

**The association between cardiac troponin,
inflammation and target organ damage:
The SABPA study**

E Jansen van Vuren

BSc Hons Physiology
22820388

Dissertation submitted in fulfilment of the requirements for the
degree *Magister Scientiae* in Physiology at the Potchefstroom
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Supervisor: Prof L Malan
Co-Supervisors: Prof NT Malan
Mrs M Cockeran

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OPSOMMING

Die verband tussen kardiaale troponien, inflammasie en eindorgaan-skade:

Die SABPA-studie

Motivering

Inflammasie is bewys betrokke te wees by die patogene en progressie van hartversaking, en meervoudige sitokiene blyk 'n rol te speel by die inflammatoriese respons deur hematopoiëse, immuun- en vaskulêre reaksies te reguleer. Die binding van die pro-inflammatoriese sitokiene, tumor nekrose faktor-alfa (TNF- α), aan die TNFR1-reseptor daarvan mag apoptosis in talle sel tipes teweegbring, insluitend kardiaale miosiete. Die doodgaan van kardiaale miosiete is getoon een van die veranderinge te wees wat tydens kardiaale hermodellering voorkom, gepaard met miosiet hipertrofie en modifiserings van die buiteseellêre matriks. Kardiaale hermodellering is 'n kompenserende meganisme wat voorkom wanneer die linker-ventrikel nie daarin slaag om 'n toereikende pols-volume in stand te hou om voldoende voldoende bloed-perfusie na die lewensorgane te voorsien nie. Meervoudige faktore wat in reaksie op linkerventrikulêre disfunksie toeneem, kan kardiaale hermodellering stimuleer, insluitend inflammasie, 'n verhoogde hemodinamiese lading, neuro-hormonale aktivering van die simpatiese senuweestelsel en die renien angiotensien-aldosteroon-sisteem asook die verhoogde produksie van reaktiewe suurstofspesies wat oksidatiewe stres tot gevolg het. Soos reeds genoem, mag die doodgaan van miosiete betrokke wees by kardiaale hermodellering. Nekrose is 'n passiewe proses wat na kardiaale besering voorkom en mag op die produksie van Troponien T (Trop T) uitloop. Trop T speel 'n betekenisvolle rol by die eksitasie-sametrekingskoppeling van skeletale en kardiaale spier, maar die presiese rol wat Trop-T by die ontwikkeling van eindorgaan-skade speel is tot nog toe nie deeglik in Afrika-bevolkings vasgestel nie. Linker-ventrikulêre hipertrofie (LVH) is getoon 'n manifestasie van eindorgaan-skade te wees. In stedelike Afrika-bevolkings is daar getoon dat linker-

ventrikulêre strukturele veranderinge met inflammasie en stil iskemie geassosieer word. Die verhoogde hemodinamiese lading gepaard met die strukturele verandering van die linker-ventrikel mag dus lei tot 'n toename in die kardialewand-stres wat die produksie van die N-terminale porsie van pro-brein natriuretiese peptide (*N-terminal portion of pro-brain natriuretic peptide* – NT-proBNP) tot gevolg kan hê. NT-proBNP is al getoon verhoog te wees in Afrika-mans in teenstelling met Koukasiër mans in wie positiewe assosiasies gedemonstreer is tussen NT-proBNP, polsdruk (PD) en C-reaktiewe proteïene. Sosio-ekonomiese status is egter nie in aanmerking geneem nie, en die verband tussen NT-proBNP, Trop T en sistemiese inflammatoriese merkers (IL-6 en TNF- α) moet nog in 'n bi-etniese geslagspopulasie met soortgelyke sosio-ekonomiese status vasgestel word.

Doelwitte

Die doel van hierdie studie was om vas te stel of assosiasies bestaan tussen inflammasie, kardiaale troponien en -hermodellering in 'n bi-etniese geslagskohort van Suid-Afrika. Ons het daarop gemik om te assesser of verbande tussen drie bekende merkers van inflammasie (CRP, IL-6 en TNF- α), Trop T en merkers van kardiaale hermodellering (NT-proBNP en LVH) bestaan.

Metodes

Hierdie studie het deel uitgemaak van die *Sympathetic activity and Ambulatory Blood Pressure in Africans*- (SABPA) studie wat in 2008 en 2009 uitgevoer is. Onderwysers, 20 tot 65 jaar oud, wat in die Dr Kenneth Kaunda Onderwysdistrik van die Noord Wes Provinsie van Suid-Afrika woonagtig is. Hierdie seleksie is gedoen om te verseker dat die deelnemers uit gelyksoortige sosio-ekonomiese status afkomstig was. Die uitsluitingskriteria vir die SABPA-studie was: swangerskap, laktasie, gebruikers van α - en β -blokkers of psigotropiese

substansie, bloedskenkers of inentings 3 maande voor kliniese assessering en 'n timpanumkoors bo 37.5°C. Bykomende uitsluitings is gemaak om vooroordeel wat met kardiometaboliese en inflammatoriese risiko verband hou, te voorkom, en deelnemers met 'n MIV positiewe status (N=19), klinies gediagnoseerde diabetes mellitus (N=10), gebruik van anti-inflammatoriese medikasie (N=24), gebruik van antistolmiddel-medikasie (N=2), gebruik van aspirien (N=11) en 'n geskiedenis van miokardiale infarksie of beroerte (N=4) is uitgesluit. Na hierdie uitsluitings het 165 manlike (76 Afrika- en 89 Koukasiër-) en 174 vroulike (80 Afrika- en 94 Koukasiër-) deelnemers oorgebly. Ingeligte toestemming is voor die aanvang van die studie van al die deelnemers bekom, en die studie voldoen aan die vereistes van die Helsinki Konvensie. Dit het voor die aanvang van die studie etiese goedkeuring van die Navorsingsetiek-komitee van die Noordwes-Universiteit bekom. Kliniese assesserings is in die loop van 'n 48-u-period verkry. Die Cardiotens CE120® (Meditech, Boedapest, Hongarye) en akselerometers is aangewend om 24-uur ambulatoriese bloeddruk (ABPM) vas te lê, 2-kabel ECG asook 24-u fisiese aktiwiteit. Die deelnemers se daaglikse fisiese aktiwiteit is oor 'n tydperk van 24 uur met die Actical® activity monitor gemoniteer. Vlak-I antropometriste het antropometriese metings geneem ooreenkomstig gestandaardiseerde prosedures. Die Mosteller-formule van $[\text{gewig (kg)} \times \text{hoogte (cm)}]^{1/2}$ is gebruik om die liggaamsoppervlak-area te bereken. Geregistreerde verpleegsters het die vastende bloedmonsters uit die ante-bragiale aar met 'n steriele gevlerkte infusie-stel om gamma-glutamiel-transferese (γ GT), kontinien, ultrahoë sensitiwiteit CRP, IL-6, hoog-sensitiewe TNF- α , hoog-sensitiewe Trop T asook NT-proBNP te meet. Die ABPM is teen 3-minuut-intervalle van 08:00 tot 22:00 en teen 60-minuut-intervalle van 22:00 tot 06:00 gemeet. Stil iskemie (profiele van ambulatoriese iskemiese voorvalle) is met twee-kanaal ECG-opnames geassesseer. 'n Rustende 12-kabel EKG is met die Norav NHH-1200® EKG vasgelê wat ook

EKG linker-ventrikulêre hipertrofie bepaal het (Cornell-produk, [RaVL+SV3]. x QRS duur). Waardes bo 244 mV.ms het LVH aangedui.

Statistiese analyses is met *Statistica version 12* uitgevoer. Onafhanklike *t*-toetse is gebruik om basislyn eienskappe van die twee etniese groepe te vergelyk. Chi-kwadraat-toetse (χ^2) is gebruik om voorkoms asook proporsies vas te stel. Die *a priori* kovariate is in al die statistiese analyses was ouderdom, liggaamsoppervlak-area, log kotinien, log γ -GT en log fisiese aktiwiteit. Enkel tweerigting-interaksies tussen hoofeffekte (etnisiteit x geslag) is vir alle merkers (24-h BP, kardiaal hermodellering, inflammasie, kardiaal troponien, stil iskemie (ST voorvalle), onafhanklik van *a priori* kovariate uitgevoer. Eenrigting-analise van kovariansie (ANCOVA) is uitgevoer om die etniese groepe te vergelyk geslagsgewys terwyl vir *a priori* kovariate aangepas word. Eenveranderlike en multi-veranderlike regressie-analises is rekenaarmatig gedoen. Voorwaartse stapsgewyse regressie-analises is in drie afsonderlike inflammatoriese modelle op rekenaar geplaas om kollineariteit (TNF alfa, IL-6 en CRP) te voorkom. Assosiasies is tussen afhanklike merkers bepaal: BP, kardiaal hermodellering (NT-proBNP, LVH) en onafhanklike veranderlikes (inflammasie, kardiaal troponien en stil iskemie, onafhanklik van *a priori* kovariate. Polsdruk is as kovariaat bygevoeg tot NT-proBNP en LVH die afhanklike veranderlikes was.

Resultate

Afrikane het hoër onaangepaste gemiddelde 24-u hipertensie, lae-graad inflammasie (CRP > 3g/l), kardiaal troponien en LVH-waardes as Koukasiërs getoon. Met inagneming van verstrengelings, het 'n soortgelyke neiging na vore getree in Afrika-mans en -vroue in die meeste gevalle, behalwe dat geen verskille vir NT-proBNP, Trop T en TNF alfa tussen etnies-geslagsgroepe waargeneem is nie. In voorwaartse stapsgewyse regressie-analises, het

geen betekenisvolle assosiasies in enige van die 3 inflammatoriese modelle vir kardiaale hermodellering by die vroue voorgekom nie. By die mans was 24-h BP egter geassosieer ($p < 0.05$) met Trop T in beide Afrikane en Koukasiërs. In die TNF- alfa en die CRP-model, was SBP met beide Trop T en stil iskemie by Afrika-mans geassosieer. Die meeste betekenisvol was die profiel wat in die sistemiese inflammasiemodel (TNF alfa model) na vore gekom het. In hierdie model is positiewe assosiasies gevind tussen kardiaale hermodellering (NT-proBNP) en 'n gekombineerde profiel [adj $R^2 = 0.45$; TNF alfa ($\beta = 0.31$; 95% CI 0.14 tot 0.48; $p < 0.001$), Trop T ($\beta = 0.48$; 95% CI 0.28 tot 0.67; $p < 0.001$) en polsdruk ($\beta = 0.28$; 95% CI 0.09 tot 0.48; $p = 0.006$)].

Gevolgtrekking

Ons het 'n assosiasie gedemonstreer tussen kardiaale troponien, inflammasie en verlaagde vlakke van miokardiale suurstofgebruik in Afrika-mans. Verhoogde voorlading op die hart en 'n geassosieerde inflammatoriese profiel in Afrika-mans verhoog hulle vatbaarheid om kardiaale hermodellering en toekomstige kardiovaskulêre situasies.

SUMMARY

The association between cardiac troponin, inflammation and end-organ damage:

The SABPA study

Motivation

Inflammation has been shown to be involved in the pathogenesis and progression of heart failure, and multiple cytokines seem to play a role in the inflammatory response by regulating haematopoiesis, immune- and vascular reactions. Binding of the pro-inflammatory cytokine, tumour necrosis factor-alpha (TNF- α), to its TNFR1 receptor may induce apoptosis in many cell types, including cardiac myocytes. The death of cardiac myocytes has been shown to be one of the changes that occur during cardiac remodelling, along with myocyte hypertrophy and modifications of the extra cellular matrix. Cardiac remodelling is a compensatory mechanism that occurs when the left ventricle fails to maintain an adequate stroke volume in order to provide adequate blood perfusion to the vital organs. Multiple factors that increase in response to left ventricular dysfunction may stimulate cardiac remodelling including inflammation, an increased hemodynamic load, neuro-hormonal activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system as well as the increased production of reactive oxygen species resulting in oxidative stress. As mentioned, the death of myocytes may be involved in cardiac remodelling. Necrosis is a passive process that occurs following cardiac injury and may result in the production of Troponin T (Trop T). Trop T plays a significant role in the excitation-contraction coupling of skeletal and cardiac muscle, but the exact role Trop T plays in the development of end-organ damage has not been thoroughly established in African populations. Left ventricular hypertrophy (LVH) has been shown to be a manifestation of end-organ damage. In urban-dwelling African populations it has been shown that left ventricular structural changes are associated with inflammation and silent ischemia. The increased hemodynamic load accompanied by the structural changes of

the left ventricle may therefore lead to an increase in the cardiac wall stress that can result in the production of the N-terminal portion of pro-brain natriuretic peptide (NT-proBNP). NT-proBNP has been shown to be increased in African men as opposed to Caucasian men in whom positive associations were demonstrated between NT-proBNP, pulse pressure (PP) and C-reactive protein. However, socio-economic status was not considered and the relation between NT-proBNP, Trop T and systemic inflammatory markers (IL-6 and TNF- α), still needs to be determined in a bi-ethnic sex population with similar socio-economic status.

Objectives

The aim of this study was to determine whether associations exist between inflammation, cardiac troponin and -remodelling in a bi-ethnic sex cohort of South Africa. We aimed at assessing whether relations exist between three known markers of inflammation (CRP, IL-6 and TNF- α), Trop T and markers of cardiac remodelling (NT-proBNP and LVH).

Methods

This study formed part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study which was conducted in 2008 and 2009. Teachers, aged 20-65 years, resided in the Dr Kenneth Kaunda Education District of the North West Province of South Africa. This selection was made to ensure that the participants were from a similar socio-economic status. The exclusion criteria for the SABPA study was: pregnancy, lactation, users of α - and β -blockers or psychotropic substances, blood donors or vaccinations 3 months prior to clinical assessment and a tympanum temperature exceeding 37.5°C. Additional exclusions were made to avoid bias pertaining to cardio-metabolic and inflammatory risk and participants with an HIV positive status (N=19), clinically diagnosed diabetes mellitus (N=10), anti-inflammatory medication usage (N=24), anti-coagulant medication usage (N=2),

aspirin usage (N=11) and history of myocardial infarction or stroke (N=4) were excluded. After these exclusions, 165 male (76 African and 89 Caucasian) and 174 female (80 African and 94 Caucasian) participants remained. Informed consent was obtained from all the participants prior to the commencement of the study, and the study complies with the requirements of the Helsinki Convention. It received ethical approval from the Research Ethics Committee of the North-West University prior to commencement. Clinical assessments were obtained over a 48-h period. The Cardiotens CE120® (Meditech, Budapest, Hungary) and accelerometers were applied to record 24-hour ambulatory blood pressure (ABPM), 2-lead ECG as well as 24-h physical activity. The participants' daily physical activity was monitored over 24-hours with the Actical® activity monitor. Anthropometric measurements were taken by registered level II anthropometrists according to standardized procedures. The Mosteller formula of $[\text{weight (kg)} \times \text{height (cm)}]^{1/2}$ was used to calculate the body surface area. Registered nurses obtained fasting blood samples from the ante-brachial vein with a sterile winged infusion set to measure gamma-glutamyl transferase (γ GT), cotinine, ultra-high-sensitivity CRP, IL-6, high sensitive TNF- α , high sensitive Trop T as well as NT-proBNP. The ABPM was measured at 30-minute intervals from 08:00 to 22:00 and at 60-minute intervals from 22:00 to 06:00. Silent ischemia (ambulatory ischemic events profile) was assessed with two-channel ECG recordings. A resting 12-lead ECG was recorded with the Norav NHH-1200® ECG which also determined ECG left ventricular hypertrophy (Cornell product, $[\text{RaVL} + \text{SV3}] \times \text{QRS duration}$). Values exceeding 244 mV.ms indicated LVH.

Statistical analyses were performed with Statistica version 12. Independent *t*-tests were used to compare baseline characteristics of the two ethnic groups. Chi-square tests (X_2) were used to determine prevalence as well as proportions. The *a priori* covariates used in all the

statistical analyses were age, body surface area, log cotinine, log γ -GT and log physical activity. Single two-way interactions between main effects (ethnicity x gender) were performed for all markers (24-h BP, cardiac remodelling, inflammation, cardiac troponin, silent ischemia (ST events), independent of *a priori* covariates. One-way analysis of covariance (ANCOVA) was performed to compare the ethnic groups by gender adjusting for *a priori* covariates. Univariate and multivariate regression analyses were computed. Forward stepwise regression analyses were computed in three separate inflammatory models to avoid collinearity (TNF alpha, IL-6 and CRP). Associations were determined between dependent markers: BP, cardiac remodelling (NT-proBNP, LVH) and independent variables (inflammation, cardiac troponin and silent ischemia, independent of *a priori* covariates. Pulse pressure was added as covariate when NT-proBNP and LVH were the dependent variables.

Results

Africans showed higher unadjusted mean 24-h hypertension, low-grade inflammation (CRP > 3g/l), cardiac troponin and LVH values than Caucasians. Considering confounders, a similar trend emerged in African men and women in most cases, except no differences for NT-proBNP, Trop T and TNF alpha were observed between ethnic-gender groups. In forward stepwise regression analyses, no significant associations were evident in any of the 3 inflammatory models for cardiac remodelling in the women. In the men though, 24-h BP was associated ($p < 0.05$) with Trop T in both Africans and Caucasians. In the TNF alpha and CRP models, SBP was associated with both Trop T and silent ischemia in the African men. Most significantly was the profile emerging in the systemic inflammation model (TNF alpha model) in these men. Positive associations were demonstrated between cardiac remodelling (NT-proBNP) and a combined profile [adj $R^2 = 0.45$; TNF alpha ($\beta = 0.31$; 95% CI 0.14 to

0.48; $p < 0.001$), Trop T ($\beta = 0.48$; 95% CI 0.28 to 0.67; $p < 0.001$) and pulse pressure ($\beta = 0.28$; 95% CI 0.09 to 0.48; $p = 0.006$).

Conclusion

We demonstrated an association between cardiac troponin, systemic inflammation and decreased levels of myocardial oxygen consumption in African men. Increased preload to the heart and an associated inflammatory profile in Africans may increase their susceptibility to cardiac remodelling and future cardiovascular events.

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LIST OF ABBREVIATIONS

24-h	24-hours
ABPM	24-hour ambulatory blood pressure
ANCOVA	Analysis of co-variance
AngII	Angiotensin II
BNP	B-type natriuretic peptide
BP	Blood pressure
cGMP	Cyclic guanine monophosphate
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECG	Electrocardiogram
ECM	Extra cellular matrix
ESH	European Society of Hypertension
ET-1	Endothelin-1
FADD	Fas-associated death domain
γ GT	Gamma glutamyl transferase
ICAM	Intracellular adhesion molecule
IL-6	Interleukin-6
LVH	Left ventricular hypertrophy
MAPK	Mitogen-activated protein kinase
MMP	Matrix metalloproteinase
NF-K β	Nuclear factor- κ B

NO	Nitric oxide
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PP	Pulse pressure
RAAS	Renin-angiotensin-aldosterone system
RIP	Receptor-interacting protein
ROS	Reactive oxygen species
SABPA	Sympathetic activity and Ambulatory Blood Pressure in Africans
SBP	Systolic blood pressure
ST-events	Ischaemic events
TNF- α	Tumour necrosis factor-alpha
TIMP	Endogenous tissue inhibitors
TNFR1	Tumour necrosis factor receptor-1
TRADD	TNF receptor-associated death domain
TRAF2	TNF-receptor-associated-factor 2
Trop T	Troponin T
VCAM	Vascular cell adhesion molecule
VWF	Von Willebrand Factor
WHO	World Health Organisation
α	Alpha
β	Beta
γ	Gamma

CHAPTER 1:
PREFACE AND OUTLINE OF THE STUDY

PREFACE AND OUTLINE OF THE STUDY

This dissertation has been completed for fulfilment of the requirements for the degree Master of Science in Physiology. It is presented in article-format as approved by the North-West University's guidelines for postgraduate studies. It consists of four chapters. The manuscript in Chapter three has been prepared in a format that meets the requirements of the peer-reviewed journal, *International Journal of Cardiology*, which is considered for submission. The reference format is also consistent with the afore-mentioned journal's guidelines, and is represented at the end of chapters two, three and four.

The four chapters consist of the following information:

Chapter one: Includes the preface and outline of the study as well as the contributions of the respective authors. It also includes a summary of the skills obtained during the postgraduate study period.

Chapter two: Consists of a general introduction, literature background, the aim and objectives of the study as well as the main hypotheses.

Chapter three: Represents the manuscript titled, *Systemic inflammation, cardiac troponin and arterial tone are associated with cardiac remodelling in African men: The SABPA study* prepared according to the guidelines of the considered journal.

Chapter four: Includes a summary of the main findings of the study as well as a conclusion and recommendations for future research.

AFFIRMATION BY THE AUTHORS

The researchers contributed to the study in the following manner:

Miss E. Jansen van Vuren (BSc Hons) conducted the literature searches and was responsible for the design, planning, statistical analyses, data interpretation, writing and presentation of the manuscript.

Prof. L. Malan (RN, PhD) as principal investigator designed the SABPA study and was involved in the initial planning and collection of data. She supervised and made recommendations regarding the initial planning of the manuscript as well as the statistical analyses, interpretation of the results and edited the writing of the manuscript and literature background.

Prof. N.T. Malan (DSc) as a co-supervisor assisted in the design and data collection phases of the SABPA study, planning and edited writing of the manuscript and literature background.

Mrs M. Cockeran (MSc) as co-supervisor assisted and made recommendations regarding all the statistical analyses and the writing of the manuscript.

Prof R. Von Känel as co-author made recommendations and edited writing of the manuscript.

I, Esmé Jansen van Vuren, hereby declare that the statement above is a true representation of my actual contribution and I give permission that the manuscript in Chapter three may be submitted for publication as part of the dissertation for the degree Master of Science in Physiology.

Miss E. Jansen van Vuren

The co-authors hereby agree that the above-mentioned statement is a true representation of each author's contribution and we give permission that the manuscript in Chapter three may be submitted for publication as part of the dissertation for the degree Master of Science in Physiology.

Prof. L. Malan

Prof. N.T. Malan

Mrs M. Cockeran

Prof R Von Känel

POSTGRADUATE STUDENT SKILLS 2015



STUDENT NAME: Esmé Jansen van Vuren	Tick if accomplished		
<i>Optional: Clinical Pharmacology course (16 credit module)</i>			
<i>Optional: Honours student mentorship (indicate number of students)</i>	N =		
Ethical consent: Sub-study application under Umbrella-study	<input checked="" type="checkbox"/>		
Obtained medical history & medications <i>Including duration of stay, education, marital status, alive family members, health (cardiometabolic, inflammation, depression, renal, arthritis, cancer, reproduction), sleep apnoea, ambulatory & dietary diary, mental stress perception</i>	<input checked="" type="checkbox"/>		
Good clinical practice: lifestyle habits; participant handling <i>Self-reported smoking & alcohol habits</i>	<input checked="" type="checkbox"/>		
<i>Dietary intake and questionnaire</i>			
Observed anthropometry measurements <i>Height, Body mass, Waist circumference, BMI</i>	<input checked="" type="checkbox"/>		
Cardiovascular assessments, download and interpretation of data Resting Blood Pressure [<i>Riester CE 0124® & 1.3M™ Littman® II S.E. Stethoscope 2205</i>] *Finometer [<i>Finapres Medical Systems®</i>] 12-lead resting ECG [<i>NORAV PC-ECG 1200®</i>] 24 ambulatory BP & -ECG [<i>Cardiotens® & Cardiovisions 1.19®, Meditech</i>] Pulse Wave Velocity and Pulse Wave Analysis [<i>Sphygmocor EXCEL, AtCor</i>]	<input checked="" type="checkbox"/>		
Laboratory skills (sample handling and analyses) 24h Urine/blood/saliva/hair: ¹ collection/ ² sampling/ ³ aliquoting/ ⁴ waste material Rapid tests (cholesterol, glucose, urine dipstick and blood type) Laboratory analyses of samples (ELISA, RIA, ECLIA, etc.) Whole blood HIV status [<i>PMC Medical, Daman, India; Pareekshak test, BHAT Bio-Tech, Bangalore, India</i>]	1	2	
	3	4	
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Accomplished training & measuring of ultrasound Carotid Intima Media Thickness (CIMT) <i>[Sonosite Micromaxx®, SonoSite Inc., Bothell, WA]</i>	<input checked="" type="checkbox"/>		
Statistical analyses ¹ Normal distribution & T-tests, ² General linear models, ³ Multiple regression analyses ⁴ ROC analyses; ⁵ prospective data analyses and risk prediction	1 <input checked="" type="checkbox"/>	2 <input checked="" type="checkbox"/>	3 <input checked="" type="checkbox"/>
	4	5	
¹ Prepared, ² submitted, ³ handled a rebuttal & ⁴ published manuscript in a peer-reviewed journal	<input checked="" type="checkbox"/> 1	2	N =
	<input checked="" type="checkbox"/> 3	4	

Sr A Burger (RN, MCur)
Head of the Hypertension
Research and Training Clinic

Prof L Malan (RN, PhD)
SABPA study project leader
Student's supervisor



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences
Department of Forensic Medicine

Our Reference: LdT-P029-15/ms

11 November 2015

TO WHOM IT MAY CONCERN

RE: MISS E JANSEN VAN VUREN (STUDENT NUMBER 14325251)

I hereby confirm that miss E Jansen van Vuren, student number 14325251, was enrolled at the University of Pretoria for the BSc Honours degree in Medical Criminalistics for 2015. This degree program is a one year full time program which entails the presence of students on most days.

During this year she attended 55 post mortem examinations at the Pretoria Medico-Legal Laboratory, one death scene and two court procedures at the North Gauteng High Court in Pretoria. Visits to the Forensic Anthropology, Forensic Odontology and Histology laboratories at the University of Pretoria were also completed.

In addition, she attended all lectures provided to the undergraduate medical students (both in medicine and law), all post graduate meetings in the department (and presented 3 seminar topics) and also completed her research (which was presented as an oral presentation at the University of Pretoria Faculty day in August 2015 where she was awarded the 2nd best oral presentation by a student).

Yours sincerely

**DOCTOR L DU TOIT-PRINSLOO
SENIOR SPECIALIST: DEPARTMENT OF FORENSIC MEDICINE**

Department of Forensic Medicine
Room 4-44, Pathology Building
Prinshof Campus
Private Bag X323, Arcadia, 0007
South Africa

Tel Number +27 012 319 2122
012 323 5296

Fax Number +27 012 323 0921

Email address:
marleta.schoitz@up.ac.za

www.up.ac.za

CHAPTER 2:
GENERAL INTRODUCTION AND LITERATURE OVERVIEW

1. General introduction

Globally, the incidence of non-communicable diseases is increasing [1-4]. Approximately 80% of all non-communicable diseases occur in low- and middle income countries, such as South Africa [1,2]. A triple burden of disease has been identified due to the increase of non-communicable diseases accompanied by the high prevalence of infectious diseases and violence-related injuries that occur annually in South Africa [5]. Cardiovascular diseases (CVDs) were shown to be the leading cause of death due to non-communicable diseases in the world [1,6]. The development of CVDs is on the rise in South Africa due to globalisation which results in an increase in known cardiovascular risk factors [1-3,5,7]. The World Health Organisation (WHO) has identified three distinct categories of risk factors that may influence the prevalence of cardiovascular diseases including behavioural risk factors, cardio-metabolic risk factors and social determinants [1,6].

1.1 Behavioural risk factors

CVDs seemed to be influenced largely by four behavioural risk factors including tobacco use, physical inactivity, alcohol abuse and an unhealthy diet [1,2,4,6]. The use of tobacco can increase the risk for CVDs either by direct consumption of tobacco or by second-hand smoking [1]. It has been postulated that there are approximately one billion smokers in the world [6]. Smoking seems to have an influence on the development in almost 10% of all CVDs [1]. The prevalence of tobacco use varies by population group and gender [8]. A study done in South Africa revealed that although men use more tobacco than women, the use of tobacco in women is still at very high levels [8]. Physical inactivity has been classified by the WHO if an individual participates in moderate intensity physical activity less than five times a week for 30 minutes or in vigorous intensity physical activity less than three times a week for 20 minutes [1]. The increased benefit due to physical activity thus depends on the

frequency, duration and intensity of an activity [9]. By not being physically active, individuals can increase their risk for all-cause mortality by 20%-30% [1]. Women seem to be more physically inactive than men and the incidence of physical inactivity seems to increase with an increase in age [1,9]. The harmful use of alcohol contributed to 3.8% of all deaths globally in 2008 [1]. More than 50% of men in South Africa seem to abuse alcohol [10]. In the year 2000, alcohol contributed to 7% of all deaths that occurred in South Africa. An unhealthy diet constitutes an excessive consumption of dietary salt, saturated fats and trans-fatty acids [1]. Dietary salt has been shown to be an important determinant of blood pressure and multiple interventions were therefore implemented to attempt to reduce the intake of salt in South Africa [1,11].

Cardio-metabolic risk factors

The above-mentioned behavioural risk factors are associated with an increased risk of developing metabolic risk factors such as hypertension, diabetes mellitus and obesity [6,11,12]. Hypertension was identified as a major risk factor for CVD development [1]. Approximately 12.8% of all worldwide deaths in 2008 were attributed to an increase in blood pressure. In South Africa, approximately 9% of all deaths were attributed to hypertension in 2000 [13]. Multiple studies have shown the positive association between increases in blood pressure and ageing [11,13,14]. Differences in blood pressure have also been demonstrated between different ethnic groups and genders where the environment and socio-economic status of an individual play an important role in the development of hypertension and thus the risk of developing CVDs later on [8,12,14]. In an urban environment, Africans move from a collectivistic cultural context towards an individualistic cultural environment where anticipated support is not forthcoming and psychosocial stress is exacerbated [15].

1.2 Social determinants

Various social determinants may influence the health profile of individuals [1,2,6]. The health profile can be negatively affected due to the negative effects of globalisation, which includes the rapid, unplanned urbanisation of individuals in low- and middle-income countries accompanied by poverty in these countries [1,14]. Accompanied by major cultural changes, urbanisation also seems to lead to changes in diet and physical activity levels that predispose individuals to a sedentary lifestyle [1-4,14]. Although urban communities run a higher risk of developing CVDs than do rural communities, these rural communities may still have an increased risk due to poor diets, limited access to healthcare and medication as well as their susceptibility to socio-economic stress [3,14]. A study done by Cois et al. reported that a higher income and education level were associated with increased blood pressure levels in South African men [12]. In contrast, higher income and education levels were associated with a decrease in blood pressure levels in South African women which indicates gender-specific patterns in the relation between blood pressure and socio-economic status. A study done on South African teachers revealed that the emerging burden among urban African men can largely be attributed to the transition to a more westernized lifestyle [16]. Hamer et al also reported that Africans had higher levels of known cardiovascular risk factors than their Caucasian counterparts. A higher level of inflammation, which may be influenced by the afore-mentioned risk factors, was also identified in this cohort [16].

2. Role of inflammation in CVDs

2.1 *The inflammation cascade*

Inflammation has been shown to be involved in the pathogenesis and progression of endothelial dysfunction and atherosclerosis that may lead to the development of cardiac remodelling [17-19]. Arterial inflammation is produced in response to stressors that may lead to the expression of adhesion molecules [17-21]. Adhesion molecules, including intracellular adhesion molecule (ICAM-1) and (VCAM-1), are responsible for the migration of leucocytes to the specific site where tissue injury had occurred [17,19]. Levels of adhesion molecules seem to differ in different ethnic groups and can be associated with increased production of pro-inflammatory cytokines [22,23]. Cytokines exert effects on their target cells by binding to specific receptors on membranes of these cells [24]. Binding of cytokines activates the receptors and leads to a downstream of signals that result in inflammatory and vascular reactions. One of the primary pro-inflammatory cytokines are tumour necrosis factor-alpha (TNF- α) [25].

2.1.1 *TNF- α*

TNF- α can be produced by various types of cells including macrophages, monocytes, endothelial cells and smooth muscle cells [17,21]. Some populations of neurons in the brain as well as microglial cells and astrocytes may also be responsible for TNF- α production [26]. TNF- α mainly binds to two distinct cell-surface receptors, namely TNF receptor one (TNFR1) and TNF receptor two (TNFR2) [26-28]. The signalling pathway for the first receptor (TNFR1) is represented in figure 1 [26]. It consists of a TNF receptor-associated death domain (TRADD) that may lead to the induction of apoptosis and transcriptional activity. TRADD recruits three additional proteins through which it exerts its effects. The Fas-associated death domain (FADD) leads to the activation of caspase-8, which in turn leads

to the activation of the relevant apoptotic machinery. The receptor-interacting protein (RIP) is responsible for the activation of the transcription factor nuclear factor- κ B (NF- κ B). The last protein, TNF-receptor-associated-factor 2 (TRAF-2) recruits anti-apoptotic proteins and activates the mitogen-activated protein kinase (MAPK) pathway to increase c-Jun N-terminal kinase activity in an attempt to increase transcription activity [26-28].

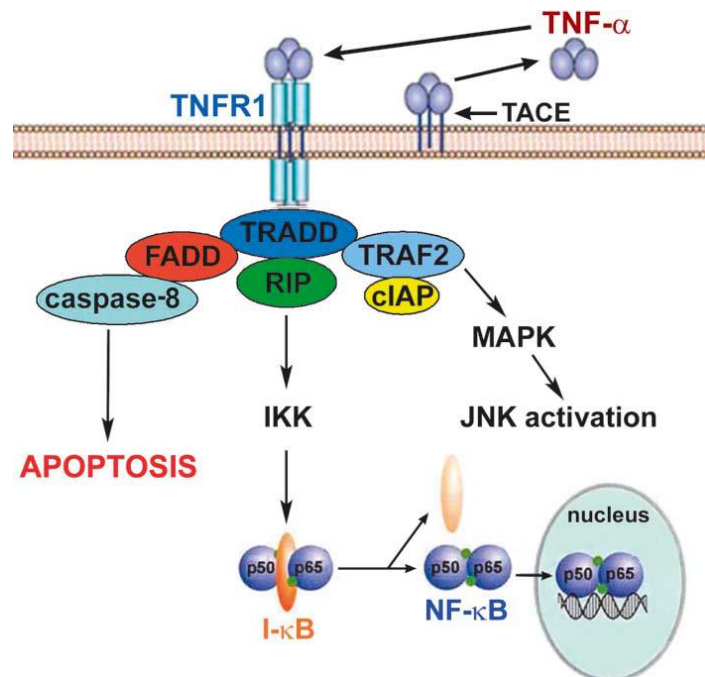


Figure 1: An overview of TNFR1 signalling pathway. Excerpt from Figiel I [26].

Binding of TNF- α to TNFR1 induces the recruitment of TRADD which then becomes a platform for binding of additional cytoplasmic adaptor proteins including TRAF2, RIP and FADD. The first two proteins are implicated in increasing the transcriptional activity. TRAF2 is involved in activation of JNK, a kinase that phosphorylates c-Jun. RIP is critical for activation of IKK (Ser/Thr protein kinases) that phosphorylate I- κ B leading to the dissociation of the I- κ B/NF- κ B complex and nuclear translocation of active transcription factor. In contrast, recruitment of FADD leads to activation of caspase-8 and apoptotic machinery.

TNF- α not only contributes to apoptosis and transcription activity, but can regulate nitric oxide (NO) induction in monocytes and lead to the expression of tissue factor in the

endothelial cells [17,21,24,29]. Indeed, Kalinowski et al showed that black individuals may have a predisposition to endothelial dysfunction due to enhanced NO inactivity [30]. The greater degree of endothelial dysfunction observed in African Americans was also reported by Brown et al [31]. They revealed that TNF- α significantly increased endothelial microparticles in African Americans, indicating the association between inflammation and endothelial dysfunction in this population group. In 36 African-Americans, TNF-alpha showed no associations with 24-h BP whilst C-reactive protein (CRP) was associated with systolic variability [32]. This in turn could facilitate early progression to target organ damage independent of absolute BP levels in African Americans. The production of interleukin-6 (IL-6), a so-called “messenger” cytokine, may also be influenced by TNF- α [21,33-35].

2.1.2 *IL-6*

IL-6 is produced by monocytes, fibroblasts, endothelial cells, macrophages and T-cells and has been shown to be associated with coronary heart disease, diabetes as well as with increased levels of smoking and body fat [34,36-38]. It has been demonstrated that IL-6 can be involved in the pathways of haemostasis and coagulation [39-40]. Binding of IL-6 to a soluble IL-6 receptor on the endothelial cells may lead to an increase in platelet production, thus enhancing platelet activation contributing to haemostasis. IL-6 may lead to an increase in multiple factors involved in the coagulation cascade including tissue factor, von Willebrand factor (VWF), factor VIII and fibrinogen [21,36,37,39,40]. It can also inhibit the production of antithrombin [39-40]. Von Känel and co-authors (2013) demonstrated higher resting levels of von Willebrand factor in Africans than in Caucasians [41]. The increased risk for the development of thrombosis has also been shown to be increased in African American individuals [42]. IL-6 may lead to the expression of acute phase reactants, including CRP, by the hepatocytes in the liver [21,34,36,43].

2.1.3 CRP

The influence of CRP in the inflammatory process is represented in figure 2 [44]. An increase in circulating CRP levels is associated with ageing, smoking, obesity and a history of diabetes mellitus [45]. These risk factors have a positive influence on the inflammatory cascade and may lead to expression of adhesion molecules. The production of these adhesion molecules are further enhanced by CRP, leading to a cycle of events that keeps on exacerbating the state of inflammation [17,20-21]. CRP can also influence the production of Angiotensin II (AngII) and endothelin-1 (ET-1). AngII and ET-1 may increase vasoconstriction as well as the production of reactive oxygen species (ROS) that decrease NO synthesis resulting in a decrease of vasodilation [17,44,46]. The resultant increase in blood pressure will act as a compensatory factor to increase oxygen supply to maintain homeostasis. In itself it will then again lead to an increased inflammatory response [21,47]. Indeed, Van der Walt et al (2013) reported that ambulatory systolic blood pressure as well as pulse pressure were associated with cardiac wall remodelling in 75 African men with low-grade inflammatory status (> 3 mg/L hs-CRP) [48]. Here, hyperdynamic blood pressure and inflammation acted in tandem as possible promoting factors to structural wall abnormalities.

The inflammatory cascade is therefore a complex process that progresses from the presence of pro-inflammatory risk factors to the release of pro-inflammatory cytokines that further enhance the cascade that may influence the function of the vasculature in multiple ways [25].

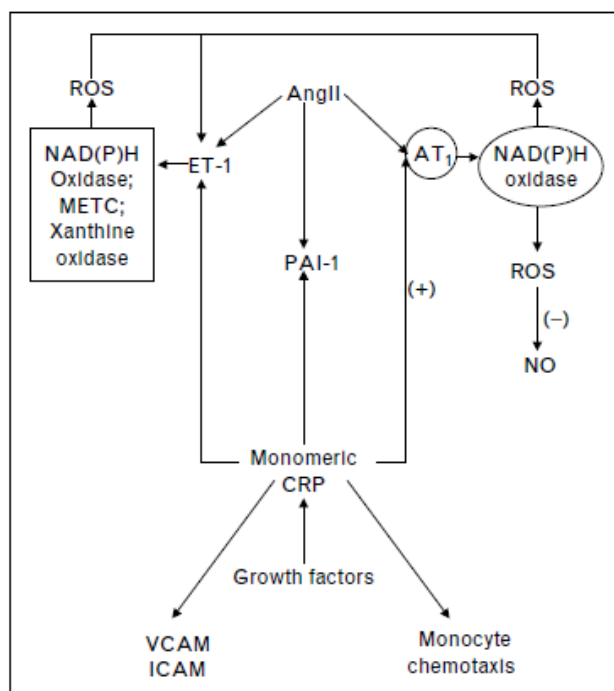


Figure 2: Pathways for C-reactive protein-induced inflammation. Excerpt from Savoia C & Schiffrin

EL [44].

2.2 Inflammation and arterial function

As mentioned, inflammatory markers can alter the function of the vasculature in multiple ways. It can alter vascular tone regulation, play an active role in the proliferation and migration of smooth muscle cells, interact with lipoproteins, promote the activity of leucocytes and even contribute to structural changes in the arterial wall [49]. Inflammation has been shown to play a crucial role in the development and progression of atherosclerosis (figure 3) [17-19,50-52]. Following the migration of leucocytes through the endothelium, monocytes are activated and differentiated into macrophages [17,19,50]. These macrophages result in the accumulation of lipids to form a fatty streak in the arteries [17,50]. Foam cells are also generated in the process [17,19,50]. As the recruitment of inflammatory cells increase, more smooth muscle cells are proliferated. The fatty streak matures into atherosclerotic plaque with a fibrous cap. Thinning of this fibrous cap may result in plaque rupture and increase the formation of thrombosis [17-19,50].

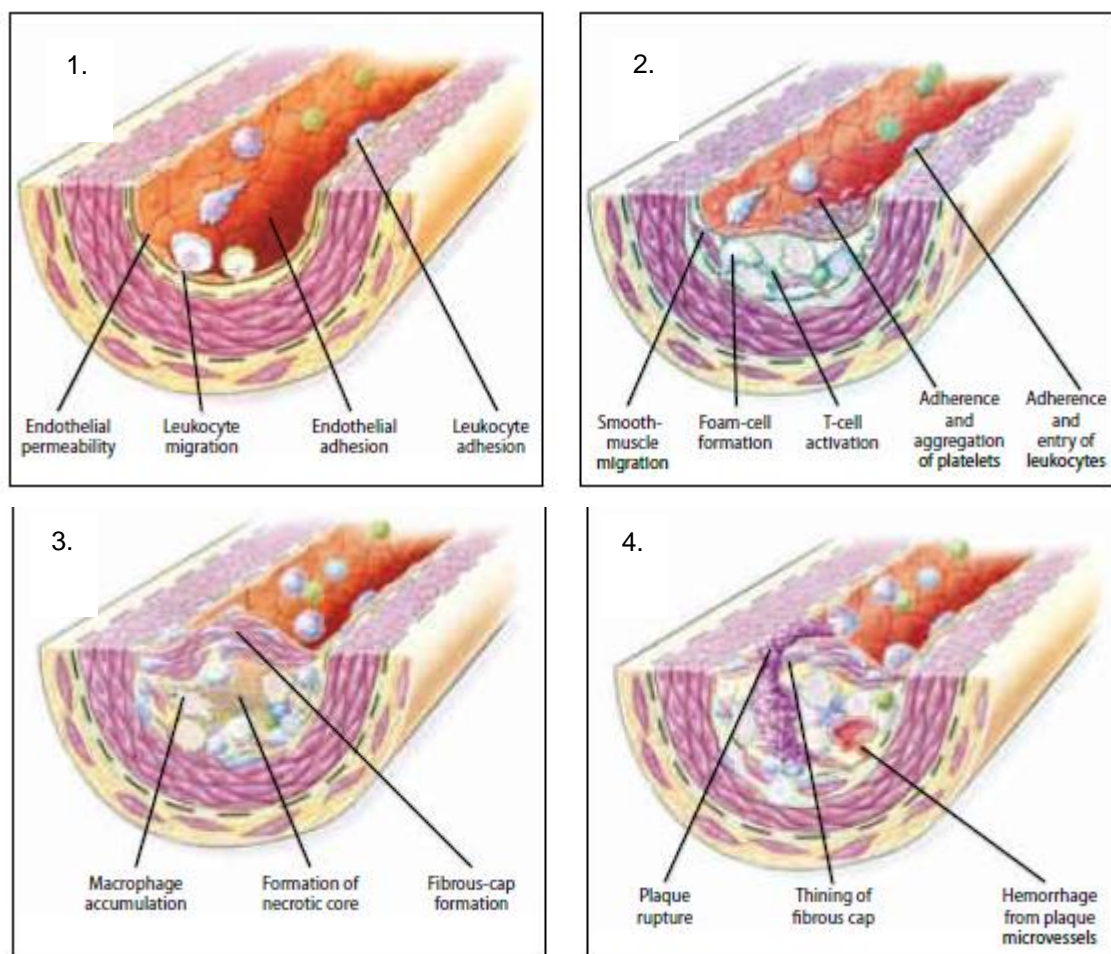


Figure 3: The influence of inflammation in the development of atherosclerosis. Stage 1, endothelial dysfunction; Stage 2, fatty streak formation; Stage 3, Fibrous cap formation and necrotic core; Stage 4, ruptured plaque. Excerpt adapted from Mendis S, Puska P & Norrving B [6].

The consequences of inflammation on the vasculature may influence the mechanical forces in the cardiovascular system [53,54]. Blood pressure, a possible cause and consequence of an increased inflammatory state, is one of the main factors that determine stretch of the blood vessels [55]. Pulse pressure (PP), the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP), have also been shown to be related to atherosclerosis and the vascular tone as it is a reflection of arterial stiffness [56,57]. Hayman et al reported that an increase in PP also seems to be associated with inflammation, as it may increase the vascular wall permeability [56]. Studies also showed that inflammation can lead to an increase in

arterial stiffness, the main cause of an increased PP [57,58]. The resulting pressure and volume overload placed on the heart were identified as the main determinants of cardiac stretch [55]. Inflammation may therefore have an unfavourable effect on the hemodynamic load of the heart resulting in an increase in cardiac wall stress [18,54,55,59].

3. Cardiac myocyte stretch

An increase in the pre- and afterload on the myocardium accompanied by the increase in cardiac wall stress during left ventricular hypertrophy (LVH), are associated with an increase in cardiac myocyte stress [55,60]. An increase in cardiac wall stress and myocyte stretch is also seemingly related to an increased production of natriuretic peptides, including the N-terminal portion of B-type natriuretic peptide (NT-proBNP) [60-62].

3.1 NT-proBNP

Three natriuretic peptides have been identified in the literature [61-63]. Atrial natriuretic peptide and B-type natriuretic peptide (BNP) are mainly released by the atria in response to an increase in cardiac wall stress [61,64-66]. The release of these peptides is, however, upregulated in the ventricles with chronic myocyte stretch. C-type natriuretic peptide is mainly released in the brain [61-62]. NT-proBNP is formed from pro-BNP when it splits into BNP and NT-proBNP under the influence of furin [61]. It binds mainly to a type-A receptor and leads to an increase in intracellular cyclic guanosine monophosphate (cGMP) production [61-62]. The effects of NT-proBNP are therefore modulated by cGMP production and include diuresis, natriuresis, inhibition of the renin-angiotensin-aldosterone system (RAAS) as well as the inhibition of cardiac and vascular myocyte growth [61-62,66-67]. When NT-proBNP binds to a type C receptor it is cleared from the circulation by renal excretion [61-

62,64]. Renal dysfunction can therefore lead to an increase in NT-proBNP levels due to a decrease in NT-proBNP clearance [61-62,67].

Multiple other factors have also been shown to have an influence on the levels of NT-proBNP [53,59,66,68]. Kruger et al has shown that levels of NT-proBNP are higher in African men than in Caucasian men of South Africa, indicating a possible role of ethnicity on NT-proBNP levels [53,65]. However, socio-economic status (SES) was not considered in these groups and could have added bias to findings as SES in Africans has been shown to be associated with an added cardiometabolic risk [69]. Women also seem to have higher NT-proBNP levels than men [60,63,66-67]. The exact mechanism responsible for this difference is still unknown, but may be attributable to the hormonal differences between men and women.

3.1 Cardiac wall stress and cardiac remodelling

Cardiac remodelling is regulated by hemodynamic stress and LVH may therefore lead to an increase in cardiac wall stress resulting in NT-proBNP production [60,66,70-71]. However, an increase in cardiac wall stress can, in turn, lead to the development of cardiac remodelling [55,72]. Excessive myocyte stretch may induce functional and structural changes in the myocardium through multiple autocrine and paracrine signal mechanisms (figure 4) [55]. These signal pathways are mostly mediated by neuro-hormonal activation and inflammatory cytokines, and include the production of ROS, NF κ B, protein kinases, phospholipases and activation of the MAPK system. ROS promotes cardiac remodelling through mechanisms related to inflammation and excessive vasoconstriction [55,73]. Indeed, Kruger et al has shown that ROS is positively associated with 24-h BP and PP in African men [74].

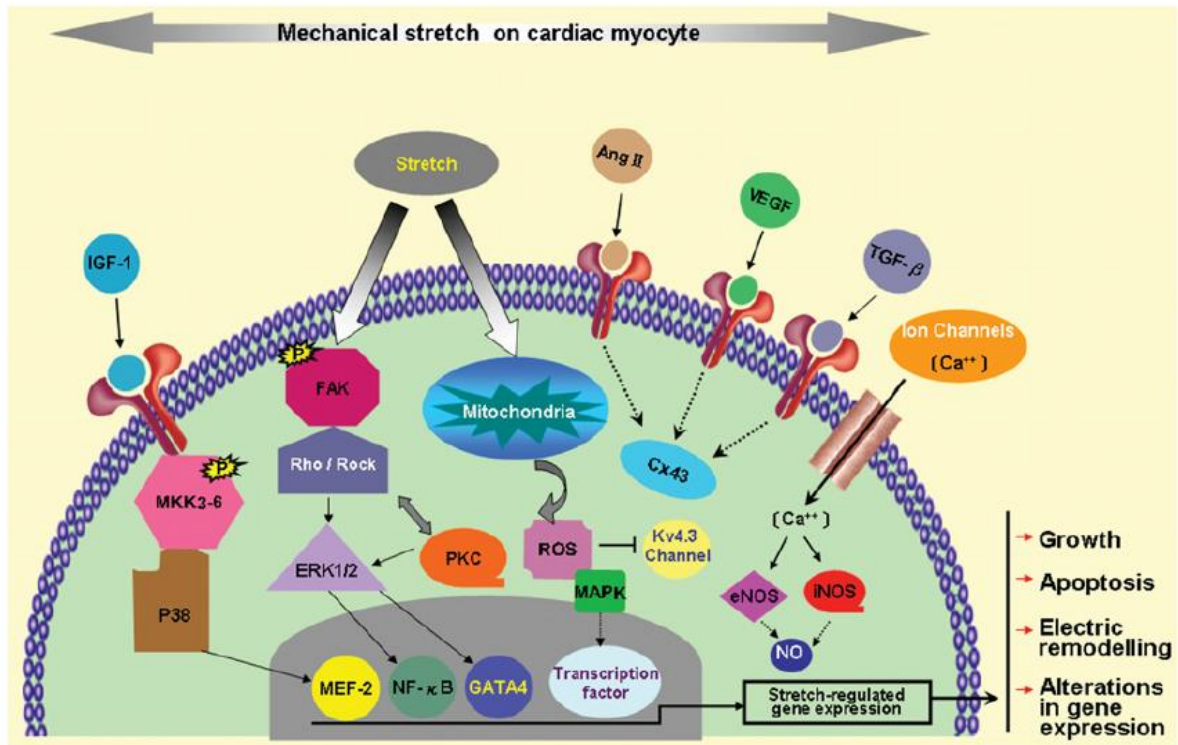


Figure 4: Response of cardiac myocytes to mechanical stretch. Excerpt from Shyu K [55].

Summary of the mechanical-stretch-induced autocrine or paracrine cytokine secretion and intracellular signalling leading to the modulation of gene expression and cellular functions, as discussed in the text. Some of the signalling pathways are observed under *in vitro* conditions only. MEF, myocyte enhancer factor; MKK, MAPK kinase.

Pathological stretch has also been shown to be related to myocyte death, one of the processes involved in cardiac remodelling [55,75-79].

4. Myocyte death

The death of cardiac myocytes can be attributed to two distinctive processes: apoptosis and necrosis [75-76,80]. Apoptosis is an active regulated process of cell death that can be induced by various factors including neuro-hormonal activation, hypoxia, nitric oxide and inflammatory cytokines. In contrast, necrosis is a passive unregulated process of cell death that occurs following cardiac injury. The death of myocytes due to myocardial injury has

been shown to be associated with production of troponin T (Trop T), a subunit of the troponin complex located in cardiac and skeletal muscle tissue [76-77,80-82].

4.1 Trop T

The troponin complex is represented in figure 5 and consists of three subunits, namely troponin T, troponin I and troponin C [76,80,83]. It has an influence on the excitation-contraction coupling mechanism of cardiac and skeletal muscles by regulating the calcium-mediated contraction through interaction of the actin monomers with the myosin heavy chains [75-76]. The main function of Trop T is the binding of the troponin complex to tropomyosin [11,80,82,84]. Trop T is present in high concentrations in the myocyte, but most of the Trop T is structurally bound in a protein pool of the myofibrils, with only a small amount freely present in the cytosol [75-76,82]. Following myocardial injury, Trop T can be released from the cytosolic compartment due to the loss of membrane integrity that results in transient leaking of Trop T [75-76,84]. The contractile apparatus can also be compromised by the actions of proteolytic enzymes as well as due to intracellular acidosis resulting in the continuous release of Trop T from the myofibril. Wallace et al has shown that levels of Trop T are increased in African American men. However, these associations could be explained by the independent associations found between Trop T and LVH in this population [85]. Hypertension, ischemia, myocyte stretch, oxidative stress and inflammation are only some of the multiple mechanisms that may lead to the release of Trop T following myocardial injury [75-76,80,82,86]. Malan et al showed that silent ischemia and LVH were facilitated by vascular responsiveness in African men [87]. A decrease in metabolic supply to the myocardial tissue may result in hypoxia of the myocardium [88]. The persistent ischemia may result in irreversible cell death and migration of inflammatory cytokines to the ischemic myocardium. An increased production of Trop T following cardiac myocyte death is but one

of the changes that occur during cardiac remodelling [55]. The myocardial cells may also increase in mass in an attempt to maintain perfusion. The resultant LVH and increased inflammatory response are related to hypertension and an increase in PP [56-58].

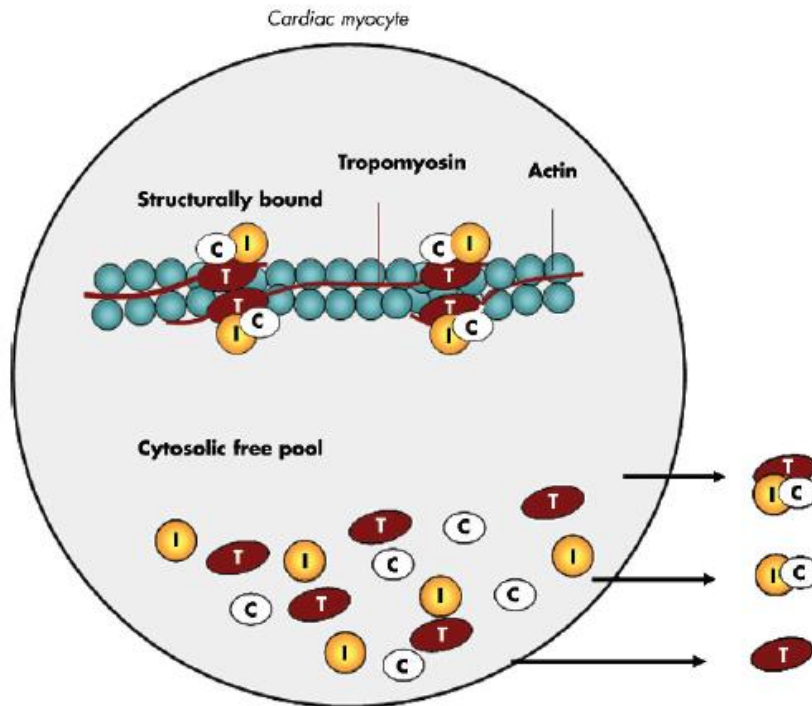


Figure 5: Schematic representation of the cardiac myofibrillar thin filament. Excerpt from Korff S, Katus HA & Giannitsis E [80].

Schematic representation of the cardiac myofibrillar thin filament. Cardiac troponins exist in a structural (bound) form and in a free cytosolic pool. Cardiac troponins are released from myocytes as complexes or as free protein as indicated on the right.

As mentioned, the death of myocytes is one of the mechanisms whereby the cardiac remodelling takes place. Increases in cardiac wall stress that may lead to the death of myocytes can also induce cardiac remodelling.

5. Cardiac remodelling

Cardiac remodelling is a compensatory mechanism that occurs when the left ventricle fails to maintain an adequate stroke volume and thus an insufficient cardiac output [75,89]. This decrease in the cardiac output leads to hemodynamic alterations [75,90]. These alterations constitute different structural and functional changes stimulated by factors that may have increased in response to left ventricular dysfunction [71,75]. These factors include recurrent ischemia, endothelial dysfunction as well as systemic inflammation [75,91]. The compensatory changes range from myocyte hypertrophy or elongation, myocyte death and even modifications to the extra cellular matrix (ECM) [70,75,79,92].

5.1 Myocyte hypertrophy

The occurrence of myocyte hypertrophy can be influenced by multiple factors [70,79,92]. The myocardium increases in mass in an attempt to provide more force in order to bear the extra load [71,75]. With an increase in pressure (afterload) more sarcomeres are added in parallel [71,90,93]. The ventricular wall will thicken accompanied by a decrease in chamber size. This is termed concentric hypertrophy. In contrast, eccentric hypertrophy is characterised by the addition of sarcomeres in series leading to a relatively thin wall accompanied by a larger chamber size. Eccentric hypertrophy occurs in response to volume overload (preload) [71,90,93].

5.2 Extra cellular matrix modifications

The ECM surrounds the myocytes and is composed of collagen and fibroblasts [92]. Alterations in the homeostasis lead to an imbalance between matrix metalloproteinase (MMP's) and endogenous tissue inhibitors (TIMP's) [60,92]. MMP's are regulated by transcription factors, such as NFκB, which are influenced by neuro-hormonal activation of

AngII as well as inflammatory cytokines such as TNF- α [26-28,92]. Excessive collagen deposition leads to the development of fibrosis which, in turn, may contribute to an increased hemodynamic load placed on the heart [70,91-92]. Yao et al reported that ECM remodelling may be a consequence of alterations in PP that have been shown to be related to inflammation [94].

6. Integration of concepts

Cardiac remodelling, characterised by myocyte death (Trop T) and myocyte hypertrophy (LVH), is a manifestation of end-organ damage [79,95]. Inflammation may contribute to an increased hemodynamic burden that may lead to an increase in cardiac wall stress and thus contribute to cardiac remodelling [70,75,80]. Remodelling of the myocardium can further enhance inflammation and cardiac wall stress indicating that all of these concepts contribute to a vicious circle that may lead to heart failure and even death [53,55,59,72].

In African populations it has been shown that left ventricular structural changes or cardiac remodelling are associated with both inflammation and silent ischemia [48,87]. NT-proBNP has been shown to be increased in African men as opposed to it being the case in Caucasian men, although socio-economic status was not considered [65,66]. Significant positive associations between NT-proBNP and SBP, PP and CRP were also demonstrated in African men, but the relation between NT-proBNP and other inflammatory markers (IL-6 and TNF- α) still needs to be established in African populations [66]. To our knowledge, no published data regarding Trop T and its relation with inflammation in African populations exist. The relation between cardiac troponin, inflammation and cardiac remodelling, therefore, still needs to be established in South African individuals.

7. Aims and Objectives

The aim of this study was to determine differences in BP, cardiac remodelling, inflammation and cardiac troponins in a bi-ethnic sex cohort of South Africa. We will also assess whether relations exist between three known markers of BP, cardiac remodelling (NT-proBNP and LVH), inflammation (CRP, IL-6 and TNF- α), Trop T and silent ischemia.

The specific objectives were:

To determine whether inflammatory markers (CRP, IL-6 and TNF- α), Trop T, silent ischemia and NT-proBNP differ in a bi-ethnic sex cohort of South Africa.

To determine whether there are any associations between markers of BP, cardiac remodelling (NT-proBNP and LVH), inflammation (CRP, IL-6 and TNF- α), Trop T and silent ischemia, in a bi-ethnic sex cohort of South Africa.

8. Hypotheses

The levels of inflammatory markers, Trop T and NT-proBNP will be higher in the African cohort than in their Caucasian counterparts.

A positive association will be evident between the BP, cardiac remodelling (NT-proBNP and LVH) and inflammatory markers and Trop T in the African sex cohort.

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CHAPTER 3:
MANUSCRIPT

INSTRUCTIONS FOR AUTHORS



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Original Articles should report original research not previously published or being considered for publication elsewhere, meeting high standards of scientific integrity. There is no maximum word count.

Sections: Title page, Structured Abstract, Key words (3-6), Introduction, Methods, Results, Discussion, Acknowledgments, References. Type double-spaced.

Title Page: Not to exceed 25 words. The full list of authors and for each author a numbered footnote. The footnote should state the author's academic affiliation and the following statement of authorship: "This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation". Any author unable to make this statement must instead state their specific contribution to the manuscript. Corresponding author and contact details Acknowledgement of grant support. Any potential conflicts of interest, including related consultancies, shareholdings and funding grants. A list of up to 6 keywords

Abstract: No more than 250 words. The preferred subheadings are Background, Methods, Results and Conclusions.

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Methods: Should be sufficiently detailed so that readers and reviewers can understand precisely what has been done. Standard methods can be referenced. Manuscripts reporting data obtained from research conducted in human subjects must include a statement of

assurance in the Methods section of the manuscript that (1) informed consent was obtained from each patient and (2) the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. A Statistical Methods Section must be included where relevant.

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Study limitations: Must be included and should disclose any reasons the findings may not be applicable more broadly.

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Tables: Should be typed with double spacing and each should be on a separate sheet. They should be numbered consecutively with Arabic numerals, and contain only horizontal lines.



Abstract: 246
Text: 3205
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Systemic inflammation, cardiac troponin and arterial tone are associated with cardiac remodelling in African men: The SABPA study

Running head: Cardiac troponin, inflammation and end-organ damage in Africans

¹E Jansen van Vuren (BSc Hons)

^{1,2}L Malan (RN, PhD), ³R von Känel (MD), ⁴M Cockeran (MSc), ¹NT Malan (DSc)

¹Hypertension in Africa Research Team (HART), School for Physiology, Nutrition, and Consumer Science, North-West University, Potchefstroom, South Africa.

²Medical Research Council Research Unit for Hypertension and Cardiovascular Disease, North-West University, Potchefstroom, South Africa.

³Department of Neurology, Inselspital, Bern University Hospital, and University of Bern Switzerland

⁴Medicine Usage in South Africa (MUSA), North-West University, Potchefstroom, South Africa.

Corresponding author: Prof L Malan, RN, PhD

Hypertension in Africa Research Team (HART), Faculty of Health Sciences, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, 2520, South Africa

Tel +27 18 2992438; Fax +27 18 2991053; Email: Leone.Malan@nwu.ac.za

ABSTRACT

Background: Inflammation can lead to an increase in cardiac wall stress through multiple pathways related to cardiac remodelling. Cardiac remodelling is a compensatory mechanism characterised by myocyte hypertrophy, myocyte death and modifications of the extra-cellular matrix. We aimed to explore associations of inflammation and cardiac troponin with cardiac remodelling in a bi-ethnic sex cohort of South Africa.

Methods: We included 165 men (76 African and 89 Caucasian) and 174 women (80 African and 94 Caucasian) participants between 20 and 65 years of age. Biochemical analyses determined C-reactive protein (CRP), interleukin-6, tumour necrosis factor-alpha (TNF- α), troponin T (Trop T) as well as the N-terminal of pro B-type natriuretic peptide (NT-proBNP). Ambulatory 24-h blood pressure (BP), -ECG and 12 lead ECG measures were obtained for determining ischemic events (ST events) and left ventricular hypertrophy (LVH).

Results: Compared to Caucasians, more Africans were 24-h hypertensive (53.85% vs. 24.59%; $p < 0.001$) with more -ischemic events ($p = 0.006$). Africans showed greater inflammation (CRP, IL-6 and TNF- α), BP, Trop T and LVH values than their Caucasian counterparts. BP was associated ($p < 0.05$) with Trop T in all men. In African men, associations were evident between cardiac remodelling (NT-proBNP), TNF alpha ($p < 0.001$), Trop T ($p < 0.001$) and pulse pressure ($p = 0.006$), adjusted $R^2 = 0.45$.

Conclusions: A risk marker profile characterized by low-grade systemic inflammation, impaired arterial tone and increased cardiac troponin is suggested to be associated with cardiac remodelling in African men. Together with the resulting preload to the heart, these findings may increase the susceptibility for future cardiovascular events.

Keywords: Africans, inflammation, cardiac troponin, arterial tone, cardiac remodelling

INTRODUCTION

Low-grade inflammation in Africans has been shown to be involved in the pathogenesis and progression of structural wall remodelling [1]. Cytokines play a role in the inflammatory response by regulating haematopoiesis, immune- and vascular reactions [2,3]. A greater degree of endothelial dysfunction related to inflammation has been reported in African Americans [4]. One key pro-inflammatory cytokine in this process is tumor necrosis factor-alpha (TNF- α) [5]. Binding of TNF- α to its TNFR1 receptor may induce apoptosis in many cell types, including cardiac myocytes [6,7].

Troponin T (Trop T) is a subunit of the troponin complex released in response to myocyte necrosis [8-10]. Levels of Trop T were found to be increased in African American men. However, these associations could be explained by the independent associations found between Trop T and left ventricular hypertrophy (LVH) [11]. In African populations it was shown that left ventricular structural changes or cardiac remodelling are associated with both inflammation and silent ischemia [1,12]. A decrease in metabolic supply to the myocardial tissue may result in ischemia of the myocardium [13]. The persistent ischemia may result in irreversible cell death and migration of inflammatory cytokines to the ischemic myocardium.

Studies also showed that inflammation can lead to an increase in arterial stiffness, the main cause of an increased pulse pressure (PP) [14-15]. This increase in hemodynamic load accompanied by the structural changes of the left ventricle may lead to an increase in cardiac wall stress [8,16-17]. The N-terminal portion of pro-brain natriuretic peptide (NT-proBNP) is released in response to an increase in cardiac wall stress following myocyte stretch, hypoxia and neuro-hormonal activation [17-18]. NT-proBNP has been shown to be increased in African men as opposed to it being the case in Caucasian men, although to what extent socio-

economic status may account for this ethnic difference is unclear [19-20]. Significant positive associations between NT-proBNP and systolic blood pressure (SBP), pulse pressure (PP) and C-reactive protein (CRP) were also demonstrated in African men, but the relation between NT-proBNP and other inflammatory markers (IL-6 and TNF- α), still needs to be elucidated in African populations [20].

Hence the aim of this study was to determine whether associations exist between inflammation, cardiac troponin and -remodelling in a bi-ethnic sex cohort of South Africa. We therefore aimed at assessing whether relationships exist between three known markers of inflammation (CRP, IL-6 and TNF- α), Trop T and markers of cardiac remodelling (NT-proBNP and LVH) under well-controlled conditions in a bi-ethnic gender cohort.

METHODS

Study design and participant selection

In 2008 and 2009 (February to May), 409 urban Caucasian and African teachers (aged 20-65 years) were enrolled in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study. Teachers resided in the Dr Kenneth Kaunda Education District of the North West Province of South Africa. This selection was made to ensure that the participants were from a similar socio-economic class [21].

The exclusion criteria for the SABPA study was: pregnancy, lactation, users of α - and β -blockers or psychotropic substances, blood donors or vaccinations 3 months prior to clinical assessment and a tympanum temperature exceeding 37.5°C. Additional exclusions were made to avoid bias pertaining to cardio-metabolic and inflammatory risk and participants

with a HIV positive status (N=19), clinically diagnosed diabetes mellitus (N=10), anti-inflammatory medication usage (N=24), anti-coagulant medication usage (N=2), aspirin usage (N=11) and history of myocardial infarction or stroke (N=4) were excluded [22]. After these exclusions 165 men (76 African and 89 Caucasian) and 174 women (80 African and 94 Caucasian) participants remained.

Informed consent was obtained from all the participants prior to the commencement of the study. The Ethics Review Board of the North-West University, Potchefstroom Campus (NWU-00036-07-S6) gave ethical approval and the study also complied with the Declaration of Helsinki's ethical guidelines (revised in 2008) [23].

Experimental methods and data collection

Research procedure

Clinical assessments were obtained over 36 hours. The Cardiotens CE120® (Meditech, Budapest, Hungary) and accelerometers were applied to measure 24-hour ambulatory blood pressure (ABPM), 2-lead ECG as well as 24-h physical activity every working day between ~07:00 and 08:00. The correct cuff sizes were applied on the non-dominant arm of each participant and thereafter the participants carried on with their normal daily activities.

At approximately 16:30, the participants were transported to the Metabolic Unit Research Facility at the North-West University. The facility was well ventilated with a comfortable temperature and each participant received his/her own private bedroom. The following day's procedures and experimental setup were explained to all the participants. Demographic and General Health questionnaires were completed and each participant received a standardised

dinner. The participants were advised to fast and rest from 22:00 for the next day's clinical measurements.

At approximately 06:00 the next morning, the 24-hour ambulatory apparatuses were disconnected, whereby the anthropometric and clinical measurements commenced. All resting ECGs and blood sampling were done after the participants had been in a semi-recumbent position for approximately 30 minutes. On completion of all the assessments, the participants received breakfast, feedback and were then transported back to their respective schools.

Lifestyle determinants

The participant's daily physical activity was monitored over 24-hours with the Actical® activity monitor (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada). Anthropometric measurements were taken, according to standardized procedures, by registered level II anthropometrists in triplicate and the mean of the three measurements were used to ensure accuracy, and inter- and intra-observer variability was found to be less than 10%. The Mosteller formula of $[\text{weight (kg)} \times \text{height (cm)}]^{.5}$ was used to calculate the body surface area [24].

Biochemical measurements

Registered nurses obtained fasting blood samples from the ante-brachial vein with a sterile winged infusion set. These samples were stored at -80°C until analysis. Gamma-glutamyl transferase (γ GT), an indicator of alcohol abuse, and serum cotinine, an indicator of nicotine levels, were analysed with the enzyme rate method (Unicel DXC 800; Beckman and Coulter;

Germany) and homogeneous immunoassay on Modular ROCHE Automized, Switzerland, respectively [25-26].

Ultra-high-sensitivity CRP was measured with the turbidimetric method (Unicel DXC 800, Beckman and Coulter, Germany). Serum IL-6 and high sensitive TNF- α were analysed with the Human IL-6 Quantikine high sensitivity Enzyme-linked immunosorbent assay (HS ELISA; R&D Systems, Minneapolis, MN USA) and the Quantikine High Sensitivity Human Tumour TNF- α Enzyme linked immunosorbent assay (HS ELISA; R&D Systems, Minneapolis, MN USA), respectively. The inter- and intra-assay variability for TNF- α was 15% and 17.8% respectively. For IL-6, the intra-assay variability was (n=60) 7.4% and the inter-assay variability (n=7), 17.02%.

High sensitive Trop T and NT-proBNP were analysed with the Electrochemiluminescence immunoassay (ECLIA), Elecsys, 2010, Roche, Basel, Switzerland. In our sample, there were 91 (26.84%) undetectable Trop T values (<3 pg/ml), which were substituted using the method of Croghan C and P. P. Egeghy (2003) for lower than detectable values [27]. The inter- and intra-batch variability was 15% and 5.6% for Trop T, and 4.6% and 4.2% for NT-proBNP.

Cardiovascular assessment procedures

The 24-h BP was measured at 30-minute intervals from 08:00 to 22:00 and at 60-minute intervals from 22:00 to 06:00. The successful inflation rates were: African men (75.7%), Caucasian men (84.9%), African women (69.5%) and Caucasian women (85.8%). The funnel-shaped arms of African women posed a problem for 24-h BP recordings. The criteria for hypertension, as defined by the European Society of Hypertension (ESH), is an average 24-hour systolic blood pressure (SBP) of ≥ 130 mmHg and diastolic blood pressure (DBP) of

≥ 80 mmHg [22]. Silent ischemia (ambulatory ischemic events profile) was assessed with two-channel ECG recordings (pre-set program recording for 20 seconds at 5 minute intervals). An ischemic event was recorded according to the 1-1-1 rule/criteria: horizontal or descending ST-segment; depression by 1 mm; duration of the ST-segment episode lasts 1 minute, and there was a 1-minute interval from the preceding episodes [12,28]. The data was analysed with the CardioVisions 1.19 Personal Edition software (Meditech, Budapest, Hungary). Throughout the day the participants had to record any abnormalities they experienced on a 24-hour diary card. The abnormalities included visual disturbances, headaches, nausea, fainting, palpitations and stress. A resting 12-lead ECG was recorded with the Norav NHH-1200® ECG (NORAV medical LTD PC 1200, Israel, Software version 5.030) and determined the ECG left ventricular hypertrophy (Cornell product, $[RaVL+SV3] \times QRS$ duration). Values exceeding 244 mV.ms were indicative of LVH [29].

Statistical analyses

Statistical analyses were performed with Statistica version 12 (Statsoft Inc., Tulsa, OK, USA). Variables with skewed distributions (γ -GT and CRP) were log-transformed. Independent *t*-tests were used to compare baseline characteristics of the two ethnic groups. Chi-square tests (X^2) were used to determine prevalence as well as proportions. The *a priori* covariates used in all the statistical analyses were age, physical activity, body surface area, cotinine and γ -GT.

Single two-way general linear model interactions on main effects (ethnicity \times gender) were computed to determine differences for 24-h BP, cardiac remodelling, inflammation, cardiac troponin, silent ischemia, independent of *a priori* covariates in the bi-ethnic cohort. Subsequently one-way analysis of covariance (ANCOVA) was performed with view to

compare differences in ethnic-gender groups adjusting for *a priori* covariates. Univariate and multivariate linear regression analyses were performed. In forward stepwise regression analyses associations were determined in three separate models between dependent variables, 24-h BP, cardiac remodelling (NT-proBNP and ECG-LVH). Independent variables included inflammation (CRP/IL-6/TNF α), cardiac troponin, silent ischemia and *a priori* covariates. The only difference between the models was that in Model 1, TNF- α was used as independent variable and in Models 2 and 3, it was replaced by IL-6 and CRP respectively. Pulse pressure was added as independent variable in cardiac remodelling analyses. For all of the above-mentioned analyses, significant values were noted when adjusted $R^2 \geq 0.25$ and $p \leq 0.05$. The F to enter was set at 2.5.

RESULTS

The baseline characteristics and overall health profile of all African and Caucasian participants are demonstrated in Table 1. Africans revealed lower body surface area ($p=0.014$), physical activity ($p=0.002$) and higher γ GT levels than their Caucasian counterparts. The Africans also showed higher levels ($p<0.001$) of inflammatory markers (CRP and TNF- α), tympanum temperature, 24-h BP, Trop T and LVH values than their Caucasian counterparts. Compared to Caucasians, more Africans were hypertensive (53.85% vs. 24.59%; $p<0.001$) and revealed a greater number of ischemic events ($p=0.006$).

Significant interaction between main effects (ethnicity x gender) were evident for CRP [F(1,326), 5.43; $p=0.02$], 24-h SBP [F(1,326), 7.85; $p=0.005$], 24-h DBP [F(1, 326), 14.74; $p<0.001$] and 24-h ST events [F(1,326), 10.44; $p=0.001$], supporting the stratification into specific ethnic-gender groups for further analysis.

Table 1: Baseline characteristics (mean \pm SD) by ethnic status

Variables	Africans (N=156)	Caucasians (N=183)	p values
<i>Confounders</i>			
Age, years	44.33 \pm 8.28	44.46 \pm 11.10	0.907
Body surface area, m ²	1.92 \pm 0.23	1.99 \pm 0.29	0.014
Physical activity, kcal/day	2647.71 \pm 730.91	3096.67 \pm 1685.29	0.002
Cotinine, ng/mL	20.46 \pm 51.15	23.86 \pm 80.36	0.651
γ GT, U/L	62.54 \pm 75.03	24.36 \pm 23.73	<0.001
<i>Markers of Inflammation</i>			
C-reactive protein, mg/L	8.49 \pm 10.13	2.89 \pm 3.33	<0.001
IL-6, pg/mL	2.18 \pm 8.23	1.11 \pm 0.88	0.08
TNF- α , pg/mL	2.99 \pm 3.25	1.82 \pm 1.94	<0.001
<i>Cardiac troponins</i>			
Trop T, pg/mL	4.61 \pm 2.52	5.46 \pm 2.85	0.004
<i>Cardiovascular measures</i>			
Temperature tympanum, $^{\circ}$ C	36.62 \pm 0.41	36.47 \pm 0.33	<0.001
24-h SBP, mmHg	132 \pm 16	124 \pm 11	<0.001
24-h DBP, mmHg	83 \pm 11	76 \pm 8	<0.001
24-h PP, mmHg	49 \pm 9	47 \pm 7	0.03
24-h ST events, count	6 \pm 15	3 \pm 6	0.006
NT-proBNP, pg/mL	44.78 \pm 48.96	43.29 \pm 45.41	0.775
Cornell product >244.0 mV.ms (ECG-LVH)	69.52 \pm 41.38	49.66 \pm 28.49	<0.001
Hypertensive, N (%)	84 (53.85)	45 (24.59)	<0.001
<i>Medications</i>			
Hypercholesterolemia, N (%)	2 (1.28)	6 (3.28)	0.227
Hypertension, N (%)	52 (33.33)	16 (8.74)	<0.001

Data are presented as mean \pm SD or number of participants (%). Abbreviations: γ GT=Gamma glutamyl transferase, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, Trop T=Troponin T, NTproBNP=N-terminal pro-Brain natriuretic peptide, ST events=Number of ischemic events in 24-h, ECG-LVH=Left Ventricular Hypertrophy.

Figure 1 shows that African men had higher CRP ($p<0.001$), IL-6 ($p=0.01$), 24h BP ($p<0.001$) and LVH ($p=0.02$) levels as well as a greater number of ischemic events ($p=0.003$) than their Caucasian counterparts. As shown in Figure 2, there was a similar trend for African women with significantly higher CRP ($p<0.001$), albeit TNF- α ($p=0.02$) rather than IL-6 ($p=0.42$), 24h BP ($p<0.001$) and LVH ($p<0.001$) levels than in their Caucasian counterparts. No differences though existed between ST events occurrence in the two ethnic female groups.

The forward stepwise regression analyses revealed no significant associations in African and Caucasian women. Therefore only associations in men will be discussed. As shown in Table 2, BP was associated ($p<0.05$) with Trop T in all three the inflammatory models of African and Caucasian men. Positive associations were also demonstrated between cardiac remodelling (NT-proBNP) and Trop T ($p<0.001$) as well as between cardiac remodelling (NT-proBNP and LVH) and PP ($p<0.05$).

Model 1 revealed positive associations in African men, between cardiac remodelling (NT-proBNP) and TNF- α ($\beta=0.31$; 95% CI 0.14 to 0.48; $p<0.001$) and between 24-h SBP and ischemic events ($\beta=0.23$; 95% CI 0.01 to 0.46; $p=0.05$). In Model 2, a positive association was demonstrated in Caucasian men between 24-h DBP and IL-6 ($\beta=0.25$; 95% CI 0.06 to 0.44; $p=0.01$). Model 3 revealed positive associations were evident in African men between 24-h SBP and ischemic events ($\beta=0.23$; 95% CI 0.01 to 0.46; $p=0.05$) and between LVH and ischemic events ($\beta=0.24$; 95% CI 0.01 to 0.47; $p=0.05$). Positive associations between 24-h DBP and ischemic events ($\beta=0.19$; 95% CI 0.01 to 0.38; $p=0.04$) and between 24-h DBP and CRP ($\beta=0.26$; 95% CI 0.06 to 0.45; $p=0.01$).

Overall, 24-h BP was associated ($p < 0.05$) with Trop T in all men. In African men, associations were evident between cardiac remodelling (NT-proBNP), TNF alpha ($\beta = 0.31$; 95% CI 0.14 to 0.48; $p < 0.001$), Trop T ($\beta = 0.48$; 95% CI 0.28 to 0.67; $p < 0.001$) and pulse pressure ($\beta = 0.28$; 95% CI 0.09 to 0.48; $p = 0.006$).

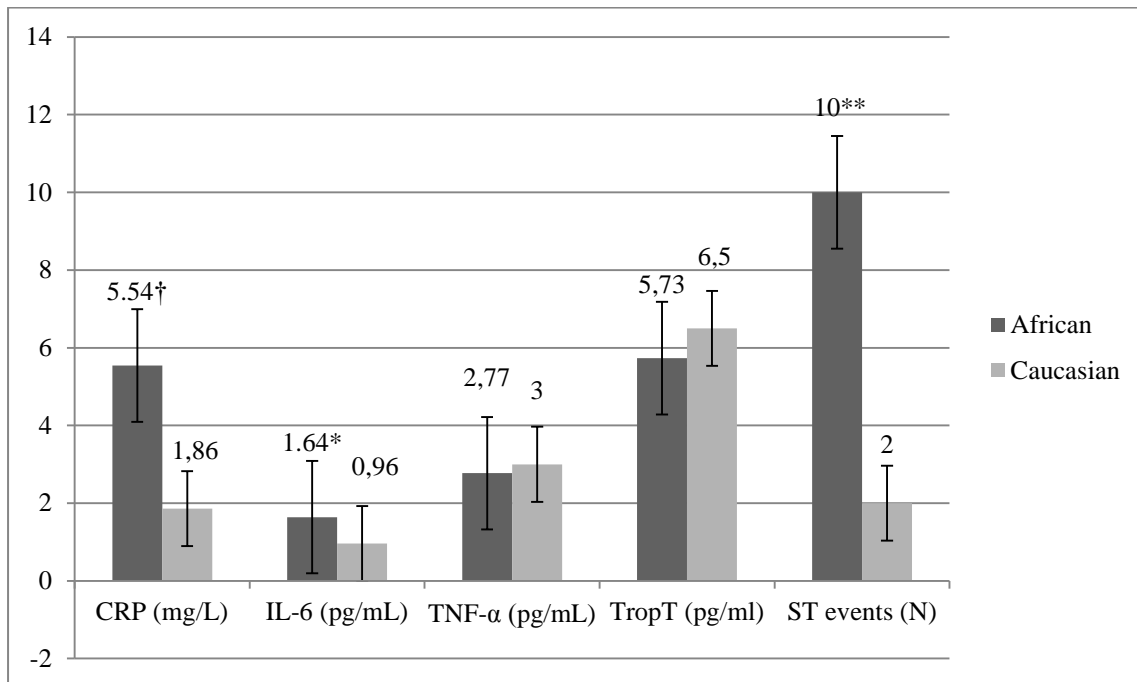
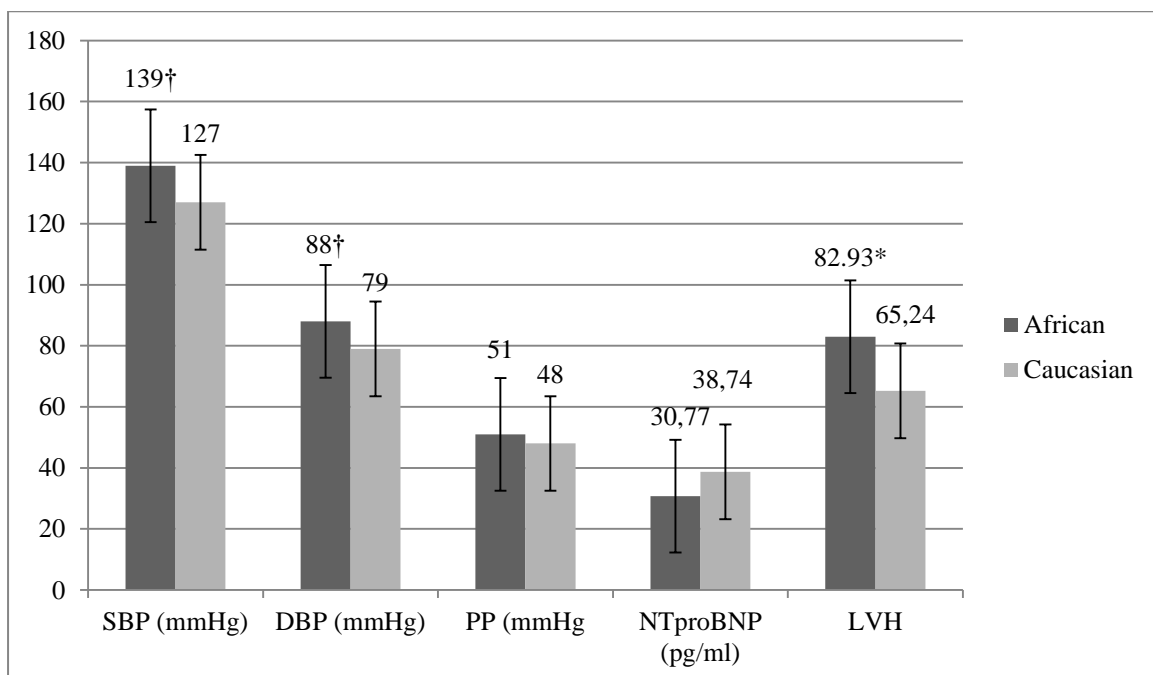
Figure 1a**Figure 1b**

Figure 1: Comparing adjusted differences in African vs. Caucasian men for inflammation, cardiac troponin and ischemic events (Fig 1a), blood pressure and cardiac remodelling (Fig 1b) values. Variables are adjusted age, body surface area, physical activity, log gamma glutamyl transferase (γ -GT) and cotinine. * $P \leq 0.05$; ** $P \leq 0.01$; † $P \leq 0.001$.

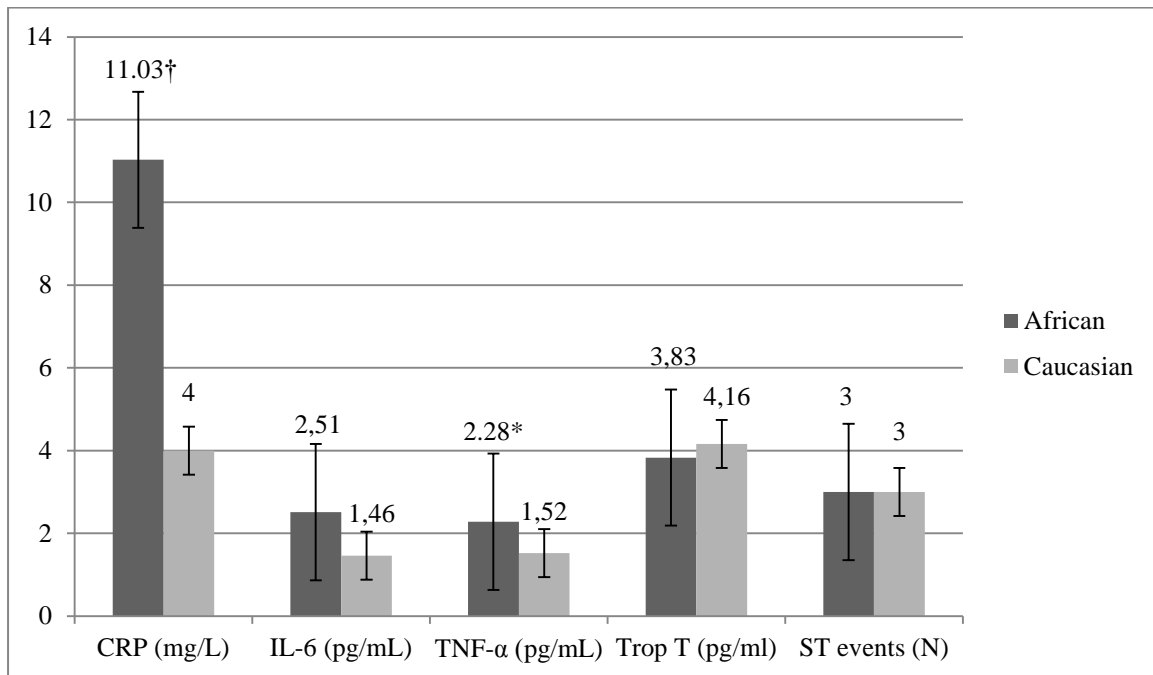
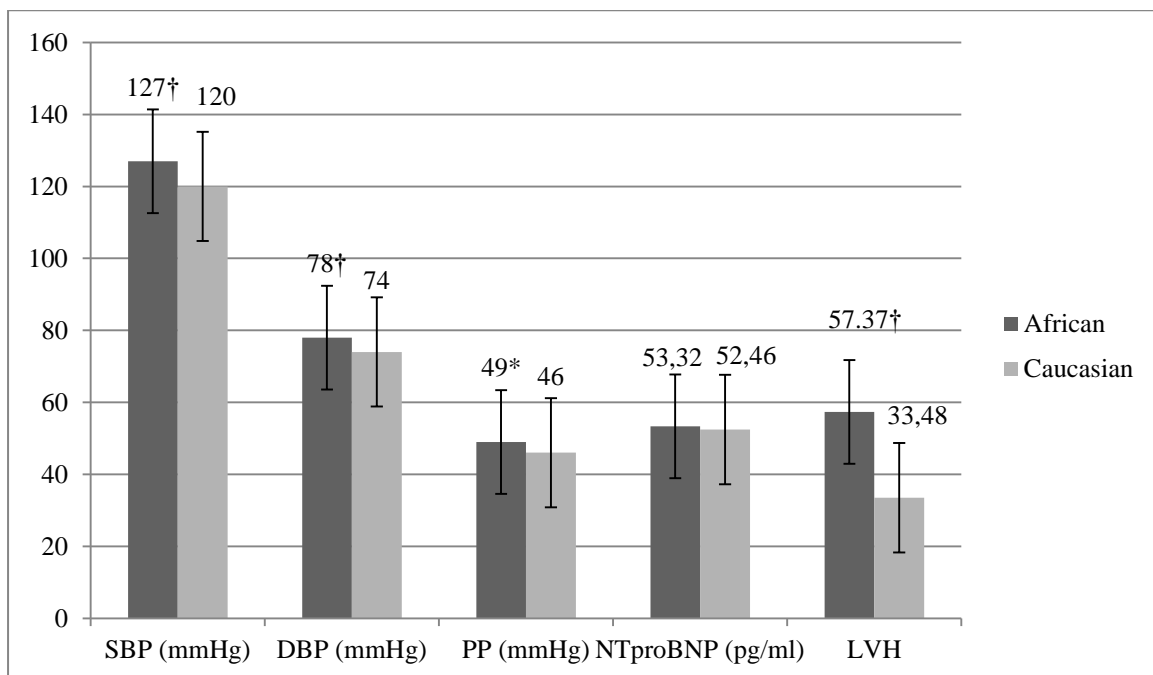
Figure 2a**Figure 2b**

Figure 2: Comparing adjusted differences in African vs. Caucasian women for inflammation, cardiac troponin and ischemic events (fig 2a), blood pressure and cardiac remodelling (fig 2b) values. Variables are adjusted for age, body surface area, physical activity, log gamma glutamyl transferase (γ -GT) and cotinine. * $P \leq 0.05$; ** $P \leq 0.01$; † $P \leq 0.001$.

Table 2: Independent associations between BP, cardiac remodelling (NT-ProBNP and ECG Left ventricular hypertrophy) and cardiovascular risk markers in African and Caucasian men.

	African men (N=76)				Caucasian men (N=89)			
	SBP β (95% CI)	DBP β (95% CI)	NT-proBNP β (95% CI)	LVH β (95% CI)	SBP β (95% CI)	DBP β (95% CI)	NT-proBNP β (95% CI)	LVH β (95% CI)
<i>Model 1</i>								
Adjusted R ²	0.35	0.24	0.45	0.23	0.23	0.24	0.09	0.07
TNF-alpha	NS	NS	0.31† (0.14;0.48)	NS	NS	NS	NS	NS
Trop T	0.25* (0.01;0.48)	0.26* (0.05;0.47)	0.48† (0.28;0.67)	NS	0.23* (0.04;0.41)	0.22* (0.03;0.41)	NS	NS
ST events	0.23* (0.01;0.46)	NS	NS	NS	NS	NS	NS	NS
PP	-	-	0.28** (0.09;0.48)	0.36** (0.11;0.61)	-	-	NS	NS
<i>Model 2</i>								
Adjusted R ²	0.36	0.24	0.41	0.28	0.23	0.27	0.09	0.08
IL-6	NS	NS	NS	-0.25* (- 0.46;-0.04)	NS	0.25* (0.06;0.44)	NS	NS
Trop T	0.29* (0.06;0.52)	0.26* (0.05;0.47)	0.41† (0.21;0.62)	NS	0.23* (0.04;0.41)	0.22* (0.03;0.40)	NS	NS
ST events	NS	NS	NS	NS	NS	NS	NS	NS
PP	-	-	0.25* (0.04;0.46)	0.39** (0.14;0.64)	-	-	NS	NS
<i>Model 3</i>								
Adjusted R ²	0.35	0.24	0.41	0.30	0.23	0.28	0.09	0.07
log-CRP	NS	NS	NS	-0.32** (- 0.54;-0.10)	NS	0.26* (0.06;0.45)	NS	NS
Trop T	0.25* (0.01;0.48)	0.26* (0.05;0.47)	0.42† (0.22;0.62)	NS	0.23* (0.04;0.41)	0.21* (0.03;0.39)	NS	NS
ST events	0.23* (0.01;0.46)	NS	NS	0.24* (0.01;0.47)	NS	0.19* (0.01;0.38)	NS	NS
PP	-	-	0.25* (0.05;0.46)	0.48† (0.24;0.72)	-	NS	NS	NS

Abbreviations: TNF- α , tumour necrosis factor-alpha; IL-6, interleukin-6; CRP, C-reactive protein; Trop T, troponin T; ST events, number of ischemic events in 24-h; PP, pulse pressure; LVH, left ventricular hypertrophy; NTproBNP, N-terminal pro brain natriuretic peptide; SBP, systolic blood pressure; DBP, diastolic blood pressure. Independent variables included age, body surface area, physical activity, log γ -GT and cotinine. *P \leq 0.05; **P \leq 0.01; †P \leq 0.001.

DISCUSSION

The main aim of our study was to determine whether inflammation and cardiac troponin are associated with cardiac remodelling in a bi-ethnic sex cohort of South Africa. A significant finding was the associations of cardiac remodelling (NT-proBNP) with inflammation (TNF- α), Trop T and PP in African men.

Cardiac remodelling marker, NT-proBNP, is a natriuretic peptide produced by the cardiac myocytes and fibroblasts in response to cardiac wall stress [17-18,30]. Inflammation can lead to an increase in cardiac wall stress through multiple pathways related to myocardial remodelling [1,12,17]. The inflammatory marker, TNF- α , not only contributes to apoptosis, but may also lead to endothelial dysfunction by enhancing NO inactivity [31-34]. Indeed, a greater degree of endothelial dysfunction was observed in African Americans [4,35]. The resulting decrease in vasodilation contributes to excessive vasoconstriction that leads to an increased afterload on the myocardium, a potent stimulus for cardiac remodelling [8]. Ambulatory SBP, as well as PP, were previously shown to be associated with cardiac wall remodelling in 75 African men with low grade inflammatory status (> 3 mg/L hs-CRP) [1]. Here, hyperdynamic BP and inflammation acted in tandem as possible promoting factors to structural wall abnormalities.

The changes that may contribute to an increase in cardiac wall stress not only include myocyte hypertrophy, but also myocyte death and modifications of the ECM, which explains the association found between NT-proBNP and Trop T in the African men [8,36-37]. Myocyte necrosis is the passive unregulated process of cell death that occurs as a result of cardiac injury and leads to the release of Trop T due to the loss of cell membrane integrity and the degradation of the contractile apparatus [8-10]. Trop T elevations were previously

associated with African ethnicity in Americans [11]. Although our study revealed no differences with regard to Trop T levels between African and Caucasian individuals, we showed that Trop T was associated with BP in African and Caucasian men. The association between SBP and ischemic events in African men can thus explain how a decrease in metabolic supply to the myocardial tissue due to hypertension may result in hypoxia of the myocardium [13]. Persistent ischemia may result in irreversible cell death leading to the release of Trop T. Indeed, Malan et al showed that silent ischemia and LVH were facilitated by vascular responsiveness in African men [12]. The sympatho-excitatory effects of indirect markers of sympathetic nervous system activity such as increased heart rate, 24-h silent ischemic events and chronic hyperglycaemia, as was shown in other SABPA sub-studies in the African cohort, could thus potentiate adrenergic overdrive reinforcing metabolic overdrive and structural alterations [38-40]. The contribution of inflammation in the pathogenesis of endothelial dysfunction can also decrease blood flow that further contributes to the development of ischemia and its involvement in cardiac cell death and thus cardiac remodelling [3,31,41].

The higher inflammatory profile has been reported in our African cohort before [1,42]. PP, an indicator of arterial stiffness, seems to be associated with inflammation, as it may increase the vascular wall permeability [43]. Studies have also shown that inflammation can lead to an increase in arterial stiffness [14-15]. The resulting pressure and volume overload placed on the heart have been identified as the main determinants of cardiac stretch, stimulating the development of cardiac remodelling [44]. Multiple mechanisms have been described on how inflammation can alter arterial stiffness. The pro-inflammatory cytokine, IL-6, can influence the coagulation cascade by increasing tissue- and von Willebrand factor production that may lead to an increase in collagen formation [45]. Indeed higher resting levels of von Willebrand

factor have been shown in Africans compared to Caucasians supporting a mechanisms by which thrombus formation could be promoted through the action of IL-6 on haemostasis [32,45-46]. TNF- α seems to induce the production of reactive oxygen species resulting in oxidative stress that may mediate the production of various adhesion molecules [2,47]. In Africans, depressed heart rate variability was shown, supporting the possibility of increased oxidative stress in our cohort [38]. Indeed, oxidative stress via glutathione levels was related to subclinical atherosclerosis in Africans [48]. This process contributes to the structural changes in the arterial wall that may contribute to arterial stiffness and increases in the hemodynamic load on the myocardium.

Multiple studies have shown that levels of NT-proBNP are higher in African men than in Caucasian men [19-20]. However, these studies did not consider socio-economic status (SES). In our study of African and Caucasian men of similar SES, we could not replicate this finding. This may suggest chronic activation of the sympathetic nervous system in individuals exposed to over-demanding situation, which could be one explanation for the previously observed ethnic difference in NT-proBNP. Despite the fact that BP and LVH were increased in African women, when compared to their Caucasian counterparts, we did not find significant associations of cardiac troponin and inflammatory measures with cardiac remodelling in the female cohort. As a proinflammatory profile was observed in both men and women of African ethnicity, there is no intuitive explanation for why there were no significant associations in the female cohort. One possibility could be the higher BP observed in the African men when compared to the women. The men also consumed significantly higher amounts of alcohol, as indicated by their γ GT values, than their female counterparts which may alter vasoconstrictiveness and subclinical vascular remodelling [38,42].

Our study has several limitations, foremost the cross-sectional design as only follow-up data could provide insight into the progression of end organ damage and the influence inflammation may have. This study only represents a cohort of the South African population and, therefore, the findings may not be applicable to the entire South African population. The observed significant associations nevertheless provide valuable information in terms of the increased cardiovascular disease risk of African men. TNF- α can be released by astrocytes, microglia and various central nervous system neurons. It has also been shown that TNF- α may induce the production of brain-derived neurotrophic factor (BDNF) which emphasises the addition of a neurotrophic marker when assessing TNF- α as an inflammatory marker [49].

In conclusion, we demonstrated an association of inflammation and cardiac troponin with cardiac remodelling in African men. Related risk markers of chronic low-grade systemic inflammation, arterial tone and cardiac troponin may further underscore the possibility of impeded myocardial oxygen supply in African men. The resulting preload to the heart and the risk marker profile may increase the susceptibility for cardiac remodelling and future cardiovascular events in the black South African population.

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CONFLICT OF INTEREST

The authors declare no conflict of interest involving the content of this article.

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CHAPTER 4:
SUMMARY, CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

3.1 Introduction

This chapter provides a brief overview of the main aims, findings and conclusions regarding the manuscript in chapter three, titled *Systemic inflammation, cardiac troponin and arterial tone associated with cardiac remodelling: The SABPA study*. It also includes the main limitations of the study as well as recommendations for future research regarding the topic.

3.2 Summary and conclusions

The aim of this study was to determine whether associations exist between inflammation, cardiac troponin and cardiac remodelling in a bi-ethnic sex cohort of South Africa. Two main hypotheses were developed for the study. Firstly: levels of inflammatory markers (CRP, IL-6 and TNF- α), Trop T and NT-proBNP will be higher in the African cohort than in their Caucasian counterparts. Secondly: a positive association will be evident between the inflammatory markers (CRP, IL-6 and TNF- α), Trop T and markers of cardiac remodelling (NT-proBNP and LVH) in the African sex cohort.

Our results revealed that, only CRP and TNF- α , but neither IL-6 nor Trop T nor NT-proBNP were higher in Africans than those of Caucasians. After adjustments were made for *a priori* covariates [age, body surface area, physical activity, log gamma glutamyl transferase (γ -GT) and cotinine], African men still revealed higher levels of CRP and IL-6 than did their Caucasian counterparts, but TNF- α differences between ethnic groups lost significance. African women revealed a more or less similar pattern with higher levels of CRP and TNF- α to those of their Caucasian counterparts. Levels of IL-6, Trop T and NT-proBNP did not differ significantly in the female cohort.

The differences concerning these markers of inflammation have been observed between Africans and Caucasians in the literature. Tolmay et al showed that CRP levels were higher in African than in Caucasian women of South Africa [1]. The same trend was reported in South African men [2-4]. Our own findings support the overall increased inflammatory profile that has been observed in African men [2]. Our study further revealed a positive association between NT-proBNP and TNF- α in African men. Studies by Kruger et al added that NT-proBNP levels were significantly higher in African men than in Caucasian men [3-5]. Our study could not replicate these findings regarding NT-proBNP in the entire cohort or in the male cohort. The fact that socio-economic status was not considered by Kruger et al (2013) might explain the lack of similarity in findings. To our knowledge no published data on levels of Trop T in South African populations currently exist, but a study done by Wallace et al revealed that Trop T elevations were associated with African ethnicity in Americans [6]. However, our study revealed no differences with regard to Trop T levels between African and Caucasian individuals. The higher inflammatory profile observed in African men and women despite no differences with regard to Trop T and NT-proBNP levels allow us to partially accept our first hypothesis.

It was shown that inflammation is involved in the pathogenesis of atherosclerosis and hypertension [7-10]. Atherosclerosis and hypertension may lead to an increased hemodynamic burden, which may initiate remodelling of the myocardium in an attempt to normalise this increased load [11]. With cardiac remodelling the cardiac myocytes are stretched, which is the stimulus for NT-proBNP production [12,13]. TNF- α may also induce cardiac remodelling through mechanisms pertaining to apoptosis, since myocyte death is one of the changes that occur during cardiac remodelling [11,14,15].

In our sub study we also showed that a positive association is present between NT-proBNP and Trop T. As mentioned, myocyte death is one of the changes that occur during cardiac remodelling. Myocyte stretch and thus an increase in cardiac wall stress may therefore be a stimulus for myocyte necrosis, which stimulates the release of Trop T [16,17]. This process is initiated by a complex signalling cascade [18]. Various autocrine and paracrine mechanisms can mediate these processes including TNF- α and Angiotensin II. The mechanisms include an increased production in reactive oxygen species, protein kinases, phospholipases and nuclear factor kappa B (NF- κ B). Activation of the mitogen-activated protein kinase (MAPK) pathway leading to transcription activity may also be an underlying mechanism [18].

The positive associations observed between NT-proBNP and TNF- α as well as between NT-proBNP and Trop T allow us to accept our second hypothesis. The mechanisms pertaining to inflammation, myocyte necrosis and cardiac wall stress are complex and intertwined. An inflammatory response may induce myocyte necrosis and -stretch through distinct mechanisms. Myocyte necrosis can lead to cardiac remodelling, increasing the cardiac wall stress. However, an increase in cardiac wall stress might also lead to myocyte necrosis. Remodelling of the myocardium can further exacerbate the inflammatory cascade. In the end a vicious cycle develops of mechanisms that may lead to the development of cardiovascular diseases and even death.

3.3 Chance and confounding

The element of chance should always be considered when interpreting the results of this study. Although this study included a bi-ethnic sex cohort from South Africa, the results cannot be seen as a true representation of the entire South African population. However, this study was well-designed and was conducted under strict protocols. The study population was

also selected from similar socio-economic class in an attempt to exclude the effect of socio-economic risk factors. If chance is present in this study, the authors would therefore not be aware thereof.

The confounders in this study included age, body surface area, physical activity, log gamma-glutamyl transferase and cotinine. The possible influence of the confounders in the associations between inflammation, cardiac troponin and end-organ damage was kept to the minimum, with adjustments made where necessary in the statistical analyses. Understanding the physiological mechanisms is therefore crucial when interpreting the associations observed in this study.

3.4 Limitations

- The cross-sectional study design: This study only represents a cohort of the South African population at a specific time period. Therefore, the findings may not be applicable to the entire South African population, but the associations demonstrated were significant and still provide valuable information in terms of the increased cardiovascular disease risk of African men.
- The absence of sympathetic activity or neurotrophin markers when evaluating TNF- α and as a low-grade inflammatory marker: TNF- α is mainly released by astrocytes, microglia and other central nervous system neurons and may induce the production of brain-derived neurotrophic factor (BDNF) [19].

3.5 Recommendations

- ❖ The use of follow-up data could provide valuable insights into the progression of end-organ damage and the influence inflammation may have in the course of time in a prospective survey.
- ❖ The addition of a neurotrophin marker, such as brain-derived neurotrophic factor (BDNF), may reveal the true extent of the influence of TNF- α on end-organ damage.

3.6 Conclusion

To conclude, related risk markers, i.e. systemic inflammation, arterial tone and cardiac troponin may underscore the possibility of impeded myocardial oxygen consumption in African men. A resulting preload to the heart and their risk markers profile increase susceptibility to cardiac remodelling and future cardiovascular events.

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APPENDICES

Appendix A: Ethical approval for the SABPA study



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: <http://www.nwu.ac.za>

Dr L Malan

Ethics Committee

Tel +27 18 299 2542
Fax +27 18 297 5308
Email Ethics@nwu.ac.za

Dear Dr Malan

6 February 2008

ETHICS APPROVAL OF PROJECT

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

Project title: SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans)	
Ethics number:	N W U - 0 0 0 3 6 - 0 7 - S 6
<small>Institution</small>	<small>Project Number</small>
<small>Status: S = Submission R = Re-Submission P = Provisional Authorisation A = Authorisation</small>	<small>Year</small>
Approval date: 12 November 2007	Expiry date: 11 November 2012

Special conditions of the approval (if any): None

General conditions:

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

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

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

Yours sincerely

Prof M M J Lowes
(chair NWU Ethics Committee)

Appendix B: Ethical approval for the sub-study



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Private Bag X6001, Potchefstroom
South Africa 2520

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

Ethics Office
Tel: 018-299 2092
Fax: 018-299 2088
Email: Minnie.Greeff@nwu.ac.za

02 November 2015

Prof L Malan
Physiology

Dear Prof Malan

HREC APPROVAL OF YOUR APPLICATION

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

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

Project title: The association between cardiac troponin, inflammation and target organ damage: the SABPA study

Project leader/supervisor: Prof L Malan

Student: E Jansen van Vuren

Application type: Sub-study

Risk level descriptor: Minimal

You are kindly informed that at the meeting held on 11/06/2015 of the HREC, Faculty of Health Sciences, the aforementioned was approved.

The period of approval for this project is from 29/10/2015 to 30/11/2016.

After ethical review:

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

The HREC requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the protocol or other associated documentation must be submitted to the HREC prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form.

A progress report should be submitted within one year of approval of this study and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC must be notified if the study is temporarily suspended

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

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

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

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

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

Yours sincerely



Prof Minnie Greeff
HREC Chairperson

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2 November 2015

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Appendix C: Confirmation of the editing of the dissertation

11 November 2015

I, **Ms Cecilia van der Walt**, hereby confirm that I took care of the editing of the dissertation of **Ms E Jansen van Vuren** titled *The Association between Cardiac Troponin Inflammation and Target-Organ Damages: The SABPA study*.

MS CECILIA VAN DER WALT

BA (*Cum Laude*)

HOD (*Cum Laude*),

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

Plus Accreditation with SATI for Afrikaans and translation


Registration number with SATI: 1000228

Email address: ceciliavdw@lantic.net

Mobile: 072 616 4943

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Appendix E: Solemn declaration



SOLEMN DECLARATION

1 Solemn Declaration by student

I, **Esme Jansen van Vuren**

hereby declare that the thesis/dissertation/article entitled

The association between cardiac troponin, inflammation and target organ damage:
the SABPA study

which I herewith submit to the North-West University, Potchefstroom campus, in compliance/partial compliance with the requirements set for the **MSc Physiology** qualification, is my own work and has been language edited and has not been submitted to any other university.

I understand and accept that the copies submitted for examination are the property of the North-West University.

22820388

Student Signature

University number

Declaration of Commissioner of Oaths

Declared before me on this **12th** day of November 2015

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The undersigned hereby declares that:

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