose and cotinine) were logarithmically transformed. Associations of NT-proBNP with markers of inflammation were tested for interaction with gender by applying the appropriate interaction terms. Chi-square tests (χ^2) were used to compare proportions and independent t-tests to compare continuous variables. We determined unadjusted associations between NT-proBNP and markers of inflammation with Pearson correlations. We divided CRP and suPAR values into tertiles to explore associations with NT-proBNP levels, while adjusting for age, body mass index and SBP with the ANCOVA analysis. Multiple linear regression analyses were performed to investigate independent associations between NT-proBNP and inflammatory markers. Several covariates were considered for entry into the regression model; however, only age, SBP, fasting glucose, γ-glutamyl transferase, and the total cholesterol, high density lipoprotein cholesterol ratio (TC:HDLC) entered the model. *P*-values of ≤0.05 were considered statistically significant.

Results

Detailed clinical and basic population characteristics are presented in Table 1. Gender groups were of similar age, but self-reported data show that the men smoked more compared to the women (73.4% vs. 47.2%; *P*=0.023), which was supported by the cotinine levels (*P*=0.016). However, inconsistencies were observed between self-reported and a reliable indicator of alcohol intake. Men reported higher use of alcohol (77.5% vs. 50.6%; p=0.004) than the women, but the levels of γ-glutamyl transferase were similar in the groups (*P*=0.23). Metabolic risk factors including BMI, waist circumference, serum glucose and lipid levels were lower in the African men. In addition, markers of inflammation (both CRP and suPAR) were significantly lower in African men compared to women. CRP and suPAR levels were also compared after adjusting for BMI, since the BMI differed significantly between these groups. After this adjustment, the significant difference between African men and women became borderline for CRP (1.36 mg/l vs. 2.50 mg/l; *P*=0.092), but remained for suPAR (2.68 ng/ml vs. 3.54 ng/ml; *P*<0.01). Although all participants were normotensive, the men exhibited higher systolic blood pressure, pulse pressure and pulse wave velocity, but higher arterial compliance compared to African women. The NT-proBNP levels were significantly lower in African men. (*P*=0.009).

In single regression analysis (Figure 1), there were positive and highly significant correlations between NT-proBNP and both CRP (r=0.38; *P*=0.001) and suPAR (r=0.46; *P*<0.0001) in African men. However, these associations were absent in women although they had higher levels of these inflammatory markers. In exploratory analysis, NT-proBNP levels were also plotted by tertiles of CRP and suPAR in both the men and women (Figure 2), while adjusting for age, BMI and SBP. The previous positive significant correlation between NT-proBNP and CRP remained in African men (*P* for trend=0.002), whereas the association between NT-proBNP and suPAR in African men became borderline significant (*P* for trend, 0.075; *P* for difference between lowest and highest tertile, <0.001). Again, no significant associations were observed in the African women.

In exploratory analysis, we tested whether cotinine and γ-glutamyl transferase drives the initial difference seen in CRP and suPAR levels between men and women, since both were initially higher in men than in women. The difference remained significant after adjusting for cotinine and γ-glutamyl transferase for both CRP (mean men vs. women: 0.97 mg/l vs. 3.38 mg/l; p<0.001) and suPAR (mean men vs. women: 2.60 ng/ml vs. 3.56 ng/ml; p<0.001) levels.

Table 1. General characteristics of normotensive African men and women

	Men *n = 78*	**Women** *n = 84*	***P*-value**
Age, years	36.7 ± 12.8	39.0 ± 11.4	0.22
Body mass index, kg/m^2	19.8 ± 3.6	27.4 ± 7.4	<0.0001
Waist circumference, cm	71.5 ± 8.4	82.3 ± 14.7	<0.0001
Biochemical analyses			
NT-proBNP, pg/ml	17.2 (12.9 – 23.0)	29.4 (22.2 – 38.8)	0.009
C-reactive protein, mg/l	1.16 (0.70 – 1.93)	3.40 (2.45 – 4.70)	<0.001
Soluble uPAR, ng/ml	2.85 ± 1.04	3.43 ± 1.57	0.006
Serum glucose, mmol/l	4.88 (4.71 – 5.06)	5.16 (4.95 – 5.38)	0.051
TC:HDLC, mmol/l	2.79 ± 0.94	3.29 ± 1.01	0.002
Triglycerides, mmol/l	0.98 ± 0.54	1.26 ± 0.96	0.026
Cardiovascular measurements			
Systolic blood pressure, mmHg	117.6 ± 10.7	110.9 ± 11.3	<0.001
Diastolic blood pressure, mmHg	76.1 ± 7.4	77.6 ± 8.5	0.25
Pulse pressure, mmHg	41.5 ± 8.5	33.3 ± 7.7	<0.0001
Heart rate, bpm	64.3 ± 14.6	72.1 ± 11.0	<0.001
Arterial compliance, ml/mmHg	1.88 ± 0.44	1.70 ± 0.44	0.010
Pulse wave velocity, m/s	7.9 ± 1.14	7.3 ± 1.34	0.002
Lifestyle			
Cotinine, ng/ml	116.1 (81.2 – 166.1)	62.8 (44.1 – 89.4)	0.016
γ-Glutamyl transferase, U/l	59.7 (43.3 – 73.9)	49.9 (40.5 – 61.4)	0.23

Values are arithmetic mean ± SD, geometric mean (95% confidence interval) or number of participants.
Abbreviations: TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio; uPAR – urokinase plasminogen activator receptor.

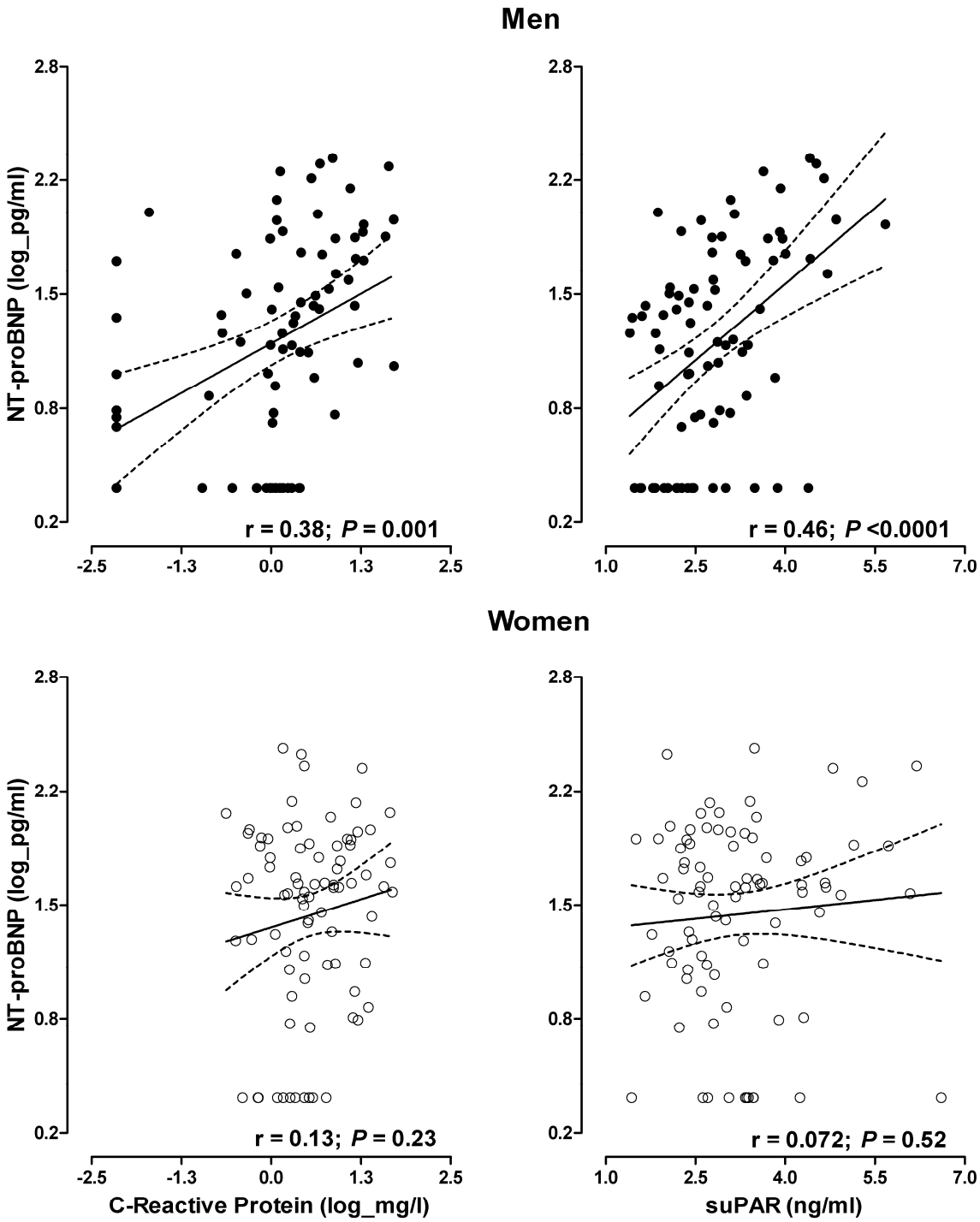

Figure 1 – Single regression analyses of NT-proBNP with markers of inflammation in African men and women.

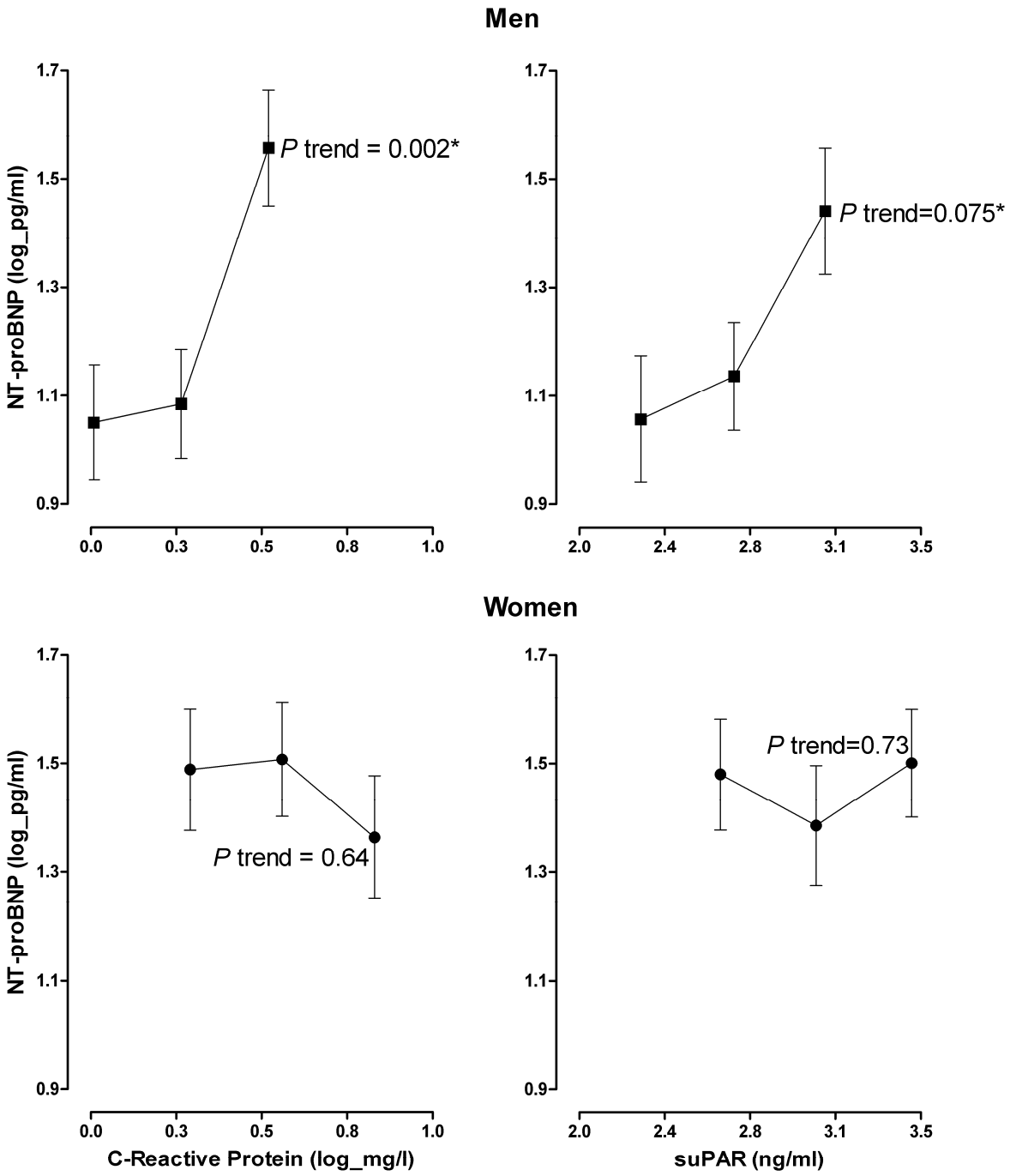

Figure 2 – NT-proBNP levels by tertiles of CRP and suPAR in African men and women adjusted for age, BMI and systolic blood pressure.
P denotes significance for trend; **P*<0.01 ($tertile_1$ vs. $tertile_3$).

In multiple regression analyses, after adjusted for significant covariates (age, SBP, γ-glutamyl transferase, fasting glucose and TC:HDLC), the associations of NT-proBNP with CRP (Table 2) and suPAR (Table 3) were confirmed in the African men only.

Table 2. Multiple regression analysis of NT-proBNP with CRP in Africans

	Men *n=78*	**Women** *n=84*
	NT-proBNP (pg/ml)	
R^2	0.27	0.23
Adjusted R^2	0.21	0.17
Std β (95% confidence interval)		
C-reactive protein, mg/l	0.292 (0.066 to 0.517)*	–0.101 (–0.320 to 0.119)
Age, years	0.348 (0.107 to 0.589)†	0.344 (0.103 to 0.584)†
Systolic blood pressure, mmHg	–0.185 (–0.396 to –0.025)*	0.140 (–0.086 to 0.366)
γ-Glutamyltransferase, U/l	–0.080 (–0.311 to 0.151)	–0.205 (–0.443 to 0.034)
Glucose, mmol/l	–0.095 (–0.316 to 0.127)	–0.291 (–0.525 to –0.056)*****
TC:HDLC, mmol/l	0.091 (–0.133 to 0.314)	–0.163 (–0.371 to 0.045)

Superscript symbol denotes significance for: *$P \leq 0.05$; †$P \leq 0.01$; ‡$P \leq 0.001$.

Abbreviations: TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio.

Table 3. Multiple regression analysis of NT-proBNP with suPAR in Africans

	Men *n=78*	**Women** *n=84*
	NT-proBNP (pg/ml)	
R^2	0.27	0.23
Adjusted R^2	0.21	0.17
Std β (95% confidence interval)		
suPAR, ng/ml	0.430 (0.201 to 0.659)‡	0.192 (–0.028 to 0.411)
Age, years	0.331 (0.107 to 0.555)†	0.318 (0.085 to 0.552)†
Systolic blood pressure, mmHg	–0.064 (–0.272 to 0.145)	0.160 (–0.061 to 0.381)
γ-Glutamyltransferase, U/l	–0.239 (–0.471 to –0.006)*****	–0.266 (–0.516 to –0.017)*
Glucose, mmol/l	–0.003 (–0.209 to 0.203)	–0.310 (–0.529 to –0.091)†
TC:HDLC, mmol/l	0.161 (–0.049 to 0.372)	–0.111 (–0.318 to 0.096)

Superscript symbol denotes significance for: *$P \leq 0.05$; †$P \leq 0.01$; ‡$P \leq 0.001$.

Abbreviations: suPAR – soluble urokinase plasminogen activator receptor; TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio.

Discussion

The aim of this study was to investigate the associations of NT-proBNP with CRP and suPAR in apparently healthy normotensive African men and women of similar age. NT-proBNP was significantly associated with both inflammatory markers in men, despite a lower inflammatory state compared to women. Also, the initial difference in CRP levels between men and women seems to be driven by obesity and not smoking or alcohol use, since this difference disappeared after adjusting for BMI, and remained after adjusting for cotinine and γ-glutamyl transferase. However, the significant difference in suPAR levels remained after adjusting for BMI, cotinine and γ-glutamyl transferase, suggesting that CRP and suPAR reflect different aspects of inflammation. It is known that CRP is a good marker of metabolic inflammation with its relation to BMI and lipids [20], while suPAR may be more related to cell-specific immune activation of the circulation [21]. Our results suggest that a low-grade inflammatory state in fairly young (median age; 35 years), normotensive African men may predispose them to premature development of cardiac damage and future cardiovascular disease as opposed to normotensive African women of similar age.

High levels of the cardiac hemodynamic volume load biomarker, NT-proBNP, reflect the risk of developing left ventricular dysfunction and congestive heart failure [22]. In addition, both CRP and suPAR are suggested to indicate active systemic as well as low-grade inflammation [8,23]. Therefore, the association of NT-proBNP with both these markers in African men suggests the pathological link between developing cardiac damage and low-grade inflammation. A cascade of contributing factors is involved in the poor cardiovascular state of the African population of which systemic inflammation forms part [24]. Indeed, systemic inflammation is described as an important pathogenetic component of heart failure [24], for the reason that the latter is not regarded as an isolated cardiac event, but more a systemic disorder involving several mechanisms of which inflammation is only one [25]. Moreover, low-grade inflammation as reflected by CRP and suPAR, may contribute to the development of the early onset of cardiovascular changes seen in the African population, especially African men.

The association of NT-proBNP with CRP and suPAR was independent of systolic blood pressure. However, in contrast to the normotensive participants in our study, patients with cardiovascular disease, such as atherosclerosis, CRP has been shown to correlate significantly with the risk of cardiovascular events [4]. Atherosclerosis as an inflammatory condition contributes to myocardial ischemia, which may lead to myocardial necrosis and dysfunction [26]. The results of this study further suggest that the African men are especially vulnerable to the effects of inflammation, as reflected by the association with NT-proBNP in subjects that were relatively young and normotensive. Discrepancies are evident regarding differences in risk among men and women from African descent, where men are more subjected to the burden of developing cardiovascular disease [27]. Moreover, several contributing factors related to lifestyle, for example smoking and alcohol intake may be possible triggers for initiating low-grade inflammatory responses, which add to the known risk of cardiovascular disease among Africans.

Elevated concentrations of inflammatory markers are generally the result of infection, whether it is a simple acute cold or flu or because of more severe chronic conditions such as tuberculosis and HIV [28,29]. In this study population, the levels of both NT-proBNP and inflammatory markers were not abnormally elevated. Yet the associations obtained in this study suggest that even low concentrations of inflammatory markers may contribute to elevated risk of cardiovascular damage and possible future cardiac events, especially in the men. Conversely, African women exhibited higher levels of CRP and suPAR (possibly due to the higher BMI) with no relationship to NT-proBNP, suggesting that some intrinsic cardioprotective mechanism(s) may be at work and warrant further investigation.

The underlying and combined mechanisms relating NT-proBNP to CRP and suPAR in cardiovascular disease are not well understood and we can therefore only speculate to explain our findings. With the development of systolic and diastolic dysfunction, due to obesity, lifestyle or predisposition, the volume preload of the ventricles increases. This elevation in volume load results

in cardiac myocyte stretch and stimulates the release of NT-proBNP [30-32]. Normally, the natriuretic peptide clearance receptor type C and the kidneys maintain the concentrations of BNP and its amino fragment to restore the equilibrium of the natriuretic peptide system and its effects [33]. However, once the heart is subjected to chronic myocyte stretch because of multiple components escalating in volume overload and augmented NT-proBNP concentrations, CRP and suPAR release is augmented by the expression of interleukin-6. The urokinase plasminogen activator will bind to its receptor (uPAR) to convert plasminogen into active plasmin [34]. Plasmin, in turn, has a direct effect on extracellular matrix degradation and can also convert pro-matrix metalloproteinases into active matrix metalloproteinases [35]. Metalloproteinases contribute to extracellular matrix degradation and ultimately to invasion of pro-inflammatory components [34]. As a result of either a systemic inflammatory or acute phase response, or also extracellular matrix remodelling, the heart is subjected to ventricular hypertrophy due to increased volume overload [36]. Cardiac myocytes therefore express even higher levels of BNP and NT-proBNP reflecting myocardial dysfunction as the end stage of this cascade. Since the latter is a speculative explanation for the connection between the natriuretic peptide system and inflammation in disease states, more evidence is needed to understand the association of NT-proBNP with inflammatory markers in this unique cohort.

Our study has certain limitations. It was cross-sectional and therefore one cannot rule out that our findings were subjected to confounding variables or unknown factors that were associated with both NT-proBNP and inflammation. The Africans in this study were from a low socio-economic class, which could have rendered these subjects as high risk for developing disease, since the availability of healthcare is limited. Data on present or past infections, such as cold, flu or tuberculosis were not available in this study. NT-proBNP has been described as a stable and sensitive marker of cardiac function, including right ventricular dysfunction [37], left ventricular systolic dysfunction, and early cardiac alterations [38]. NT-proBNP was therefore used only as a marker of possible cardiac risk, since left ventricular hypertrophy data was not available for this study. This was a well-

designed study supervised under controlled conditions, and was the first to our knowledge that investigated NT-proBNP and its associations with inflammatory markers in Africans from South Africa.

In conclusion, levels of NT-proBNP, CRP and suPAR were lower in normotensive African men than women. However, NT-proBNP was prominently associated with CRP and suPAR in African men only. These results suggest that in a low-grade inflammatory state, young normotensive African men are more at risk to develop early subclinical cardiovascular damage and possible future cardiac events than young normotensive African women. These findings need confirmation with prospective and experimental studies.

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Disclosure

No competing interests were reported.

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CHAPTER 6

RESEARCH ARTICLE 4

NT-proBNP and alkaline phosphatase in African and Caucasian men: the SAfrEIC study.

R Kruger[a], R Schutte[a], HW Huisman[a], MH Olsen[b], AE Schutte[a]

[a] *Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa.*
[b] *Cardiovascular Research Unit, Division of Cardiology, Department of Internal Medicine, Glostrup Hospital, University of Copenhagen, Denmark.*

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References should be prepared according to style guidelines based on Uniform Requirements style and presented in the *American Medical Association Manual of Style*, 9th edition (1997). Two examples of the most commonly used citations follow; please note and precisely employ text enhancements, capitalization, spacing, and punctuation.

Abstract

Objective: The N-terminal prohormone B-type natriuretic peptide (NT-proBNP) is a reliable marker of cardiac strain. In hypertensive heart disease, NT-proBNP levels increase and may lose its protective function. Simultaneously, the vasculature is also subject to hemodynamic stress, resulting in vascular matrix remodelling and stiffening which contribute to further cardiac alterations. Alkaline phosphatase (ALP) is a marker of osteoblast activity and is involved in vascular calcification. We explored the link between NT-proBNP and ALP in African and Caucasian men.

Design and main outcome measures: This study included 128 African (mean age, 41.1 years) and 118 Caucasian (mean age, 36.4 years) men. Conventional measurements were acquired along with serum NT-proBNP and ALP.

Results: NT-proBNP correlated positively with ALP ($r=0.29$; $p<0.001$) in Africans, but inversely in Caucasians ($r=–0.20$; $p=0.024$). After minimal adjustment (age, body mass index, SBP and arterial compliance), the positive significant correlation of NT-proBNP with ALP remained in African men ($r=0.225$; $p=0.014$), whereas significance was lost in Caucasian men. Multiple regression analyses confirmed the independent association of NT-proBNP with ALP in African men ($R^2=0.37$; $\beta=0.248$; $p=0.005$), as well as in younger African men ($R^2=0.26$; $\beta=0.375$; $p<0.001$; $n=96$), with no significance in Caucasians.

Conclusions: NT-proBNP is independently and positively associated with ALP in African men. This was however not evident in Caucasian men. These results suggest that African men are susceptible to early vascular calcification and may develop increased cardiac afterload prematurely.

Key Words: NT-proBNP, alkaline phosphatase, vascular calcification, ethnicity, arterial stiffness.

Introduction

The increasing prevalence of hypertensive heart disease is a major concern in black South Africans.[1, 2] One of the contributing risk factors is the progression of arterial stiffness, which seems to be more prominent among Africans in comparison with Caucasians.[3] Africans are also subjected to early vascular alterations,[3] making this population group more vulnerable for eminent stiffening of blood vessels and resultant cardiac damage. The subsequent increased hemodynamic afterload on the heart is reliably assessed by the N-terminal prohormone B-type natriuretic peptide (NT-proBNP).[4, 5] An elevated level of NT-proBNP indicates left ventricular dysfunction and heart failure.[4-6] NT-proBNP levels are also elevated in the presence of lower-extremity arterial calcification.[7]

Alkaline phosphatase (ALP) is a marker of osteoblast activity[8] and elevated expression of this enzyme may initiate spontaneous calcification in blood vessels.[9] Vascular calcification is more prominent in disease states such as kidney disease, diabetes and hypertension. Under these conditions, vascular smooth muscle cells gain osteoblast-like characteristics and express augmented production of ALP.[10, 11] As a result, arterial stiffness increases which in turn augments myocardial afterload and stress.[7] This is supported by NT-proBNP levels, which are elevated in high-risk patients with lower-extremity artery calcification and stiffness.[7] The majority of data on vascular calcification in terms of ALP is available only from animal studies[12] and high risk Caucasian populations,[13] or Africans Americans.[14] Little is known about ALP and cardiac function in the African population of South Africa.

Therefore, since the prevalence of arterial stiffness is increasing along with hypertensive heart disease in Africans, this study aimed to explore the association between NT-proBNP and a marker of osteoblastic activity in the cardiovascular system in African and Caucasian men.

Methods

Study population

186 African and 160 Caucasian men were selected from a larger South African investigation on the role of Sex, Age and Ethnicity on Insulin sensitivity and Cardiovascular function (SAfrEIC) study from the North West province of South Africa. Men infected with HIV (n=55), those with missing data of relevant variables (n=4) and those using medication (n=41) for comorbidities such as diabetes and hypertension, were excluded from the study. A total of 128 African and 118 Caucasian men were included in this study. The Ethics Review Board of the North-West University approved the SAfrEIC study and the protocol conformed to the revised ethical guidelines of the Declaration of Helsinki (revised in 2008) for investigation of human participants.

Clinical procedures

In 2007, from March to July, approximately 20 participants visited the Metabolic Unit facility daily at the Potchefstroom campus of the North-West University. Each participant was informed about all procedures included in the protocol and gave written informed consent to participate. Basic health and demographic questionnaires were completed during the course of the morning. Each participant received an informative description regarding the results of the health assessment. In the event where abnormalities were identified (e.g. hypertension or diabetes), the participant was advised to visit their physician.

Cardiovascular measurements

The OMRON HEM-757 apparatus (Omron, Kyoto, Japan) was used to determine systolic (SBP) and diastolic blood pressure (DBP) with the cuff on the left upper arm in the sitting position. The first blood pressure recording was taken after an initial 10 minute rest and the second after five minutes. Pulse pressure was subsequently calculated by subtracting the DBP from SBP. Participants with a SBP ≥140 mmHg and/or DBP ≥90 mmHg were considered hypertensive.[15]

Windkessel arterial compliance in diastole and heart rate were determined with the Finometer apparatus (FMS, Finapres Measurement Systems, Amsterdam, the Netherlands).[16, 17]

Anthropometric measurements

Body height was measured to the nearest 0.1 cm by using the Invicta Stadiometer (Invicta Plastics Ltd. 1465, London, UK) and body weight to the nearest 0.1 kg (Precision Health Scale, A & D Company, Japan), according to standard procedures. Subsequently, the body mass index (BMI) was calculated for each participant as weight (kg) divided by height (m) squared.

Biochemical measurements

Participants were requested to fast for a minimum of eight hours. In serum, fasting lipids, glucose, γ-glutamyl transferase, ALP, albumin, creatinine and high sensitivity C-reactive protein (CRP) were determined with the Konelab 20i autoanalyzer (Thermo Fisher Scientific, Vantaa, Finland). The Cockcroft–Gault formula was used to determine estimated creatinine clearance.[18] We determined serum cotinine with the IMMULITE 2000 nicotine metabolite assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA) and insulin (ST AIA-PACK IRI, Cat. No. 025260) with a two-site immunoenzymometric assay on the TOSOH AIA System analyzer (San Francisco, CA, USA). The Elecsys proBNP sandwich immunoassay was used on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany) to determine serum NT-proBNP.

Statistical analyses

Statistica software 10.0 (StatSoft, Inc., Tulsa, OK, USA) was used for database management and statistical analyses. We established the normal distribution of all variables prior to any further statistical analyses. Variables that deviated from normality (NT-proBNP, CRP, insulin, glucose, cotinine and γ-glutamyl transferase) were logarithmically transformed. The association of NT-proBNP with ALP was tested for interaction with ethnicity by introducing the appropriate

interaction terms in multiple regression analysis. T-tests and chi-square tests were performed to compare means and proportions between groups, respectively. We investigated associations between NT-proBNP and ALP using single and partial correlations. Forward stepwise multiple regression analyses were plotted to illustrate independent associations between NT-proBNP and ALP in both groups. Several covariates were considered for entry into the model including age, BMI, SBP, heart rate, arterial compliance, albumin, estimated creatinine clearance, glucose, CRP, γ-glutamyl transferase, cotinine and the total cholesterol to high density lipoprotein cholesterol ratio (TC:HDLC). Of these variables, heart rate, serum albumin, estimated creatinine clearance, glucose and cotinine did not enter the model. Probability values of ≤0.05 were considered statistically significant.

Results

The descriptive characteristics of the African and Caucasian men are presented in Table 1. African men had significantly lower BMI, triglycerides, HOMA index and TC:HDLC ratio compared to Caucasian men. Despite the favorable metabolic profile in Africans, they had significantly higher mean systolic and diastolic blood pressures and a higher prevalence of hypertension ($p<0.0001$). Self-reported smoking was significantly higher in Africans ($p<0.0001$), which was supported by the cotinine levels ($p<0.0001$). Self-reported alcohol use did not differ between the two groups. The mean γ-glutamyl transferase level was higher in Africans compared to Caucasians ($p<0.0001$). The mean ALP level was higher in African men ($p<0.0001$) than Caucasian men. ALP levels were also above the normal reference range of 30–120 U/L in African men, whereas Caucasian men's mean ALP levels were within the normal physiological range. NT-proBNP levels were also significantly higher in African men compared to Caucasian men ($p<0.0001$).

Table 1: Characteristics of African and Caucasian men

	Africans n=128	**Caucasians** n=118	**p-value**
Age, years	41.1 ± 13.6	36.4 ± 11.7	<0.01
Body mass index, kg/m^2	20.4 ± 4.1	27.8 ± 4.9	<0.0001
Biochemical measurements			
NT-proBNP, pg/mL	24.4 (2.5 – 178.3)	8.4 (2.5 – 47.9)	<0.0001
Alkaline phosphatase, U/L	126.5 ± 45.9	100.0 ± 24.1	<0.0001
γ-Glutamyl transferase, U/L	78.7 (20.8 – 485.9)	35.3 (17.7 – 86.4)	<0.0001
Serum albumin, g/L	45.0 ± 7.2	48.7 ± 8.4	<0.001
Serum creatinine, µmol/L	64.3 ± 10.0	71.3 ± 10.5	<0.0001
Creatinine clearance, ml/min	1.25 ± 0.38	1.85 ± 0.42	<0.0001
C-reactive protein, mg/L	1.55 (0.01 – 22.9)	1.03 (0.01 – 9.23)	0.104
Insulin, µU/mL	5.1 ± 5.2	10.1 ± 8.9	<0.0001
Serum glucose, mmol/L	5.0 (4.0 – 6.4)	5.6 (4.6 – 7.2)	<0.0001
HOMA-IR	1.22 ± 1.37	2.63 ± 2.67	<0.0001
Triglycerides, mmol/L	1.11 ± 0.58	1.56 ± 0.95	<0.0001
TC:HDLC, mmol/L	2.93 ± 1.10	5.24 ± 2.10	<0.0001
Cotinine, ng/mL	126.1 (9.0 – 500.0)	19.3 (9.0 – 371.0)	<0.0001
Cardiovascular measurements			
Systolic blood pressure, mmHg	130.4 ± 20.6	121.8 ± 11.0	<0.0001
Diastolic blood pressure, mmHg	84.4 ± 13.8	77.9 ± 8.1	<0.0001
Pulse pressure, mmHg	46.0 ± 11.4	43.9 ± 8.5	0.11
Heart rate, bpm	67.4 ± 14.5	66.1 ± 9.0	0.41
Arterial compliance, mL/mmHg	1.68 ± 0.50	2.53 ± 0.50	<0.0001
Hypertension status, n (%)	50 (39.1)	9 (7.6)	<0.0001
Lifestyle			
Current smoking, n (%)	97 (75.8)	26 (22.0)	<0.0001
Alcohol use, n (%)	106 (83.0)	89 (75.4)	0.13

Values are arithmetic mean ± SD, geometric mean (5th and 95th percentile interval) or number of participants.
Abbreviations: HOMA-IR – Homeostatic model assessment insulin resistance score; TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio.

In univariate analysis, we plotted the NT-proBNP by ALP levels for African and Caucasian men (Figure 1). We found a strong positive correlation between NT-proBNP and ALP in African men, but a significant inverse correlation was observed in Caucasian men. Furthermore, after partially adjusted for age, BMI, systolic blood pressure and arterial compliance (Table 2), the positive association between NT-proBNP and ALP remained in African men while the negative association in the Caucasians became non-significant.

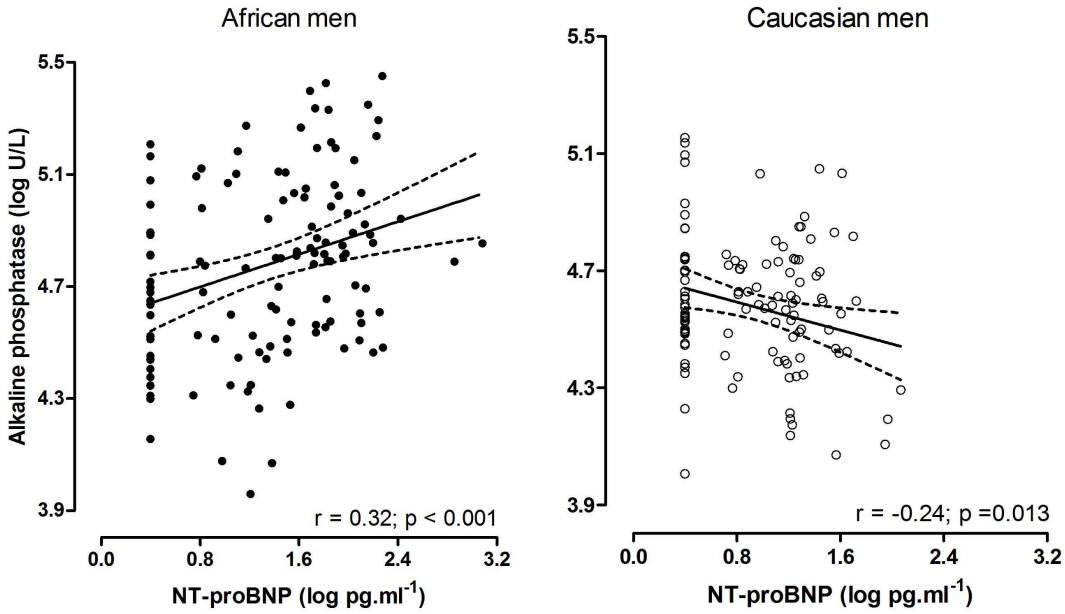

Figure 1: Unadjusted correlations between NT-proBNP and ALP in both the African and Caucasian men.

Table 2: Adjusted correlations of NT-proBNP with ALP and other biochemical measures

	Africans n=128	**Caucasians** n=118
	NT-proBNP ($pg.ml^{-1}$)	
Alkaline phosphatase, U/L	**r = 0.225; p = 0.014**	r = –0.147; p = 0.14
γ-Glutamyl transferase, U/L	r = –0.092; p = 0.32	r = –0.051; p = 0.61
Fibrinogen	r = –0.066; p = 0.47	r = 0.074; p = 0.45
Serum albumin, g/dL	**r = –0.235; p = 0.010**	r = 0.054; p = 0.59
C-reactive protein, mg/L	**r = 0.236; p = 0.009**	r = –0.011; p = 0.91
Creatinine clearance, ml/min	r = 0.078; p = 0.40	r = –0.012; p = 0.91
TC:HDLC, mmol/L	r = 0.009; p = 0.93	r = –0.053; p = 0.60
Serum glucose, mmol/L	r = –0.064; p = 0.49	r = 0.026; p = 0.80

Adjustments applied for: age, body mass index, systolic blood pressure and arterial compliance.
Abbreviations: TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio.

In multiple regression analysis, we confirmed the positive relationship between NT-proBNP and ALP in African men (R^2=0.37; β=0.248; p=0.005) (Figure 2). In sensitivity analysis to unravel this association and the possibility of early vascular ageing, we repeated the analyses in young men. We therefore arbitrarily excluded all men aged 55 years and older. Within the younger African group, the association between NT-proBNP and ALP remained significant (R^2=0.26; β=0.375; p=0.0008; n=96) and again we observed no association in the Caucasian men (R^2=0.11; β=–0.144; p=0.14; n=106). Lastly, even after excluding all hypertensives the results for Africans (R^2=0.34; β=0.386; p=0.004; n=70) and Caucasians (R^2=0.10; β=–0.167; p=0.11; n=98) were again confirmed.

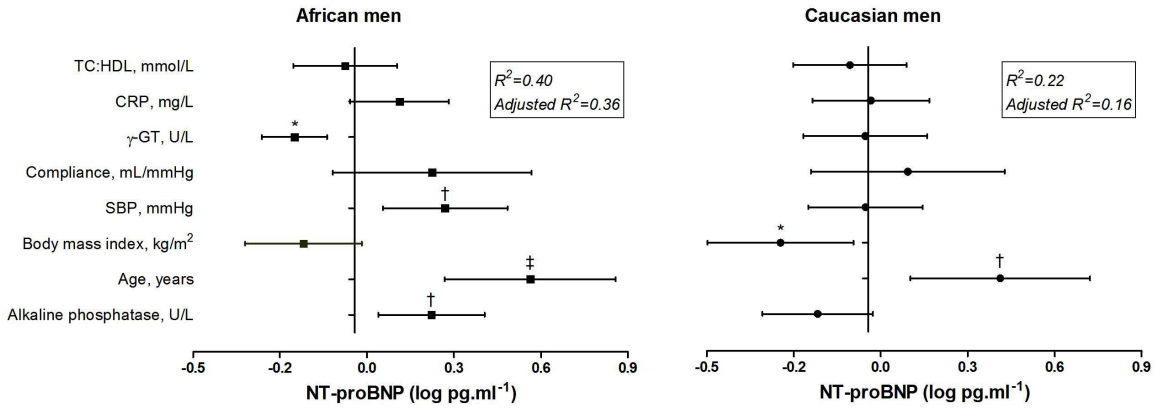

Figure 2: Multiple regression analyses of NT-proBNP with ALP in both African and Caucasian men. Values are indicated as standardized β (± 95% confidence interval). CRP – C-reactive protein; γ-GT – gamma glutamyl transferase; SBP – systolic blood pressure; TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio. * p ≤0.05; † p ≤0.01; ‡ p ≤0.001.

Discussion

We explored the possible link between NT-proBNP as a marker of cardiac strain and ALP as a marker of osteoblastic activity in African and Caucasian men. Our study showed that both NT-proBNP and ALP were higher in African compared to Caucasian men and were independently associated. This independent relationship between NT-proBNP and ALP was consistent with the total African population as well as in a younger normotensive group. It is noteworthy to mention that this association was completely absent in Caucasian men. Our results suggest that possible vascular calcification and associated cardiac load occurs relatively early in the life of African men, even under normotensive conditions. This may contribute to the high cardiovascular risk known to this group.

Most of the health profile variables (such as body mass index, CRP, HOMA index and TC:HDLC) were within their reference ranges.[19] However, the positive association of NT-proBNP with ALP together with SBP in African men may suggest that the blood vessels are possibly subjected to adverse ectopic osteoblastic activity. This may lead to arterial stiffening due to ectopic calcification, which can escalate into cardiac impairment due to increased afterload on the heart.[20]

The same association of NT-proBNP with ALP was also confirmed in younger normotensive African men. This result once again underlines the potential cardiovascular risk that is present at a relatively young age, independent of a hypertensive state. Studies indicated that NT-proBNP is directly associated with calcified aortic valves or aortic stenosis,[6, 21, 22] which merely signifies that NT-proBNP is a reliable biomarker of cardiovascular damage. However, vascular calcification is a complex process of multifactorial origin of which little is known. Beddhu *et al*. and others[14, 20, 23] concluded that ALP is independently associated with increased mortality in patients subjected to chronic kidney disease. Although kidney function was not extensively measured in our study, the

mean serum creatinine levels and estimated creatinine clearance were in the normal physiological range of the groups studied. Vascular calcification contributes to chronic kidney disease, but also plays a role in other conditions including diabetes, hypertension and pressure-related hypertrophy of cardiac tissue.[24, 25] However, we found the association between NT-proBNP and ALP in sub-groups without these conditions.

Mineralization *in vivo* is limited to tissues that express both type I collagen and ALP.[26] Type I collagen is present in normal artery walls, but not ALP. Both these components confine as mineral deposits in calcific atherosclerosis[27] and are produced in vascular cells *in vitro*.[28] Therefore, co-expression of these proteins induces ectopic calcification.[29] This ectopic mineralization refers to calcification not taking place in normal bone, but adverse mineralization in blood vessel walls. Membrane-bound ALP normally reduces the expression of inhibitor pyrophosphates and contributes to hydroxyapatite formation by producing free phosphates in bone.[30] However, during this mineralization process matrix vesicles are released, which are rich in ALP and other phospholipids.[31, 32] Ectopic calcification is due to increasing uptake of phosphates into cells by the type III sodium-phosphate co-transporter and up-regulation of type 1 pituitary-specific transcription factor by means of a positive feedback system.[33] This will in turn support osteoblast conversion as well as possible vascular calcification.[11] Calcification of the tunica media is normally the result of adverse alterations in the elastin network of arteries, because elastin acts as a nucleation scaffold for hydroxyapatite deposition.[34] In the process of vascular calcification, the end products of elastin degradation initiate the conversion of smooth muscle cells and dermal fibroblasts into osteoblast-like cells.[35, 36] These newly formed cells may be accountable for pathological calcification of blood vessels increasing the risk of cardiovascular disease and mortality as seen in the African men.[37, 38]

Although the aforementioned mechanism is merely speculative, the association of NT-proBNP with ALP in our cohort corroborates the possibility of early vascular calcification. Based on our finding, African men are at risk of developing cardiovascular-related calcification, even in a young normotensive state. Whether this association is merely a reflection of the environmental or classic risk factors contributing to hypertensive heart disease, or whether this population is predisposed to early onset vascular changes remains unanswered. However, the association between a marker of cardiac strain and a marker of calcification in this population may require urgent intervention to curb the ever-increasing trend of cardiovascular morbidity and mortality of black South Africans.

The findings of this study need to be interpreted within the context of its limitations and strengths. As mentioned previously, this was a cross-sectional study and therefore cause and effect cannot be determined. Furthermore, data on end-organ damage (carotid intima-media thickness, left ventricular hypertrophy, echocardiographic imaging of calcified valvular or vascular sclerosis) were not available in this study. This study was well designed and performed under strong controlled conditions. To our knowledge this was the first study that focused on the association between NT-proBNP and ALP in African and Caucasian men from South Africa.

In conclusion, we observed a persistent association between NT-proBNP and a marker of calcification in relatively young and normotensive African men. This may suggest that these men are subjected to early onset cardiac load, possibly due to enhanced vascular calcification which may escalate in cardiac damage. These findings are clinically relevant and need confirmation in larger prospective studies.

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Disclosure

There are no conflicts of interest to disclose.

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CHAPTER 7

GENERAL FINDINGS AND CONCLUSIONS

1. INTRODUCTION

In this conclusive chapter, a summary of the main findings from the four research articles are stated. The results from each article are also discussed, interpreted, explained and compared to relevant literature. Conclusions are drawn and recommendations are made to researchers investigating associations of NT-proBNP with markers of cardiovascular function, extracellular matrix and inflammation in a sample South African population.

2. SUMMARY OF MAIN FINDINGS

The relevant findings reported in the four studies of this thesis were:

2.1 NT-proBNP levels and its associations with cardiovascular function in African and Caucasians (Chapter 3)

This sub-study aimed to compare NT-proBNP levels between an African and Caucasian population and also to explore the associations between NT-proBNP and cardiovascular variables. It was hypothesised that NT-proBNP levels are lower in Africans compared to Caucasians and that NT-proBNP is positively associated with markers of cardiovascular function in both Africans and Caucasians.

The results of this study indicated that the Africans had higher NT-proBNP levels compared to the Caucasians, independent of gender, body mass index and pulse wave velocity. However, when additionally adjusted for systolic blood pressure and arterial compliance, the significant difference disappeared. Hence, the first hypothesis is rejected. Moreover, arterial compliance and systolic blood pressure to some extent appear to drive the initial difference seen in NT-proBNP levels. NT-proBNP was also positively associated with both systolic blood pressure and pulse pressure in the Africans, but not in the Caucasians. Also, even after adjustments were made to significant covariates, the positive significant association remained between NT-

proBNP, systolic blood pressure and pulse pressure in the Africans only. Therefore, the second hypothesis is partially accepted since we found a significant association between NT-proBNP and cardiovascular measures in the Africans, but not in the Caucasians.

2.2 NT-proBNP is associated with fibulin-1 in Africans (Chapter 4)

The aim of this study was to investigate the associations between NT-proBNP and an extracellular matrix component, fibulin-1, as well as markers of arterial function (pulse wave velocity and arterial compliance) in African and Caucasian men and women. The hypothesis was that NT-proBNP is positively associated with fibulin-1 and pulse wave velocity, and negatively associated with arterial compliance in both African and Caucasian men and women.

The results indicated that both the African men and women had higher levels of fibulin-1 and lower arterial compliance as opposed to the Caucasian men and women. In addition, NT-proBNP was positively associated with fibulin-1 in the African men only, after adjustments were made to age, body mass index, systolic blood pressure, heart rate and estimated creatinine clearance. No significant association was observed in the Caucasian men or in the African and Caucasian women. In addition, no significant association appeared between NT-proBNP, arterial compliance and pulse wave velocity in all the Africans (men and women) as well as in the Caucasian men, but a positive correlation existed between NT-proBNP and arterial compliance in the Caucasian women (which disappeared after multivariate analysis). After full adjustments, the positive association between NT-proBNP and fibulin-1 was confirmed in the African men only. The same multiple regression analysis was performed on younger subjects (age < 55 years). The African men showed similar results; however this association also became significant in the younger African women. Again no association was observed in the Caucasians.

Therefore, the hypothesis is partially accepted for the positive significant association that existed between NT-proBNP and fibulin-1 in the African men only. In addition, a new result emerged when this association was also present in the younger Africans, but with still no association present in the Caucasian men and women.

2.3 NT-proBNP and inflammatory markers in normotensive Africans (Chapter 5)

This sub-study investigated the associations of NT-proBNP with the pro-inflammatory cytokine, C-reactive protein (CRP) and also the soluble bioactive form of urokinase plasminogen activator receptor (suPAR) in normotensive African men and women. The aim was to explore the possible contribution of low-grade inflammation to early cardiovascular load in young African men and women still in the normotensive state. The hypothesis was that NT-proBNP is positively associated with inflammation as depicted by the CRP and suPAR, in both African men and women, in the absence of a hypertensive state and/or known infectious disease.

The African men had lower levels of NT-proBNP, CRP and suPAR compared to the African women. However, NT-proBNP is positively associated with both CRP and suPAR in the normotensive African men as opposed to their female counterparts. However, unadjusted and adjusted correlations indicated no significant association in the normotensive African women. Even after full adjustments in multiple regression analysis, the positive significant association of NT-proBNP with CRP and suPAR was confirmed in the African men only. The hypothesis is therefore partially accepted as NT-proBNP was positively associated with inflammation in the African men, but not in the women. This result, in combination with previous findings, indicates a distinct pattern of cardiovascular risk manifested in the African male population.

2.4 NT-proBNP and vascular calcification in African and Caucasian men (Chapter 6)

The aim of this particular study was to investigate the possible link between a marker of cardiac load and a marker of osteoblastic activity in African and Caucasian men. The results indicated that the African men had higher mean levels of both NT-proBNP and ALP compared to the Caucasian men. An opposite result in the two ethnic groups appeared with respect to the correlation between NT-proBNP and ALP, where a strong positive correlation existed in the African men and a negative correlation in the Caucasian men. However, after minimal adjustments, the positive relationship remained in the African men and all significance was lost in the Caucasian men. To determine whether this association persisted, we performed a forward stepwise multivariate regression analysis and confirmed the association of NT-proBNP with ALP. We explored this result even further by excluding older participants (> 55 years) and those who were hypertensive, and again confirmed our previous result in the African men only.

The hypothesis of this study was therefore partially accepted, since the relationship between NT-proBNP and ALP remained after multiple adjustments, in the younger men and also independent of a hypertensive state.

3. COMPARISON OF FINDINGS WITH THE LITERATURE

It is essential to compare these results with those found in the literature regarding other population groups. Certain findings confirmed and others contradicted the results of earlier studies. In addition, certain findings from this study contribute to the existing literature.

The results from this study confirmed findings in the literature regarding the elevated levels of NT-proBNP with increasing blood pressure.[1] The findings also confirm reports in literature regarding the confounding determinants of circulating levels of NT-proBNP, which include age, gender and systolic blood pressure.[2] From the literature, it is evident that NT-proBNP is a reliable marker of heart failure. Studies also reported that heart failure (as reflected by NT-proBNP levels) is associated with the remodelling of the brachial artery, which is characterised by morphological, mechanical and functional changes of the vessel wall.[3] The findings of this study confirm the possibility of vascular remodelling in the African population due to the significant positive association between NT-proBNP and fibulin-1. Certain studies also reported that the combination of NT-proBNP and CRP is essential in risk stratification and a reliable predictor of cardiovascular risk, damage, morbidity and mortality.[4] The present results confirm the association between NT-proBNP and markers of inflammation and its contribution to adverse cardiovascular function.

Findings of the current study that contradict those in literature were that NT-proBNP levels were initially higher in the African compared to the Caucasian population before adjustments were made, while Bekelman et al. reported that NT-proBNP levels were similar between African-Americans and Caucasians.[5] Abdullah et al. also reported that plasma NT-proBNP levels were slightly, but significantly, lower in African-Americans compared to Caucasians.[2] The higher NT-proBNP levels of the Africans in this study are possibly due to early asymptomatic left ventricular systolic/diastolic dysfunction, asymptomatic myocardial ischemia and possible early hypertensive heart disease in some patients.

The findings from this study also add the following information to existing knowledge regarding NT-proBNP and its associations with cardiovascular function, vascular integrity, remodelling and inflammation. The African population in this study revealed significant positive associations between NT-proBNP, systolic blood pressure and pulse pressure even after full adjustments were made to significant covariates. These associations were not present in the Caucasian population. Our results therefore add valuable information regarding future intervention strategies that could target these aspects to curb on-going trends in hypertensive heart disease among black South Africans. Moreover, this study was the first to investigate the association between NT-proBNP, fibulin-1 and arterial function in South Africa. Little is known about the associations between NT-proBNP and measures of arterial stiffness. However, no significant associations were established between NT-proBNP and arterial stiffness indices in this study, but the association that existed between NT-proBNP and fibulin-1 adds to the evidence in literature that vascular remodelling does indeed exist even in younger Africans, independent of a hypertensive state and/or any known infectious disease.

The higher NT-proBNP levels and fragile cardiovascular state of the young normotensive African population is to some extent driven by inflammation. Although the association between NT-proBNP and CRP is known and used as a reliable combination in risk stratification, this was also to the best of our knowledge the first study that investigated the association between NT-proBNP and suPAR in a South African context. The association between NT-proBNP and suPAR was also much stronger than that of CRP, suggesting that suPAR may be a more reliable and sensitive marker of either vascular-specific or systemic inflammation.

In the literature, it is clear that NT-proBNP is a good predictor of either cardiac load or damage.[6] It is also known that ALP reflects an osteoblast-like activity, also in vascular smooth muscle cells.[7-9] However, to our knowledge, the link between these two biochemical components regarding

cardiovascular function has never been reported in a South African bi-ethnic population. This is a rather distinct addition to current literature on the topic of ectopic calcification. Although ALP is not the only biomarker for ectopic calcification, it is an additional component which should be considered in populations subjected to the early onset of vascular alterations. Unfortunately our study was limited to only certain biochemical data along with other measures of cardiovascular function, which did not include sonar imaging of the carotid intima-media or echocardiographic data of cardiac valves. However, the association obtained in this study regarding cardiac load (reflected by NT-proBNP levels) and ALP as a component of the calcification pathway (whether physiological or pathological) still add to the current knowledge of ectopic calcification in the cardiovascular system. This encourages further investigations to determine the exact mechanistic involvement of the metastatic calcification process and its link to possible cardiac damage.

A very important concern arises from literature as well as from the new findings in this thesis. The findings among the African population, especially the men, indicate that adverse processes may be at work. There is, however, not only one component that drives this occurrence of growing cardiovascular disease, but rather a vast spectrum of aspects of which this thesis only covered a few, including cardiovascular, extracellular matrix, osteogenic and inflammatory components. All these results provide mechanistic support for the increasing burden of cardiovascular disease observed in Africans and highlight the need for early intervention.

4. CHANCE AND CONFOUNDING

It is essential to indicate possible determinants that might have affected the results of this study. With regards to methodology, this was a cross-sectional study and therefore one cannot infer causality. The results obtained stemmed from a particular target population, and do not necessarily reflect the status of the entire African and Caucasian populations of South Africa. The number of participants included in each study provided adequate statistical power; however, again these groups only reveal the general health of a particular geographical area of South Africa and not the entire population.

Moreover, the number of African men (n=78) and women (n=84) in the study population of the third article is relatively small, yet associations obtained were independent of significant covariates and confounders. It is also important to mention that the African population stemmed from a lower socio-economic background (although from urban regions), which could explain the reason for some differences of the findings in this study. Furthermore, pulse pressure, the dorsalis-pedis pulse wave velocity and Windkessel arterial compliance were used as measures of arterial function and stiffness as opposed to the golden standard measurements, that is, 24h ambulatory blood pressure, distensibility measurements and the carotid-femoralis pulse wave velocity, which were not available for this study. Also, left ventricular hypertrophy measurement was not available for this study. NT-proBNP was therefore used as a reliable biomarker of cardiovascular risk and cardiac strain. Overall, the SAfrEIC study was well controlled following a strict protocol that initially consisted of 756 participants.

With reference to the results, the possibility of chance should also be considered. By using partial correlations and forward stepwise regression analyses, the statistics indicated that one out of twenty significant correlations may be due to chance. By adjusting for appropriate covariates such as age, gender, body mass index, SBP, heart rate, serum glucose, TC:HDLC ratio, CRP, γ-

glutamyltransferase and creatinine clearance, it is possible that these covariates and confounders could have influenced the results by causing over- or underestimation of the associations between NT-proBNP and the various variables investigated in this study. However, a rule was followed that only one covariate per 15 subjects was allowed in the final regression analysis. It was also necessary to interpret all the statistical results from a physiological perspective, which entails that all statistical significance does not necessarily indicate physiological significance and vice versa.

5. DISCUSSION OF MAIN FINDINGS

It is known that NT-proBNP levels are elevated in patients with left ventricular hypertrophy and congestive heart failure,[10] and an early increase in plasma levels of NT-proBNP is related to both cardiovascular morbidity and mortality.[11] Recent epidemiological evidence showed that NT-proBNP is a very powerful predictor of mortality in hypertensive patients, even in those without left ventricular hypertrophy.[12] Since the levels of NT-proBNP were higher in the African compared to the Caucasian population, the concern is raised whether the Africans from our study are subjected to a significant increased risk for future cardiovascular events.

Although it is difficult to extrapolate the results to the general African population of South Africa, the findings of this study provide a starting point for larger scale prospective studies with a study population consisting of randomly selected participants, in order to curb or address the early development of cardiovascular disease in this population. The initial higher NT-proBNP levels, probably due to a chronically elevated cardiac afterload in the African population, possibly indicate early vascular changes that may already be present at a relatively young age. This is supported by the higher SBP and pulse pressure observed in this group. This emphasises the importance of early intervention or counteractive measures to lower blood pressure and reduce cardiac strain.

Patients with left ventricular hypertrophy and/or systolic and diastolic dysfunction experience increased myocardial stress load, causing NT-proBNP levels to elevate,[13] which in turn can result in changes of cellular structure and function. The ECM is considered to be a highly adaptive and dynamic structure and plays a primary role in myocardial ventricular remodelling as it is regulated by mechanical stress, neurohormonal activation, inflammation and oxidative stress.[14] In this study, African men and women exhibited higher fibulin-1 levels, as well as higher pulse wave velocity and lower arterial compliance compared to Caucasian men and women. The African men also had higher NT-proBNP levels compared to the Caucasian men, with no difference between the African and Caucasian women. Although no associations between NT-proBNP and arterial stiffness indices were evident in all groups of this study, the association between NT-proBNP and fibulin-1 in Africans suggests that vascular alteration is present in this young normotensive African population. This further suggests that the African men and women are at higher risk to develop cardiovascular disease as opposed to the Caucasians. In addition, the results of this study add to current knowledge that a connection exists between NT-proBNP and the expression of ECM proteins in blood vessels and the heart, as reflected by both fibulin-1 and NT-proBNP, respectively.

It became evident that low-grade inflammation may be a driving component of these early vascular changes observed in the African population, especially the African men. Although NT-proBNP, CRP and suPAR levels were lower in the normotensive African men, NT-proBNP was independently associated with these markers of inflammation in the African men and also the younger African women. Risk factors of cardiovascular disease provoke the biological effects of pro-inflammatory cytokines, which partly elucidate the conducts of heart failure.[15] These results suggest that in a low-grade inflammatory state, normotensive African men are more at risk to develop early cardiovascular alterations as opposed to normotensive African women. The results also indicate that both the African men and women are already subjected to the adverse effects of inflammation

on cardiovascular function at a relatively young age, independent of hypertension and known infection.

It is still unclear whether ALP is associated with athero- or arteriosclerosis. It is evident that ALP is expressed from VSMCs, which suggests that ALP may be involved in atherosclerotic plaque formation. However, in more progressive disease states such as coronary heart disease and adverse stiffening, ALP may play an important role in Mönckeberg's arteriosclerosis. It is evident that ALP is expressed in the media of arterial walls,[16-18] but it is not clear whether calcification is initiated from the luminal intima or from the ECM basal layer to the arterial media.

Although ALP is not a recognised marker of vascular calcification as opposed to vitamin K-dependent proteins such as circulating matrix γ-carboxyglutamate (Gla) proteins, it is at least known as a marker of osteoblastic activity.[19, 20] Structural measures of calcification also include electron beam and multi-detector computed tomography.[21] Nonetheless, the fact that a marker of cardiac strain was associated with a marker of osteoblastic activity indicates that early adverse calcification is taking place in the cardiovascular system of the African group under study.

6. CONCLUSIONS

NT-proBNP is positively associated with markers of cardiovascular function as well as fibulin-1 in a fairly young African population, but not in Caucasians, suggesting that vascular alterations develop at an earlier stage in Africans as opposed to Caucasians. Moreover, the significant positive association established between NT-proBNP, CRP and suPAR in the normotensive African men, indicates that low-grade inflammation may already present increased cardiovascular risk in this young African group, independent of a hypertensive state and/or infectious disease. Together with the previous findings, the link between cardiac strain and a marker of osteogenic smooth muscle cell turnover highlights the importance of curbing modifiable risk factors augmenting arteriosclerotic activity in this population and by doing so limiting the increasing trend of cardiovascular morbidity and mortality in Africans.

7. RECOMMENDATIONS

The following recommendations are intended to improve cardiovascular health in the African population, especially in African men:

- NT-proBNP screening is quite expensive and not available to the general population. Therefore, it is essential to develop a more cost-effective screening method for this risk marker that may be able to predict possible cardiac events in future and thereby decrease the escalating cardiovascular disease rates.

- Numerous factors (whether intrinsic, modifiable or genetic) contribute to adverse cardiovascular function and although human physiology is complex and interconnected, it is recommended that early interventions are implemented to address the onset of hypertension and low-grade inflammation in the African population.

In this study a few limitations were evident and the following are recommendations which would help to improve further research in this regard:

- To improve the quality of future studies, more clinical markers of end organ damage are needed to investigate cause and effect in prospective studies. These include markers of endothelial dysfunction such as micro-albuminuria, carotid intima-media thickness, echocardiographic data of the posterior wall of the left ventricle as well as electrocardiographic information.

- Larger cohort population studies, longitudinal studies and prospective experimental intervention studies are needed to determine the cause of this cardiovascular burden. By doing so, recommendations will be made to policy makers to provide funding for early intervention strategies in order to improve the overall cardiovascular health burden in this population.

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