Cardiac stress and cardiovascular risk markers: The SABPA study

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

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PREFACE

This thesis has been completed in fulfilment of the requirements for the degree *Philosophiae Doctor* in Physiology. It is presented in article-format as approved by the North-West University's guidelines for postgraduate studies and consists of six chapters:

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

Chapter two: Elaborates on the detail of the SABPA study recruitment protocol, methods of data collection and statistical analyses performed.

Chapter three: Presents the manuscript titled: Longitudinal changes of cardiac troponin and inflammation reflect progressive myocyte stretch and likelihood for hypertension in a Black male cohort: The SABPA study. This manuscript has been prepared in a format that meets the requirements of the peer-reviewed journal, Hypertension Research, in which the manuscript was accepted for publication.

Chapter four: Presents the manuscript titled: BDNF, cardiac stress and cognitive interference in Black men: The SABPA prospective study. This manuscript has been prepared in a format that meets the requirements of the peer-reviewed journal, European Journal of Clinical investigation, to which the manuscript was submitted for publication.

Chapter five: Presents the manuscript titled, Prospective associations between cardiac stress, glucose dysregulation and executive cognitive function in Black men: The SABPA study. This manuscript has been prepared in a format that meets the requirements of the peer-reviewed journal, Diabetes and Vascular Disease Research, in which the manuscript was accepted for publication.

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

Note: The relevant references are provided at the end of each chapter. The reference format of Chapters three to five was prepared according to the instructions for authors (summarised in each Chapter) of the journal chosen for each manuscript. Throughout the rest of the thesis, the *Vancouver* reference format was adapted to ensure uniformity.

AFFIRMATION BY THE AUTHORS

The researchers contributed to the study in the following manner:

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

Prof L Malan (RN, PhD) as principal investigator designed the SABPA study and was involved in the initial planning and collection of data. As promoter she made recommendations regarding the initial planning of the manuscripts as well as the statistical analyses, interpretation of the results and edited the writing of the manuscripts and thesis.

Prof R von Känel (MD) as a co-promoter made recommendations and edited the writing of the manuscripts and thesis.

Prof NT Malan (DSc) as a co-promoter assisted in the design and data collection phases of the SABPA study, and edited writing of the manuscripts and literature thesis.

Dr L Lammertyn (PhD) as a co-promoter made recommendations and edited the writing of the manuscripts and thesis.

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

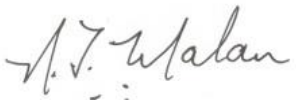
Prof. M. Magnusson (MD, PhD) as co-author gave input and edited the writing of final two manuscripts.

I, Esmé Jansen van Vuren, hereby declare that the statement above is a true representation of my actual contribution and I gave permission that the manuscripts in Chapters three to five be submitted for publication as part of the thesis for the degree Doctor of Philosophy 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 manuscripts in Chapters three to five be submitted for publication as part of the thesis for the degree Doctor of Philosophy in Physiology.

Prof L Malan



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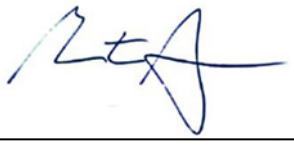

Prof M Magnusson

SUMMARY

Motivation

Pathological cardiac remodelling is a manifestation of end-organ damage and can be characterised by myocyte death and myocyte hypertrophy. An increased hemodynamic burden may follow the cardiac remodelling process leading to an increase in cardiac stress. It has been reported that the cardiac stress risk markers, cardiac troponin T (cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), may explain the increased susceptibility to subclinical vascular disease in African populations. Hence the interlinked associations of these cardiac stress risk markers with various other cardiovascular risk markers were investigated in a South African cohort. Indeed, a worsening of cardiovascular prognosis was found in South Africans individuals with the risk profile differing between Blacks and Whites. Compared to Whites of the *Sympathetic activity and Ambulatory Blood Pressure in Africans* (SABPA) study, Blacks were proven to reveal an increased cardio-metabolic vulnerability that related to subclinical wall remodelling, as well as an increased risk for silent myocardial ischemia, coupled with compensatory inflammatory and blood pressure (BP) increases. The possible increased hemodynamic burden may further interfere with normal neurotrophin (brain-derived neurotrophic factor, BDNF) and glucose homeostasis. Maintenance of BDNF and glucose is not only needed for cardiovascular health, but also for optimal brain health and executive cognitive functioning.

Aims

The main aim of this thesis was to determine whether cardiac stress changed in a bi-ethnic gender cohort over a period of three years and to determine whether cardiac stress risk markers associate with other cardiovascular risk markers, including inflammation (Manuscript 1), BP, left ventricular hypertrophy (LVH), BDNF (Manuscript 2), executive cognitive function and glucose dysregulation (Manuscript 3) over a follow-up period.

Methods

This prospective study is embedded within the SABPA cohort study that was conducted in 2008/2009 (baseline) and again in 2011/2012 (follow-up). Urban Black and White teachers (N=409, aged between 20 and 63 years), that resided in the Dr Kenneth Kaunda Education District of the North West Province of South Africa were enrolled at baseline. At follow-up, 359 Black and White participants were included, with reasons for non-participation being pregnancy, lactation, deceased, having moved too far away from the data collection site or having chosen not to participate. For purposes of this thesis we included individuals who participated in both phases of the study and additionally excluded participants with an HIV positive status to avoid bias pertaining to cardio-metabolic risk. Therefore we included 152 Black and 186 White participants in this study.

A well-controlled protocol was followed to obtain the various measurements in accordance with standardised procedures. Data were obtained concerning lifestyle factors (alcohol use, smoking status and physical activity), cardiovascular assessments (24-hour ambulatory blood pressure and electrocardiogram (ECG)-LVH and biochemical analyses of cTnT, NT-proBNP, C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), BDNF, glycated haemoglobin (HbA1c) and the homeostatic model assessment-insulin resistance (HOMA-IR). The STROOP-color-word conflict test (STROOP-CWT) was applied to assess response inhibition capacity of executive cognitive function.

Hypotheses were tested by computing general linear models with interactions of main effects (ethnicity x gender) for cardiac stress and all cardiovascular risk markers. Statistical analyses comprised independent *t*-tests and Chi-square tests, which were used to compare baseline characteristics of the ethnic groups and prevalence as well as proportions at baseline.

Differences over time (Δ) in each ethnic cohort were calculated via dependent *t*-tests and one-way covariance analyses. Multivariate linear regression analyses and logistic regression analyses were performed to determine associations between main variables.

Results

Interactions between main effects (ethnicity × gender) were revealed for ΔBDNF [F(1,309), 9.86, p=0.002], ΔTNF-α [F(1,323), 4.91, p=0.03], STROOP-CWT [F(1,324), 97.20, p<0.001], [F(1,324), 21.73, p<0.001] and ΔHOMA-IR [F(1,320), 14.28, p<0.001], [F(1,320), 3.99, p=0.046]. Furthermore interactions between main effects (ethnicity) were shown for ΔNT-proBNP [F(1,306), 5.74, p=0.02] that motivated further stratification into ethnic-gender groups.

At baseline, no differences were observed between Blacks and Whites concerning the cardiac stress risk markers, cTnT and NT-proBNP. NT-proBNP significantly increased in Blacks and Whites over the three-year period with cTnT remaining constantly high (≥4.2ng/L) over the three-year follow-up period.

Pertaining to the cardiovascular risk markers, more Blacks were hypertensive with higher inflammation, HbA1c and insulin levels than were Whites at baseline. Over the three-year follow-up period BDNF and systolic blood pressure (SBP) increased while TNF-α and HOMA-IR decreased in Blacks.

In contrast, Whites revealed higher cTnT and BDNF levels with a higher STROOP-CWT score than Blacks at baseline. Over the three-year follow-up period, TNF-α increased while cTnT, diastolic blood pressure (DBP) and HOMA-IR decreased in Whites.

In Black men, NT-proBNP and BDNF showed increases, TNF-α decreased, with other markers remaining constant over the three-year period. No significant associations emerged in Black women.

Chronic increased cTnT levels were positively associated with increased ΔNT-proBNP and with decreases in ΔTNF-α (Manuscript 1) in Black men only. In these men, ΔNT-proBNP increased the likelihood of 24-hour hypertension (Manuscript 1). Furthermore, ΔBDNF increased the likelihood of cTnT levels being lower than 4.2ng/L (Manuscript 2). Again in Black men, Hyperglycaemia (HbA1c≥5.7%) was positively associated with moderate IR (HOMA-IR>3) and with increases in ΔNT-proBNP (Manuscript 3). Lastly, baseline STROOP-CWT score was inversely associated with chronic higher cTnT (Manuscript 2) and moderate IR levels (Manuscript 3).

Conclusion

Myocyte injury, hyperglycaemia and insulin resistance were accompanied by progressive myocardial stretch in Black men that may be reflective of cardiac metabolic over-demand increasing the likelihood of hypertension and ischemic heart disease risk. However, central neural control mechanisms potentially may have upregulated BDNF and down-regulated TNF-α in these men as a way of protecting against these demands and of improving processes related to interference control.

Key words: cardiac stress, cardiac troponin T, NT-proBNP, inflammation, hypertension, BDNF, cognitive interference, hyperglycaemia, insulin resistance, cardio-metabolic risk.

OPSOMMING

Motivering

Patologies kardiale hermodellering is ’n manifestasie van eindorgaan-skade en kan gekenmerk word deur miosietsterfte en miosiethipertrofie. ’n Verhoogde hemodinamiese las kan na die kardiale hermodelleringsproses volg en tot ’n toename in kardiale stres lei. Daar is gerapporteer dat die kardiale stres risikomerker, kardiale troponien T (kTnT) en N-terminale pro-B-tipe natriuretiesc peptide (NT-proBNP), die verhoogde vatbaarheid vir subkliniese vaskulêre siekte in Afrikabevolkings kan verklaar. Daarom is die onderlinge verbande tussen hierdie kardiale stres risikomerkers met verskeie ander kardiovaskulêre risikomerkers in ’n Suid-Afrikaanse kohort ondersoek. Inderdaad is ’n verergering van kardiovaskulêre prognoses in Suid-Afrikaanse individue gevind met die risikoprofiel wat tussen Swartes en Wittes verskil. Vergeleke met Wittes van die *Sympathetic activity and Ambulatory Blood Pressure in Africans*- (SABPA) studie, is bewys dat Swartes ’n verhoogde kardiometaboliese vatbaarheid toon wat verband gehou het met subkliniese wand-hermodellering, asook ’n verhoogde risiko vir stil hartspier iskemie, gepaard met kompensatoriese inflammatoriese en bloeddruk- (BD) verhogings. Die moontlike verhoogde hemodinamiese las kan moontlik verder met normale neurotrofien (brein-afkomstige neurotrofien-faktor, BANF) en glukose-homeostase inmeng. Onderhoud van BANF en glukose in nie alleen vir kardiovaskulêre gesondheid nodig nie, maar ook vir optimale breingesondheid en uitvoerende kognitiewe funksionering.

Doelstellings

Die hoofdoel van hierdie proefskrif was om vas te stel of kardiale stres in ’n bi-etniese gelagskohort oor ’n periode van drie jaar heen verander het, en om te bepaal of kardiale stres risikomerkers oor ’n opvolgperiode heen in verband gebring kan word met ander

kardiovaskulêre risikomerkers, inbegrepe inflammasie (Manuskrip 1), BD, linkerventrikulêre hipertrofie (LVH), BANF (Manuskrip 2), uitvoerende kognitiewe funksie en glukose-disregulering (Manuskrip 3).

Metodes

Hierdie voorgenome studie is ingebed in die SABPA kohort-studie wat in 2008/2009 (basislyn) uitgevoer is en weer in 2011/2012 (opvolg). Stedelike Swart en Wit onderwysers (N=409, tussen die ouderdomme 20 en 63 jaar), wat in die Dr. Kenneth Kaunda Onderwysdistrik van die Noordwes Provinsie van Suid-Afrika woonagtig was, is op basislyn gewerf. Met die opvolg is 359 Swart en Wit deelnemers ingesluit, met redes vir nie-deelname nie synde swangerskap, laktasie, oorlede, na plekke ver weg van die data-insamelingsgebied verhuis, of verkies het om nie deel te neem nie. Vir doeleindes van hierdie proefskrif het ons individue ingesluit wat in beide fases van die studie deelgeneem het en het bykomstig ook deelnemers met 'n HIV positiefstatus uitgesluit om vooroordeel met betrekking tot kardiometaboliese risiko te voorkom. Ons het dus 152 Swart and 186 Wit deelnemers ingesluit in hierdie studie.

'n Goedbeheerde protokol is gevolg om die onderskeie metings ooreenkomstig gestandaardiseerde prosedures te bekom. Data is bekom met betrekking tot leefstylfaktore (alkoholgebruik, rookstatus en fisiese aktiwiteit), kardiovaskulêre assesserings (24-uur bloeddruk en elektrokardiogram (EKG)-LVH) en biochemiese analises van kTnT, NT-proBNP, C reaktiewe proteïen (CRP), tumornekrose faktor-alfa (TNF-α), BANF, gegliseerde hemoglobien (HbA1c) en die hemostatiese model assesseringsinsulien-weerstand- (HOMA-IW). Die Stroop-toets (STROOP-color-word conflict test [STROOP-CWT]) is toegepas om responsinhiberingskapasiteit van uitvoerende kognitiewe funksie te assesseer.

Hipoteses is getoets deur algemene lineêre modelle met interaksies van hoofeffekte (etnisiteit x geslag) te bereken vir kardiale stres en alle kardiovaskulêre risikomerkers. Statistiese analises het bestaan uit onafhanklike t-toetse en Chi-kwadraattoetse, wat gebruik is om basislyn-kenmerke van die etniese groepe en voorkomssyfer asook proporsies op basislyn te vergelyk. Verskille oor tyd heen (Δ) in elke etniese kohort is bereken via afhanklike t-toetse en eenrigting-kovariansieanalises. Meerveranderlike lineêre regressie-analises en logistieke regressie-analises is uitgevoer om verbande tussen hoofveranderlikes te bepaal.

Resultate

Interaksies tussen hoofeffekte (etnisiteit × geslag) is aan die lig gebring vir ΔBANF [F(1,309), 9.86, p=0.002], ΔTNF-α [F(1,323), 4.91, p=0.03], STROOP-CWT [F(1,324), 97.20, p<0.001], [F(1,324), 21.73, p<0.001] en HOMA-IR [F(1,320), 14.28, p<0.001], [F(1,320), 3.99, p=0.046]. Voorts is interaksies tussen hoofeffekte (etnisiteit) aangedui vir ΔNT-proBNP [F(1,306), 5.74, p=0.02] wat verdere stratifikasie in etnies-geslagsgroepe gemotiveer het.

Op basislyn is geen verskille tussen Swartes en Wittes opgemerk wat betref die kardiale stres risikomerkers, kTnT en NT-proBNP nie. NT-proBNP het betekenisvol in Swartes en Wittes toegeneem oor die drie-jaarperiode heen met kTnT wat konstant hoog gebly het (≥4.2ng/L) oor die drie-jaar opvolgperiode heen. Met betrekking tot die kardiovaskulêre risikomerkers was meer Swartes hipertensief met hoër inflammasie, HbA1c en insulienvlakke by basislyn as wat die geval met Wittes was. Oor die drie-jaar opvolgperiode het BANF en sistoliese bloeddruk (SBD) verhoog terwyl TNF-α en HOMA-IR in Swartes gedaal het. Hierteenoor, het Wittes hoër kTnT- en BANF-vlakke getoon met ’n hoër STROOP-CWT-telling as Swartes by basislyn. Oor die drie-jaar opvolgperiode het TNF-α toegeneem terwyl kTnT,

diastoliese bloeddruk (DBD) en HOMA-IR in Wittes gedaal het. In Swart mans het NT-proBNP en BANF toenames getoon, TNF-α het gedaal, met ander merkers wat konstant gebly het oor die drie-jaarperiode heen. Geen betekenisvolle assosiasies het in Swart vroue voorgekom nie.

In slegs Swart mans is kronies verhoogde cTnT-vlakke positief met verhoogde ΔNT-proBNP geassosieer en met afname in ΔTNF-α (Manuskrip 1). In hierdie mans het ΔNT-proBNP die waarskynlikheid van 24-uur hipertensie verhoog (Manuskrip 1). Verder het ΔBANF die waarskynlikheid dat kTnT-vlakke laer as 4.2ng/L sou wees, verhoog (Manuskrip 2). Weereens, in Swart mans is Hiperglikemie (HbA1c≥5.7%) positief met matige IR (HOMA-IR>3) geassosieer en met toenames in ΔNT-proBNP (Manuskrip 3). Laastens is basislyn STROOP-CWT-telling omgekeerd met kroniese hoër kTnT geassosieer (Manuskrip 2) en matige IR-vlakke (Manuskrip 3).

Gevolgtrekking

Miosiet-besering, hiperglikemie en insulienweerstand het gepaard gegaan met progressiewe miokardiale rekking in Swart mans wat moontlik weerspieëlend is van kardiale metaboliese ooraanvraag wat die waarskynlikheid van hipertensie en iskemiese hartsiekte-risiko verhoog. Nietemin, sentrale neurale kontrolemeganismes kan potensieel in hierdie mans BANF opgereguleer en TNF-α afgereguleer het as ’n wyse om teen hierdie eise te beskerm en om prosesse wat verband hou met inmeningsbeheer te verbeter.

Sleutelwoorde: *cardiac stress, cardiac troponin T, NT-proBNP, inflammation, hypertension, BDNF, cognitive interference, hyperglycaemia, insulin resistance, cardio-metabolic risk*

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LIST OF ABBREVIATIONS

24-h	24-hour
ABPM	Ambulatory blood pressure
ANCOVA	Analysis of co-variance
ANP	Atrial natriuretic peptide
BDNF	Brain-derived neurotrophic factor
BNP	B-type natriuretic peptide
BP	Blood pressure
cGMP	Cyclic guanosine monophosphate
CI	Confidence interval
CRP	C-reactive protein
cTnT	Cardiac troponin T
CVD	Cardiovascular disease
CWT	Color-Word Conflict test
DBP	Diastolic blood pressure
ECG	Electrocardiogram
ECLIA	Electrochemiluminescence immunoassay
ECM	Extracellular matrix
ELISA	Enzyme linked immunosorbent assay
ESC	European Society of Cardiology

FADD	Fas-associated death domain
γGT	Gamma glutamyl transferase
HART	Hypertension in Africa Research Team
HbA1c	Glycosylated haemoglobin A1c
HOMA	Homeostatic model assessment
IR	Insulin resistance
LVH	Left ventricular hypertrophy
MAPK	Mitogen activated protein kinases
NFκb	Nuclear factor kappa B
NO	Nitric oxide
NPR-A	A-type natriuretic peptide receptors
NRF	National Research Foundation
NT-proBNP	N-terminal portion of B-type natriuretic peptide
p75NTR	p75 Neurotrophin receptor
PI3-K	Phosphatidylinositol-3 kinase
pro-BNP	Pro-B-type natriuretic peptide
RAAS	Renin-angiotensin-aldosterone system
RIP	Receptor-interacting protein
SABPA	Sympathetic activity and Ambulatory Blood Pressure in Africans
SBP	Systolic blood pressure

SES	Socio-economic status
TNF-α	Tumour necrosis factor-alpha
TNFR1	Tumour necrosis factor receptor-1
TNFR2	Tumour necrosis factor receptor-2
tPA	Tissue plasminogen activator
TRADD	TNF receptor-associated death domain
TRAFF	TNF-receptor-associated-factor 2
TrkB	Tropomyosin-related kinase
α	Alpha
B	Beta
γ	Gamma

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE OVERVIEW

1.1. General introduction

The incidence of non-communicable diseases is on the rise globally with developing countries being especially vulnerable.[1,2] Studies show a substantial burden of specifically cardiovascular diseases (CVD) in South Africa.[3] More studies are therefore being conducted to determine the contributing factors to the development of CVD in African populations.

The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study is one such study that was conducted in the North West Province of South Africa.[4] The study included participants from a similar socio-economic status (SES) in order to minimize the effect of SES on the distribution of various cardiovascular risk factors. This study reported a worsening cardiovascular profile in Black and White individuals over a three-year follow-up period.[5] A different distribution regarding cardiovascular risk markers was also evident, which may explain the excess burden of subclinical vascular disease evident in Black Africans.[6] It is thus of paramount importance to investigate the longitudinal influence of various cardiovascular risk markers on CVD development in African populations.

The main aim of the SABPA prospective study was to determine the role of the brain-heart link and neural response pathways in the development of CVD in African individuals.[4] Therefore, in this study, we not only investigated associations between cardiac stress with other well-known cardio-metabolic risk factors such as hypertension, inflammation and glucose dysregulation,[2,7] but also with risk markers for brain health and cognition.

1.2. Cardiac stress

The cardiovascular system is continually subjected to haemodynamic forces due to the pulsatile nature of blood flow through the cardiovascular system.[8] Sustained haemodynamic influences such as volume- and pressure overload to the heart may result in structural and functional vascular changes in an attempt to diminish the haemodynamic burden.[8-10] These structural and functional changes determine the cardiac remodelling process that occurs in an attempt to maintain stroke volume for sustained oxygen delivery to the myocardium as well as various other tissues throughout the body.[9,11,12] Cardiac remodelling may comprise modifications of the extracellular matrix (ECM), myocyte injury as well as myocyte hypertrophy.[13-15]

The ECM surrounds cardiac myocytes and is composed of collagens and fibroblasts.[16] Excessive collagen deposition leads to the development of fibrosis that may contribute to an increased hemodynamic load placed on the heart.[9,14,16] Early-onset ECM alterations were shown to be present in Africans.[17] Also in Africans, fibrosis was positively associated with myocardial ischaemia[18] that can further contribute to cardiac alterations including myocyte injury. Injury to cardiac myocytes has been identified as a major contributor to cardiac dysfunction and -failure.[19,20] Three distinct processes of myocyte injury have been identified in literature, namely apoptosis, autophagy and oncosis.[19-22] Apoptosis and autophagy were described as self-programmed cell death as it involves regulated self-induced processes that lead to cardiomyocyte injury.[13,19,20] In contrast, oncosis is a passive form of cell death as it is induced through external stimuli.[20,23] Once the injury becomes irreversible with cell degradation, the process is defined as necrosis.[19,20] Various cellular and molecular pathways were identified in the process leading to cardiomyocyte necrosis.[24] This includes oxidative stress, hypertension, inflammation and myocardial ischemia.[24-26] Activation of the renin-

angiotensin-aldosterone system (RAAS) and sympathetic nervous system may also contribute to myocyte injury and the release of cardiac troponin T (cTnT).[27]

1.2.1. Cardiac troponin T

cTnT forms part of the troponin complex that binds tropomyosin to actin in cardiac muscle (Figure 1.1).[28,29] Myocyte injury is the main stimulus for the release of cTnT from the myofibrils, as 94% of cTnT is structurally bound to the troponin protein complex with only 6% being free in the cytosol.[10,22,27] With myocyte injury, a loss in the integrity of the cell membrane can lead to transient leakage of cTnT from the cytosolic compartment.[10,30] Proteolytic enzymes may also disintegrate the contractile apparatus leading to the release of cTnT from the bound protein pool.

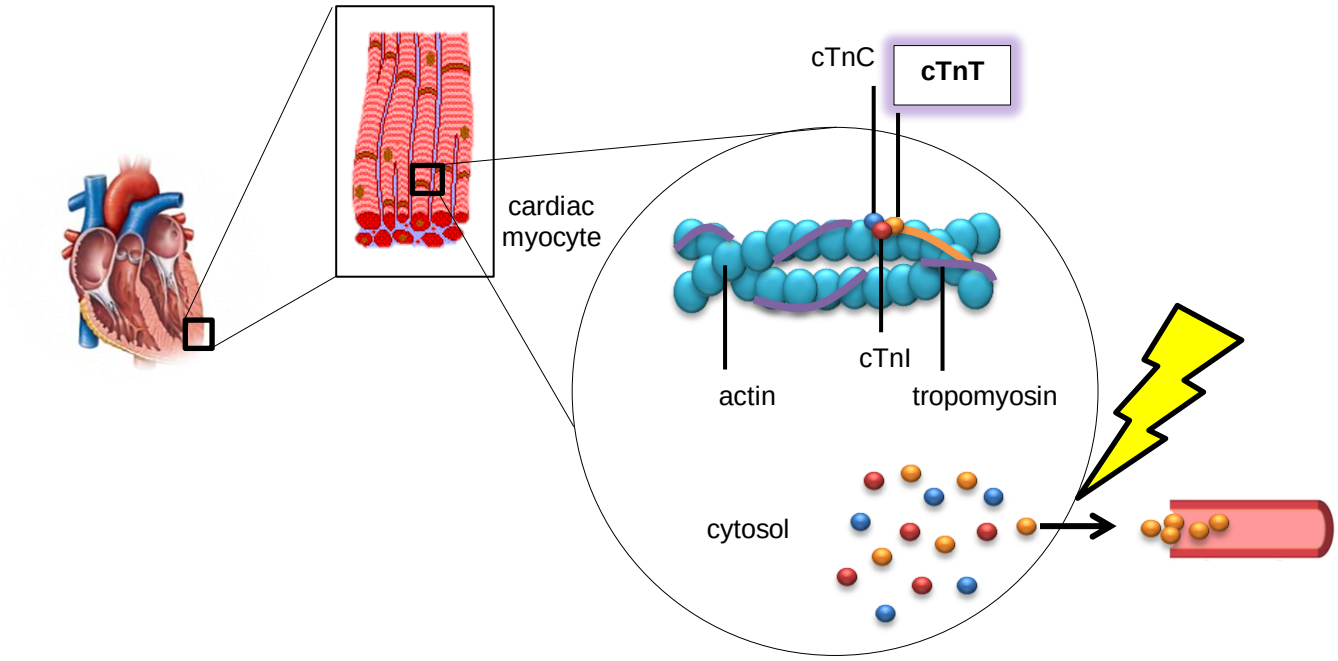

Figure 1.1. Cardiac troponin release in response to myocyte injury. *Abbreviations: cTnT, troponin T; cTnI, troponin I; cTnC, troponin C. (Excerpt developed with information from Del Carlo & O'Connor. 1999; Jabbar. 2013).*[10,30]

Elevations in cTnT have been shown to increase the risk for cardiovascular mortality three-fold.[31] Wallace, et al. (2006)[32] reported that levels of cTnT are higher in African-Americans than in White Americans. In contrast, during cross-sectional investigation, the SABPA study previously showed that Whites have higher levels than Blacks.[33] McEvoy, et al. (2015)[34] further identified factors such as age, hypertension, diabetes and obesity that may determine longitudinal increases in cTnT. An association between cTnT and cardiac wall stress have been reported in individuals with metabolic syndrome.[35] Similar observations were found in Blacks from the SABPA cohort, which may indicate an increased cardiac wall strain in these individuals.[33] Also in these individuals, cTnT positively associated with blood pressure and silent myocardial ischemic events.[36,37] Ischemia has also been shown to be associated with the development of left ventricular structural changes or electrocardiogram-left ventricular hypertrophy (ECG-VLH).[38] Other studies also revealed cTnT to be associated with left ventricular wall thickness and -mass that may be indicative of myocyte hypertrophy.[39,40]

Myocyte hypertrophy occurs in an attempt to increase myocardial mass and provide more force in order to increase oxygen delivery and bear the extra load of haemodynamic stress.[41] It was proposed that markers of haemodynamic stress can augment risk prediction in various CVD.[42] A well-known haemodynamic marker is the natriuretic peptide, N-terminal portion of B-type natriuretic peptide (NT-proBNP), released by cardiac myocytes in response to cardiac wall distention, myocardial stretch and neuro-hormonal activation.[42,44]

1.2.2. N-terminal pro-B-type natriuretic peptide

In literature, three natriuretic peptides were identified, namely atrial natriuretic peptide (ANP), brain- or B-type natriuretic peptide (BNP) and C-type natriuretic peptide.[43,45] The atrium is normally the main source for ANP and BNP production, but the ventricles may also

produce BNP with chronic myocyte stretch.[43,46] BNP is released from a prohormone, pro-B-type natriuretic peptide (pro-BNP) that is deglycosylated under the influence of furin into active BNP and an inactive fragment, NT-proBNP (Figure 1.2).[43,45] BNP elicits its function by binding to A-type natriuretic peptide receptors (NPR-A).[43,45] During haemodynamic stress binding to NPR-A leads to cyclic guanosine monophosphate (cGMP) production inducing diuresis, natriuresis, vasodilation and inhibiting adrenergic activity as well as the RAAS.[43-46]

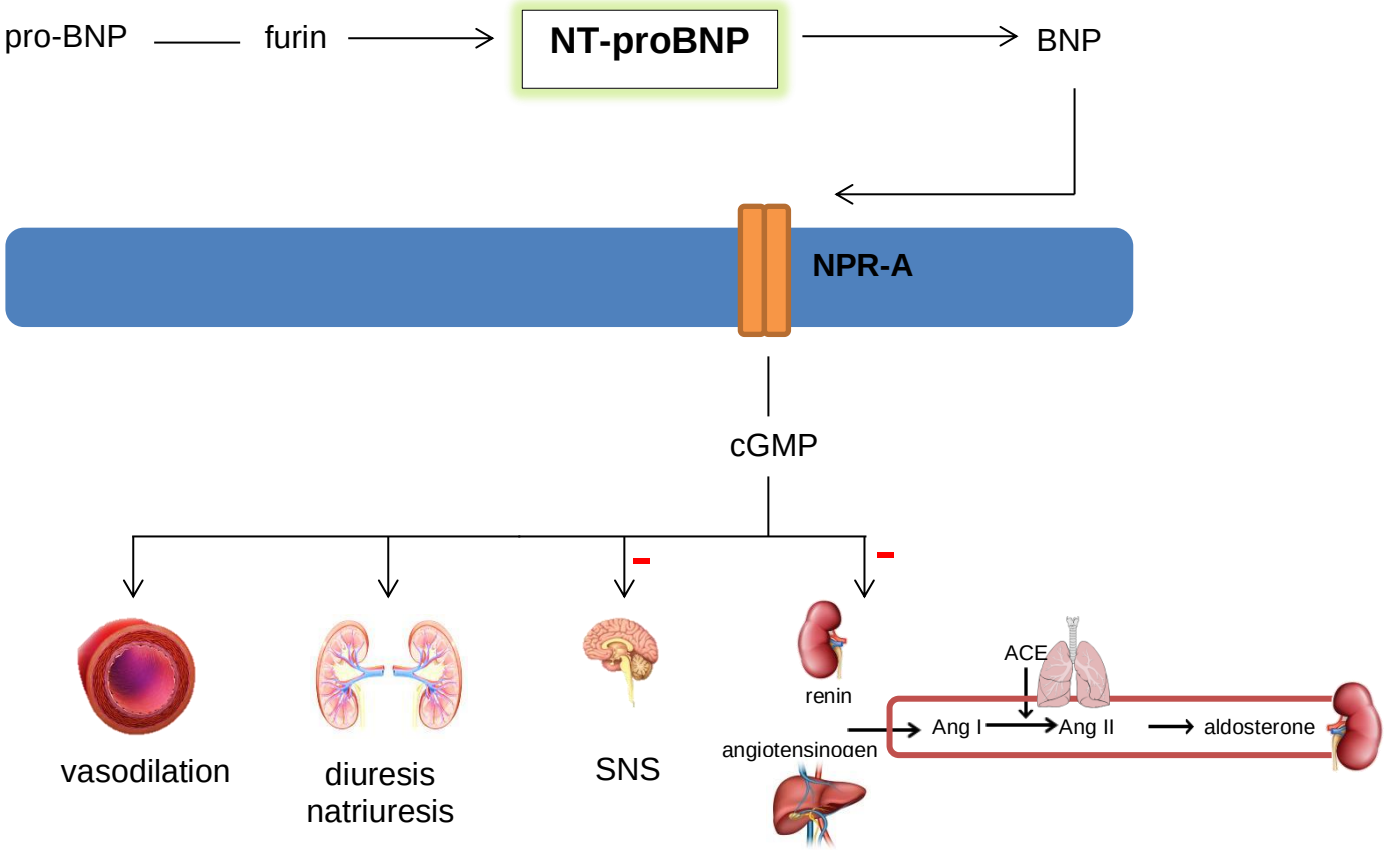

Figure 1.2. Release and physiological functions of NT-proBNP and BNP. *Abbreviations: BNP, b-type natriuretic peptide; NT-proBNP, N-terminal pro-BNP; NPR, natriuretic peptide receptor; cGMP, cyclic guanosine monophosphate; SNS, sympathetic nervous system; Ang, angiotensin; ACE, angiotensin converting enzyme. (Excerpt developed with information from Daniels & Maisel. 2007; Hall. 2005; Palazzuoli, et al. 2010).*[43-46]

Levels of NT-proBNP seem to be higher in women than in men.[46-47] Daniels and Maisel (2007)[45] additionally reported that African Americans manifest higher levels than their White counterparts and stated that the higher blood pressure levels observed in African Americans may be one driving factor for the higher NT-proBNP levels observed. In contrast, we did not find any significant NT-proBNP differences between races and genders in a previous study.[33] Higher NT-proBNP levels were also observed with other factors including advanced ageing, diabetes, atrial fibrillation and an increased resting heart rate.[46,48] In a South African Black cohort NT-proBNP showed positive associations with low-grade inflammation.[33]

1.3. Cardiovascular risk markers

1.3.1. Inflammation

1.3.1.1. Inflammation and cardiac stress

Inflammatory mechanisms are essential to restore and maintain tissue homeostasis.[49,50] When tissue injury cannot be repaired in a short period of time, a chronic systemic inflammatory response prevails that may contribute to end-organ damage and therefore increases the risk for the development of CVD.[51-53] Inflammation may increase CVD risk by affecting endothelial function.[51,54] A decrease in vasodilation and increase in vasoconstriction may lead to the production of reactive oxygen species and a resultant decrease in nitric oxide availability and synthesis.[55,56] A higher degree of endothelial dysfunction was reported in African Americans.[57] In agreement, disturbed endothelial responses were revealed in Blacks from South Africa when habitually applying defensive coping.[58,59] Furthermore Mels, et al. (2016)[60] showed different mechanisms regarding NO bioavailability in Black and White men, leading to endothelial dysfunction.

Inflammation also leads to the expression of intracellular- and vascular adhesion molecules as well as monocyte chemotactic protein-1 which attracts monocytes to the vascular intima to initiate their differentiation into macrophages and the formation of foam cells.[51,61-63] Inflammation therefore plays a very important role in the initiation and progression of atherosclerosis.[51,62,64] Furthermore, chronic low-grade inflammation in Blacks was associated with structural wall abnormalities and blood pressure.[18,65] Therefore various pro-inflammatory cytokines as mediators of inflammation may be released in response to hypertension and other cardiovascular risk markers including smoking, alcohol abuse, increased cholesterol levels and vascular injury.[51,66,67] A well-known marker of inflammation is the acute phase reactant C-reactive protein (CRP). CRP is released from hepatocytes upon stimulation from various other pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-6.[51,61]

1.3.1.2. Tumour necrosis factor-alpha

TNF-α is a pro-inflammatory cytokine released by a variety of cells including monocytes, macrophages, endothelial cells and smooth muscle cells.[61-63] Different groups of neurons were also shown to produce TNF-α.[68] Mechanisms have been described on how TNF-α may contribute to cardiovascular disease development.[69,70] They suggest that TNF-α may regulate nitric oxide induction in monocytes and lead to depressed myocardial contractile function in a nitric oxide (NO) dependent and -independent manner. The NO independent phase leads to immediate contractile dysfunction through TNF-induced increases in sphingosine that decreases calcium transients.[70,71] Sustained contractile dysfunction relates to increases in NO production that desensitizes the myofilament to calcium.[69,70]

TNF-α may also induce myocardial apoptosis through TNF receptor one (TNFR1) or Fas activation as shown in Figure 1.3.[68,72] Once TNF-α binds to TNFR1 or Fas, conformational changes occur in the cytoplasmic proteins referred to as death domains.[68,70] These proteins include a TNF receptor-associated death domain (TRADD) and a Fas-associated death domain (FADD). These two death domains interact with each other through a receptor-interacting protein (RIP) that initiates intracellular communication leading to nuclear DNA degradation.[68,70] FADD may further leads to caspase-8 activation also leading to apoptosis.

In turn, bondage of TNF-α to TNF receptor two (TNFR2) activates TNF receptor-associated factors (TRAFs), involved in transcription factor activation, which includes nuclear factor kappa B (NFκB).[68-70] NFκB activity was shown to be increased in patients with essential hypertension.[73] With chronic inhibition of NFκB in the paraventricular nucleus, sympathetic hyperactivity, cardiac remodelling and hypertension were reduced, as it seemed to restore the imbalance between anti- and pro-inflammatory cytokines that resulted due to hypertension.[73]

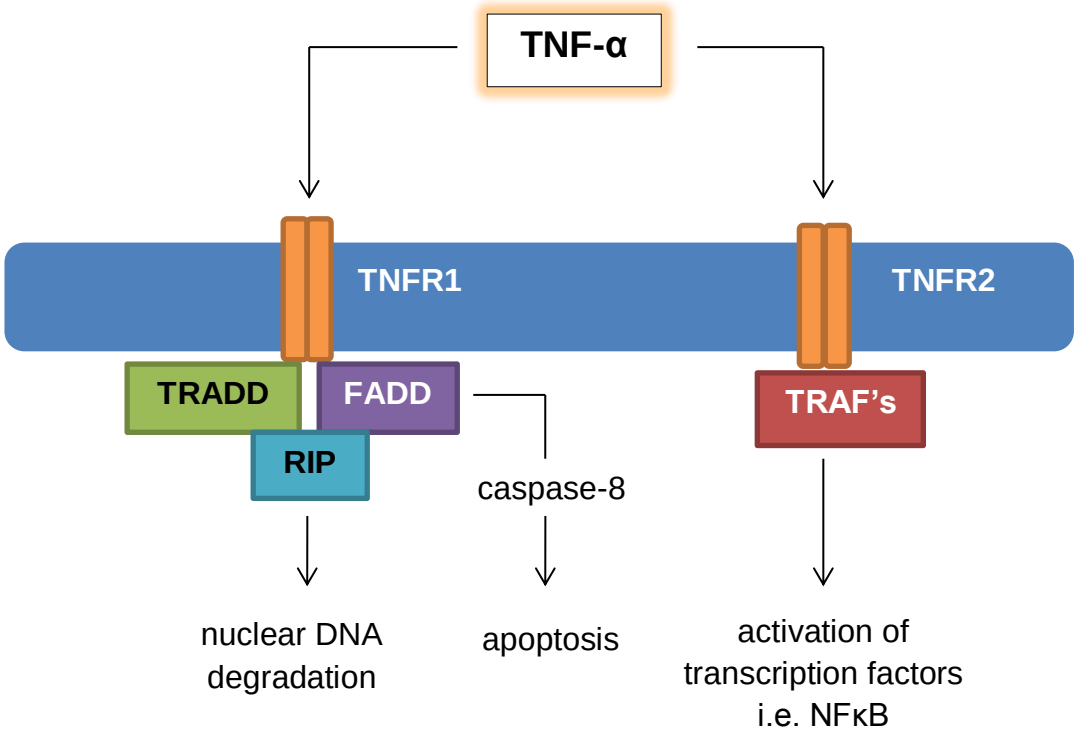

Figure 1.3. Signalling pathways of TNF-α receptor binding. *Abbreviations: TNF-α, tumour necrosis factor-alpha; TNFR1, TNF receptor one; TNFR2, TNF receptor two; TRADD, TNF receptor-associated death domain; FADD, Fas-associated death domain; RIP, receptor-interacting protein; TRAF's, TNF receptor-associated factors; NFκB, nuclear factor kappa-B. (Excerpt developed with information from Blaser, et al., 2016; Figiel. 2008; Meldrum. 1998; Micheau & Tschopp, 2003)* [68-70,72]

1.3.2. Hypertension

1.3.2.1. Hypertension and cardiac stress

Hypertension was shown to be an important risk factor in CVD development.[74,75] Studies showed that lowering blood pressure to the recommended levels reduces the risk for vascular injury and all-cause mortality.[76,77] Over a ten-year period, blood pressure decreased in hypertensive adults from the United States.[78] However, the opposite was evident in Black

individuals from South Africa[5] where greater increases in 24-hour systolic and diastolic blood pressure were observed over a three-year period.

Therefore studies are attempting to identify the underlying pathophysiology leading to blood pressure increases in Black individuals. In the SABPA study, Blacks demonstrated more adverse lifestyle behaviours than did their White counterparts.[6] Furthermore, they revealed hyperactivity of the sympathetic nervous system[37,79], silent ischemia[36] and suppressed RAAS activity[80] that were associated with cardiac remodelling[38] and compensatory ambulatory blood pressure increases.[37] In Black men specifically, cardiac remodelling was positively associated with decreases in levels of brain-derived neurotrophic factor (BDNF).[81]

1.3.3. Brain-derived neurotrophic factor

BDNF forms part of the neurotrophin family secreted by neurons, glial cells and peripheral immune cells in the brain.[82] A pre-pro neurotrophin is cleaved into pro-BDNF which is converted into active mBDNF.[83-85] This conversion into mBDNF occurs under the influence of furin and proconvertases in secretory vesicles before the active BDNF is released as shown in Figure 1.4. In neurons, tissue plasminogen activator (tPA) is responsible for this conversion.[83-85] BDNF can bind to two receptors, a high-affinity tropomyosin-related kinase B (TrkB) receptor or a low affinity p75 neurotrophin receptor (p75NTR).[85-87] TrkB activation leads to an intracellular cascade that activates PLC-gamma (γ) and phosphatidylinositol-3 kinase (PI3-K).[88-89] This in turn activates mitogen-activated protein kinases (MAKP's), activating various transcription factors involved in neuronal survival.[85,89]

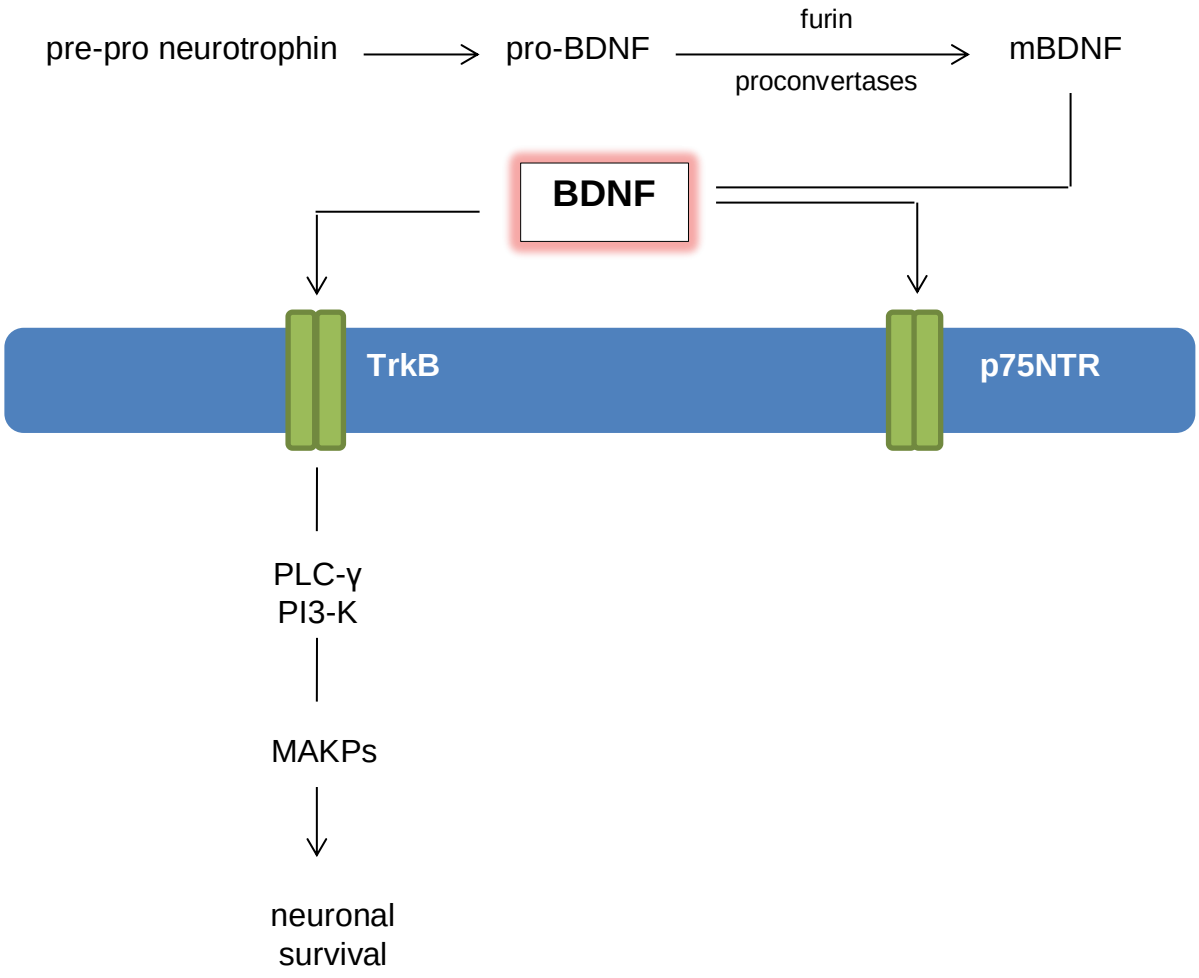

Figure 1.4. Release and signalling pathways of BDNF receptor binding. *Abbreviations: BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin-related kinase B; p75 neurotrophin receptor; PLC-γ, phospholipase C-gamma; PI3-K, phosphatidylinositol-3 kinase; MAKPs, mitogen activated protein kinases. (Excerpt developed with information from Marosi & Mattson. 2014; Pius-Sadowska & Machaliński. 2017; Tasci, et al. 2012 and Xiao, et al. 2016.)* [85,87-89]

1.3.3.1. BDNF and cardiac stress

Levels of BDNF have been shown to be decreased in men and African individuals.[81] It has been shown that BDNF plays a central role in neuronal survival and differentiation as well as

synaptic development and maintenance.[82,90] The function of BDNF is however not limited to the nervous system, since circulating BDNF is stored in platelets and plays a role in the development of adipose tissue, inflammation and atherosclerosis.[87,90,91] In the cardiovascular system, BDNF is released in response to myocyte injury and shear stress as a compensatory response to increase the survival of cardiac myocytes.[90,92-94] Increased levels of BDNF have been shown to improve angiogenesis and left ventricular function in the ischemic myocardium.[90,93]

As aforementioned, BDNF plays an essential role in neuronal survival and synaptic growth in the central nervous system.[82,90] Therefore studies commenced with the possibility of BDNF influencing cognition. Indeed, it was shown that BDNF levels were decreased in various diseases associated with cognitive decline.[95] In sleep-deprived patients, increased BDNF levels might be related to normal prefrontal cognitive functions.[96] Decreases in BDNF were associated with lower cognitive test scores and mild cognitive impairment.[97,98]

1.3.4. Cognition

Cognitive control has been defined as "the ability to coordinate thought and action and direct it toward obtaining goals".[99] It encompasses processes such as attention, memory, language, reasoning, problem-solving and decision making. The brain involves cognitive processing when perceiving the environment in order to monitor changes.[100] When changes in the internal and external environment occur, the brain evaluates the significance thereof by using existing knowledge and generating new knowledge in order to prepare appropriate responses to these changes.[100] During chronic exposure to stress, areas of the brain responsible for cognition processes can be damaged leading to the development of various neurodegenerative states.[101,102] The observation of cognitive deficits mostly involves executive function

documentation that includes inhibitory functions, working memory and cognitive flexibility.[103,104] Fronto-striatal network integrity is crucial for the maintenance of executive cognitive functioning, since processes related to interference control depend on the anterior cingulate and dorsolateral prefrontal areas (Figure 1.5).[104,105] One test that can be used for determining an individual's executive cognitive function is the STROOP-Color Word Conflict test (CWT).[106-107]

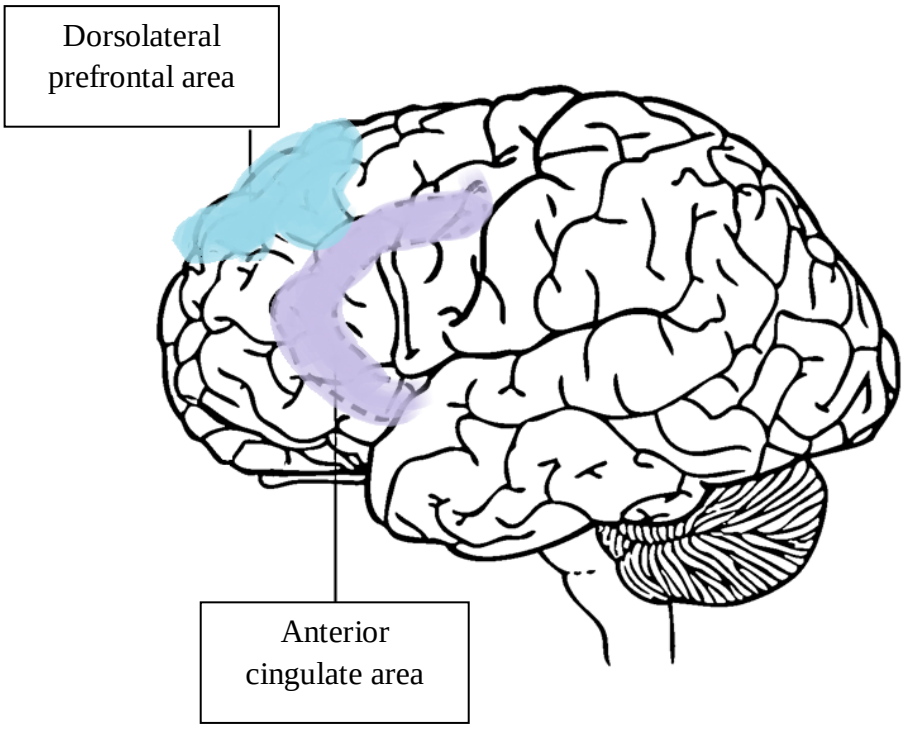

Figure 1.5. Areas of the brain involved in executive cognitive functioning. *(Excerpt developed with information from Culpepper, 2015; Etkin et al., 2013 and McCalla A, 2018).*[104,105,108]

1.3.4.1. STROOP-Color Word Conflict test

The STROOP-CWT test involves a series of five words being presented in a random order describing a specific colour.[109] However, these words are written in different colours displayed on a cardboard (Figure 1.6). It is required of individuals to verbally identify the

colour of a given word (ink colour) and not to read the colour represented by the word.[109,110] This simultaneous presentation of two stimuli causes interference when the processing of one stimulus interferes with the simultaneous processing of the second stimulus.[109,110] The more automated task (reading the colour represented by the word) interferes with the performance of the less automated task (naming the ink colour) and participants are required to inhibit this interference.[109,110] Therefore, the test assesses the ability to inhibit cognitive interferences in an incongruent manner.[109]

Figure 1.6. The STROOP-CWT cardboard.

1.3.4.2. Executive cognitive function and cardiac stress

Cognitive deficits are at the core of neurodegenerative disease development. However, different mechanisms leading to cognitive decline have been identified including neuronal apoptosis and inflammatory responses.[104,111] Lower cognitive performance was also positively associated with various cardiovascular risk factors including high blood pressure[112], left ventricular mass[113] and cardiac autonomic dysfunction.[114] Risk markers for cardiac stress were also shown to be independently associated with cognitive decline.[115,116] Indeed, higher NT-proBNP levels associated with too poorer executive cognitive functioning

assessed in multiple cognitive tests including the STROOP-CWT.[117] Furthermore, Umegaki, et al. (2017)[118] showed an association between cognitive decline and hyperglycaemia.

1.3.5. Glucose regulation

The importance of blood glucose regulation in CVD prevention has previously been described.[119,120] Plasma glucose concentration is determined by the rate of glucose production into the circulation and the rate of glucose absorption into the cells.[121,122] Glucose is absorbed into the cells via insulin-mediated or non-insulin-mediated pathways.[123,124] The neuroendocrine system plays an important role in glucose regulation under normal conditions[123,125] When high blood glucose levels exist, neurons in the ventromedial hypothalamus is stimulated to activate the vagus nerve (pancreatic branch) in order to increase insulin production.[123,125] Insulin enhances glucose uptake as well as glycogen synthesis.[121,123] However, when subjected to stress or injury, more glucose needs to be mobilized to meet the increased cellular metabolic demands.[123,126] Here, the sympathetic nervous system plays a crucial role by increasing the production of certain hormones, including catecholamines, growth hormone and cortisol.[123,125]

1.3.5.1. Glucose dysregulation and cardiac stress

Hyperglycaemia persists when chronic stress or injury leads to disrupted glucose control and has been identified as an independent risk factor for CVD.[127-129] Studies showed that hyperglycaemia was positively associated with incident heart failure and subclinical myocyte injury.[130-132] Kirk, et al. (2006)[133] reported that higher glycosylated haemoglobin (HbA1c), indicative of high long-term plasma glucose level,[134] exist in African-Americans when than is the case with Whites. Similar trends were found in Black individuals from South Africa.[135]

The pathogenesis of diabetes has been assessed to improve an understanding of the role of insulin resistance (IR) in the development of hyperglycaemia. Indeed, IR is the driving force in the development of type 2 diabetes (T2D), where the tissues show reduced sensitivity to insulin-mediated biological activity.[122,136] However, IR also plays a detrimental role in CVD development.[136,137] In patients with T2D, it was shown that the homeostatic model assessment (HOMA)-IR independently predicted prevalent and incident cardiovascular disease development.[138] Certain risk markers predicting CVD were also identified which amplified IR.[139,140] Indeed, Park, et al. (2009)[141] reported IR to be positively associated with inflammation in patients without diabetes. In an African population, the markers that were identified included isolated diastolic blood pressure, low high-density-lipoprotein concentrations and smoking.[140]

Hyperglycaemia and insulin resistance seems to be involved not only in the progression of CVD and diabetes. Evidence is increasing regarding the influence of altered blood glucose regulation even on cognitive processes and memory.[142,143] Brain regions involved with cognition, such as the hippocampus in the limbic system, were shown to contain high numbers of insulin receptors,[144,145] and reductions in the volume of these regions were reported in diabetics.[146] Patients with diabetes seem to have an increased risk for the development of diseases that involve memory and cognitive function, such as Alzheimer disease.[142,145,147] Insulin is important for neuronal survival, synaptic plasticity and -function in the brain.[148,149] It was also shown that insulin regulates neurotransmitter expression, triggers signal transduction cascades and increases cortical glucose metabolism in the brain, especially in regions involved in cognition and memory.[145,150] Indeed, impaired glucose regulation associated with executive cognitive functioning,[151] which was shown to differ between individuals with type 2 diabetes and individuals with normal glucose metabolism.[152]

1.4. Integration of concepts (Figure 1.7)

During cross-sectional analyses of the SABPA study it was shown that more Blacks than Whites were hypertensive, had higher levels of inflammatory cytokines, cardiac remodelling indices and lower neurotrophin levels.[6,18,33,81] Black individuals also revealed adrenergic overdrive which was enhanced via chronic hyperglycaemia inducing a pressure overload state.[58,153] However, the increased vascular responsiveness profile in Blacks rather suggests volume overload, further potentiating cardiac remodelling.[38] Indeed, myocardial stretch (volume overload) has been shown to accompany inflammation and injury to cardiomyocytes potentiating cardiac remodelling in Black men of the SABPA cohort.[33] Cardiac remodelling also associated with attenuated BDNF.[81] Furthermore, optimal BDNF levels and glucose control are needed for optimal response inhibition capacity so as to maintain executive cognitive functioning.[96,154]

To our knowledge, no published data exist regarding the longitudinal relationships of cardiac stress risk markers (cTnT and NT-proBNP) and its relation with cardiovascular risk markers (inflammation, BDNF, cognitive interference and glucose dysregulation) in African populations. The lack of longitudinal studies on cardiac stress and cardiovascular risk markers motivated the investigation.

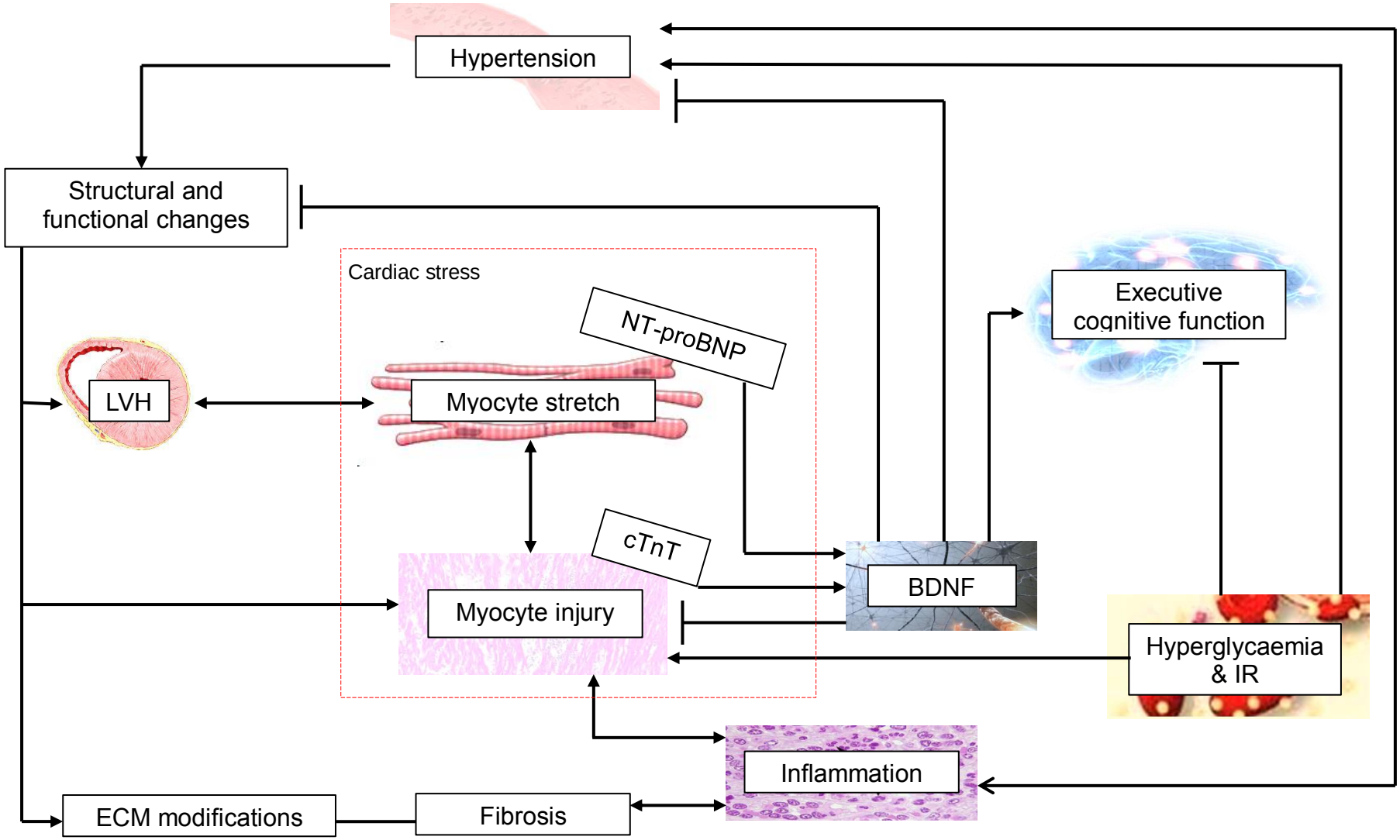

Figure 1.7. Illustration of the main interconnected concepts of this thesis. *Abbreviations: cTnT, cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; BDNF, Brain-derived neurotrophic factor, IR, insulin resistance; LVH, left ventricular hypertrophy.*

1.5. Aims and Hypotheses

1.5.1. Main aim of this study

The main aim of this study was to determine whether cardiac stress, as indicated by cTnT and NT-proBNP, will change in a bi-ethnic gender cohort over a period of three years and to determine whether these cardiac stress risk markers associate with various cardiovascular risk markers over the three-year period.

1.5.2. Main hypothesis of this study

The main hypothesis of this study was that the cardiac stress risk markers will increase in Blacks and be associated with various cardiovascular risk markers over a three-year period.

1.5.3. Detailed aims and hypotheses of each manuscript

1.5.3.1. Longitudinal changes of cardiac troponin and inflammation are associated with progressive myocyte stretch that predicts hypertension in a Black male cohort: The SABPA study.

Aim:

The aim of the first manuscript was to determine whether longitudinal changes of cTnT, NT-proBNP and inflammation (CRP and TNF-α) occur and were associated with the development of subclinical wall remodelling (electrocardiogram (ECG)-left ventricular hypertrophy (LVH)) and hypertension in a bi-ethnic gender cohort over a three-year follow-up period.

Hypotheses:

- *Hypothesis 1.1*: Levels of cTnT, NT-proBNP and inflammation will increase in Blacks over the three-year period.

- *Hypothesis 1.2*: Changes of NT-proBNP will positively associate with changes of cTnT and TNF-α in Black men only.

1.5.3.2. BDNF and attenuated inflammation as defence response to cardiac stress and cognitive interference in Black men: The SABPA prospective study.

Aim:

The aim of the second manuscript was to determine whether changes in cardiac stress markers (cTnT and NT-proBNP) occur and are associated with changes in BDNF, TNF-α and with cognitive interference in a bi-ethnic cohort, over a period of three years.

Hypotheses:

- *Hypothesis 1.3*: Levels of BDNF will remain low in Blacks compared to those in Whites over the three-year period.
- *Hypothesis 1.4*: Changes in BDNF will inversely associate with markers of cardiac stress, TNF-α and with cognitive interference in Blacks.

1.5.3.3. Prospective associations between cardiac stress, glucose dysregulation and cognitive interference in Black men: The SABPA study

Aim

The aim of the last manuscript was to determine whether changes in cardiac stress risk markers (cTnT and NT-proBNP) occur and are associated with changes in insulin resistance, hyperglycaemia and baseline cognitive interference in a bi-ethnic male and female cohort over a three-year period.

Hypotheses

- *Hypothesis 1.5*: Levels of IR and hyperglycaemia will increase in Blacks over the three-year period.

- *Hypothesis 1.6*: Changes in cardiac stress risk markers will significantly associate with changes in IR and hyperglycaemia in Blacks.
- *Hypothesis 1.7*: Changes in IR and hyperglycaemia will inversely associate with cognitive interference in Blacks and Whites.

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CHAPTER 2

METHODOLOGY

2.1. Ethical approval

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.[1] Prior to commencement of the study, consent was obtained from the North-West Department of Education, North-West University, the South African Democratic Teacher's Union and the headmasters of the respective school. Informed consent was obtained from all the participants prior to commencement of the study.

2.2. Study design and participant selection

The *Sympathetic activity and Ambulatory Blood Pressure in Africans* (SABPA) prospective cohort study was conducted on urban Caucasian and African teachers who resided in the Dr Kenneth Kaunda Education District of the North West Province of South Africa (Figure 2.1).[2] This selection was made to ensure that the participants were from a similar socio-economic class, but we could not control for cultural diversity. Teachers (2170), aged 20-63 years, were invited to participate and 471 participants were assessed for eligibility (Figure 2.2). The exclusion criteria for the SABPA study were: 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.

Ultimately 409 participants (200 Africans and 209 Caucasians) were enrolled in phase I of the study which was conducted from February to May (2008 and 2009). Stretched across a three-year period, the rate of successful follow-ups was 87.8%; thus Phase II was conducted from February to May (2011 and 2012) on 359 participants (173 Africans and 186 Caucasians). Reasons for non-participation in phase II of the study were pregnancy (N=2), deceased (N=6) and bailed outs (N=42).

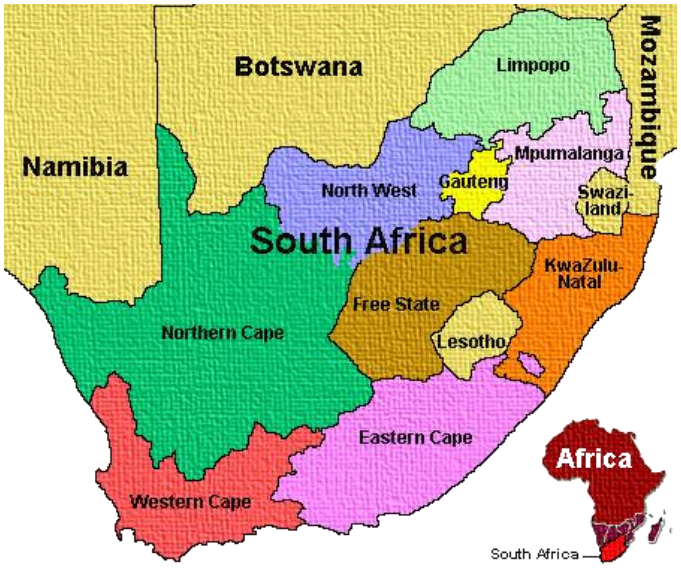

Figure 2.1. The Dr Kenneth Kaunda Education District of the North West Province of South Africa.

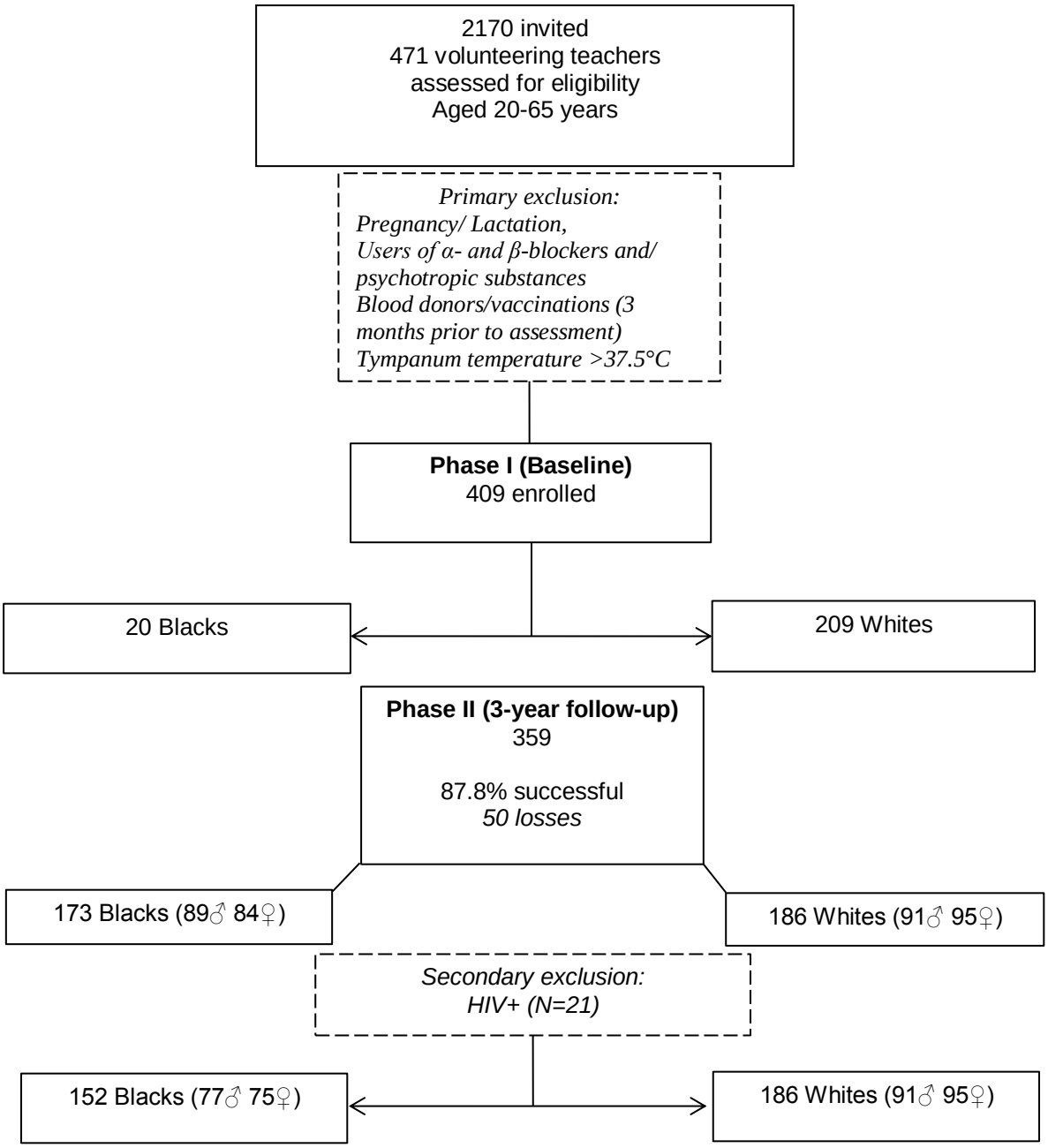

Figure 2.2. Longitudinal study design assessing a South African bi-ethnic gender cohort.

The protocol for phases I and II of the study are described in the following methodological description. The protocol was kept as similar as possible for both phases. However, differences are discussed where the protocols differ.

2.3. Experimental methods and data collection

2.3.1. Research procedure

Clinical assessments were obtained over a stretch of 36 hours. The Cardiotens CE120® (Meditech, Budapest, Hungary) and accelerometers were applied to measure 24-hour (24-h) ambulatory blood pressure (ABPM), 2-lead electrocardiogram (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.

In phase I, at approximately 16:30, the participants were transported to the Metabolic Unit Research Facility at North-West University. In contrast, in phase II, the participants were first transported to North-West University (at approximately 15:00) for other clinical assessments and thereafter (at approximately 17:00) they were transported to the Metabolic Unit Research Facility. 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 standardized dinner. The participants were advised to fast and rest from 22:00 for the next day's clinical measurements.

In phase 2, at approximately 07:30 the next morning, the 24-h ambulatory apparatuses were disconnected, whereby the anthropometric and clinical measurements commenced. All resting ECGs and blood samplings were done after the participants had been in a semi-recumbent position for approximately 30-45 minutes. On completion of all the assessments, the participants received breakfast, feedback and were then transported back to their respective schools.

2.3.2. Lifestyle determinants

Each participant's daily physical activity was monitored over a 24-h period with the Actical® activity monitor (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada). Anthropometric measurements were taken by registered level II anthropometrists, according to standardized procedures in triplicates 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 [weight (kg) x height (cm) ÷ 3600].½ was used to calculate the body surface area.[3]

2.3.3. Biochemical measurements

Registered nurses obtained fasting blood samples from the ante-brachial vein with a sterile winged infusion set. These samples were separated and stored at -80°C until analysis could take place. 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.[4,5]

Cardiac Troponin T (cTnT) and N-terminal portion of B-type natriuretic peptide (NT-proBNP) were analysed using the high sensitive Electrochemiluminescence immunoassay (ECLIA), Elecsys, 2010, Roche, Basel, Switzerland. In our sample, there were 91 (26.84%) undetectable cTnT values (<3 pg/ml).[6] The inter- and intra-batch variability was 15% and 5.6% for cTnT, and 4.6% and 4.2% for NT-proBNP. Previously, an established cut-point of 4.2ng/L cTnT was shown to predict 24h and clinic hypertension in this SABPA cohort[7] and was used as an indicator of ischemic heart disease risk.[8]

C-reactive protein (CRP) was measured with the ultra-high sensitivity turbidimetric method (Unicel DXC 800, Beckman and Coulter, Germany). An established cut-point of 3mg/L CRP was used to indicate high inflammation.[9] Serum tumour necrosis factor-alpha (TNF-α) was analysed with the Quantikine High Sensitivity Human Tumour TNF-α Enzyme linked to immunosorbent assay (HS ELISA; R&D Systems, Minneapolis, MN USA). The inter- and intra-assay variability for TNF-α was 15% and 17.8% respectively. Serum brain-derived neurotrophic factor (BDNF) was analysed with a Quantikine Colorometric-Sandwich Immunoassay. (Catalogue #: DBD00). The intra-assay precision was between 3.8 and 6.2%, and the inter-assay precision was between 7.6 and 11.3%.

Fasting blood glucose samples were collected in sodium fluoride tubes and analysed using the timed-end-point method (Unicel DXC 800, Beckman Coulter, Germany). Glycosylated haemoglobin (HbA1c) was determined by means of turbidometric inhibition immunoassay (Integra 400; Roche, Basil, Switzerland). The electrochemiluminescence immunoassay was used to analyse serum insulin (ECLIA; Elecsys 2010, Roche, Basel, Switzerland) with an intra-assay- and inter-assay precision of 2% and 2.8% respectively. The homeostatic model assessment (HOMA) was applied to indicate insulin resistance (IR) and was measured using the following formula: fasting glucose x fasting insulin/405.[10]

2.3.4. Cardiovascular assessment procedures

The 24-h blood pressure (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 European Society of Cardiology (ESC) criteria for hypertension were employed [average 24-h systolic blood pressure (SBP) of ≥130mmHg and diastolic blood pressure (DBP) of ≥80mmHg].[9] The data were 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-h 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 (LVH) (Cornell product, [RaVL+SV3]. x QRS duration). Values exceeding 244 mV.ms were indicative of LVH.[11]

2.3.5. Executive cognitive function

Executive cognitive function was assessed using the STROOP-Color-Word-Conflict test (CWT).[12] The participants were shown a cardboard containing series of five words in random order describing a specific colour, but written in different colours. The ink colour of a given word had to be identified verbally. Participants had to guard against reading the colour represented by the word. They were also encouraged to progress as fast as possible within 1 minute, and had to correct wrong answers. An interference score was calculated that represented the number of correct answers produced during the fixed period of 1 minute. A lower score thus indicates that the individual found it more difficult to inhibit the interference. This is used to determine the individual's cognitive flexibility and reaction to cognitive stress.[13] On completion of the task participants received a monetary incentive in accordance with their performance.

2.3.6. Statistical analyses

Statistical analyses were performed with Statistica version 13 (TIBCO Software Inc., Palo Alto, USA, 2018). Normal distributions were computed to reveal symmetrical data. Variables with skewed distributions were log-transformed. Initial single two-way general linear modelling tested interactions on main effects (ethnicity x gender) for all main markers,

independent of a priori covariates. A priori covariates included age, physical activity, body surface area, γ-GT and cotinine.[9] Independent *t*-tests were used to compare characteristics of the two ethnic groups. Chi-square tests (X^2) were used to determine prevalence as well as proportions. Single two-way general linear model interactions on main effects (ethnicity x gender) were computed for all cardiovascular risk markers independent of a priori covariates.[9]

Dependent sample T tests compared unadjusted differences over time in each ethnic group. Adjusted differences over time were calculated via analysis of co-variance (ANCOVA's), independent of a priori covariates. Multivariate linear regression analyses were performed and percentage changes (%Δ) were calculated with the following formula: [(follow-up - baseline)/baseline] ×100. Forced entry stepwise forward regression analyses determined associations between dependent variables and independent variables. Independent variables included a priori and baseline values of the respective variables. Lastly, logistic regression analyses were performed to determine the odds of main markers to increase likelihood for hypertension and ischemic heart disease risk, independent of a priori covariates. 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 will be set at 2.5.

2.4. References

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CHAPTER 3

MANUSCRIPT 1

The abstract of Manuscript 1 was presented at the 45th Conference of the Physiology Society of Southern Africa from 27-31 August 2017 in Pretoria, South Africa.

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3.1. INSTRUCTIONS FOR AUTHORS

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Aims and scope:	Hypertension Research is the official publication of the Japanese Society of Hypertension. The journal publishes papers reporting original clinical and experimental research that contribute to the advancement of knowledge in the field of hypertension and related cardiovascular diseases. The journal publishes original articles, reviews and correspondence including case reports.
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Paragraph spacing:	Double
Font:	12
Sections:	Title page: Authors and affiliations (not qualifications), Corresponding author contact details, Grants. Abstract, Introduction, Methods, Results, Discussion, Acknowledgements, Conflict of interest, References, Figure legends, Tables, Figures, Supplementary information
Title:	Concise informative
Abstract:	250 words 3-5 keywords
Tables and Figures:	In separate files.
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Longitudinal changes of cardiac troponin and inflammation reflect progressive myocyte stretch and likelihood for hypertension in a Black male cohort: The SABPA study

Running head: Hypertension, inflammation and cardiac stress

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3.2. ABSTRACT

Inflammation was cross-sectionally associated with subclinical wall remodeling and hypertension. Whether longitudinal changes (Δ) in inflammation, myocyte injury (troponin T) and stretch (N-terminal-pro-B-type natriuretic peptide) are associated with hypertension and ECG left ventricular hypertrophy (ECG-LVH), are unclear. The first prospective analysis in Africa assessing these associations, included a Black and White teachers' cohort (N=338; aged 20-63 years). Fasting blood samples were obtained to measure tumor necrosis factor-alpha (TNF-α), cardiac troponin T (cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP). Ambulatory blood pressure, -2-lead ECG and resting 10-lead ECG values were obtained. A higher mean hypertensive status (62%) was evident in Blacks compared to Whites (44%, p=0.007). Over 3 years, NT-proBNP increased in both ethnic groups. No associations were evident in women and in White men. In Black men, ECG-LVH at follow-up was positively associated with baseline cTnT (Adj R2 0.43; β= 0.48; 95% CI 0.28 to 0.68, p<0.001) and baseline SBP (Adj R2 0.43; β= 0.29; 95% CI 0.09 to 0.49, p=0.006). Again in Black men, baseline TNF-α (OR=1.49, 95% CI 1.05 to 2.14, p=0.03) and decreased ΔTNF-α (OR=2.07, 95% CI 1.26 to 3.40, p=0.004) increased likelihood for cTnT levels ≥4.2ng/L. Here, Baseline NT-proBNP (OR=1.12, 95% CI 1.01 to 1.23, p=0.03) and ΔNT-proBNP progression (OR=1.09, 95% CI 1.00 to 1.81, p=0.04) increased likelihood for 24-hour hypertension. In conclusion, chronically increased levels of markers of myocyte injury accompanied by progressive myocardial stretch, reflective of cardiac metabolic over-demand, may ultimately increase hypertension and ischemic heart disease risk in a Black male cohort.

Keywords: hypertension; NT-proBNP; cardiac troponin T; tumor necrosis factor-α; Black men

3.3. INTRODUCTION

The development of hypertension is an ongoing phenomenon worldwide, especially in developing countries and is modified by various risk factors.[1] Indeed, inflammation can induce hypertension through mechanisms involving atherosclerosis and endothelial dysfunction.[2-5] The progression of cardiovascular risk factors over time appears to be accelerated in South African individuals and also differs between Blacks and Whites.[6] In teachers from the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective study, a hypertensive status was evident in Blacks in cross-sectional analysis.[7] Further increases in 24h blood pressure (BP) were observed in these individuals over a period of three-years.[6]

In Black men, a pro-inflammatory profile was shown to be associated with the development of subclinical cardiac remodeling.[8-9] Regulation of chronic inflammation may therefore play a crucial role in the treatment of myocardial hypertrophy.[10] Interlinked mechanisms are involved in the development of cardiac remodeling.[11-13] Structural and functional alterations occur in an attempt to maintain cardiac output when the left ventricle fails to deliver sufficient oxygen.[13] For instance, the myocardium can increase in mass to increase the contraction force leading to the development of left ventricular hypertrophy (LVH).[13] Insufficient oxygen delivery due to impaired blood flow can also lead to cardiac injury characterized by the death of cardiac myocytes leading to the release of cTnT from the myofibril.[14-15] Levels of cTnT have been shown to be increased in African American men.[15] However, we previously reported on Whites having higher cTnT levels than Blacks at baseline.[8] Despite these findings, Malan, et al. (2017) showed that a cTnT cut-point as low as 4.2ng/L predicted clinical and ambulatory hypertension in Blacks whereas a higher cut-point (5.6ng/L) predicted hypertension in Whites.[16] Support for hypertensive susceptibility in

Blacks was evident in the positive cross-sectional associations between cTnT, blood pressure (BP) and NT-proBNP of this cohort.[8] NT-proBNP, the inactive precursor of brain natriuretic peptide (BNP), is released in response to excessive myocyte stretch.[17-19] African Americans were reported to have higher levels of NT-proBNP than Whites driven by the higher BP levels observed in the former.[20] Indeed, elevated levels of NT-proBNP or positive changes in NT-proBNP levels over time preceded the development of hypertension in a multi-ethnic population from the United States.[21]

The aim of our study was to determine whether longitudinal changes of cTnT, NT-proBNP and inflammation were associated with the development of subclinical wall remodeling (ECG-LVH) and hypertension in a Black cohort over a three-year follow-up period.

3.4. METHODS

The SABPA prospective cohort study was conducted on urban Black and Whites teachers, which resided in the Dr Kenneth Kaunda Education District of the North-West Province of South Africa.[22] This selection ensured a similar socio-economic class.[23] Teachers (2170), aged 20-63 years were invited to participate and 471 participants were assessed for eligibility, as shown in Figure 3.1. After a period of three years, phase II of the study was conducted from February to May (2011 and 2012) including 359 participants. The successful follow-up rate was 87.8%. Reasons for non-participation in phase II of the study were pregnancy (N=2), death (N=6) and bailed outs (N=42). For the purpose of this study, additional exclusion criteria were added to avoid bias pertaining to cardio-metabolic and inflammatory risk.[23] Therefore, participants with a HIV positive status (N=21) were excluded. Ultimately 338 (152 Black and 186 White) participants remained for the present study.

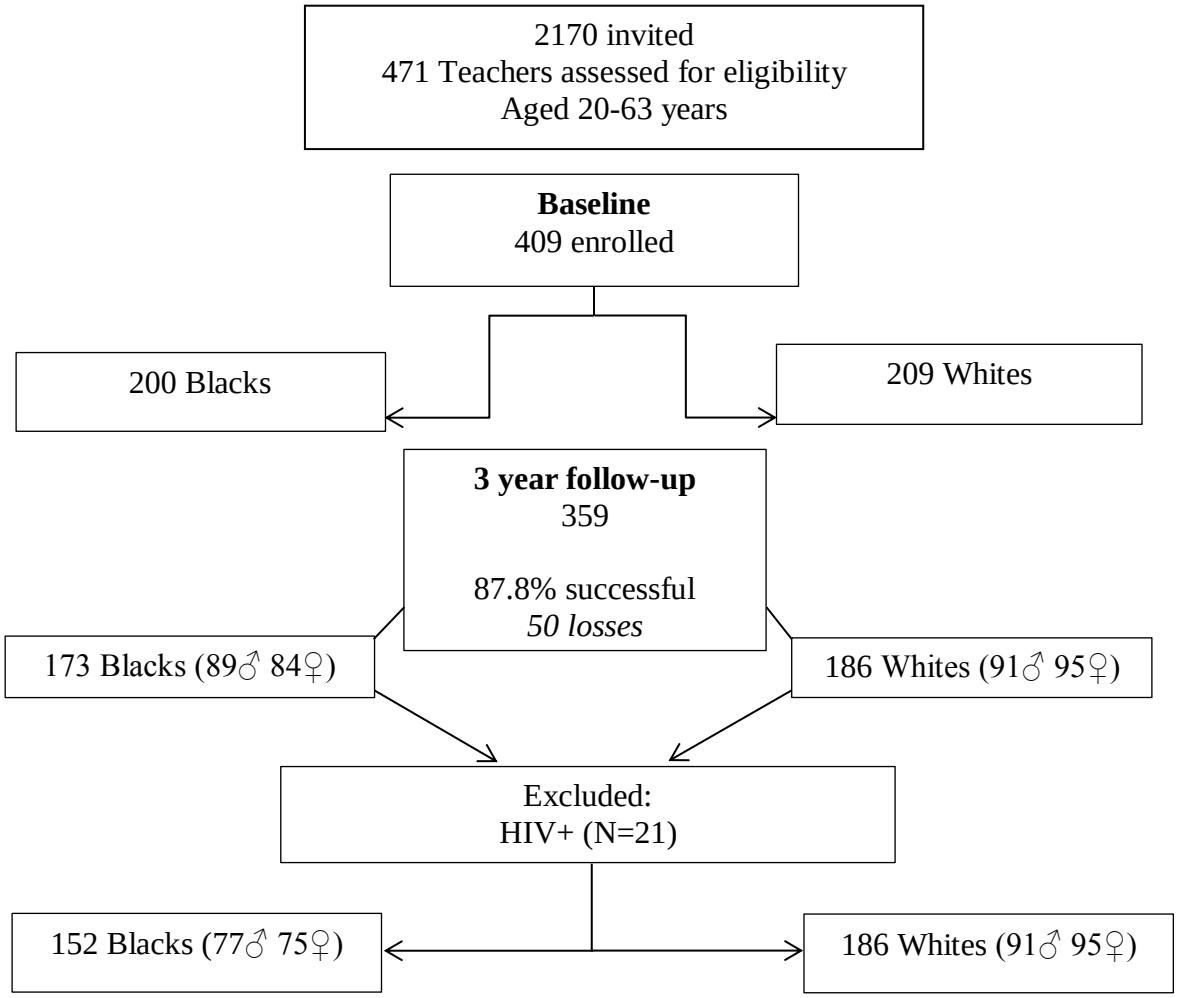

Figure 3.1. A South African bi-ethnic sex cohort.

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.[22]

3.4.1. Experimental methods and data collection

Clinical assessments were obtained over 48 hours. The Cardiotens CE120® (Meditech, Budapest, Hungary) and accelerometers were applied to each participant at his/her school of employment to measure 24-hour ambulatory blood pressure (ABPM), electrocardiogram (ECG), as well as 24-h physical activity, between ~07:00 and 08:00. The correct cuff sizes were applied to 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 and introduced to the experimental set-up. The facility was well ventilated with a comfortable temperature and each participant received his/her own private bedroom. Demographic and General Health questionnaires were completed and each participant received a standardized dinner. The participants were advised to fast and rest from 22:00 for the next day's clinical measurements.

The next morning, at approximately 07:30, the 24-hour ambulatory devices were disconnected after completion of the last BP measurement, followed by the anthropometric and clinical measurements. All resting ECG's and blood sampling were done after the participants had been in a semi-recumbent position for approximately 30-45 minutes. On completion of all the assessments, the participants received breakfast, immediate available feedback and were transported back to their respective schools.

3.4.2. 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 in triplicate, by registered level II anthropometrists according to standardized procedures. The mean of the three measurements was used to ensure accuracy. Inter- and intra-observer variability was found to be less than 10%. The Mosteller formula [weight (kg) x height (cm) ÷ 3600]^{½} was used to calculate the body surface area.

3.4.3. Biochemical measurements

Registered nurses obtained fasting blood samples from the ante-brachial vein utilizing a sterile winged infusion set. These samples 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 analyzed with the enzyme rate method (Unicel DXC 800; Beckman and Coulter; Germany) and homogeneous immunoassay on Modular ROCHE Automized (Basel, Switzerland), respectively. High sensitive cTnT and NT-proBNP were analyzed with the Electrochemiluminescence immunoassay (ECLIA), Elecsys (ROCHE, Basel, Switzerland). In our sample, there were 91 (26.84%) undetectable cTnT values (<3pg/ml), which were substituted using the method of Croghan and Egeghy (2003) for lower than detectable values.[24] The inter- and intra-batch variability was 15% and 5.6% respectively for cTnT, and 4.6% and 4.2% for NT-proBNP. CRP was measured with an ultra-high-sensitivity turbidimetric method (Unicel DXC 800, Beckman and Coulter, Germany). High sensitive methodology was utilized for TNF-α, and samples were centrifuged within 30 minutes after sample collection and analyzed with the Quantikine High Sensitivity Human TNF-α Enzyme

linked immunosorbent assay (HS ELISA; R&D Systems, Minneapolis, MN USA). The inter- and intra-assay variability for TNF-α was 15% and 17.8% respectively.

3.4.4. Cardiovascular assessment procedures

Addressing the design of the SABPA study, the 24-hour BP was measured at 30-minute intervals from 08:00 to 22:00 and at 60-minute intervals from 22:00 to 06:00.[25] The European Society of Cardiology criteria for hypertension were employed [average 24-hour systolic blood pressure (SBP) of ≥130mmHg and/or diastolic blood pressure (DBP) of ≥80mmHg].[23] The data was analyzed 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. The resting 10-lead ECG was recorded with the Norav NHH-1200® ECG (NORAV medical LTD PC 1200, Israel, Software version 5.030) and determined the ECG-LVH (Cornell product, [RaVL+SV3] x QRS duration). Values exceeding 244 mV.ms were indicative of ECG-LVH.[26]

3.4.5. Statistical analyses

Statistical analyses were performed with Statistica version 13.3 (TIBCO Software Inc., Palo Alto, USA, 2018). Normal distributions were computed to reveal symmetrical data. Variables with skewed distributions were log- or box cox-transformed. Independent t-tests were used to compare characteristics between the two ethnic groups. Chi-square tests (X^2) were used to determine prevalence as well as proportions. Single two-way general linear model interactions on main effects (ethnicity x gender) were computed for all cardiovascular risk markers, independently of a priori defined covariates.[23] Differences over time in each ethnic cohort were calculated via dependent t-tests as well as one-way covariance analyses

(ANCOVA's), the latter independent of priori covariates. Percentage changes over time (%Δ) were calculated by using the formula: [(follow-up - baseline)/baseline] ×100. McNemar's case-control tests were used to demonstrate changes when participants without hypertension (negative) at baseline become positive at follow-up; and hypertension-positive people at baseline recover to negative at follow-up. Forward stepwise regression analyses determined associations between dependent variables (SBP, ECG-LVH, cTnT and NT-proBNP at follow-up as well as %ΔSBP, %ΔECG-LVH, %ΔcTnT and %ΔNT-proBNP) and independent variables referring to baseline and/or %Δ of cTnT, NT-proBNP, inflammation (either TNF-α or CRP); SBP, ECG-LVH and additional covariates in separate models. Lastly, logistic regression analyses were performed to determine the odds of baseline and/or changes (Δ: follow-up – baseline) of cTnT, ΔNT-proBNP and inflammatory markers (either ΔTNF-α or ΔCRP) to associate with cTnT levels ≥4.2ng/L and the development of hypertension (SBP/DBP ≥130/80mmHg), independent of a priori covariates. For all of the above-mentioned analyses, significant values were noted when adjusted $R^2 \geq 0.25$ and $p \leq 0.05$.

3.5. RESULTS

Table 3.1 shows the clinical characteristics of the bi-ethnic cohort at baseline. Black individuals showed significantly higher alcohol abuse (γGT), inflammation (CRP and TNF-α), BP and LVH values than White individuals. In contrast, higher body surface area, cTnT and physical activity levels were present in Whites. Blacks were also more often hypertensive than Whites (62% vs. 44%, p=0.007).

Table 3.1. Clinical characteristics of a South African bi-ethnic sex cohort at baseline.

Variables	Blacks (N=152)	Whites (N=186)	p-values
Confounders			
Age, years	44.10 ± 8.27	46.13 ± 9.83	0.060
Body surface area, m^2	1.91 ± 0.23	2.00 ± 0.29	0.003
TEE, kcal/day	2577.69 (731.32;918.45)	2967.98 (1517.87;1861.98)	0.007
Cotinine, ng/mL	0.01 (54.67;68.65)	0.01 (73.53;90.20)	0.701
γGT, U/L	42.94 (74.11;93.00)	18.50 (32.02;3.28)	<0.001
Markers of Inflammation			
CRP, mg/L	5.01 (8.92;11.19)	1.50 (3.60;4.41)	<0.001
TNF-α, pg/mL	2.63 (2.95;3.71)	1.29 (1.73;2.13)	<0.001
Cardiovascular characteristics			
24h SBP, mmHg	132 ± 17	124 ± 13	<0.001
24h DBP, mmHg	82 ± 11	77 ± 8	<0.001
NT-proBNP, pg/mL	28.23 (40.91;51.49)	34.98 (42.63;52.45)	0.785
cTnT, pg/mL	5.66 (37.83;47.54)	6.92 (36.41;44.69)	0.033
LVH >244.0, mV.ms	61.94 (37.57;47.81)	44.64 (26.02;32.37)	<0.001
Hypertensive, N (%)	94 (61.84)	81 (43.55)	<0.001
Medication usage			
Hypertension, N (%)	57 (37.50)	26 (13.98)	<0.001
Anti-inflammatory, N (%)	11 (7.24)	8 (4.30)	0.244

Data are presented as mean ± SD, median (95% CI) or number of participants (%). Abbreviations γGT, Gamma glutamyl transferase; TEE, total energy expenditure; CRP, C-

reactive protein; TNF-α, Tumor necrosis factor-alpha; SBP, systolic blood pressure; DBP, diastolic blood pressure; NT-proBNP, N-terminal pro-b-type-natriuretic peptide; cTnT, cardiac troponin T; ECG-LVH, Cornell product Left Ventricular Hypertrophy; Hypertensive, SBP ≥ 130 and/or DBP ≥ 80 mmHg.

The comparisons of adjusted differences in inflammatory and cardiac-related markers over time are shown in Figure 3.2. Significant differences were revealed between Blacks and Whites, where TNF-α decreased in Blacks but increased in Whites over the three-year period (Figure 3.2a). Blacks had greater decreases in DBP (-1.44 vs -0.08) values than Whites (Figure 3.2b). In contrast, Whites showed greater increases in SBP (+0.61 vs +0.92) and NT-proBNP than Blacks.

Stratification into specific ethnic-gender groups was motivated by the significant ethnicity-by-gender interactions for NT-proBNP [F(1,282), 6.89, p=0.009], cTnT [F(1,287), 7.14, p=0.008], CRP [F(1,226), 17.05, p≤0.001];[F(1.226), 46.61, p≤0.001] and TNF-α [F(1,290), 7.26, p=0.007].

Table 3.2 shows the unadjusted differences between Black and White men over the three year period. In Black men NT-proBNP (p=0.039) increased, whereas decreases were seen for TNF-α (p=0.02). In White men, decreases were seen in cTnT (p<0.001), whereas increases were seen for NT-proBNP (p<0.001) and ECG-LVH (p<0.001). In Black men, 24-h hypertension incidence did not change over the three-year follow-up period (%Δ 2.77 [OR=1.5 (0.9, 2.6)], p=0.50) as only 5 participants recovered from baseline to follow-up.

The unadjusted differences between Black and White women showed decreases for CRP and increases for NT-proBNP and SBP in both ethnic groups (Supplementary Table 3.1). In White women increases were observed for TNF-α and ECG-LVH whilst DBP decreased. The incidence of 24-h hypertension remained unchanged in both Black and White women.

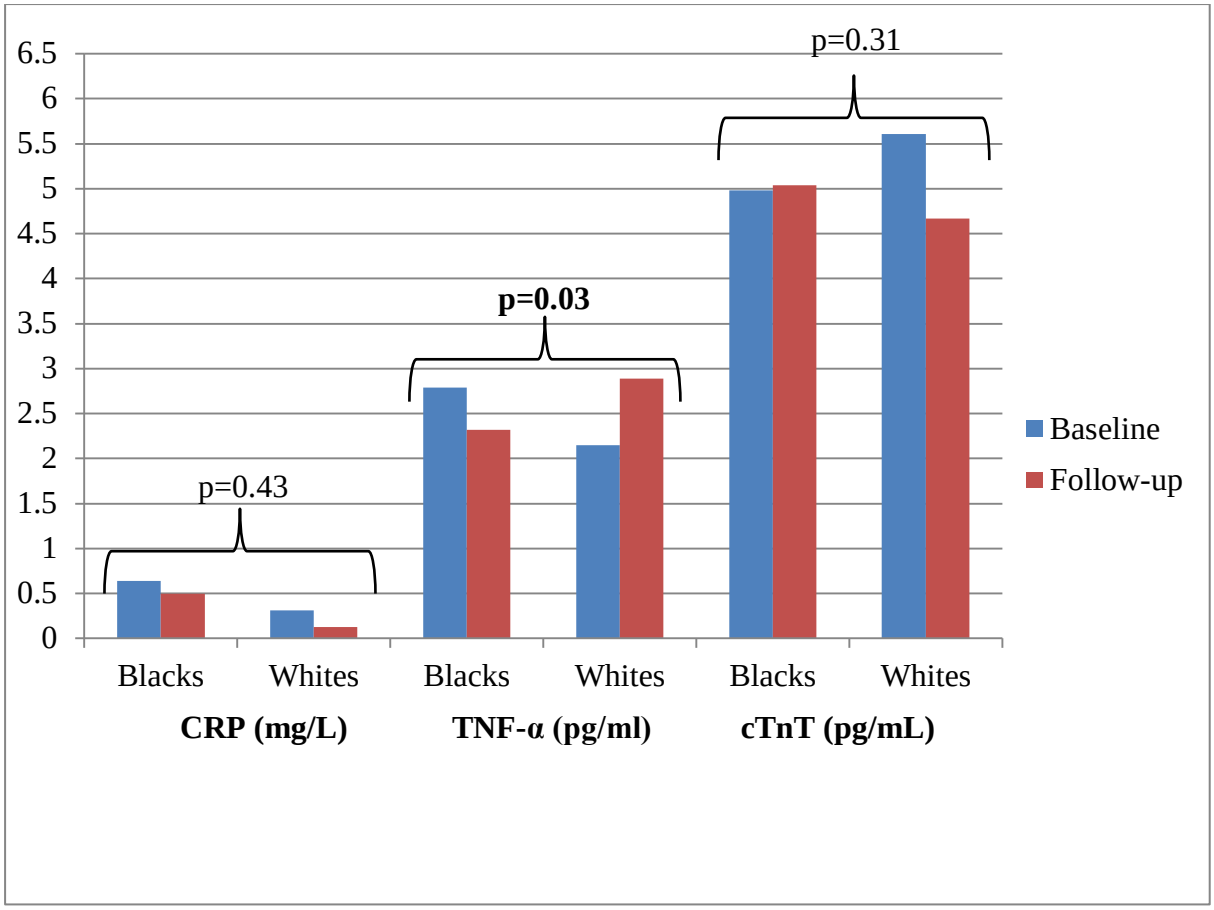

Figure 3.2a. Adjusted differences for inflammation and cardiac troponin between Blacks and Whites over a three-year period. Data presented is adjusted for age, total energy expenditure, body surface area, cotinine, gamma-glutamyl transferase. Abbreviations: CRP, C-reactive protein; TNF-α, tumor necrosis factor alpha; cTnT, cardiac troponin T.

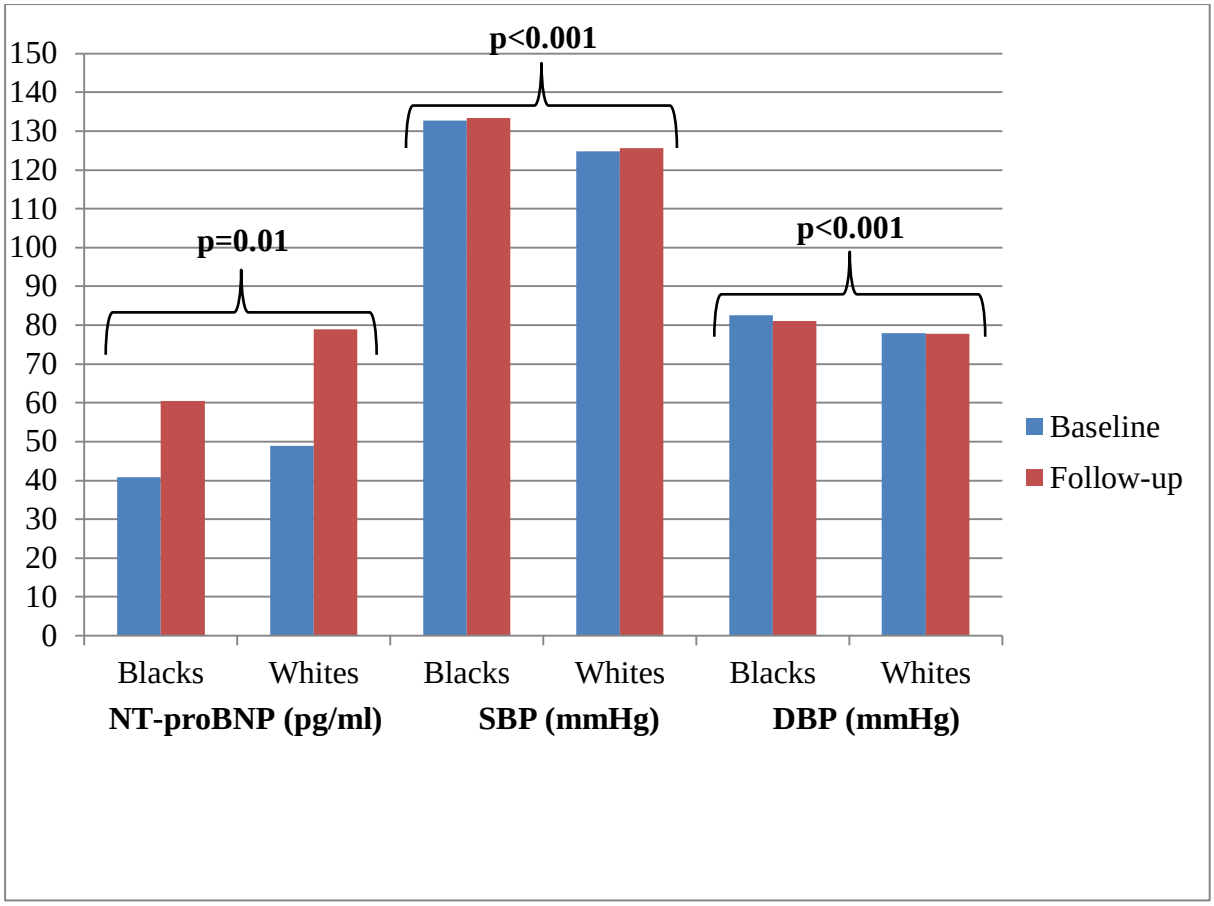

Figure 3.2b. Adjusted differences for NT-proBNP and blood pressure between Blacks and Whites over a three-year period. Data presented is adjusted for age, total energy expenditure, body surface area, cotinine, gamma-glutamyl transferase. Abbreviations: NT-proBNP, N-terminal pro-B-type natriuretic peptide; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3.2. Changes over a period of 3-years in a bi-ethnic male cohort.

	Black men (N=77)			White men (N=91)		
Variables	**Baseline; Follow-up**	**Difference**	**p-value**	**Baseline; Follow-up**	**Difference**	**p-value**
CRP, mg/L	5.53; 6.30	+0.78	0.630	2.37; 2.72	+0.35	0.723
TNF-α, pg/mL	3.71; 2.74	-0.97	0.020	2.17; 2.60	+0.44	0.094
IL-6, pg/mL	1.51; 1.91	+0.40	0.226	1.11; 0.97	-0.14	0.151
cTnT, pg/mL	5.59; 5.36	-0.23	0.570	6.86; 5.92	-0.94	<0.001
NT-proBNP, pg/mL	36.12; 47.85	+13.01	0.039	35.83; 64.91	+29.08	<0.001
24h SBP, mmHg	137; 139	+2	0.120	129; 128	-1	0.400
24h DBP, mmHg	87; 88	+1	0.529	80; 79	-1	0.097
ECG-LVH, mV.ms	83.76; 90.46	+6.70	0.156	64.06; 79.90	+15.83	<0.001
†24h Hypertension: % Δ [OR (95% CI)], p	0.50 [0.5 (0.43, 2.51)], 0.48			2.54 [2.71 (1.14, 6.46)], 0.02		
†24h Hypertension *(BL +, FU -) / (BL -, FU +)*	3 / 5			19 / 7		

Data presented is unadjusted dependent sample T-tests. Abbreviations: CRP, C-reactive protein; TNF-α, Tumor necrosis factor-alpha; cTnT, Troponin T; NT-proBNP, N-terminal pro-Brain natriuretic peptide.SBP, Systolic blood pressure; DBP, Diastolic blood pressure; ECG-LVH, left

ventricular hypertrophy. †McNemar chi-square equation values are presented as percentage difference over three years' time followed by the Odds Ratio (±95% Confidence Interval). (BL +, FU -), frequency at baseline positive but negative at follow-up; (BL -, FU +), frequency at baseline negative but positive at follow-up.

Forward stepwise regression analyses determined associations between 24h blood pressure, subclinical cardiac remodeling, cTnT and NT-proBNP at follow-up (*Model 1*) with baseline cTnT, NT-proBNP and inflammation. In *Model 2*, associations were determined between %Δ or progression in 24h blood pressure, subclinical cardiac remodeling, cTnT and NT-proBNP with %Δ in cTnT, NT-proBNP and inflammation over a three-year period. No associations were evident in women and in White men; therefore, we will only report associations found in Black men as shown in Table 3.3.

In *Model 1*, positive associations were revealed in Black men of SBP at follow-up with baseline ECG-LVH (Adj R^2 0.20; β=0.24, 95% CI 0.01 to 0.46, p=0.05). Here associations were also revealed between ECG-LVH at follow-up with baseline cTnT (Adj R^2 0.43; β= 0.48; 95% CI 0.28 to 0.68, p<0.001) and baseline SBP (Adj R^2 0.43; β= 0.29; 95% CI 0.09 to 0.49, p=0.006). NT-proBNP at follow-up associated with baseline cTnT (Adj R^2 0.30; β=0.29, 95% CI 0.07 to 0.52, p=0.01).

In *Model 2*, %ΔSBP were positively associated with %ΔNT-proBNP progression (Adj R^2 0.22; β=0.50, 95% CI 0.27 to 0.73, p<0.001) in Black men. Here, chronic high %ΔcTnT (≥4.2ng/L) positively associated with %ΔNT-proBNP progression (Adj R^2 0.23; β=0.33, 95% CI 0.10 to 0.56, p=0.007) and with %ΔTNF-α decreases (Adj R^2 0.23; β=0.25, 95% CI 0.02 to 0.48, p=0.04). Also in Black men, %ΔNT-proBNP progression inversely associated with %ΔTNF-α decreases (Adj R^2 0.34; β= -0.25, 95% CI -0.48 to -0.05, p=0.02) and with %ΔECG-LVH (Adj R^2 0.34; β= -0.27, 95% CI -0.46 to -0.04, p=0.02).

Table 3.3. Independent associations between BP, subclinical cardiac remodelling, cTnT, NTproBNP and inflammation in Black men.

Model 1	**SBP at follow-up**	**ECG-LVH at follow-up**	**cTnT at follow-up**	**NTproBNP at follow-up**
	β (95%CI)	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**
Adjusted R^2	0.20	0.43	0.34	0.30
Baseline cTnT	NS	0.48 (0.28; 0.68)†	-	0.29 (0.07; 0.52)*
Baseline SBP	-	0.29 (0.09; 0.49)**	NS	NS
Baseline LVH	0.24 (0.01; 0.46)*	-	0.35 (0.15; 0.56)**	NS
Model 2	**%ΔSBP**	**%ΔECG-LVH**	**%ΔcTnT**	**%ΔNTproBNP**
	β (95%CI)	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**
Adjusted R^2	0.22	<0.10	0.23	0.34
%ΔNT-proBNP	0.50 (0.27; 0.73)†	NS	0.33 (0.10; 0.56)**	-
%ΔTNF-α	NS	NS	0.25 (0.02; 0.48)*	-0.25 (-0.48; -0.05)*
%ΔSBP	-	NS	NS	0.44 (0.22; 0.65)†
%ΔECG-LVH	NS	-	NS	-0.27 (-0.46; -0.04)*

Data presented represents percentage differences (%Δ) over time and is adjusted for a priori covariates. Abbreviations: cTnT, cardiac troponin T; NT-proBNP, N-terminal pro-b-type-natriuretic peptide; CRP, C-reactive protein; TNF-α, Tumor necrosis factor-alpha; SBP, systolic blood

pressure; DBP, diastolic blood pressure; ECG-LVH, ECG-Left ventricular hypertrophy. A priori covariates included age, total energy expenditure, body surface area, cotinine, gamma glutamyl transferase. Either CRP or TNF-α was added in separate models to avoid collinearity. Superscript symbol shows the trend of significance:*$p \leq 0.05$; **$p \leq 0.01$; †$p \leq 0.001$.

The odds ratios of baseline levels and ΔcTnT, ΔNT-proBNP and ΔTNF-α to increase the likelihood of cTnT levels ≥4.2ng/L as well as 24h hypertension (≥130/80mmHg) over a period of three years are shown in Table 3.4. Baseline NT-proBNP (OR=1.12, 95% CI 1.01 to 1.23, p=0.03) and ΔNT-proBNP increases (OR=1.09, 95% CI 1.00 to 1.81, p=0.04) associated with 24h hypertension in Black men only. Also in Black men, baseline TNF-α (OR=1.49, 95% CI 1.05 to 2.14, p=0.03) and ΔTNF-α decreases (OR=2.07, 95% CI 1.26 to 3.40, p=0.004) significantly increased the likelihood of cTnT ≥4.2ng/L.

Table 3.4. Probability of inflammation and cardiac stress markers predicting myocyte injury and 24h ambulatory hypertension in Black men.

	Black men (N=77)				
	Nagelkerke R^2	**Odds Ratio**	**5th Percentile**	**95th Percentile**	**p-value**
			cTnT ≥4.2pg/mL		
Baseline TNF-α	0.42	1.49	1.05	2.14	0.030
ΔTNF-α	0.42	2.07	1.26	3.40	0.004
			24h Hypertension (≥130/80mmHg)		
Baseline NT-proBNP	0.55	1.12	1.01	1.23	0.032
ΔNT-proBNP	0.55	1.09	1.00	1.18	0.038

Data presented represents differences (Δ) over time (follow-up – baseline) and is adjusted for a priori covariates (age, body surface area, physical activity, cotinine, γGT and baseline levels of the respective risk factors. Abbreviations: TNF-α, Tumor necrosis factor-alpha; CRP, C-reactive protein; cTnT, Troponin T; NT-proBNP, N-terminal b-type-natriuretic peptide; ECG-LVH, left ventricular hypertrophy; SBP, systolic blood pressure.

3.6. DISCUSSION

We aimed to determine whether longitudinal changes in cTnT, NT-proBNP and markers of inflammation are associated with subclinical wall remodeling and the development of hypertension in a bi-ethic cohort from South Africa. Black men already showed susceptibility for ischemic heart disease risk (mean cTnT≥4.2pg/mL) and a pro-inflammatory profile at baseline, which reflected subclinical cardiac remodeling and hypertension at follow-up. Over a three-year period, chronic levels of myocyte injury were accompanied by rises in myocardial stretch, particularly in Black men. It may be reflective of cardiac metabolic over-demand, which increased the likelihood for hypertension and ultimately ischemic heart disease risk in a Black male cohort.

Levels of cTnT in Blacks remained constant with no significant changes occurring over the three-year period. However, cTnT at baseline and after a three-year follow-up was higher than the stress-related 4.2pg/L cut point determined by Malan, et al., which had predicted hypertension.[16] The high cTnT levels at baseline were positively associated with LVH at follow-up possibly indicating a role of myocyte injury in the development of LVH. Positive associations were previously shown between cTnT and NT-proBNP, indicating that myocyte injury may be associated with myocardial stretch in Black men.[8] We now were able to demonstrate that cTnT was prospectively associated with NT-proBNP over a period of three years in Black men. NT-proBNP, released with excessive myocardial metabolic demands, was also shown to be higher in African-Americans when compared to Whites at baseline.[20] Although we could not fully replicate this finding in cross-sectional analysis, we did find that mean levels of NT-proBNP increased over 3 years in both Black and White individuals. The protection of BNP thus seems to be increased in an attempt to counteract myocardial injury due to the higher amount of silent ischemic events in the Black men.[27] BNP have also been

shown to inhibit the sympathetic nervous system.[17,20] Therefore, NT-proBNP may have increased in response to the hyper-sympathetic response reported in the Black men.[22] However, baseline and increased NT-proBNP rather increased the likelihood for the development of 24-hour hypertension in these men. Sanchez, et al. (2015) reported similar findings, where not only positive changes, but also elevated levels of NT-proBNP at baseline preceded the development of hypertension.[21] We argue that progressive myocyte stretch accompanied by chronic cardiomyocyte injury may increase susceptibility of hypertension and metabolic demands in the Black men.

Furthermore, NT-proBNP revealed a negative association with decreased inflammation over the three-year period. Inflammatory mechanisms are essential to restore and maintain tissue homeostasis.[28,29] When tissue injury cannot be repaired in a short period of time, a chronic systemic inflammatory response prevails that may contribute to end organ damage.[28,30] Previously, a pro-inflammatory profile, utilizing CRP, was observed in the Black cohort, predisposing these individuals to early development of cardiovascular diseases.[7-9] However, in this study the consistently higher (≥ 3mg/L) [23] levels of CRP revealed no associations with BP, cardiac remodeling, myocyte injury or –stretch over the three-year follow-up period. The decreases in TNF-α rather increased likelihood for high levels of cTnT (≥4.2pg/L) and revealed a negative association with progression of myocyte stretch. This may indicate that increases in volume overload leading to myocardial stress may be related to the recruitment of inflammatory cells into the cardiac tissue to decrease blood pressure and promote restoration of myocardial tissue injury.[28,31] Studies have shown raised TNF-α to have a deleterious effect on cardiomyocytes by increasing myocyte injury leading to further increases in myocardial stretch.[30,32] Pei, et al. (2015) further showed that inhibition of TNF-α may promote cardiomyocyte survival and diminish myocardial ischemic injury in mice.[33] In

our study however, decreasing levels of TNF-α over 3 years were not protective and were associated with cardiomyocyte injury at a level as low as cTnT 4.2pg/L. Clearly more research is needed.

Levels of TNF-α decreased in Black men over the three-year follow-up period. Indeed, an unsuspected finding. Central neural inflammatory control reflexes or control mechanisms may be responsible for the decreases in TNF-α while other inflammatory markers remained constant.[34] Multiple studies revealed that stimulation of the beta-adrenergic receptors located on macrophages leads to decreases in specifically TNF-α.[34-37] In contrast stimulation of alpha-adrenergic receptors stimulates pro-inflammatory signaling thereby increasing TNF-α production. The specific pro- or anti-inflammatory pathway that catecholamine binding will initiate is said to be determined by the concentration of the catecholamines or adrenergic receptor agonists present.[35] Hyperactivity of the sympathetic nervous system was reported in this cohort before.[22] Here, Black men revealed augmented α-adrenergic and diminished β-adrenergic responses.[38] The augmented α-adrenergic responses may therefore have attributed to the increased levels of TNF-α in the black men, which however was not the case.

Production of inflammatory cytokines can also be diminished through stimulation of the vagus nerve.[35,39] A study done by Sloan, et al. (2007) showed that R-R interval variability, an index of vagus nerve modulation, was inversely associated with inflammation.[40] However, once again it was shown that vagus-nerve mediated autonomic control of the heart was lower in Black men of the SABPA cohort.[23,38] The reason for the decrease of TNF-α over the three-year period thus warrants further investigation as current known anti-inflammatory (TNF-α) pathways seems unlikely in our cohort.

Despite the observed increases for 24h SBP, NT-proBNP and ECG-LVH in White men and women, no associations existed between inflammation, cTnT, NT-proBNP and BP. Hamer et al., (2011) showed that the excess burden of subclinical vascular disease in Blacks can be explained by detrimental health behaviors and conventional risk factors that are increased in Blacks rather than in Whites.[41] Black women also had fewer cardiovascular risk factors compared to Black men, which was also found in African-Americans.[22,42] Although Black women showed increases for SBP and NT-proBNP, the absence of associations in the Black women could imply a resiliency pertaining to underlying mechanisms which may be responsible for these increased values.[43]

Although this study is the first of its kind performed in a Black cohort, it also has its limitations. The study only represents a cohort of the South African population and the results can therefore not be attributed to the African population at large. Longitudinal trends beyond three years regarding adverse cardiovascular outcomes cannot be projected.

To conclude, the first prospective analysis in Africa assessing inflammatory-cardiac stress and hypertension associations showed that chronic myocyte injury accompanied by progressive increases in NT-proBNP were positively associated with the development of hypertension in a Black male cohort. Ultimately, this concurs with an increased likelihood of hypertension and ischemic heart disease in a Black male cohort.

3.7. CONFLICT OF INTEREST

The authors declare no conflict of interest pertaining to the information of this manuscript.

3.8. ACKNOWLEDGEMENTS

This study would not have been possible without all the individuals who volunteered to participate in this study.

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Supplementary Table 3.1. Changes over a period of 3-years in a bi-ethnic female cohort.

	Black women (N=75)			White women (N=95)		
Variables	**Baseline; Follow-up**	**Difference**	**p-value**	**Baseline; Follow-up**	**Difference**	**p-value**
CRP, mg/L	10.78; 7.07	-3.71	<0.001	3.82; 2.44	-1.39	0.003
TNF-α, pg/mL	2.52; 2.15	-0.37	0.144	1.48; 2.88	+1.40	<0.001
cTnT, pg/mL	4.17; 3.86	-0.32	0.461	4.54; 4.21	-0.33	0.132
NT-proBNP, pg/mL	51.12; 66.35	+15.22	0.029	56.75; 99.07	+42.32	<0.001
24h SBP, mmHg	132; 128	+4	0.017	119; 121	+2	0.036
24h DBP, mmHg	79; 80	+1	0.309	74; 72	-2	<0.001
ECG-LVH, mV.ms	83.76; 90.46	+6.70	0.156	64.06; 79.90	+15.83	<0.001
†24h Hypertension						
%Δ [OR (95% CI)], p	0.80 [0.67 (0.27, 1.63)], 0.37			0.89 [1.57 (0.61, 4.05], 0.35		
(BL+, FU-) / (BL-, FU+)	8 / 12			11 / 7		

Data presented is unadjusted dependent sample T-tests. Abbreviations: CRP, C-reactive protein; TNF-α, Tumor necrosis factor-alpha; cTnT, Troponin T; NT-proBNP, N-terminal pro-Brain natriuretic peptide.SBP, Systolic blood pressure; DBP, Diastolic blood pressure; ECG-LVH, left ventricular hypertrophy. †McNemar chi-square equation values are presented as percentage difference (%Δ) over three years' time followed by

the Odds Ratio (±95% Confidence Interval). (BL +, FU -), frequency at baseline positive but negative at follow-up; (BL -, FU +), frequency at baseline negative but positive at follow-up.

CHAPTER 4

MANUSCRIPT 2

The abstract of Manuscript 2 was presented as a Poster at the 19th Annual SA Heart Conference from 4-7 October 2018 in Sun City, South Africa and published in the SA Heart Journal.

Manuscript 2 was submitted to the European Journal of Clinical Investigation.

4.1. INSTRUCTIONS FOR AUTHORS

European Journal of Clinical Investigation

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Impact factor:	3.086
Publisher:	Wiley Online Library
Aims and scope:	EJCI considers any original contribution from the most sophisticated basic molecular sciences to applied clinical and translational research and evidence-based medicine across a broad range of subspecialties. High-quality research pertaining to the genetic, molecular, cellular, or physiological basis of human biology and disease, as well as research that addresses prevalence, diagnosis, course, treatment, and prevention of disease. We are primarily interested in studies directly pertinent to humans. Interdisciplinary work and research using innovative methods and combinations of laboratory, clinical and epidemiological methodologies and techniques is of great interest to the journal.
Language:	English (UK)
Margins:	1-inch
Paragraph spacing:	Double
Font:	12
Sections:	Title page: Authors and affiliations in lower case superscript letter (not qualifications), Corresponding author contact details, Grants.
Abstract:	250 words; 6 keywords
Tables and Figures:	Placed next to the relevant text or in a separate file.
References:	Example: King VM, Armstrong DM, Apps R, Trott JR. Numerical aspects of pontine, lateral reticular, and inferior olivary projections to two paravermal cortical zones of the cat cerebellum. J Comp Neurol 1998;390:537-551.
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Hypertension in Africa Research Team

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BDNF, cardiac stress and cognitive interference in Black men: The SABPA prospective study

Running head: BDNF, cognition and cardiac injury

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4.2. ABSTRACT

Background

Brain-derived neurotrophic factor (BDNF) modulates brain health and cognition, which can interfere with executive cognitive function. BDNF was implicated with microcirculatory ischemia and may reflect cardiac stress and inflammation. We aimed to determine whether prospective % changes (Δ) in BDNF are associated with Δcardiac stress risk markers (troponin T and N-terminal-pro-B-type natriuretic peptide), Δtumor necrosis factor-alpha (TNF-α) and executive cognitive function in a bi-ethnic cohort.

Design

This SABPA prospective sub-study was conducted in a bi-ethnic gender cohort (aged 20-65 years) from South Africa. Ambulatory blood pressure and fasting serum blood samples for BDNF, cardiac troponin T (cTnT), N-terminal brain natriuretic peptide (NT-proBNP) and TNF-α were obtained. The STROOP color-word conflict test (CWT) was applied to assess executive cognitive function.

Results

In Black men, no changes occurred in cTnT, but BDNF ($p<0.001$) and NT-proBNP ($p=0.009$) increased over the three-year period. In Black men only, chronic raised cTnT (≥4.2 ng/L) were associated with increased ΔBDNF (β=0.25; 95% CI 0.05 to 0.45; p=0.02), ΔNT-proBNP (β=0.29; 95% CI 0.09 to 0.49; p=0.006) and decreases in ΔTNF-α (β=0.24; 95% CI 0.04 to 0.44; p=0.02). Again in Black men, chronic raised cTnT were inversely associated with baseline STROOP-CWT (β= -0.33; 95% CI -0.53 to -0.12; p=0.003). Here, ΔBDNF associated with cTnT levels ≤4.2 ng/L (OR=2.35, 95% CI 1.12 to 4.94, p=0.02).

Conclusions

Central neural control mechanisms may have upregulated BDNF and down-regulated TNF-α in Black men as a way to protect against myocardial stress progression and to possibly improve processes related to interference control.

Keywords BDNF; myocardial injury; cognition; TNF-α; Black men

4.3. INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor secreted by neuronal tissue in the central and peripheral nervous system [1]. Originally BDNF was only seen as a pro-survival factor for neurons [2,3] as it facilitated cognitive processes in the hippocampus [4]. Furthermore, the release of neurotrophins, such as BDNF, modulates the functional integrity of the fronto-striatal networks [5]. The integrity of these networks is crucial to maintain executive cognitive functioning, as processes related to interference control depend on the anterior cingulate and dorsolateral prefrontal areas [6,7]. The STROOP-color-word conflict test (STROOP-CWT) was shown to be a valid test to assess cognitive interference or executive cognitive functioning [8,9]. During exposure to the STROOP-CWT the presentation of a word stimulus and a colour stimulus simultaneously causes interference when the processing of one stimulus interferes with the simultaneous processing of the second stimulus [8,9]. Giacobbo et al. (2016) suggested that higher levels of BDNF were optimal for response inhibition capacity assessed by the STROOP-CWT [10].

Increasing evidence has shown that BDNF may have a protective effect on the cardiovascular system [11]. Indeed, Smith et al. (2015) have shown that BDNF decreases atherosclerotic plaque formation in the carotid artery of Black South African men [12]. In turn, oxidative myocyte injury can also be influenced by BDNF [13,14]. A marker that can be used to assess myocyte injury is cardiac Troponin T (cTnT) [15,16]. In the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) cohort conducted on Black and White teachers, we previously reported positive associations between cTnT and N-terminal of pro-brain natriuretic peptide (NT-proBNP) levels in Black men [17]. NT-proBNP, the precursor to brain natriuretic peptide, is mainly released following increased cardiac wall strain or -stress [18,19].

In Black men, NT-proBNP was also associated with the inflammatory marker, tumour necrosis factor-alpha (TNF-α) [17]. Inflammation is a defence mechanism that occurs in order to restore and maintain tissue homeostasis [20,21]. Once tissue injury occurs independent of repair, a chronic systemic inflammatory response prevails that is detrimental to cardiovascular health [22,23,24]. Low-grade systemic inflammation was additionally associated with poorer executive cognitive functioning [25]. Indeed, TNF-α is a pro-inflammatory cytokine produced by various types of cell including populations of neurons in the brain, microglial cells and astrocytes [26]. Inhibition of TNF-α prevents cognitive decline and BDNF decreases in the hippocampus [27]. Therefore the pro-inflammatory profile previously reported in the SABPA cohort may not only have damaging effects in the cardiovascular but also in the central nervous system.

Hence, we aimed to assess prospective associations between BDNF, cardiac stress (cTnT and NT-proBNP), inflammation (TNF-α) and cognitive interference (STROOP-CWT) in a bi-ethnic gender cohort from South Africa. We hypothesized that changes in BDNF will inversely associate with markers of cardiac stress, inflammation and cognitive interference in Blacks.

4.4. METHODS

4.4.1. Study design and participant selection

The SABPA prospective cohort study was conducted in the North West Province of South Africa and is well described elsewhere [28]. Urban-dwelling Black and White teachers (N=2170) residing in the Dr Kenneth Kaunda Education District, were invited to participate. Only 471 volunteering participants complied with the exclusion criteria and were assessed for

eligibility as shown in Figure 4.1. Teachers had similar socio-economic class but we could not control for cultural diversity.

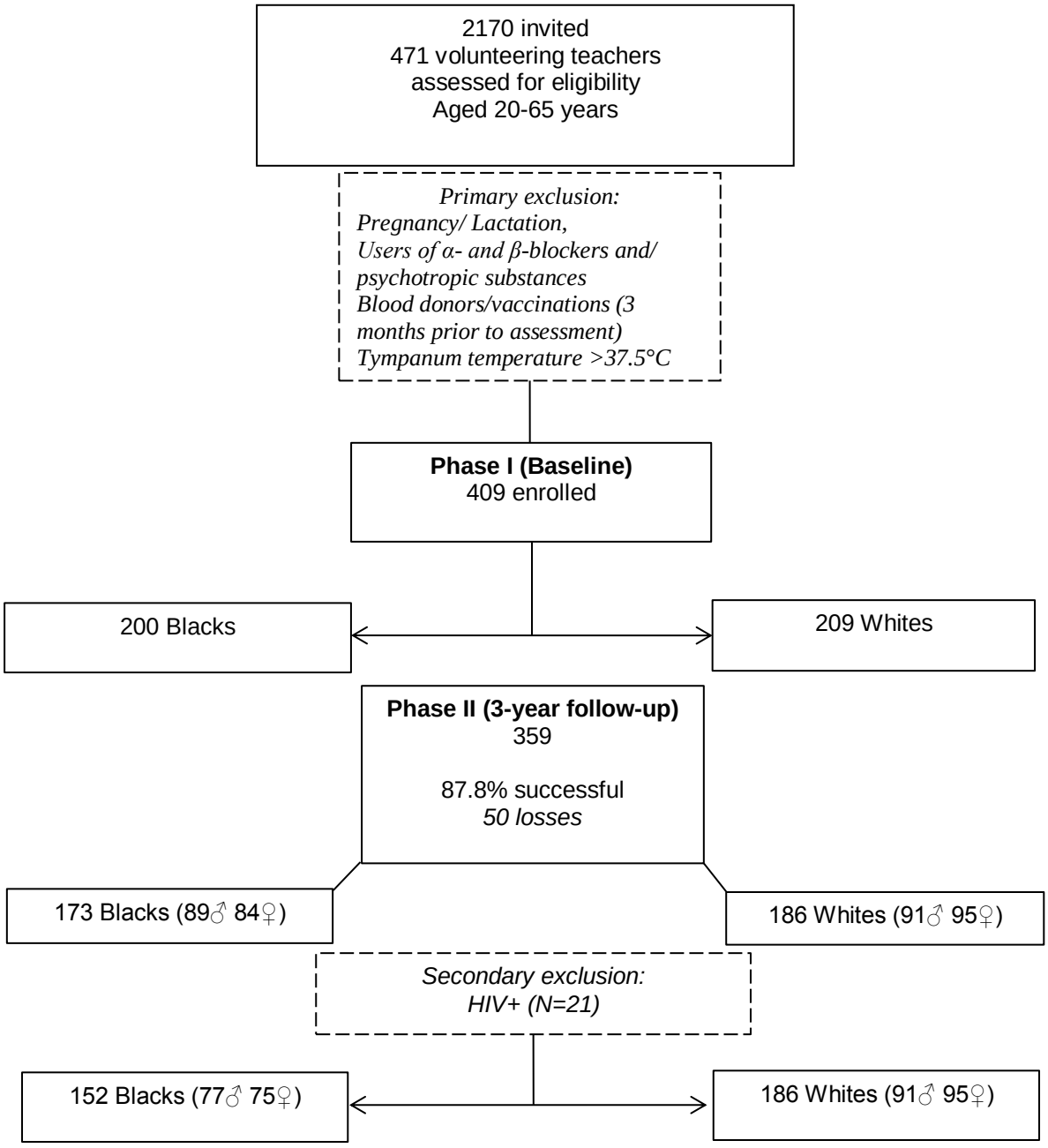

Figure 4.1. Longitudinal study design assessing a South African bi-ethnic gender cohort.

The exclusion criteria for the SABPA study were: pregnancy, lactation, users of α- and β-blockers or psychotropic substances, blood donors or vaccinations within 3 months prior to clinical assessment and a tympanum temperature exceeding 37.5°C. Phase I of the study included 409 participants (200 Blacks and 209 Whites, aged between 20 and 65 years) and was conducted from February to May (2008 and 2009). Phase II of the study commenced after a period of three years and was conducted from February to May (2011 and 2012). The successful follow-up rate was 87.8% (359 participants) with reasons for non-participation being pregnancy (N=2), deceased (N=6), emigrated (N=4), moved too far away from the data collection site (N=10), chose not to participate (N=28).

Additional exclusions were made for the purpose of this sub-study to avoid bias pertaining to cardio-metabolic risk [29]. Therefore participants with an HIV positive status (N=21) were excluded. A total of 35 participants had incomplete data for the main variables, which included BDNF (N=14), STROOP-CWT (N=1), cTnT (N=6), NT-proBNP (N=11) and TNF-α (N=3). Participants with incomplete data (N=35) were however included in all analyses as their exclusion did not change the outcome of the results. Ultimately, 338 (152 Black and 186 White) participants remained.

4.4.2. Experimental procedure and data collection

The exact well-controlled protocol was followed for both phases of the study to minimize bias when comparing data. Every working day between ~07:00 and 08:00 (Monday to Thursday) researchers arrived at the respective schools to check informed consent and to programme the Cardiotens CE120® (Meditech, Budapest, Hungary) and Actical® activity monitor (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada) apparatuses for the measurement of 24-hour ambulatory blood pressure (ABPM), 2-lead electrocardiogram

(ECG) and daily physical activity (monitored over 24-hours). 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 the end of the working day at approximately 16:30, the participants were transported to the Metabolic Unit Research Facility at North-West University where each participant was accommodated in a private bedroom. They were also informed about the following day's procedures and experimental setup. Demographic and general health questionnaires were completed and a standardized dinner was served to participants who were advised to fast and rest from 22:00 for the next day's clinical measurements.

The next morning at approximately 07:00, the 24-hour ambulatory apparatuses were disconnected where after the anthropometric and clinical measurements (blood pressure, ECG's and blood sampling as well as stressor exposure) commenced. Before resting ECG's and blood sampling were performed, the participants were positioned in a semi-recumbent manner for approximately 30-45 minutes. All the blood samples were obtained before 09:00 to ensure that the sampling times of both phases corresponded. After the assessments breakfast was served, feedback was provided and the participants were transported back to their respective schools.

4.4.3. Lifestyle determinants

Registered level II anthropometrists measured the anthropometric variables in triplicate according to standardized procedures. The mean of the three measurements was used to maximize accuracy. Inter- and intra-observer variability was found to be less than 10%. The

body surface area was calculated with the Mosteller formula of [weight (kg) x height (cm) ÷ 3600]^{½}.

4.4.4. **Biochemical measurements**

Fasting blood samples were obtained by registered nurses from the ante-brachial vein using a sterile winged infusion set. All samples were dealt with in accordance with standardized procedures and stored at -80°C until analysis. Gamma-glutamyl transferase (γ-GT), an indicator of alcohol abuse, was analysed with the enzyme rate method (Unicel DXC 800; Beckman and Coulter; Germany). Serum cotinine, an indicator of nicotine levels, was analysed with the homogeneous immunoassay on Modular ROCHE Automized, Switzerland.

The electrochemiluminescence immunoassay [(ECLIA), Elecsys, 2010, Roche, Basel, Switzerland] was used to analyse serum cTnT and NT-proBNP. In our sample, there were 91 (26.84%) undetectable cTnT values (<3 pg/ml) at baseline, which were substituted using the method of Croghan and Egeghy (2003) for lower than detectable values [30]. Inter- and intra-batch variability was 15% and 5.6% for cTnT, and 4.6% and 4.2% for NT-proBNP. C-reactive protein was measured with the ultra-high-sensitive turbidimetric method (Unicel DXC 800, Beckman and Coulter, Germany). Serum TNF-α was stored for 6 years and thawed once for analyses at an accredited laboratory with the Quantikine High Sensitivity Human TNF-α enzyme-linked immunosorbent assay (HS ELISA, Catalogue number: HSTAOOD; R&D Systems, Minneapolis, MN USA) [31]. The inter- and intra-assay variability for TNF-α was 15% and 17.8%, respectively. BDNF was analysed with a Quantikine Colorometric-Sandwich Immunoassay (Catalogue number: DBD00). The intra-assay precision was between 3.8 and 6.2%, and the inter-assay precision was between 7.6 and 11.3%.

4.4.5. Cardiovascular assessment procedures

Ambulatory 24-h BP measurements were performed on the non-dominant arm at 30-minute intervals from 08:00 to 22:00 and at 60-minute intervals from 22:00 to 06:00 using suitable cuff sizes [29]. The European Society of Cardiology (ESC) criteria for hypertension were employed [average 24-hour systolic blood pressure (SBP) ≥130mmHg and/or diastolic blood pressure (DBP) ≥80mmHg] [29]. Participants were required to note any abnormalities they experienced during the day on a 24-hour diary card. Typical abnormalities included visual disturbances, headaches, nausea, fainting, palpitations, physical activity and stress.

4.4.6. Cognitive interference

The STROOP-color-word conflict test (CWT) was applied to assess executive cognitive function. A series of five words in a random order describing a specific colour, but written in different colours (total of 10 columns) displayed on a cardboard, were shown to the participants. The participants had to verbally identify the colour (ink colour) of a given word and not to read the colour represented by the word, within 1 minute. The more automated task (reading the colour represented by the word) interferes with the performance of the less automated task (naming the ink colour) and participants are required to inhibit this interference [8,9]. In order to increase pressure or tension, the participants were encouraged to progress as fast as possible. Mistakes were identified and participants were asked to repeat answers that were wrong. An interference score (STROOP-CWT) was calculated that represents the number of correct answers produced during the fixed period of 1 minute. A lower score thus indicates that the individual found it more difficult to inhibit the interference. The same two scientists (medical doctor and registered nurse) obtained STROOP-CWT scores of all teachers at baseline. On completion of the task participants received a monetary incentive in accordance with their performance.

4.4.7. Statistical analyses

Statistical analyses were performed using Statistica version 13.1 (Statsoft Inc., Tulsa, OK, USA). Initial single two-way general linear modelling tested interactions on main effects (ethnicity x gender) for BDNF, TNF-α, cognitive interference and cardiac stress (cTnT and NT-proBNP) markers, independent of a priori covariates. A priori covariates included age, physical activity, body surface area, γ-GT and cotinine [29]. Variables with skewed distributions were log-transformed. Main risk markers BDNF, STROOP-CWT, cTnT, NT-proBNP and TNF-α data were compared in participants with (N=303) and without a complete data set (N=35) by using independent *t*-tests. For all analyses, significant values were noted when adjusted $R^2 \geq 0.25$ and $p \leq 0.05$.

4.4.7.1. Cross sectional data analyses

Independent *t*-tests were used to compare characteristics of the two ethnic groups; whilst Chi-square tests determined prevalence as well as proportions at baseline.

4.4.7.2. Longitudinal data analyses

Dependent sample T tests compared unadjusted differences over time in each ethnic group. Multivariate linear regression analyses were performed and percentage changes (Δ) were calculated with the following formula: [(follow-up - baseline)/baseline] ×100. Forced entry stepwise forward regression analyses in several models determined associations between dependent variables (ΔcTnT), and independent variables (ΔNT-proBNP, ΔBDNF, ΔTNF-α, STROOP-CWT score and selected *a priori* covariates). Logistic regression analyses were performed independent of a priori covariates, to determine the odds of ΔBDNF, ΔNT-proBNP, ΔTNF-α and baseline STROOP-CWT to predict levels of cTnT using the established 4.2ng/L cTnT cut-point. This cut point was previously shown to predict 24-h and clinic hypertension in this SABPA cohort [32].

4.5. RESULTS

When comparing main risk markers BDNF, STROOP-CWT, cTnT, NT-proBNP and TNF-α in participants with (N=303) and without complete data (N=35) significant differences were observed only in STROOP-CWT scores (57 vs. 52, p=0.046). However, participants with incomplete data (N=35) were included in all analyses as their exclusion did not change the outcome of the results. Interactions on main effects (ethnicity x gender) were computed and motivated further stratification into specific ethnic and gender groups. Interactions between main effects (ethnicity × gender) were revealed for ΔBDNF [F(1,309), 9.86, p=0.002], ΔTNF-α [F(1,323), 4.91, p=0.03] and STROOP-CWT [F(1,324), 97.20, p<0.001], [F(1,324), 21.73, p<0.001]. Furthermore interactions between main effects (ethnicity) was shown for ΔNT-proBNP [F(1,306), 5.74, p=0.02].

4.5.1. Cross-sectional data analyses

The clinical characteristics of the study participants are presented in Table 4.1. Blacks had significantly higher levels of γGT (p<0.001), inflammation (p<0.001) and 24-h BP than Whites. The prevalence of hypertension was also higher in Blacks than in Whites (58% vs. 43%, p=0.008). In turn, Whites revealed a larger body surface area as well as increased physical activity, BDNF, cTnT levels and STROOP-CWT scores than was the case with Blacks.

4.5.2. Longitudinal data analyses

The unadjusted differences over time are shown in Table 4.2. BDNF and SBP increased in Blacks whereas cTnT and DBP decreased in Whites. NT-proBNP increased in Blacks and Whites over the three-year period. In Figure 4.2, the unadjusted differences for every ethnic and gender group are shown. In Black men, BDNF (p<0.001) increased while TNF-α (p=0.02) decreased. In contrast, TNF-α (<0.001) increased in White women. cTnT (p<0.001) showed decreases in White men.

Table 4.1. Clinical characteristics of a South African bi-ethnic gender cohort at baseline.

Variables	Blacks (N=152)	Whites (N=186)	p-values
Confounders			
Age, years	44.10 ± 8.27	46.13 ± 9.83	0.060
Body surface area, m^2	1.91 ± 0.23	2.00 ± 0.29	0.003
TEE, kcal/day	2577.69 (731.32;918.45)	2967.98 (1517.87;1861.98)	0.007
Cotinine, ng/mLl	0.01 (54.67;68.65)	0.01 (73.53;90.20)	0.701
γGT, U/L	42.94 (74.11;93.00)	18.50 (32.02;3.28)	<0.001
Neurotrophins			
BDNF, ng/L	1.49 ± 0.66	1.67 ± 0.85	0.048
Markers of inflammation			
CRP, mg/L	5.01 (8.92;11.19)	1.50 (3.60;4.41)	<0.001
TNF-α, pg/mL	2.63 (2.95;3.71)	1.29 (1.73;2.13)	<0.001
Cognitive interference			
STROOP-CWT	48 ± 11	63 ± 13	<0.001
Cardiovascular characteristics			
24h SBP, mmHg	132 ± 17	124 ± 13	<0.001
24h DBP, mmHg	82 ± 11	77 ± 8	<0.001
NT-proBNP, pg/mL	28.23 (40.91;51.49)	34.98 (42.63;52.45)	0.785
cTnT, pg/mL	5.66 (37.83;47.54)	6.92 (36.41;44.69)	0.033
Hypertensive, N (%)	94 (61.84)	81 (43.55)	<0.001
Medication usage			

Hypertension, N (%)	57 (37.50)	26 (13.98)	<0.001

Data are presented as mean ± SD, median (95% CI) or number of participants (%). Abbreviations γGT, Gamma glutamyl transferase; TEE, total energy expenditure; BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; TNF-α, tumour necrosis factor-alpha; STROOP-CWT, STROOP-color-word conflict test score for cognitive interference; SBP, systolic blood pressure; DBP, diastolic blood pressure; NT-proBNP, N-terminal pro-b-type-natriuretic peptide; cTnT, cardiac troponin T; Hypertensive, SBP ≥ 130 and/or DBP ≥ 80 mmHg.

Table 4.2. Unadjusted differences over a period of 3-years.

	Blacks (N=152)			Whites (N=186)		
Variables	**Baseline; Follow-up**	**Difference**	**p-value**	**Baseline; Follow-up**	**Difference**	**p-value**
BNDF, ng/mL	1.50; 1.85	+0.35	<0.001	1.67; 1.60	-0.07	0.331
TNF-α, pg/mL	3.12; 2.45	-0.67	0.006	1.82; 2.74	+0.93	<0.001
cTnT, ng/L	4.79; 4.43	-0.36	0.204	5.47; 4.89	-0.58	<0.001
NT-proBNP, pg/mL	44.48; 57.84	+13.36	0.009	46.50; 83.29	+36.79	<0.001
24h SBP, mmHg	132; 134	+2.00	0.011	124; 123	-1.00	0.113
24h DBP, mmHg	82; 83	+1.00	0.547	77; 76	-1.00	0.001

Data presented are unadjusted dependent sample *t*-tests. Abbreviations: BDNF, Brain-derived neurotrophic factor; TNF-α, Tumour necrosis factor-alpha; cTnT, Troponin T; NT-proBNP, N-terminal pro-Brain natriuretic peptide; SBP, Systolic blood pressure; DBP, Diastolic blood pressure.

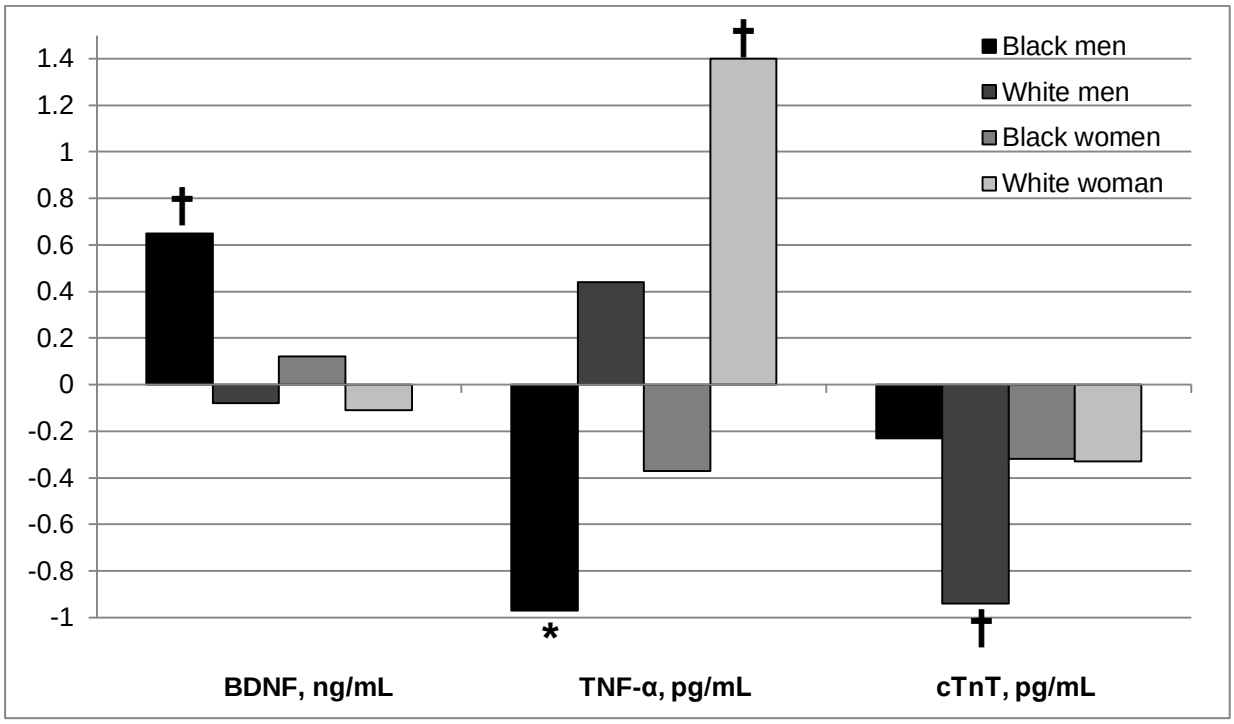

Figure 4.2. Unadjusted differences over a period of 3-years in a Black and a White cohort. Abbreviations: BDNF, brain-derived neurotrophic factor; TNF-α, tumour necrosis factor-alpha; cTnT, cardiac troponin T. Superscript symbol shows the trend of significance:*p ≤ 0.05; **p ≤ 0.01; †p ≤ 0.001.

No significant associations existed between BDNF, cTnT, TNF-α or STROOP-CWC in whites (men and women) and will not be discussed further. Table 4.3, shows the independent associations (Δ) between cTnT, BDNF, NT-proBNP and the STROOP-CWT. In Black men, chronic raised cTnT levels were positively associated with ΔNT-proBNP (Adj R^2 0.35; β=0.29; 95% CI 0.09 to 0.49; p=0.006), ΔBDNF (Adj R^2 0.35; β=0.25; 95% CI 0.05 to 0.45; p=0.02) and ΔTNF-α (Adj R^2 0.35; β=0.24; 95% CI 0.04 to 0.44; p=0.02). In turn, chronic raised cTnT inversely associated with cognitive interference (Adj R^2 0.35; β= -0.33; 95% CI -0.53 to -0.12; p=0.003). In Black women, constant cTnT levels inversely associated with constant TNF-α levels (Adj R^2 0.25; β= -0.25; 95% CI -0.46 to -0.04; p=0.02).

Odds ratios showed probability of BDNF increases reflecting lower cTnT (≤4.2 ng/L) levels (Table 4.4) (OR=2.35, 95% CI 1.12 to 4.94, p=0.02). In Black women, ΔTNF-α (OR=0.63, 95% CI 0.43 to 0.93, p=0.02) and baseline STROOP-CWT (OR=1.08, 95% CI 1.11 to 1.15, p=0.02) significantly associated with cTnT levels ≤4.2ng/L.

Table 4.3. Longitudinal associations between BDNF and markers of cardiac stress, inflammation and cognitive interference in a bi-ethnic gender cohort.

	Black men (N=66)		**Black women (N=64)**	
	ΔBDNF, ng/mL β (95% CI)	**ΔcTnT, pg/mL β (95% CI)**	**ΔBDNF, ng/mL β (95% CI)**	**ΔcTnT, pg/mL β (95% CI)**
Adjusted R^2	0.20	0.35	<0.10	0.25
ΔBDNF, ng/mL	-	0.25* (0.05; 0.45)	-	-
ΔcTnT, pg/mL	0.32** (0.08; 0.55)	-	NS	-
ΔNT-proBNP, pg/mL	NS	0.29** (0.09; 0.49)	NS	NS
ΔTNF-α, pg/mL	NS	0.24* (0.04; 0.44)	NS	-0.25* (-0.46; -0.04)
STROOP-CWT	NS	-0.33** (-0.53; -0.12)	NS	NS

Data presented are adjusted for a priori covariates age, physical activity, body surface area, cotinine, gamma glutamyl transferase. Abbreviations: BDNF, Brain-derived neurotrophic factor; cTnT, Troponin T; NT-proBNP, N-terminal pro-Brain natriuretic peptide; TNF-α, Tumour necrosis factor-alpha; STROOP-CWT, STROOP-color-word conflict test score. Superscript symbol shows the trend of significance:*$p \leq 0.05$, **$p \leq 0.01$.

Table 4.4. Probability of risk marker changes using an established cTnT 4.2pg/L cut point (Malan et al., 2017) in Blacks.

	cTnT ≤4.2pg/L	
	Men (N=77)	**Women (N=75)**
Nagelkerke R^2	0.47	0.33
ΔBDNF, ng/mL	2.35 (1.12; 4.94)*	1.51 (0.95; 2.40)
ΔTNF-α, pg/mL	0.71 (0.39; 1.31)	0.63 (0.43; 0.93)*
STROOP-CWT	0.93 (0.80; 1.07)	1.08 (1.01; 1.15)*

Data presented represent differences over time (Δ) and are adjusted for a priori covariates (age, physical activity, body surface area, cotinine, gamma glutamyl transferase). Abbreviations: BDNF, Brain-derived neurotrophic factor; cTnT, Troponin T; TNF-α, tumour necrosis factor-alpha; STROOP-CWT, STROOP-color-word conflict test score. Superscript symbol shows the trend of significance:*$p \leq 0.05$.

4.6. DISCUSSION

The aim of this study was to determine whether changes in cardiac stress markers were associated with changes in BDNF, TNF-α and with executive cognitive function in a bi-ethnic cohort from South Africa. Overall, Black men showed higher cognitive interference at baseline with increases in BDNF over time to possibly regulate executive cognitive functioning and presumably cardiac function. Central neural control may thus have upregulated BDNF in the brain as a way to protect against myocardial stress and probably to improve processes related to interference control in Black men.

BDNF was shown to have a protective effect in the central nervous [2] and cardiovascular systems as low levels of BDNF particularly are associated with future cardiovascular events and mortality [11,33,34]. Indeed, BDNF has been demonstrated to protect the myocardium against ischemic-related injury, thereby promoting the survival of cardiomyocytes [14,35]. It was also shown that BDNF may lead to a decrease in atherosclerotic plaque formation of the carotid artery in the SABPA cohort [12]. In the current cohort, levels of BDNF were higher in Whites than in Blacks at baseline. However, this increased level of BDNF in White individuals decreased over the three-year period, thereby presumably also reducing the protective role of BDNF in the Whites. The contrary was seen in Blacks, where BDNF increases or upregulation over the three-year period suggested cardiovascular protection and potentially improves executive cognitive functioning. The upregulation of BDNF was associated with changes in cTnT in Black men – something that did not occur in the White men. Levels of cTnT remained constant in Blacks over the three-year period. However, the levels at follow-up were higher than the cut-point of 4.2pg/mL in Blacks from the same cohort. Although cTnT levels decreased in Whites over the three-year period, the levels remained higher than in Blacks. These levels might have decreased more if BDNF did not

decrease in these individuals. In Black men specifically, BDNF increases were positively associated with lower cTnT levels (≤ 4.2pg/L) indicating that BDNF may possibly act as a compensatory mechanism to protect against myocyte injury. However, it may not be sufficient to decrease cTnT in Blacks.

Previously we showed associations between cTnT, NT-proBNP and TNF-α in cross-sectional analysis [17]. The current study shows that upregulation of NT-proBNP was associated with cTnT over a period of three years in Black men, which may be indicative of the fact that cardiac injury increases the release of NT-proBNP in an attempt to repair the damage sustained to the myocardium. NT-proBNP is the inactive precursor of BNP and BNP has cardio-protective effects including decreasing myocardial wall stress and thus myocardial injury [18,19]. However, upregulation of NT-proBNP seems insufficient to decrease cTnT over the three-year period as the high cTnT level in Black men was sustained. Chronic high cTnT levels were also associated with decreased TNF-α in Black men. Binding of TNF-α to its TNF receptor one may induce apoptosis and necrosis leading to myocyte death [26,36]. Indeed, it has been shown that inflammation can further contribute to myocyte death by promoting the development of atherosclerotic plaque in coronary arteries [22,37,38], that can induce hypoxia, a main stimulus for myocyte death [39,40]. Neuro-inflammation may also impair cognitive function by inhibiting neurogenesis [41]. It thus seems as if the brain down-regulated inflammation not only in an attempt to prevent further myocardial damage, but also to prevent cognitive interference as cTnT inversely associated with the STROOP-CWT in Black men.

Although levels of myocyte injury and inflammation remained constant in Black women, an inverse association was shown between cTnT and TNF-α. It thus seems as if inflammation is

still protective in Black women as chronic inflammation may prevent the increase in myocyte injury over a period of three years.

At baseline, we showed Blacks to have a lower STROOP-CWT score than their White counterparts. This may indicate that Blacks had more difficulty inhibiting the automated process than Whites; thus leading to a greater cognitive interference in the Blacks. This interference may possibly be affected by high cTnT levels as shown in Black men. In Black women, STROOP-CWT associated with lower (≤4.2pg/mL) cTnT levels possibly indicating that better inhibitory ability associates with less myocardial damage. Indeed, elevated levels of cTnT at baseline were shown to be associated with lower cognitive test scores and increase the risk for dementia in the future [42].

The associations between cTnT with BDNF, TNF-α and NT-proBNP in Black men may indicate defence or compensatory responses. The brain aims to maintain homeostasis to decrease myocardial stress and restore the damage to the myocytes as BDNF has protective cardiac and central nervous system effects [4,14,34]. Smith, et al. (2014) supported this finding as BDNF was inversely associated with the development of atherosclerotic plaque in the carotid artery in the SABPA Blacks [12] and in other studies [22,43]. An impact on the microcirculation is suggested where down-stream signalling of the carotid artery when atherosclerosis is present may induce inflammation and cortical ischemia [44], a main stimulus for myocyte injury, and the release of cTnT [45,46]. Myocyte injury also seems to be related to cognitive impairment [42]. Upregulation of BDNF may thus be apparent in Black men to protect against myocardial injury and which will also improve neurotrophin and cognitive health over a three-year period in Black men. BDNF was indeed shown to increase neurogenesis that may lead to improved cognitive function [41,47].

This study is the first of its kind to assess the role of BDNF in cardiac injury over a period of time. However, the study still poses several limitations as it only represents a small prospective cohort of the South African population. The results can therefore not be attributed to the African population at large. No causal inferences can be drawn from the observed correlations and associations. The presence of only peripheral biomarkers reflecting central levels and absence of central biomarkers may also limit the exact role of these markers in neural protection. Longitudinal trends beyond three years may also help to relate the observed physiology to adverse cardiovascular outcomes, thereby emphasizing the clinical relevance of our findings.

Our hypothesis of BDNF being inversely associated with markers of cardiac stress, inflammation and executive cognitive function in Blacks is partially rejected. Only BDNF increases were positively associated with lower cTnT levels (≤ 4.2pg/L) possibly indicating that BDNF may act as a compensatory mechanism to protect against myocyte injury. In conclusion, central neural control mechanisms may have facilitated upregulation of BDNF in the brain to maintain homeostasis, as a way to protect against myocardial stress, and possibly to improve neural processes related to interference control in Black men.

4.7. AKNOWLEDGEMENTS

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recommendations expressed in this material are those of the author(s) and therefore funders do not accept any liability in regard thereto.

4.8. CONFLICTS OF INTEREST

The authors declare no conflict of interest pertaining to the information of this article.

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CHAPTER 5

MANUSCRIPT 3

The abstract of Manuscript 3 was presented as a Poster at the 19th Annual SA Heart Conference from 4-7 October 2018 in Sun City, South Africa.

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5.1. INSTRUCTIONS FOR AUTHORS

Diabetes and Vascular Disease Research

Impact factor:	3.340
Publisher:	SAGE Journals
Aims and scope:	Diabetes & Vascular Disease Research is the first international peer-reviewed journal to unite diabetes and vascular disease in a single title. It mirrors the increasing recognition that diabetes and cardiovascular disease are a single entity in which diabetes and related disorders, such as insulin resistance, are directly linked with assaults on the vessel wall and the development of vascular risk clustering. • Links diabetes, its metabolic consequences and vascular outcomes • Original research in fields of insulin resistance and metabolic disorders • Promotes understanding of pathology, aetiology and management of thrombosis, hyperglycaemia, hypertension, dyslipidaemia and micro- and macrovascular consequences.
Language:	English (UK)
Word limit:	Not specified
Margins:	Wide
Paragraph spacing:	Double
Font:	12, Arial or Times New Roman
Sections:	Title page: Title of article, author names - forename, initials, surname - author affiliations, author for correspondence - title, address, telephone and fax numbers, email Abstract, Introduction, Methods, Results, Discussion, Acknowledgements, Conflict of interest, References, Tables, Figures, Supplementary information
Title:	Concise informative
Abstract:	200 words, 6 keywords max
Key messages:	Max 5
References:	Ideally 30, Vancouver
Submission:	http://mc.manuscriptcentral.com/dvdres

Hypertension in Africa Research Team

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Prospective associations between cardiac stress, glucose dysregulation and executive cognitive function in Black men: The SABPA study

Running head: Cardiac stress, insulin resistance and cognition

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5.2. ABSTRACT

Objective

Glucose dysregulation is an independent risk factor for cardiovascular and neurodegenerative disease development through synaptic dysfunction resulting in cognitive decline. The aim of this study was to study the interplay between impaired glycaemic metabolism (hyperglycaemia and insulin resistance (IR)), cardiac stress (cardiac troponin T (cTnT) and N-terminal brain natriuretic peptide (NT-proBNP)) and executive cognitive function prospectively, in a bi-ethnic gender cohort.

Methods

Black and White teachers (N=338, aged 20-63 years) from the Sympathetic Activity and Ambulatory Blood Pressure in Africans study were monitored over a three-year period. Fasting blood samples were obtained for cTnT, NT-proBNP, glycated haemoglobin (HbA1c) and the homeostatic model assessment (HOMA-IR) for insulin resistance. The STROOP-color-word conflict test (STROOP-CWT) was applied to assess executive cognitive function at baseline.

Results

Over the three-year period Black men revealed constant high levels of cTnT (≥4.2ng/L), pre-diabetes (HbA1c>5.7%) and IR (HOMA-IR>3). HbA1c was associated with IR ($p<0.001$) and increases in ΔNT-proBNP ($p=0.02$) in Black men only. In the latter, baseline STROOP-CWT was inversely associated with cTnT ($p=0.001$) and IR levels ($p=0.01$).

Conclusion

Progressive myocyte stretch and chronic myocyte injury, coupled with glucose dysregulation may interfere with processes related to interference control in Black men.

Keywords: NT-proBNP; cardiac troponin; hyperglycemia; insulin resistance; cognition

5.3. INTRODUCTION

The importance of blood glucose regulation in cardiovascular disease (CVD) prevention has been emphasized.[1] Hyperglycaemia persists during conditions of chronic stress and vascular injury.[2,3] One of the causes of hyperglycaemia, insulin resistance (IR), plays a detrimental role in CVD, as it independently predicts prevalent and incident CVD development.[4] Further, studies also show that chronic hyperglycaemia (glycosylated haemoglobin (HbA1c) ≥5.6)) is associated with subclinical myocyte injury.[5]

Injury to cardiac myocytes has been identified as a major contributing factor to cardiac dysfunction and -failure.[6] Myocyte injury is the main stimulus for the release of cardiac troponin T (cTnT) from the myofibrils in cardiac muscle.[7] An association between cTnT and N-terminal pro-B-type natriuretic peptide (NT-proBNP) was reported in individuals with the metabolic syndrome.[8] In Black men from the SABPA cohort, cTnT was also associated with NT-proBNP, which may indicate increased cardiac wall strain in our population group.[9]

There is increasing evidence to suggest a role of cardiac stress in the development of neurodegenerative diseases as cTnT and NT-proBNP are independently associated with cognitive decline.[10,11] Mirza et al. (2015) reported higher NT-proBNP is related to poorer performance in multiple cognitive tests (STROOP-color-word conflict test (STROOP-CWT)) while Gluck et al. (2013) further showed that the STROOP-CWT, was associated with impaired glucose regulation.[10,12] Furthermore, executive cognitive function, assessed with the STROOP-CWT, differed significantly between individuals with type 2 diabetes and individuals with normal glucose metabolism.[13]

The aim of this study was therefore to study the interplay between impaired glycaemic metabolism, cardiac- and cognitive function. We assessed the relationship between changes (Δ) in cardiac stress risk markers (cTnT and NT-proBNP), glycaemic metabolism (HbA1c, IR) and a cognitive test score in a bi-ethnic gender cohort over a three-year period.

5.4. METHODS

The Sympathetic and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study included urban Black and Whites teachers from the Dr Kenneth Kaunda Education District of the North West Province of South Africa.[14] This selection was chosen to ensure a similar socio-economic class.[15] From February to May (2008 and 2009), 2170 teachers were invited to participate (Figure 5.1). Participants aged 20-65 years were assessed for eligibility, with 407 being included in phase I of the study. Phase II of the study was conducted after a period of three years from February to May (2011 and 2012). The successful follow-up rate was 87.8% (359 participants; aged between 20 and 63 years). Reasons for non-participation in phase II of the study were pregnancy (N=2), death (N=6) and drop outs (N=42). To avoid bias pertaining to cardio-metabolic risk, participants with an HIV positive status (N=21) were excluded for the purpose of this study.[15] Ultimately 338 (152 Black and 186 White) participants remained for the present study.

Informed consent was obtained from all the participants prior to commencement of the study. The Ethics Review Board of 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.[16]

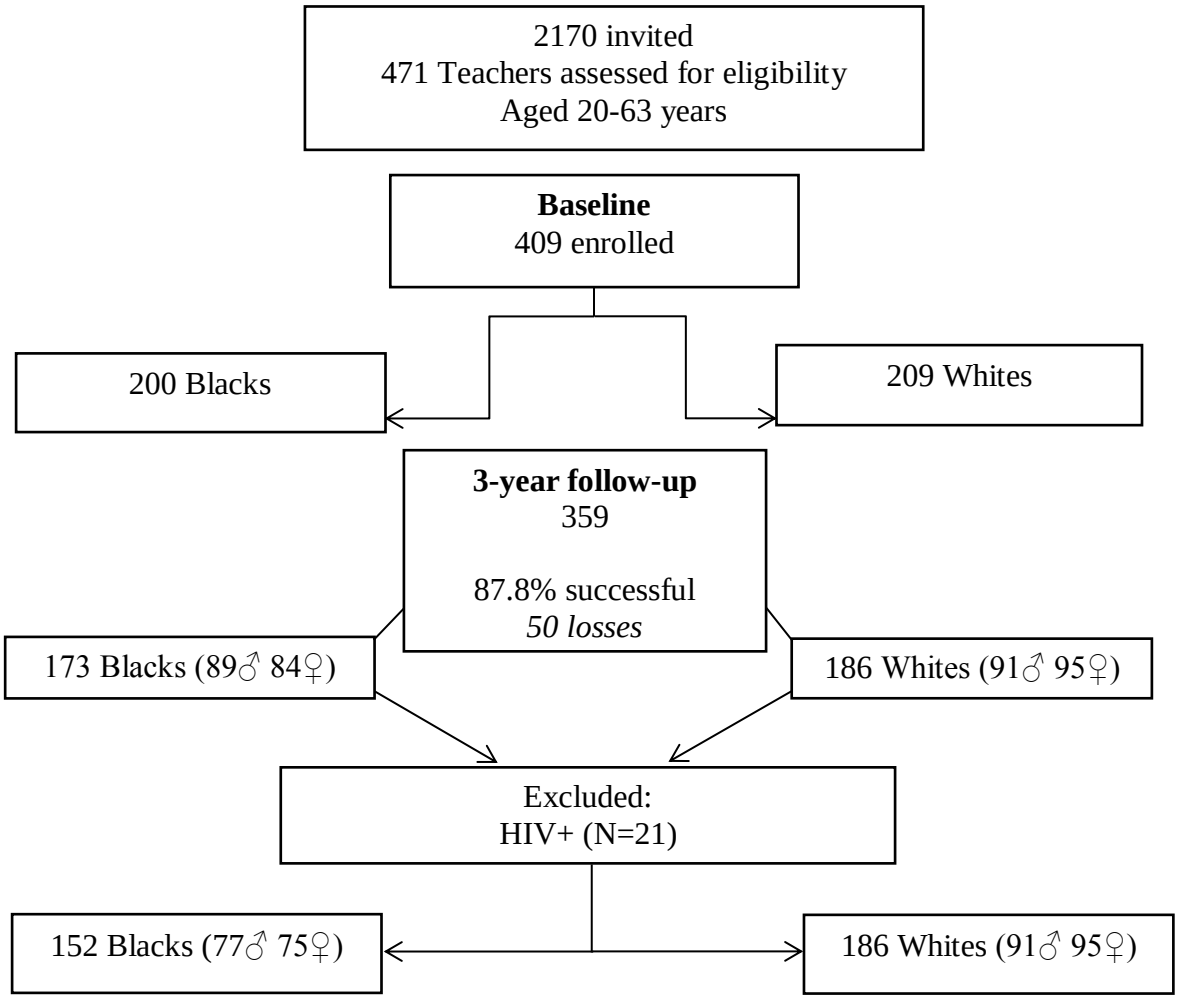

Figure 5.1. A South African bi-ethnic gender cohort.

5.4.1. Experimental methods and data collection

All clinical assessments were obtained over 48 hours. Each participant was connected to the Cardiotens CE120® (Meditech, Budapest, Hungary) apparatus and accelerometers in order to measure 24-hour ambulatory blood pressure (ABPM), electrocardiogram (ECG), as well as 24-h physical activity. The correct cuff sizes were applied to the non-dominant arm of each participant. The participants were transported to the Metabolic Unit Research Facility at North-West University at approximately 16:30 where they were introduced to the experimental set-up. Each participant received his/her own private bedroom and a standardized dinner. Demographic and General Health questionnaires were also completed. The participants were advised to fast and rest from 22:00 for the next day's clinical measurements.

At approximately 07:30 the following morning, the 24-hour ambulatory devices were disconnected where after anthropometric and clinical measurements followed. All resting ECG's, blood sampling and STROOP-CWT measures were obtained after the participants had been in a semi-recumbent position for approximately 30-45 minutes. Once the assessments were completed, the participants received breakfast and were transported back to their respective schools. Immediate feedback was also provided.

5.4.2. Lifestyle determinants

Registered level II anthropometrists obtained the anthropometric measurements according to standardized procedures. The mean of three measurements was used to ensure accuracy. Inter- and intra-observer variability was found to be less than 10%. The Mosteller formula [weight (kg) x height (cm) ÷ 3600]½ was used to calculate the body surface area. Each

participant's daily physical activity was also monitored (Actical® activity monitor; Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada).

5.4.3. Biochemical measurements

Fasting blood samples were obtained by registered nurses from the ante-brachial vein utilizing a sterile winged infusion set and handled according to standardized procedures for storage at -80°C until analysis. Gamma-glutamyl transferase (γ-GT), an indicator of alcohol abuse, was analysed with the enzyme rate method (Unicel DXC 800; Beckman and Coulter; Germany). Serum cotinine, an indicator of nicotine levels, was analysed with the homogeneous immunoassay on Modular ROCHE Automized (Basel, Switzerland). Serum cTnT and NT-proBNP was analysed with the high sensitive electrochemiluminescence immunoassay (ECLIA), Elecsys (ROCHE, Basel, Switzerland). In our sample, there were 91 (26.84%) undetectable cTnT values (<3 pg/ml) that were substituted for lower than detectable values. The inter- and intra-batch variability was 15% and 5.6% respectively for cTnT, and 4.6% and 4.2% for NT-proBNP. An ultra-high-sensitivity turbidimetric method (Unicel DXC 800, Beckman and Coulter, Germany) was used to analyse C-reactive protein. Serum tumour necrosis factor-alpha (TNF-α), was analysed with the Quantikine High Sensitivity Human TNF-α Enzyme linked immunosorbent assay (HS ELISA; R&D Systems, Minneapolis, MN USA). Despite serum handling within 30 minutes, the inter- and intra-assay variability for TNF-α was 15% and 17.8% respectively.

Fasting blood glucose samples were collected in sodium fluoride tubes and analysed with the timed-end-point method (Unicel DXC 800, Beckman Coulter, Germany). The turbidometric inhibition immunoassay was used to determine HbA1c (Integra 400; Roche, Basil, Switzerland). Serum insulin was analysed with the electrochemiluminescence immunoassay

(ECLIA; Elecsys 2010, Roche, Basel, Switzerland) with an intra-assay- and inter-assay precision of 2% and 2.8% respectively. The homeostatic model assessment (HOMA) was used to indicate insulin resistance (IR) and was measured using the following formula: fasting glucose x fasting insulin/405.[17]

5.4.4. Cardiovascular assessment procedures

As part of the SABPA study design 24-hour BP was measured at 30-minute intervals from 08:00 to 22:00 and at 60-minute intervals from 22:00 to 06:00.[18] The European Society of Cardiology criteria for hypertension were employed [average 24-hour systolic blood pressure (SBP) of ≥130mmHg and/or diastolic blood pressure (DBP) of ≥80mmHg].[15] The data were analysed with the CardioVisions 1.19 Personal Edition software (Meditech, Budapest, Hungary). Participants had to record any abnormalities they experienced throughout the day, on a 24-hour diary card. The abnormalities included visual disturbances, headaches, nausea, fainting, palpitations and stress. The Norav NHH-1200® ECG (NORAV medical LTD PC 1200, Israel, Software version 5.030) was used to record the resting 10-lead ECG.

5.4.5. Executive cognitive function

Executive cognitive function was assessed by means of the STROOP-color-word conflict test (STROOP-CWT). The participants were shown a cardboard containing series of five words in random orders describing a specific colour, but written in different colours. The ink colour of a given word had to be identified verbally. When participants are faced with the task to name the ink colour of a word instead of reading the word, an interference is caused by the more automated task (reading the colour represented by the word).[19,20] In order to perform the less automated task it is therefore required by the participants to inhibit this interference caused by the more automated task.[19,20] Participants had to guard against reading the colour

represented by the word. They were also encouraged to progress as fast as possible within 1 minute, and were interrupted to correct wrong answers. An interference score (STROOP-CWT) was calculated that represented the number of correct answers produced during the fixed period of 1 minute. A lower score thus indicates that the individual found it more difficult to inhibit the interference.[19] The same two scientists obtained STROOP-CWT scores of teachers at baseline. On completion of the task participants received a monetary incentive in accordance with their performance.

5.4.6. Statistical analyses

Statistical analyses were performed with Statistica version 13 (TIBCO Software Inc., Palo Alto, USA, 2018). Normal distributions were computed to reveal symmetrical data. Logarithmic transformations were used for variables with skewed distributions. Baseline characteristics of the two ethnic groups were determined with independent *t*-tests. Chi-square tests (X^2) were used to determine prevalence as well as proportions. Single two-way general linear model interaction on main effects (ethnicity x gender) were computed for all cardiovascular risk markers, independently of a priori defined covariates.[15] Dependent t tests were used to calculate differences over time in each ethnic group. Percentage changes over time (%Δ) were calculated by using the formula: Δ = (follow-up – baseline)/baseline*100. McNemar's case-control tests were used to demonstrate changes when participants without diabetes (negative) at baseline become positive at follow-up; and diabetes-positive people at baseline recover to negative at follow-up. Forward stepwise regression analyses determined associations between dependent variables (ΔcTnT, ΔNT-proBNP, ΔHbA1c, ΔHOMA-IR and baseline STROOP-CWT) and independent variables (ΔcTnT, ΔNT-proBNP, ΔHOMA-IR and baseline STROOP-CWT) and additional covariates (inflammation) in several separate

models. For all of the above-mentioned analyses, significant values were noted when adjusted $R^2 \geq 0.25$ and $p \leq 0.05$.

5.5. RESULTS

5.5.1. Cross-sectional data analyses

Table 5.1 depicts the clinical characteristics of the South African bi-ethnic gender cohort at baseline. Blacks had higher γGT, HbA1C, insulin and blood pressure values than whites. In turn, higher body surface area, physical activity, STROOP-CWT scores and cTnT levels were found in the Whites. More Blacks than Whites showed hyperglycaemia (HbA1c>5.7%), compatible with a pre-diabetic state (62% vs. 31%; p<0.001).

Significant interactions with gender were revealed for NT-proBNP [F(1,316), 8.19, p=0.005], cTnT [F(1,322), 12.44, p<0.001] and with ethnicity for insulin [F(1,324), 4.40, p=0.04]. Furthermore, interactions between ethnicity and gender were revealed for cognitive interference [F(1,324), 97.20, p<0.001]; [F(1, 324), 21.73, p<0.001] that motivated further stratification into ethnic-gender groups.

5.5.2. Longitudinal data analyses

A total of 33 participants had incomplete data for the main variables that included HbA1c (N=8), HOMA-IR (N=4), cTnT (N=9), NT-proBNP (N=11) and STROOP-CWT (N=1). Participants with incomplete data (N=33) were however included in all analyses as their exclusion did not change the outcome of the results.

Table 5.1. Clinical characteristics of a South African bi-ethnic gender cohort at baseline.

Variables	Blacks (N=152)	Whites (N=186)	p-values
Confounders			
Age, years	44.65 ± 8.13	46.58 ± 9.87	0.053
Body surface area, m^2	1.92 ± 0.22	2.01 ± 0.29	0.001
TEE, kcal/day	2678.06 ± 814.21	3155.06 ± 1672.30	0.002
Cotinine, ng/mL	26.71 ± 60.86	24.05 ± 81.01	0.739
γGT, U/L	65.74 ± 82.48	28.02 ± 38.28	<0.001
Potential diabetes risk markers			
Glucose, mmol/L	5.71 ± 2.17	5.71 ± 0.83	0.976
HbA1C, %	6.08 ± 1.19	5.53 ± 0.43	<0.001
Insulin, μU/mL	14.96 ± 10.35	12.26 ± 8.70	0.010
HOMA-IR	3.87 ± 3.26	3.29 ± 2.90	0.083
Cognition			
STROOP-CWT score	48 ± 11	63 ± 13	<0.001
Cardiovascular characteristics			
24h SBP, mmHg	132 ± 17	125 ± 12	<0.001
24h DBP, mmHg	83 ± 11	77 ± 8	<0.001
NT-proBNP, pg/mL	43.50 ± 45.59	46.35 ± 47.03	0.580
cTnT, pg/mL	4.90 ± 2.81	5.68 ± 3.60	0.031
C-reactive protein, mg/L	8.23 ± 9.96	3.19 ± 4.08	<0.001
TNF-alpha, pg/mL	2.95 ± 3.45	1.83 ± 1.90	<0.001

Hyperglycaemic, N (%)	93 (62.00)	57 (30.65)	<0.001
Moderate IR, N (%)	71 (47.33)	76 (41.08)	0.252
Medication usage			
Hypertension, N (%)	57 (37.50)	26 (13.98)	<0.001
Anti-diabetic, N (%)	9 (5.92)	8 (4.30)	0.358

Data are presented as mean ± SD, median (95% CI) or number of participants (%). Abbreviations: γGT, Gamma glutamyl transferase; TEE, total energy expenditure; HbA1C, glycated haemoglobin; HOMA-IR, Homeostatic model assessment-Insulin resistance; STROOP-CWT, STROOP-color-word conflict test score; SBP, systolic blood pressure; DBP, diastolic blood pressure; NT-proBNP, N-terminal pro-b-type-natriuretic peptide; cTnT, cardiac troponin T; TNF-alpha, tumour necrosis factor alpha. Hyperglycaemic defined as HbA1c>5.7% and moderate insulin resistance defined as HOMA-IR>3 (33).

Over the three-year period, insulin and IR decreased whereas NT-proBNP increased in Blacks and Whites (Table 5.2). Additionally, Whites revealed decreases in cTnT and resting glucose. In Black men, ΔNT-proBNP increased over the three-year period, whereas no significant % changes (Δ) were revealed for cTnT, HbA1c and IR (Table 5.3). In Black men, incidence of diabetes changed significantly over the three-year follow-up period (%Δ 7.36 [OR 0.1 (0.01, 0.78)], p=0.007). Here, 10 participants developed diabetes over the three-year period, with only 1 participant recovering from baseline to follow-up.

Forward stepwise regression analyses determined associations between changes (%Δ) in cardiac stress markers (NT-proBNP and cTnT), STROOP-CWT, IR and HbA1c over a three-year period. No associations were evident in women (of either ethnicity) and in White men; therefore, we will only report associations found in Black men as shown in Table 5.4. In Black men, constant high HbA1c (>5.7%) was associated with constant high IR (HOMA-IR>3) (Adj R^2 0.28, β=0.43; 95% CI 0.22 to 0.64; p<0.001) as well as increases in ΔNT-proBNP (Adj R^2 0.28, β=0.26; 95% CI 0.05 to 0.48; p=0.02). Also in Black men, baseline STROOP-CWT score was inversely associated with constant high cTnT (>4.2pg/mL) (Adj R^2 0.24, β= -0.36; 95% CI -0.57 to -0.15; p=0.001) and constant high IR (Adj R^2 0.24, β= -0.28; 95% CI -0.49 to -0.06; p=0.01).

Table 5.2. Unadjusted differences over a period of 3 years in a Black and a White cohort.

	Blacks (N=152)			Whites (N=186)		
Variables	**Baseline; Follow-up**	**Difference**	**p-value**	**Baseline; Follow-up**	**Difference**	**p-value**
Glucose, mmol/L	5.71; 5.62	-0.09	0.523	5.71; 4.43	-1.28	<0.001
HbA1C, %	6.07; 6.18	+0.11	0.204	5.53; 5.59	+0.05	0.120
Insulin, μU/mL	14.80; 12.19	-2.60	<0.001	12.26; 10.32	-1.94	<0.001
HOMA IR	3.83; 3.08	-0.75	0.004	3.29; 2.16	-1.13	<0.001
NT-proBNP, pg/mL	43.57; 57.67	+14.11	0.003	46.50; 83.29	+36.79	<0.001
cTnT, pg/mL	4.89; 4.62	-0.27	0.352	5.47; 4.89	-0.58	<0.001

Data presented are unadjusted dependent sample *t*-tests. Abbreviations: HbA1C, glycated haemoglobin; HOMA IR, Homeostatic model assessment-Insulin resistance; NT-proBNP, N-terminal pro-b-type-natriuretic peptide; cTnT, cardiac troponin T.

Table 5.3. Unadjusted differences over a period of 3-years in Black men.

	Black men (N=77)		
Variables	**Baseline; Follow-up**	**Difference**	**p-value**
Glucose, mmol/L	6.13; 6.07	-0.07	0.727
Insulin, μU/mL	15.69; 13.41	-2.28	0.086
HOMA-IR	4.38; 3.59	-0.79	0.074
HbA1C, %	6.27; 6.31	+0.04	0.784
cTnT, pg/mL	5.59; 5.36	-0.23	0.570
NT-proBNP, pg/mL	36.12; 49.13	+13.01	0.039
†Diabetes: % Δ [OR (95% CI)], p	7.36 [0.1 (0.01, 0.78)], 0.007		
†Diabetes *(BL +, FU -) / (BL -, FU +)*	1 / 10		

Data presented are unadjusted dependent sample *t*-tests. Abbreviations: HOMA-IR, Homeostatic model assessment-Insulin resistance; HbA1C, glycated haemoglobin; cTnT, Troponin T; NT-proBNP, N-terminal pro-Brain natriuretic peptide. †McNemar chi-square equation values are presented as percentage difference over three years' time followed by the Odds Ratio (±95% Confidence Interval). (BL +, FU -), frequency at baseline positive but negative at follow-up; (BL -, FU +), frequency at baseline negative but positive at follow-up.

Table 5.4. Independent associations between cardiac stress markers, insulin resistance and cognitive interference scores in a Black male cohort.

	Black men (N=77)				
	ΔNTproBNP β (95%CI)	**ΔcTnT** β (95%CI)	**STROOP-CWT** β (95%CI)	**ΔHOMA-IR** β (95%CI)	**ΔHbA1C**
Adjusted R^2	0.11	0.25	0.24	0.10	0.28
ΔNTproBNP	-	0.31** (0.10; 0.52)	NS	NS	0.26* (0.05; 0.48)
ΔcTnT	0.30** (0.07; 0.52)	-	-0.36** (-0.57; -0.15)	NS	NS
STROOP-CWT	NS	-0.32** (-0.53; -0.11)	-	-0.26* (-0.50; -0.02)	NS
ΔHOMA-IR	NS	NS	-0.28* (-0.49; -0.06)	-	0.43† (0.22; 0.64)

Data presented are adjusted for a priori covariates and tumour necrosis factor-alpha. Abbreviations: NT-proBNP, N-terminal pro-b-type-natriuretic peptide; cTnT, cardiac troponin T; STROOP-CWT, STROOP-color-word conflict test score at baseline; HOMA IR, Homeostatic model assessment-Insulin resistance; HbA1C, glycated haemoglobin. Superscript symbol shows the trend of significance:*$p \leq 0.05$; **$p \leq 0.01$; †$p \leq 0.001$.

5.6. DISCUSSION

The aim of this study was to determine whether changes in markers of cardiac stress were associated with changes in insulin resistance, hyperglycaemia and a cognitive test score in a bi-ethnic male and female cohort. Over a three-year period cardiac stress (NT-proBNP and cTnT) associated with dysregulated glucose metabolism (IR and hyperglycaemia) may interfere with the ability to inhibit cognitive interference.

5.6.1. Executive cognitive function, cardiomyocyte injury and glucose metabolism

In this study, Blacks revealed a lower STROOP-CWT score than Whites indicating that Blacks had more difficulty to inhibit cognitive interference than Whites. One of many reasons why Blacks revealed lower STROOP-CWT scores than Whites might be explained by the inverse association found between STROOP-CWT with constant high (≥4.2pg/L) cTnT levels[21] in this study, as higher cTnT might lead to subclinical cerebral injury expressed as silent brain infarcts and white matter lesions on magnetic resonance imaging.[22] Wijsman et al., reported that participants with higher hs-cTnT levels had steeper STROOP-CWT declines over a period of 3.2 years.[23] Indeed, in Black men high levels of cTnT >4.2pg/L indicative of ischemic heart disease risk[24] was sustained that associated with reduced cardiac output[25] possibly leading to cerebral hypoperfusion resulting in executive functional decline.[21]

Other factors may also influence cognitive interference scores such as neural activation[26] rather than increased cerebral perfusion in low-level alcohol users when compared with non-users.[27] As alcohol use increases, the difficulty score pertaining to the STROOP-CWT increases[28] indicating that high levels of alcohol abuse[29] and neural activity[23] previously

described in the Black cohort should be considered for the lower cognitive interference scores evident in the Blacks.

Cognitive interference was also inversely associated with IR in the current Black male cohort. Studies have shown IR to be associated with memory impairments and atrophy of brain regions leading to cognitive deficits.[30] Usually atrophy of these brain regions occur in early onset Alzheimer's disease (AD).[30] Indeed, individuals with diagnosed AD showed reduced resistance to cognitive interference[31] and hypo-activation of brain areas involved in this inhibitory control.[32] The exact mechanism of how peripheral IR affects cognitive inhibitory control is however unclear. Numerous mechanisms leading to impaired cognition may apply including excessive amyloid beta accumulation and tau hyper-phosphorylation.[33] Therefore, regulation of blood glucose and correction of IR in a clinical setting may be of crucial importance for improved cognitive control and health.

5.6.2. Dysregulated glucose and myocyte stretch

IR has been reported in Black women of South Africa before.[34] We expand current findings by showing moderate IR (HOMA-IR >3, <5)[35] also in Black men. Different cellular mechanisms may be involved in the pathogenesis of IR that includes inflammation.[36] The Insulin Resistance Atherosclerosis Study reported associations between the inflammatory marker, TNF-α with higher glucose levels and IR.[37] However, statistical adjustments of TNF-α in our study indicated that moderate IR was evident in Black men, independent of inflammation.

The association found between hyperglycaemia and IR in our Black male/female cohort may underscore their pre-diabetic state (HbA1c >5.7%)[35] previously reported by Lammertyn et al., (2011).[38] Insulin is responsible for the regulation of metabolism as it promotes glycogen synthesis and glucose uptake into the cells while inhibiting glucose release into the circulation.[36] With IR, the tissues thus show reduced sensitivity to insulin-mediated biological activity.[36] This results in a disrupted balance between glucose uptake and release into the circulation leading to excessive glucose accumulation in blood vessels.[2,3]

Again increased neural activity or depressed heart rate variability in the SABPA Black cohort, particularly men[23,39] might be one mechanism to explain the chronic hyperglycaemia. Indeed, non-dipping Black men revealed higher HbA1c values, and associations were demonstrated between chronically elevated blood glucose and a blunted nocturnal blood pressure dipping.[38] The latter is indicative of autonomic dysfunction and volume overload.[40] NT-proBNP is a valuable marker to assess volume overload as it is released in response to myocyte stretch along with BNP that may lead to sympathetic nervous system inhibition, as well as the induction of natriuresis and diuresis.[41] In this study, we saw that constant elevated cTnT and HbA1c levels were associated with NT-proBNP increases. Other studies reported an association between chronic hyperglycaemia and subclinical myocardial injury, as indicated by increased cTnT levels.[5] We cautiously suggest that progressive increases in NT-proBNP may act as compensatory protective mechanism against chronic hyperglycaemia and cardiomyocyte injury in the current Black male cohort over a three-year period.

5.6.3. Limitations and Conclusion

Although this study is the first to present associations between cardiac stress, hyperglycaemia, IR and cognitive interference in Blacks, there are limitations. The results cannot be attributed to the African population at large as participants consisted of a small prospective cohort in South Africa. We did not perform a mechanistic study, so even plausible inferences about the interplay of discussed relationships must remain speculative. The clinical relevance of our findings needs to be shown beyond a three-year observation period.

In conclusion, as depicted in Figure 5.2, we propose that progression of mycoyte stretch is associated with chronic myocyte injury and hyperglycaemia in Black men. Furthermore, hyperglycaemia is associated with insulin resistance that may interfere with the inhibition of cognitive interference in these men. Therefore in Black men, progressive myocyte stretch and chronic myocyte injury, coupled with dysregulation of glucose metabolism may interfere with processes related to interference control.

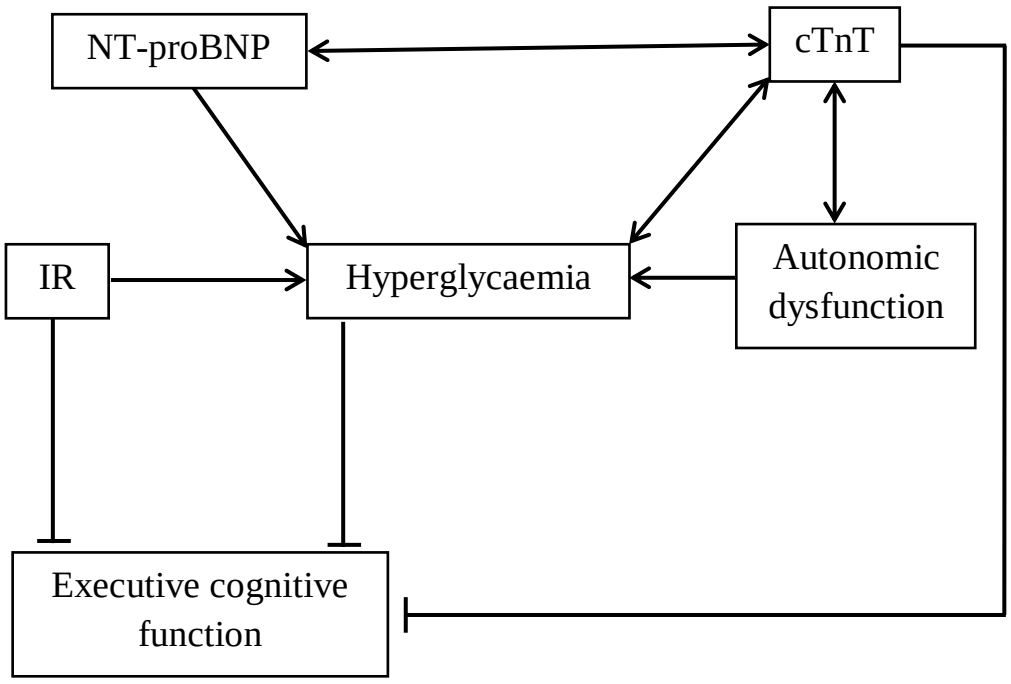

Figure 5.2. Proposed mechanism of cardiac stress markers and glucose dysregulation associating with executive cognitive function in Black men. Abbreviations: cTnT, cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; ⟶ Arrows indicate that one variable leads to an increase of the next variable; ⟷Arrows indicate that positive associations exist between these variables; ⟙ Arrows indicate that one variable may lead to a decrease of the next variable.

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5.8. DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest pertaining to the information of this manuscript.

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CHAPTER 6

GENERAL FINDINGS AND CONCLUDING REMARKS

6.1. Introduction

This chapter provides an overview of the main aims and findings regarding the three manuscripts that form part of this thesis. The results and conclusions of each manuscript will be interpreted and compared with the relevant literature. The main limitations of the study will also be discussed as well as recommendations made for future research regarding cardiac stress and cardiovascular risk markers in African populations.

6.2. Discussion and summary of main findings

The main aim of this study was to determine whether cardiac stress, as indicated by cardiac troponin (cTnT) and N-terminal pro-Brain natriuretic peptide (NT-proBNP) levels, will change in a bi-ethnic gender cohort over a period of three years. Furthermore, we aimed to determine whether these cardiac stress risk markers will be associated with various cardiovascular risk markers including inflammation, brain-derived neurotrophic factor (BDNF), glucose dysregulation and executive cognitive function over the three-year period.

The main integrative hypothesis of the thesis comprises two parts. In the first part we hypothesised that cardiac stress risk markers (cTnT and NT-proBNP) will increase in Blacks over the three-year period, as Hamer et al. (2015)[1] reported that Blacks demonstrated greater increases in cardiovascular risk factors such as 24-hour blood pressure when compared with that of Whites. Only NT-proBNP increased in Blacks over the three-year period. Increases for NT-proBNP were also revealed in the Activity and Function in the Elderly in Ulm study.[2] Here, longitudinal increases of NT-proBNP were associated with an increased mortality risk in asymptomatic older individuals. Although cTnT showed no changes over the three-year period, levels remained higher than the reported cut-point of cTnT ≥4.2pg/L which

predicted hypertension in Blacks.[3] Pareek et al. (2017)[4] reported that roughly 10% of 695 apparently healthy individuals had abnormally high cTnT levels. The first part of our integrative hypothesis is partly confirmed, as only NT-proBNP, but not cTnT increased in Blacks over the three-year period.

In the second part of our integrative hypothesis we proposed that markers of cardiac stress will be associated with various cardiovascular risk markers (inflammation, BDNF, executive cognitive function and glucose dysregulation) over the three-year period. Neither NT-proBNP nor cTnT associate with all the cardiovascular risk markers investigated in this study; hence we only partially confirmed part two of our integrative hypothesis.

In the following sections we elaborate on the specifics of our integrative hypothesis by mentioning the associations found in each manuscript and whether the specific hypotheses of each manuscript (hypothesis 1.1 - 1.7) can be confirmed or rejected:

6.2.1. Longitudinal changes of cardiac troponin and inflammation are associated with progressive myocyte stretch that predicts hypertension in a Black male cohort: The SABPA study

The role of inflammation in the development of cardiovascular diseases (CVD) has been reported before.[5,6] Various pro-inflammatory cytokines are released in response to cardiovascular risk markers including hypertension, vascular and cardiac injury.[5,7,8] In Blacks from South Africa, a low-grade inflammatory profile was evident that was positively associated with blood pressure and structural wall abnormalities.[9,10] We therefore proposed (*hypothesis 1.1*) that levels of inflammation, as indicated by elevated circulating level of

C-reactive protein (CRP) and tumour necrosis factor-alpha (TNF-α), will increase over a three-year period in Blacks. Our hypothesis (*hypothesis 1.1*) had to be rejected. CRP showed no changes with levels remaining constantly high (CRP ≥3mg/L) over the three-year period. Similarly, Parinello et al. (2015)[11] reported that 29% of the 10160 participants of the Atherosclerosis Risk in Communities (ARIC) study revealed sustained elevated (>3mg/L) CRP levels over a six-year period. These individuals were more likely to be obese and have hypertension, diabetes or the presence of CVD. An interesting finding was that TNF-α remained constant in all ethnic-gender groups except in Black men where it decreased over the three-year period. One would expect TNF-α to increase with increases of NT-proBNP as these factors were positively associated at baseline.[12] Other studies showed opposite findings, as inflammation was reported to increase with increases in age.[13] Furthermore, TNF-α increased in elderly patients with type 2 diabetes over a period of two years.[14]

Our second hypothesis of manuscript 1 (*hypothesis 1.2*) indicated that changes of cTnT will positively associate with changes of NT-proBNP and TNF-α in Black men only, as these associations were evident at baseline in Black men only.[12] In this study novel findings indicated that chronically increased levels of markers of myocyte injury were positively associated with progressive myocardial stretch that may be reflective of cardiac metabolic over-demand, ultimately increasing hypertension and ischemic heart disease risk in the Black male cohort. Chronic high cTnT levels (≥4.2ng/L) may be a marker of silent myocardial ischemia.[15] Therefore, the association between chronic high cTnT levels and increased NT-proBNP is not surprising as post-mortem levels of NT-proBNP are higher in individuals who had suffered from chronic cardiac ischemia.[13] Evidence also showed that NT-proBNP elevations may be predictive of long-term cardiovascular risk.[4] In our study we found that

raised NT-proBNP increased the likelihood for 24-h hypertension development. Sanchez et al. (2016)[16] found that the development of hypertension was indeed preceded by substantial increases in NT-proBNP. Therefore we can confirm *hypothesis 1.2*.

6.2.2. BDNF and attenuated inflammation as defence response to cardiac stress and cognitive interference in Black men: The SABPA prospective study

Brain-derived neurotrophic factor (BDNF) is a neurotrophin mainly secreted by neurons, glial cells and peripheral immune cells in the brain.[17] However, circulating BDNF is also stored in platelets and studies showed that BDNF plays a role in the development of inflammation and atherosclerosis.[18-20] Smith et al. (2015)[21] reported that BDNF decreases atherosclerotic plaque formation in the carotid artery of Black South African men. The authors further stated that Blacks revealed significantly lower levels of BDNF than their White counterparts at baseline.[21] Due to the adverse cardiometabolic profile previously reported in Blacks,[22,23] we hypothesised (*hypothesis 1.3*) that levels of BDNF will remain lower than in Whites after the period of three years. This was however not the case and *hypothesis 1.3* had to be rejected as BDNF significantly increased over the three-year period in Blacks only. It thus seems as if BDNF upregulated as a compensatory mechanism in order to protect against myocyte injury and possibly to improve executive cognitive functioning as will be described in the following paragraph. We found no other studies reporting similar results.

Our second hypothesis of manuscript 2 (*hypothesis 1.4*) was that BDNF will be inversely associated with cardiac stress risk markers (cTnT and NT-proBNP), inflammation (TNF-α) and executive cognitive function in Blacks. Our findings support results from a study conducted on dementia patients[24] where no associations were revealed between BDNF and

TNF-α. Studies done in rodents showed that BDNF may be beneficial to limit myocardial ischemic injury,[25] the main stimulus for cTnT release.[26,27] Our results support these findings as progression of BDNF increased the likelihood for cTnT levels lower than the cTnT cut-point (≤4.2pg/L) for silent ischemic risk.[15] BDNF is also known to have protective effects in the central nervous system as BDNF modulates the functional integrity of the fronto-striatal networks involved in executive cognitive functioning.[28,29] Indeed, higher BDNF levels were shown to be optimal for response inhibition capacity assessed by the STROOP-color-word conflict test (CWT).[30] Although we found no direct association between BDNF and STROOP-CWT in this study, the fact that the STROOP-CWT revealed an inverse association with cTnT might indicate that the brain increased BDNF in an attempt to maintain executive cognitive functioning through prevention of further myocardial ischemic injury. *Hypothesis 1.4* was therefore partially confirmed as BDNF only revealed positive associations with cTnT and not with NT-proBNP, TNF-α or STROOP-CWT.

6.2.3. Prospective associations between cardiac stress, glucose dysregulation and cognitive interference in Black men: The SABPA study

The regulation of blood glucose levels is an important factor that has to be taken into consideration when assessing diabetes and CVD risk.[31,32] Studies have shown that Black individuals have higher glycosylated haemoglobin (HbA1c) levels indicative of hyperglycaemia, when compared with their White counterparts.[33,34] Furthermore, African-Americans revealed higher prevalence of insulin resistance (IR) when compared with Whites.[35] Therefore we hypothesised (*hypothesis 1.5*) that levels of Insulin Resistance (IR), indicated by the homeostatic model assessment (HOMA-IR), and HbA1c will increase in Blacks over the three-year period. Similar to Hamer et al., (2015)[1] we found that levels of

HbA1c in Blacks did not change over the three-year period, but remained constantly higher than the proven cut-point (HbA1c ≥5.7%) for hyperglycaemia compatible with a pre-diabetic state.[36] This was accompanied by constantly high HOMA-IR levels (>3, <5) indicating moderate IR,[36] especially in Black men, leading to the rejection of *hypothesis 1.5*.

Secondly, we proposed (*hypothesis 1.6*) that changes in cardiac stress risk markers (cTnT and NT-proBNP) will significantly associate with changes in IR and hyperglycaemia in Blacks. In this study, a positive association was revealed between hyperglycaemia and NT-proBNP progression in Black men only. In contrast, Olsen et al. (2005)[37] found that higher NT-proBNP levels were associated with lower plasma glucose levels. *Hypothesis 1.6* is therefore partially confirmed.

Increasing evidence exists to suggest a role of glucose dysregulation in the development of neurodegenerative diseases[38,39] as insulin is important for neuronal survival, synaptic plasticity and -function in the brain.[40,41] Executive cognitive function, assessed by means of the STROOP-CWT, differed significantly between individuals with type 2 diabetes and individuals with normal glucose metabolism.[42] Our third hypothesis of manuscript 3 (*hypothesis 1.7*) was therefore that changes in IR and hyperglycaemia will inversely associate with STROOP-CWT in Blacks and Whites. We could not fully replicate findings of Gluck et al. (2013)[43] as only moderate IR and not hyperglycaemia was inversely associated with STROOP-CWT in Black men and therefore our *hypothesis 1.7* can only be partially confirmed.

6.2.4. Summary

In Black men the following chain of events may possibly explain our observations (Figure 6.1). Chronic myocyte injury prevailed in Black men over the three-year period [Manuscripts 1-3]. This may have contributed to an increased NT-proBNP production in an attempt to repair damage sustained to the myocardium. However, further increases in blood pressure may occur [Manuscript 1] and contributed to hyperglycaemia [Manuscript 3] indicating a vicious cycle of events where glucose dysregulation and hypertension reflected myocyte injury. The brain may also attempt to repair cardiomyocyte injury through other defence mechanisms that include down-regulation of inflammation (TNF-α) and BDNF upregulation [Manuscripts 1-2]. Indeed BDNF increases raised the likelihood for lower levels of cTnT [Manuscript 2]. Therefore, BDNF increases may have prevented further myocyte injury. Although inflammation seems to contribute to myocyte stretch at baseline it now seems that decreases of inflammation might not be protective as TNF-α decreases were associated with higher levels of cTnT [Manuscript 1]. The chronically elevated levels of cTnT along with glucose dysregulation may also have damaging effects on the central nervous system. Indeed, the inverse associations between cTnT and IR with cognitive interference [Manuscript 3] may indicate that response inhibition capacity might be impaired in individuals with a pre-diabetic state and increases the risk for myocardial ischemia. Therefore BDNF might also have increased in an attempt to maintain executive cognitive functioning in these individuals.

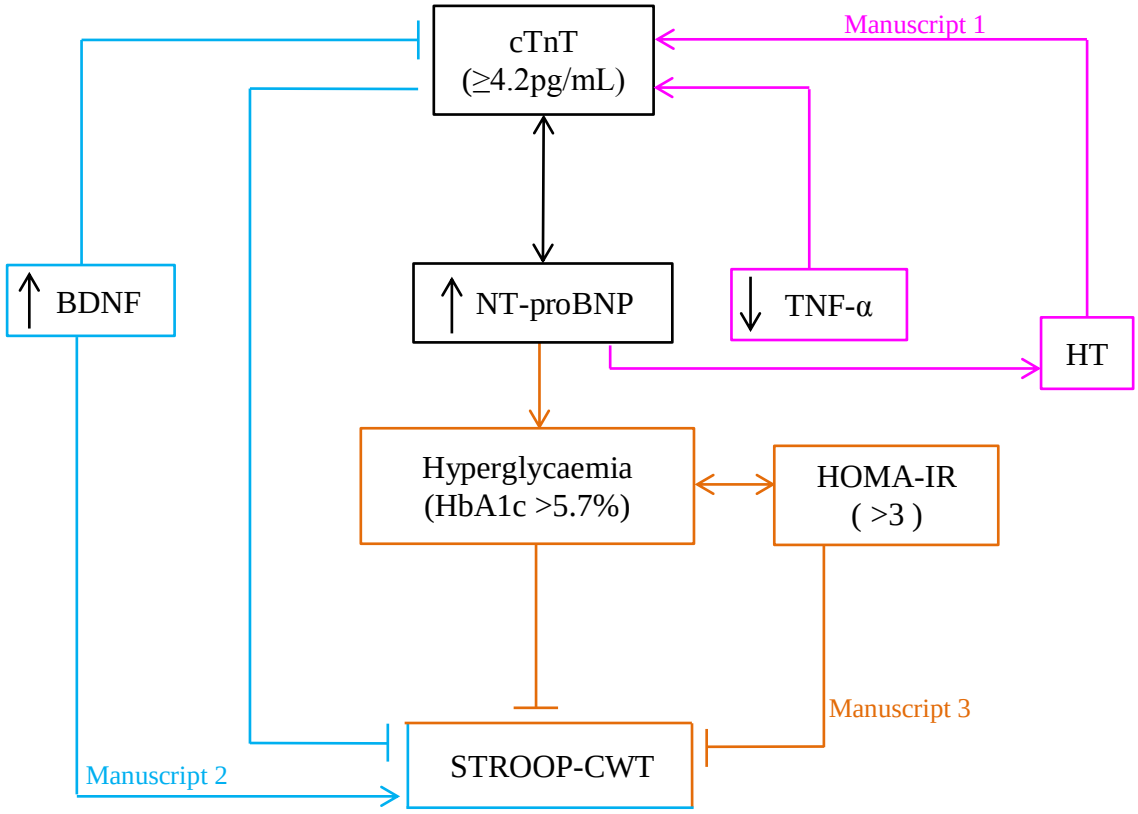

Figure 6.1. Proposed mechanism depicting the results of this PhD thesis regarding cardiac stress and cardiovascular risk markers in Black men. *Abbreviations: cTnT, troponin T; NT-proBNP; BDNF, brain-derived neurotrophic factor; TNF-α, tumour necrosis factor-alpha; HT, hypertension; HOMA-IR, insulin resistance; STROOP-CWT, STROOP-color-word conflict test.*

6.3. 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 gender cohort from South Africa, we could not control for cultural differences and 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.

We did not perform an intervention study, so even plausible inferences about the interplay of discussed relationships must remain speculative. However, the associations demonstrated were statistically significant and were investigated from a physiological perspective. Understanding the physiological mechanisms is therefore crucial when interpreting the associations observed in this study. Hence this study still provides valuable information in terms of the increased CVD risk of Black South Africans.

Studies have reported that BDNF measured in the periphery changes parallel to BDNF measured in the cerebrospinal fluid[44] and reflect brain-tissue BDNF levels across various species.[45] However, the presence of only peripheral biomarkers reflecting central levels and absence of central biomarkers may also limit the exact role of these markers in neural protection.

The use of the STROOP-CWT as the only measure of executive cognitive function may limit the scope of inhibitory control assessment. The STROOP-CWT was only assessed at baseline.

The confounders in this study included age, body surface area, physical activity, log gamma-glutamyl transferase and cotinine. Additionally participants with an HIV positive status were excluded from all analyses. The possible influence of the confounders in the associations between main markers was kept to the minimum, with adjustments made where necessary in the statistical analyses. We also followed a hypothesis-driven approach reflecting a brain-heart link in order to avoid multiple comparisons.

6.4. Recommendations

1. In order to validate and extrapolate our findings to the entire South African population further longitudinal studies are to be recommended in African populations.
2. Longitudinal trends beyond three years may also help to relate the observed physiology to adverse cardiovascular outcomes, thereby emphasizing the clinical relevance of our findings.
3. Assessment of biomarkers of the central nervous system along with peripheral biomarkers may provide valuable information to determine the exact role of these markers in neural protection.
4. Additional executive cognitive function measurements including neuroimaging correlates (i.e. functional magnetic resonance imaging, positron emission tomography, diffusion tensor imaging), as well as other neuropsychological battery assessments (i.e. Trail Making Test, Verbal Fluency Test) may assist in determining the exact role of cardio-metabolic risk in processes related to interference control.
5. Experimental and intervention studies are also needed to determine cause and effect so as to make recommendations with regard to prevention and treatment strategies in Black populations.

6.5. Final conclusion

To the best of our knowledge, this study is the first to investigate longitudinal relationships between cardiac stress and other cardiovascular risk markers in a Black and White African population. In Black men, chronically increased levels of markers of myocyte injury, hyperglycaemia and insulin resistance accompanied by progressive myocardial stretch may be reflective of cardiac metabolic over-demand increasing the likelihood for hypertension and ischemic heart disease risk. However, central neural control mechanisms potentially may have upregulated BDNF and down-regulated TNF-α in these men as a way to protect against these demands and to improve processes related to interference control.

6.6. References

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APPENDICES

POSTGRADUATE STUDENT SKILLS

STUDENT NAME:	**Tick if accomplished**	
Undergraduate teaching *(indicate number of courses)*	N =	
Optional: Clinical Pharmacology course *(16 credit module)*	√	
Optional: Honours student mentorship *(indicate number of students)*	N = **2**	
Ethical consent: *Sub-study application under Umbrella-study*	√	
Obtained and interpreted medical history, medication status: *Socio-economic (medical aid access, education; job), marital, family history, health and cardio-metabolic incidents/events; medications*	√	
Dietary habits questionnaire		
Good Clinical Practice (GCP) course: *Year obtained*	**2018**	
[1]***Observed collection/***[2]***Interpreted psychosocial battery measures:*** *Measures with known heritability: Life orientation, Personality*	[1]	[2]
Predictors of developing/worsening hypertension: Coping, Depression, Cognitive distress		
Moderating effects of the environment: Fortitude, Mental Health, Self-regulation, Job stress		
Performed/Interpreted anthropometry measurements *Skinfolds, Height, Body mass, Waist circumference, Physical activity*	√	
[1]***Cardiovascular assessments,*** [2]***download and*** [3]***interpretation of data*** *Resting Blood Pressure [Riester CE 0124® & 1.3M ™ Littman® II S.E. Stethoscope 2205]*	√	
**Finometer [Finapres Medical Systems®]*		
12-lead resting ECG [NORAV PC-ECG 1200®]	√	
24 ambulatory BP & -ECG [Cardiotens® & Cardiovisions 1.19®, Meditech]	√	
Pulse Wave Velocity and Pulse Wave Analysis [Sphygmocor EXCEL, AtCor]	√	
Laboratory skills (sample handling and analyses)	[1]√	[2]√
24h Urine/blood/saliva/hair: [1]*collection/*[2]*sampling/*[3]*aliquoting/*[4]*waste material*	[3]√	[4]√
Rapid tests (cholesterol, glucose, urine dipstick and blood type)	√	
Laboratory analyses of samples (ELISA, RIA, COBAS Integra, E411)		
Whole blood HIV status *[PMC Medical, Daman, India; Pareekshak test, BHAT Bio-Tech, Bangalore, India]*	√	
[1]**Accomplished training &** [2]**measuring of ultrasound Carotid Intima Media Thickness (CIMT)** *[Sonosite Micromaxx®, SonoSite Inc., Bothell, WA]*	[1]√	[2]√
[3]**Retinal Vessel Assessment,** [4]**Data download & Interpretation (***Imedos®*)	[3]	[4]
Statistical analyses	[1]√ [2]√ [3]√	
[1]*Normal distribution & T-tests,* [2]*General linear models,* [3]*Multiple regression analyses* [4]*ROC analyses;* [5]*Prospective data analyses and risk prediction*	[4]	[5]√
Successful grant/funding application/s: *NRF*[1]*/MRC*[2] *South Africa*	[1]N = **3**	[2]N =
Publications: *Prepared, submitted, handled rebuttal of manuscript in a peer-reviewed journal*	N= **5**	
Conference meetings: [1]*National,* [2]*International* [3]*oral/*[4]*poster presentation*	[1]N= **2**	[2]N= **2**
	[3]N= **3**	[4]N= **3**

N=number; *Inclusive of sympathetic nervous system (SNS) responses (acute mental laboratory stressors e.g. cold pressor & colour-word-conflict).

Prof L Malan (RN, HED, PhD)
PI, SABPA study

Dr CMC Mels (PhD Biochemistry)
Manager HART Laboratory

Sr A Burger (RN, MCur)
Manager Hypertension Research and Training Clinic

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Dr L Malan

Dear Dr Malan

6 February 2008

ETHICS APPROVAL OF PROJECT

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

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

***Ethics* number**:

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

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

Approval date: 12 November 2007 **Expiry date**: 11 November 2012

Special conditions of the approval (if any): None

General conditions:

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

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

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

Yours sincerely

Prof M M J Lowes
(chair NWU Ethics Committee)

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To whom it may concern

31 August 2012

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

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

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

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

Kind regards

Prof. H.H. Vorster
Chair person

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13 September 2017

Dear Prof Malan

FEEDBACK ON HREC ANNUAL MONITORING REPORT: NWU-00036-07-A6

We would like to thank you for submitting the annual monitoring report for your project entitled, ***"The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study"*** to the Health Research Ethics Committee (HREC) in a timely manner. Please find below the decision of the HREC regarding the continuation of your project.

Classification	Mark with X	Comment	
Clarification			
Completion (Final report)			
Suspended			
Continuation	X	Date of next monitoring report:	30 September 2018
Termination			

Should you have any further queries, please feel free to contact Ms Leanie van Ronge at your earliest convenience (E-mail: Ethics-HRECMonitoring@nwu.ac.za; Tel: 018 299 2197). We wish you well in your future endeavours.

Yours sincerely

Prof Minrie Greeff
Head of Health Sciences Ethics
Office for Research, Training and Support

Dr Wayne Towers
Chairperson: HREC

Original details: (23239522) C:\Users\NWUUSER\Google Drive\HREC Monitoring\2017\NWU-00036-07-A6 (L Malan)\Continuation Letter 13_09 13 September 2017

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2016/09/02

ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by **Health Research Ethics Committee (HREC)**, after being reviewed at the meeting held on **08/06/2016**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Cardiac stress and cardiovascular risk markers: The SABPA study.

Study Leader/Supervisor: **Prof L Malan**
Student: **E Jansen van Vuren**

Ethics number: N W U - 0 0 0 5 1 - 1 6 - A 1
Institution | Study Number | Year | Status

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

Application Type: Single study
Commencement date: 2016-08-05 **Risk:** **Minimal**

Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years.

Special conditions of the approval (if applicable):

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

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 study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,*
    - *the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *HREC can be contacted for further information or any report templates via Ethics-HRECApply@nwu.ac.za or 018 299 1206.*

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

Yours sincerely

Prof LA Du Plessis
Digitally signed by Prof LA Du Plessis
Date: 2016.09.05 17:24:44 +02'00'

Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

Turnitin Originality Report

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12 November 2018

I, **Ms Cecilia van der Walt**, hereby declare that I took care of the **editing** of the **thesis** of **Ms Esmé Jansen van Vuren** titled ***Cardiac Stress and Cardiovascular Risk Markers: The SABPA study.***

MS CECILIA VAN DER WALT

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