Electrical and cardiac stress reactivity associations with pre-clinical target organ damage: The SABPA study

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Dissertation submitted in fulfilment of the requirements for the degree Masters of Science in Physiology at the Potchefstroom Campus of the North-West University

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November 2016
Alles is vir Hom en deur Hom geskep.
Voor alles was Hy al daar en deur Hom bly alles in stand.
~ Kol 1:16-17
I am indebted to many individuals that shared with me their time, expertise and support to make this study possible. I am privileged to express my sincere appreciation and profound gratitude to the following:

- My supervisor, Prof. Leoné Malan – an exceptional visionary and mentor which taught me to strive, not for success, but, to be of value – for her imperative guidance, moral support, availability and inspiration.

- Prof. Nicolaas T Malan, my co-supervisor – a scientist to whom I compare all others – for his inestimable expertise, encouragement, patience and for teaching me to always practise sound science with integrity and to stand firmly for what one believes, even if one stands alone.

- Dr Shani Botha, co-supervisor, for her unwavering encouragement, positive feedback, and belief that every contribution we make has the possibility to change the world.

- Prof Dr Roland von Känel, my co-supervisor – the personification of humility – for his vital guidance, constant support and positive attitude throughout this entire study.

- The NRF for providing me with a scholarship to pursue this study.

- My mother and brothers, for their unconditional love, support and tolerance.

- My Heavenly Father for blessing me with the opportunity and privilege to explore His creation, as well as strength and perseverance to complete this study – it is all but for the Grace of God.
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Opsomming

Titel
Elektriese en kardiale stres reaktiwiteit assosiasies met pre-kliniese teiken orgaan skade: Die SABPA studie

Sleutelwoorde
Stressor reaktiwiteit; etnisiteit; Suid-Afrika; Kardiale Troponien T; NT-proBNP; KVS; QTc

Motivering
Mense se fisiologiese aanpassingsreaksies op voortdurende blootstelling aan spanningsvolle omgewings word geassosieer met 'n volgehou toename in die voorkoms van kardiovaskulêre siektes en ko-morbiditeite. 'n Onvermoë om suksesvol te reageer op beide psigiese en fisiese stressors word geassosieer met die toenemende voorkoms van hipertensie, koronêre-arterie-siekte (KAS), beroerte en kardiale strukturele hermodellering. Hierdie aanpassingsresponse is nie alleenlik afhanklik van die individu se persoonlikheid en vorige ervarings nie; veranderlikes soos ouderdom, geslag en etnisiteit is ook bepalend. Die skakel tussen kardiovaskulêre risiko en die ontwikkeling van kardiovaskulêre siektes mag binne die outonne senuweestelsel (OSS) lê. Boonop kan die OSS se reaktiwiteit, soos gedurende akute psigiese strestoetsing, spesifieke reaktiwiteitsresponse vergesel. Voorheen is daar gedemonstreer dat gedurende akute psigiese strestoetsing, verstedelikte Swartes (hierna verwys as Afrikane) verhoogde bloed druk-(BD) waardes en α-adrenergiese vaskulêre response toon. Daarenteen blyk dit duidelik dat Blankes (hierna verwys as Kaukasiërs) hoofsaaklik 'n sentrale kardiale β-adrenergiese respons
toon, met gepaardgaande normale BD-waardes. Verhoogde kardiovaskulêre risiko kan dus moontlik gekoppel wees aan ‘n α-adrenergie respons in diegene wat meer stres ervaar. Dit is egter steeds onduidelik of hierdie spesifieke hemodinamiese response assosieer met identifiseerbare kardiale stres en elektriese reaktiwiteitsmerkers gedurende akute psigiese strestoetsing. Merkers van kardiale stres sluit onder andere kardiale troponien T (cTnT) en N-terminaal pro-brein natriuretiese peptied (NT-proBNP) in. Hierdie merkers word normaalweg gebruik om kardiale hipertrofie, ischemie en hartversaking te identifiseer. Onlangs is verhoogde vlakke van hierdie merkers met versteurde OSS-funksie asook akute psigiese stres geassosieer. Elektriese merkers wat verband hou met kardiale outonome funksie, soos die gekorrigeerde QT interval (QTc), kan ook moontlik ‘n aanduiding wees van OSS-veranderinge gedurende akute psigiese stres, aangesien die QTc as ‘n maatstaf van kardiale simpatiese tonus geldidentifiseer is.

Doelstellings

Geen vergelykende etniese data ten opsigte van BD, elektriese en kardiale stres-merker reaktiwiteit is tans in Suid-Afrika beskikbaar nie. Die doelstellings was dus, eerstens, om die etnies-spesifieke verskille in BD, QTc en kardiale stres reaktiwiteit gedurende akute psigiese strestoetsing, te identifiseer en tweedens, om aan te dui dat α-adrenergiee BD response geassosieer is met ‘n toename in QTc-verlenging en kardiale-stresmerkers in Afrikane. Derdens, om daarop te wys dat α-adrenergiee BD response, QTc en kardiale stres reaktiwiteit sal dui op pre-kliniese veranderinge in die ladingstoestande en struktuur van die hart.

Metodologie

Hierdie dwarsdeursnit, vergelykende, teikenpopulasie-studie maak deel uit van die Simpatiese aktiiteit en Ambulatoriese Bloeddruk in Afrikane (SABPA) studie. Die SABPA-studie is gedurende die somer en herfs van beide 2008 (Afrikane) en 2009
(Kaukasiërs) uitgevoer, om sodoende enige seisoenale variasie te voorkom. Die Navorsing in Gesondheidswetenskappe Etiëkomitee (HREC) van die Noordwes-Universiteit se Potchefstroom kampus het hierdie studie as ook die huidige sub-studie goedgekeur. Alle vrywillige deelnemers het geskrewe ingeligte toestemming onderteken en ingedien voordat hulle by die studie ingesluit kon word. Alle prosedures het voldoen aan die geskikte institusionele riglyne soos uiteengesit in die Verklaring van Helsinki. Uitsluitingskriteria het behels die gebruik van α-, β-blokker en psigotropiese substans-gebruikers, inenting of bloedskenking binne drie maande voor die studie, oorkanaalteemperature van >37.5°C en swanger of borsvoedende vroue. Deelnemers is verder uitgeskakel indien daar by hulle atriale fibrillasie (N=16), ’n geskiedenis van miokardiale infarksie (N=4), elektrokardiogram linker ventrikulêre hipertrofie (EKG-LVH) (N=1) en ventrikulêre ektopiese episodes (uitgesluit tydens rekenaarverwerking), teenwoordig was. Die finale sub-studie-populasie het bestaan uit 388 onderwysers onder wie 193 verstedelikte Afrikaners en 195 Kaukasiërs. 24 uur Ambulatoriese BD-metings (24H ABPM) is opgeneem deur die Cardiotens CE120®. Actical® accelerometers is toegerus om fisieke aktiwiteit te meet. Deelnemers is in enkelkamers van die Metaboliese Navorsingseenheid van die Noordwes-Universiteit gehuisves en versoek om om 22h00 te gaan slaap. Bogenoemde apparate is die volgende dag ontkoppel, waarna antropometriese metings en bloedmonsteromtrekking deur ’n geregistreerde verpleegkundige geskied het. Vastende glukose, heel-bloed gegliseerde hemoglobien, totale cholesterol, hoë digtheid lipoproteïen (HDL-cholesterol), asook leefstil-merkers soos gamma glutamiel transferase (vir alkohol-gebruik) en kotinien (vir sigaretrook), is bepaal. Die Finapres het kontinue slag-tot-slag BD-veranderinge gedurende psigiese stresstoetsing geregistreer. Rustende slag-tot-slag BD en 10-afleiding EKG metings is vir 5 min lank geregistreer, gevolg deur veneuse bloedmonsteromtrekking. Na ’n tydperk van 5-10 min, is die Stroop kleur-woorde-konfliktoets vir 1 min toegepas, en slag-tot-slag BD en EKG-response is gedurende dié tyd geregistreer. Nog ’n bloedmonster is 10 min na stresstoetsing verkry. Hiërdie
bloed monsters is onder andere vir kardiale-stresmerkers cTnT en NT-proBNP via 'n elektrochemiluminisensie tegniek geanalyser. Die normaalverspreiding van veranderlikes is geverifieer en beskrywende t-toetse het etniese verskille uitgelig. Met chi-kwadraat statistiek is proporsies en voorkoms bepaal. ANCOVAs het die kleinste kwadraat gemiddelde verskil in reaktiwiteits-merkers tussen etnisiteite, onafhanklik van a priori veranderlikes, bereken. Regressie-analises is in drie modelle uitgevoer. Die statistiese betekenisvolheid van al die bogenoemde analises is gestel as $p \leq 0.05$ en die invoerings-F-waarde is as 2.5 vasgestel. Receiver-operated characteristics (ROC) analises het etnies-spesifieke cTnT afsny punte, wat voorspellend van 24 uur diastoliese hipertensie (24H DBD HT) is, bepaal. 'n Ratio vir verschillende modelle is ook bereken om die verband van KVS-risiko met 'n toename in die R-golf van die aVL-afleiding van die EKG (RaVL-amplitude), bokant die minimum opspoorbare vlak van hierdie hoë sensitwiteitsmetode cTnT kategorie, vir elke etnisiteit respektiewelik vas te stel.

Resultate

Afrikane het 'n hoër risiko vir kardiovaskulêre siektes, sowel as verhoogde RaVL amplitude, EKG-LVH voorkoms en 'n hoër gemiddelde getal ischemiese episodes getoon. Rustende waardes vir kardiovaskulêre merkers was grotendeels dieselfde tussen etnisiteite, maar die graad waartoe hierdie merkers gedurende akute psigiese strestoetsing verander het, het egter noemenswaardig tussen hulle verskil. Akute psigiese stresresponse in Afrikane het gepaardgegaan met 'n tipiese α-adrenergieuse respons-profiel. Daarenteen het Kaukasiers hoofsaaklik 'n sentrale kardiale β-adrenergieuse respons-patroon getoon. 'n Positiewe assosiasie was duidelik tussen cTnT en NT-proBNP reaktiwiteit in beide etniese groep voorgekom, alhoewel hierdie assosiasie aansienlik sterker in die Kaukasiese groep voorgekom het. ROC-analises het 'n hoër cTnT afsny punt gedurende akute psigiese strestoetsing in Afrikane (4.19 pg/mL) blootgelê, wat spesifiek beduidend is van 'n verhoogde risiko van 24H
DBD HT. In Kaukasiërs was die afsnypt 3.24 pg/mL. In beide etnisiteite is ‘n verhoogde RaVL amplitude egter geassosieer met verhoogde vlakke van cTnT gedurende akute stres – ‘n assosiasie wat ‘n waarskynlikheidsratio van ongeveer 11 vir beide etnisiteite aandui.

**Gevolgtrekking**

Kardiale stres (cTnT en NT-proBNP) en QTc-reactiwiteit was onafhanklik geassosieer met ‘n verhoogde pre-kliniese risiko vir strukturele en mekaniestieke veranderinge, spesifiek in die Afrikane van die SABPA-studie. In hierdie Afrikaangroep, waar kardio-metaboliese vatbaarheid en α-adrenergiestieke reaktiwiteit oorheersend is, mag die voorgenoomde veranderinge uiterst nadelig wees, soos dit duidelik blyk uit die verhoogde risiko vir DBD HT, KVS, ischemie en KAS. Verhoogde kardiale stres en QTc reaktiwiteit, spesifiek geassosieer met α-adrenergiestieke reaktiwiteit, kan bydra tot die vroeë sensitisering van en skade aan die miokardium, asook tekens van KAS, veral in ‘n populasie wat ‘n hoë risiko toon.
Summary

Title

Electrical and cardiac stress reactivity associations with pre-clinical target organ damage: The SABPA study

Keywords

Stressor reactivity; ethnicity; South Africa; Cardiac Troponin T; NT-proBNP; CVD

Motivation

People’s physiological adaptive responses to chronic stressful environments have been persistently associated with an increased incidence of cardiovascular diseases (CVD) and co-morbidities. An inability to successfully respond to both mental and physical stressors is associated with an increased incidence of hypertension, coronary artery disease (CAD), stroke and cardiac structural remodelling. These adaptive responses not only depend on one’s personality and previous experiences, but also on factors such as age, gender and ethnicity. The link between cardiovascular risk and the development of CVD may be presented by reactivity of the autonomic nervous system (ANS), such as during acute mental stress application, and may accompany specific reactivity patterns. It has been demonstrated that during acute mental stress exposure, urban-dwelling Blacks (hereafter referred to as Africans) present elevated blood pressure (BP) values and exhibit α-adrenergic vascular responses, whilst their White (hereafter referred to as Caucasian) counterparts predominantly presented a central cardiac, β-adrenergic response accompanied by essentially normal BP values. Therefore, an increased cardiovascular risk may be linked to α-adrenergic vascular responses in those experiencing greater stress. However, whether these specific
haemodynamic responses are linked to identifiable cardiac stress and electrical reactivity markers during acute mental stress has yet to be determined. Markers of cardiac stress include cardiac troponin T (cTnT) and N-terminal pro-Brain natriuretic peptide (NT-proBNP). These markers are traditionally used to indicate cardiac hypertrophy, ischemia and heart failure. However, recently increased levels of these markers have been associated with disrupted autonomic function and acute mental stress. Electrical markers pertaining to cardiac autonomic function, such as the corrected QT interval (QTc), may also indicate autonomic alterations during acute mental stress, seeing that the QTc has been shown to be a measure of cardiac sympathetic tone.

Objectives

No ethnic-comparative data regarding BP, electrical or cardiac stress marker reactivity are available in sub-Saharan African individuals. Therefore, the objectives were firstly, to indicate and compare ethnic-specific differences in BP, QTc and cardiac stress reactivity during acute mental stress application. Secondly, to signify that α-adrenergic BP responses will associate with increased QTc prolongation and cardiac stress levels in Africans. Thirdly, to illustrate that an α-adrenergic BP response, QTc and cardiac stress markers' reactivity will indicate pre-clinical alterations in the loading conditions and structure of the heart.

Methodology

This cross-sectional, comparative target population study forms part of the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SAPBA) study. The SABPA study was conducted between late summer until autumn in both 2008 (Africans) and 2009 (Caucasians) so as to avoid seasonal variations. The Health Research Ethics Committee (HREC) of the North-West University Potchefstroom Campus approved this study and all voluntary participants gave written informed
consent prior to their inclusion in the study. All procedures pertained to the applicable institutional guidelines as stated by the Declaration of Helsinki. Exclusion criteria entailed the use of α-, β-blocker and psychotropic substance users, vaccination or blood donation within three months prior to the investigation, tympanum temperatures >37.5°C and pregnant or lactating women. Participants were additionally excluded if they presented any sign of atrial fibrillation (N=16), history of myocardial infarction (N=4), electrocardiographic left ventricular hypertrophy (ECG-LVH) (N=1) and ventricular ectopic episodes (computationally excluded). The final sub-study sample comprised 388 teachers of whom 193 were urban dwelling Africans and 195 Caucasians. 24 hour Ambulatory BP measurements (24H ABPM) were recorded with the Cardiotens CE120®. Actical® accelerometers were equipped to attain physical activity recordings. Participants were requested to go to bed at 22h00, fasting overnight. The mentioned apparatus were removed the following day, followed by anthropometric measurements and blood sampling by a registered nurse. Fasting glucose, whole blood glycated haemoglobin, total cholesterol, high-density lipoproteins (HDL), as well as lifestyle markers such as gamma glutamyl transferase (alcohol consumption) and cotinine (smoking) were determined. The Finapres continuously assessed beat-to-beat BP changes throughout psychophysiological testing. Resting beat-to-beat BP and 10-lead ECG measurements were obtained for 5 min, followed by venous blood sampling. After a period of 5-10 min, the Stroop colour-word-conflict test was administered for 1 min, during which beat-to-beat BP and ECG responses were obtained. Another blood sample was obtained 10 min post-stress application. These blood samples (both prior and post-stress) were analysed for cardiac stress markers, cTnT and NT-proBNP, via electrochemiluminescence. The normality of all variables was verified and descriptive t-tests depicted ethnic characteristics. Chi-square statistics determined proportions and prevalence. Two-way ANCOVAs determined the least square mean difference in reactivity markers between ethnic groups, independent of a priori covariates. Regression analyses were performed in three
models and F to enter was set at 2.5. For all the aforementioned analyses, significance was set at a $p \leq 0.05$. Additionally, receiver-operated characteristics (ROC) analyses determined ethnic-specific cTnT cut-point values predicting 24 hour diastolic hypertension (24H DBP HT). Odds ratios (OR) were also calculated for several models to establish CVD risk relation to RaVL amplitude increases in the detectable cTnT category in each ethnicity respectively.

**Results**

A higher risk of cardiovascular vulnerability was observed in Africans as well as an increased RaVL amplitude, ECG-LVH prevalence and greater average number of ischemic events. Resting values for cardiovascular markers were quite similar between ethnicities. However, the degree to which these values changed during acute mental stress testing differed significantly. Acute mental stress responses of Africans were accompanied by a typical α-adrenergic response profile, whereas Caucasians predominantly presented a central cardiac β-adrenergic response pattern. A positive association existed between cTnT and NT-proBNP reactivity, in both ethnic groups, yet it was greater in the Caucasian group. ROC analyses revealed a higher cTnT cut-point during acute mental stress predicting 24H DBP HT in Africans (4.19pg.mL) compared to that of Caucasians (3.24pg/mL). An increased RaVL amplitude was associated with increased levels of cTnT during acute stress, in both ethnicities, giving rise to an OR of approximately 11.

**Conclusion**

Cardiac stress (cTnT and NT-proBNP) and QTc reactivity were independently associated with an increased pre-clinical risk of structural and mechanistic alterations, specifically in the SABPA African cohort. In this African group, where cardio-metabolic vulnerability and α-adrenergic reactivity are predominant, the aforementioned modifications may be detrimental, evidenced by an increased DBP HT, CVD, ischemia
and CAD. Increased cardiac stress and QTc reactivity, associated with α-adrenergic reactivity, may contribute to early sensitization and damage to the myocardium as well as signs of CVD, especially in an at-risk population.
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Chapter 3

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## Nomenclature

<table>
<thead>
<tr>
<th>Symbol/Abbreviation</th>
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<tr>
<td>α</td>
<td>Alpha</td>
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<tr>
<td>β</td>
<td>Beta</td>
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<td>Δ</td>
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<td>°C</td>
<td>Degrees Celsius</td>
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<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>mmHg</td>
<td>Millimetre of mercury</td>
</tr>
<tr>
<td>γGT</td>
<td>Gamma glutamyl transferase</td>
</tr>
<tr>
<td>ABPM</td>
<td>Ambulatory Blood Pressure Monitoring</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of Covariance</td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic Nervous System</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BSA</td>
<td>Body Surface Area</td>
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<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
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<tr>
<td>CI</td>
<td>95 % Confidence interval</td>
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<tr>
<td>CO</td>
<td>Cardiac Output</td>
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<td>cTnT</td>
<td>Cardiac Troponin T</td>
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<tr>
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<td>24 hour Diastolic hypertension</td>
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<td>Double Product</td>
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<td>Di-peptidyl peptidase 4</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>ECG-LVH</td>
<td>Electrocardiographic Left Ventricular Hypertrophy</td>
</tr>
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<td>F</td>
<td>Female</td>
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<td>HbA&lt;sub&gt;1c&lt;/sub&gt;</td>
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<td>High Density Lipoproteins</td>
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<td>HR</td>
<td>Heart Rate</td>
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<tr>
<td>Abbreviation</td>
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<tr>
<td>IDE</td>
<td>Insulin Degrading Enzyme</td>
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<td>Left Ventricular Hypertrophy</td>
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<td>Renin-Angiotensin-Aldosterone System</td>
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<td>R-wave of the aVL lead</td>
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<tr>
<td>ROC</td>
<td>Receiver Operated Characteristics</td>
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<tr>
<td>SABPA</td>
<td>Sympathetic Activity and Ambulatory Blood Pressure in Africans</td>
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<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SE</td>
<td>Standard Error of the Mean</td>
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<td>SNS</td>
<td>Sympathetic Nervous System</td>
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<td>SV</td>
<td>Stroke Volume</td>
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<td>TEE</td>
<td>Total Energy Expenditure</td>
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<td>TPR</td>
<td>Total Peripheral Resistance</td>
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<td>QTc</td>
<td>Corrected QT-interval</td>
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<td>U/L</td>
<td>Units per Litre</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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Chapter 1

Preface

Outline of Study

Author contributions
1.1 Preface

This study forms part of the program for the degree Master of Science (MSc) in Physiology. The manuscript presented in Chapter 3 has been submitted for peer-reviewing in the Journal of Hypertension (JH-D-2016-06092016). Chapter 2 contains a comprehensive literature overview of all the variables, including a detailed discussion of the sympathetic nervous system’s role in mental stress reactivity, as well as markers pertaining to such reactivity, cardiac stress and cardiac electrical activity. The focus was specifically on the corresponding ethnic differences in reactivity profiles and their relation to pre-clinical target organ damage. At the ends of chapters two, three and four, the relevant references are consistent with the author guidelines for publishing in the aforementioned journal, according to the Vancouver bibliographical style.

1.2 Study outline / outline of the study

This study, divided into four chapters, entails the following information:

Chapter 1 explains the preface to and outline of the study as well as the respective author’s contributions, whereas the second chapter discusses the general introduction, literature overview of the investigated variables, published data and questions arising from the literature. The motivation, objectives and hypotheses of the study are also included in this chapter.

Chapter 3 contains the manuscript of the study, specifically titled: Electrical and cardiac stress reactivity associations with pre-clinical target organ damage: The SABPA study. All the findings of the study, as well as its limitations, and comparison to literature are discussed in the fourth and final chapter. Chapter 4 also includes the general conclusion and recommendations for future research pertaining to similar investigations.
### 1.3 Author’s contributions

The role of each researcher involved in this study is as follows:

<table>
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<th>Role</th>
<th>Contribution</th>
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<tr>
<td><strong>Student</strong></td>
<td>Miss Annemarie Wentzel (BSc Honours Physiology) was responsible for all literature searches, statistical computations and interpretation of the results, together with the planning and writing of the manuscript. The clinical research skills obtained by the student during the course of this study, in ongoing projects in the Hypertension Research and Training Clinic, are similar to the SABPA study (illustrated in the <em>Post-graduate competency form</em> on the next page).</td>
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<tr>
<td><strong>Supervisor</strong></td>
<td>Prof Leoné Malan (RN, HED, WBI, PhD), as supervisor, contributed to the design and collection of data for the SABPA study, assisted in the initial planning of the manuscript and supervised the analytical as well as writing processes.</td>
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<tr>
<td><strong>Co-supervisor</strong></td>
<td>Prof Nico T Malan (DSc), as co-supervisor, contributed to the design and collection of data for the SABPA study, assisted in the initial planning of the manuscript, supervised the writing of the manuscript and was responsible for its critical review.</td>
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<tr>
<td><strong>Co-supervisor</strong></td>
<td>Dr Shani Botha (PhD), as co-supervisor, contributed to data collection and supervised the writing of the manuscript.</td>
</tr>
<tr>
<td><strong>Co-supervisor</strong></td>
<td>Prof Dr Roland von Känel (MD), as co-supervisor, supervised the writing of the manuscript and was responsible for its critical review.</td>
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I, Annemarie Wentzel, hereby declare that the aforementioned is representative of my actual contribution and that I hereby give my consent that this manuscript may be published as part of the dissertation for the degree Master of Science in Physiology.

Miss A Wentzel

The aforementioned statements confirm the individual roles of the four co-authors respectively and hereby Profs L Malan, NT Malan, Dr S Botha and Prof Dr R von Känel give permission that this manuscript may form part of the dissertation.

Prof L Malan  Prof NT Malan  Dr S Botha  Prof Dr R von Känel
POSTGRADUATE STUDENT SKILLS 2016

<table>
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<th>STUDENT NAME: Annemarie Wentzel (BSc Hons)</th>
<th>Tick if accomplished</th>
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</table>

Optional: Clinical Pharmacology course (16 credit module)

Optional: Honours student mentorship (indicate number of students)

Grant writing and submission

Ethical consent: Sub-study application under Umbrella-study

Obtained and interpreted medical history & medications

Including duration of stay, education, marital status, alive family members, health (cardiometabolic, inflammation, depression, renal, arthritis, cancer, reproduction), sleep apnoea, ambulatory & dietary diary, mental stress perception

Good clinical practice: lifestyle habits; participant handling

Objective & Self-reported smoking & alcohol habits

Dietary intake and questionnaire

Obtained Collection of psychosocial battery measures

Measures with known heritability: Life orientation, Personality

Predictors of developing/worsening hypertension: Coping, Depression, Cognitive distress

Moderating effects of the environment: Fortitude, Mental Health, Self-regulation, Job stress

Observed anthropometry measurements

Height, Body mass, Waist circumference, BMI

Cardiovascular assessments, download and 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)

24h Urine/blood/saliva/hair: 1 collection/2 sampling/3 aliquoting/4 waste material

Rapid tests (cholesterol, glucose, urine dipstick and blood type)

Laboratory analyses of samples (ELISA, RIA, ECLIA, etc.)

Whole blood HIV status [PMC Medical, Daman, India; Pareekshak test, BHAT Bio-Tech, Bangalore, India]

Accomplished training & measuring of ultrasound Carotid Intima Media Thickness (CIMT)

[Sonosite Micromaxx®, SonoSite Inc., Bothell, WA]

Statistical analyses

Normal distribution & T-tests, 2 General linear models, 3 Multiple regression analyses

ROC analyses; 4 prospective data analyses and risk prediction

Prepared, 5 submitted, 6 handled a rebuttal & 7 published manuscript in a peer-reviewed journal

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*Including sympathetic nervous system (SNS) responses (laboratory stressors namely the cold pressor & colour-word-conflict tests)
Chapter 2

General Introduction

Literature overview

Aims

Objectives

Hypotheses
2. General introduction

It has become abundantly clear that humans of the 21st century are being exposed to a multitude of stressful scenarios. Our ability to respond to these challenges has a key influence on our health and wellbeing. An increase in incident cardiovascular diseases (CVD) and co-morbidities has been associated with biological responses to stressful environments. Failure to successfully respond to both mental and physical stressors is associated with an increased prevalence of hypertension, coronary artery disease (CAD), stroke and cardiac structural remodelling [1-3]. Autonomic control of the heart is imperative for maintaining normal cardiac function. In turn, change in cardiac autonomic activity identifies autonomic control of the heart as an essential pathophysiological pathway that links cardiovascular risk factors with the development of CVD [1]. Mental stress alters autonomic responses of the heart in both chronic and acute conditions and is a recognized risk factor for CVD [4].

This observed link between the brain and the heart is not novel. In 1929, William Harvey stated that ‘For every affection of the mind that is attended with either pain or pleasure, hope or fear, is the cause of an agitation whose influence extends to the heart’. [5]. The brain-heart-link has been extensively studied over the past half century, both anatomically and physiologically, and its complexity is undebated [6-8]. Activation of the autonomic nervous system (ANS) not only alters heart rate, haemodynamics and electrical conduction, but also cellular and subcellular properties of cardiomyocytes [8]. However, these alterations do not necessarily occur in a similar manner among all individuals, but relate to demographic factors such as age, ethnicity and gender. It was shown that acute and chronic stress responses are associated with sympathetic nervous system (SNS) hyperactivity, leading to hypertension, pressure overload and myocardial ischemic events in Africans [2] and African Americans [9]. The accumulative effect of hypertension, ischemic events and increased pressure load may lead to repolarization abnormalities, regardless of whether or not left ventricular
hypertrophy (LVH), CAD and mechanistic complications such as diastolic dysfunction are present. Malan and co-workers demonstrated that Africans who reside in urban environments tend to have an increased prevalence of hypertension and they also exhibit α-adrenergic vascular responses, while their Caucasian counterparts present a central cardiac β-adrenergic response accompanied by normal blood pressure (BP) values [2, 3]. The latter response was also found in rural dwelling Africans [10, 11]. Increased cardiovascular risk is therefore observed amongst Africans experiencing greater psychosocial stress [11]. The exact mechanisms by which mental stress, electrical activity of the heart, cardiac stress, elevated BP and appraisal responses are related to each other remain unclear [2]. In an effort to elucidate these mechanisms, it is imperative to acknowledge and investigate the role of the brain as the element of central control, and investigate feasible biomarkers that can be utilized to assess autonomic control of the heart. An overview of the literature regarding such biomarkers follows.

2.1 Central control

The brain is the central command centre of the human body. It fundamentally shapes stress reactivity, coping and recovery processes and it is also the primary mediator and target of stress vulnerability and resilience [12]. The brain alone differentiates between threatening and non-threatening scenarios, subsequently regulating appropriate physiological and behavioural responses to these perceived situations [13]. It does this via a distributed circuitry of fundamental systems that involve the hippocampus, amygdala and specific areas of the prefrontal cortex (Figure 2.1) [14].
Figure 2.1: The physiological responses that follow limbic activation. These are the means through which the brain launches and integrates biological responses, resulting in effective adaptation to environmental stressors which are processed as sensory stimuli. Where: NS, nervous system; $\Delta$ reactivity change.

These systems regulate physiological and behavioural stress responses, which may be adaptive or maladaptive depending on the duration and frequency of activation. Such stress responses originate from bidirectional communication between the brain and sympathetic and cardiovascular systems [14]. This communication is maintained via endocrine and neural mechanisms sustaining cognition, behaviour, and perception and influenced by previous experience [14]. Demographic factors such as age, gender and ethnicity also shape these highly integrated and sophisticated processes that ensure effective psychophysiological responses [13, 15]. Therefore, the brain processes input from the external environment, ensuring that the body adjusts accordingly. Some of the systems promoting such adaptation include the ANS,
elements of the metabolic system (thyroid axis, insulin), the kidneys and immune system [13]. The bio-mediators of these systems, such as the sympathetic and parasympathetic systems, cytokines and metabolic hormones operate as a non-linear interactive network. In this network the mediators up-and-down regulate one another in feedback loops and the activity of these mediating systems closely relate to the developmental, behavioural and mental state of the individual [13]. This (the ANS) bidirectional communication network promotes short-term adaptation and protection of the body – to ensure maintenance of allostasis – yet the very same network of mechanisms may lead to long-term dysregulation of allostasis [13]. Such dysregulation promotes a maladaptive strain on the body, when chronically exposed to stressful conditions, thereby increasing the allostatic load, the cumulative result of an allostatic state, eventually leading to allostatic overload. Allostasis is defined as achieving stability through change [16]. Therefore, it is seen as a process that maintains homeostasis – defined as those physiological parameters essential for maintaining life – even though the boundaries and set-points may change with environmental conditions [17]. Romero and co-workers stated that allostasis can, therefore, be defined as the active process of maintaining homeostasis [18]. In this context allostasis is referred to as the ability of the body to produce changes in physiological activity such as the production of hormones (e.g. cortisol, adrenalin), haemodynamic changes (blood pressure) and other mediators (such as cytokines and autonomic activity) [18]. All these changes occur to assist the individual in adapting to the new challenge or environment – this includes predictable and unpredictable changes [17].

When stress resiliency is compromised, it affects the overall health of an individual. Changes mediated by these systems are easily observed in the cardiovascular system [15]. Therefore, identifying markers of electrical or biochemical activity pertaining to adaptation, or in many cases, maladaptation, is of utmost importance.
2.2 The concept of ‘stress’ and ‘stressor’

The ability of people to react and adapt to a specific threat or change in the environment is governed by a psychophysiological stress response [19], serving physiological adaptation through several biochemical pathways. This specific threat or change in an individual’s environment has been defined as a “stressor” by Hans Selye in 1936 [20]. Selye played a crucial role in the development of the stress concept. Initially, it was thought that the neuroendocrine response to non-specific stress was restricted to the release of catecholamines [20]. However, Selye appreciated the central role of the adreno-cortico- hypophysial axis during stress responses [20]. Later, in 1946, Selye provided a comprehensive and elaborate framework, describing the specific response to stress as the ‘general adaptation syndrome’, according to which the initial reaction to stress is shock, followed by a counter-shock phase and the development of gradual resistance to the particular stressor [21]. However, in the event that a stressor persists, this resistance may progress into exhaustion. Therefore, the definition of biological stress, according to Hans Selye is ‘the nonspecific response of the body to any demand made upon it’ [20, 21].

Over the past few decades, several definitions of stress have been adapted and applied. However, the essence still remains the same. This being that agents, very diverse in nature, such as chemical, physical, biological or psychological means may elicit a response, depending on whether they are perceived as benign or threatening. This response depends on an individual’s adaptive coping resources [13, 15, 22-24]. In accordance with this description, not all stress reactions are identical, as these reactions elicit different autonomic responses based on resource availability, previous experiences and scenario perception [24]. The result of these responses may be monitored by observing changes in certain physiological systems.
When mental stressors were applied to subjects within a laboratory environment, they elicited a cardiovascular response – identifying such methods as a valuable tool to evaluate acute stress responses [15]. Recently it was proven that the reaction to a stressor recorded in a laboratory environment is similar to the reaction observed during everyday, real-life stress situations [25]. This might be due to the fact that an individual’s response to a stressor – regardless of its origin or nature – is the result of genetic, epigenetic and constitutional vulnerability [25, 26]. This host of responses, both peripheral and central are defined as reactivity. Reactivity includes the activation of the neuroendocrine/hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous as well as the cardiovascular systems. These specific systems' reactivity reflects the way by which the brain sets certain bodily processes in motion to support the apparent behaviours required to ensure a successful response to the environmental demands [15, 25, 26]. These systems are hierarchically controlled – from the initial thoughts and emotions being generated, shaped and stored in the cortical regions of the brain and integrated with visceral output by the hypothalamus and then to the brainstem, where our fundamental respiratory, cardiac and haemodynamic rhythm originates (Figure 2.1). As illustrated in Figure 2.1, the physiological responses that follow limbic activation include an increase in blood pressure, heart rate and cardiac contractility, also referred to as haemodynamic reactivity [27, 28]. These are the means through which biological reactions and brain activity are integrated, resulting in an effective response to environmental stressors. Therefore, numerous regions of the central nervous system are interconnected, both neuro-anatomically and functionally, creating a network that culminates in sympatho-adrenal reactivity (Figure 2.2).
Figure 2.2: A schematic presentation of the location and key functions of limbic areas that play an essential integrated role in control processes important for maintaining allostasis.

To summarize, the brain’s response to specific mental stressors is not only a highly integrated and sophisticated process via which sensory input is evaluated, but it is also related to perceptions, previous conditioning and experiences relative to present goals [15]. This hierarchical response entails an organized and coordinated array of specific functional neuro-anatomic regions and peripheral responses working together in an effort to ensure that the individual elicits an effective response.

2.3 The autonomic nerve projections innervating the heart

To improve comprehension regarding the manner by which the ANS regulates the heart, we will now focus our attention on the autonomic nerve innervation of the heart. Janes and colleagues were the first to describe the anatomy of cardiac sympathetic nerve projections in 1986 [29]. Although the conduction and impulse-generating systems of the heart establish an endogenous rhythm, the rate and contractile force is
determined by neural input [30]. Both branches of the ANS supply non-myelinated postganglionic fibres to the heart. Even though the innervation is bilaterally derived, the functionality is asymmetrical. These sympathetic and parasympathetic nervous systems are components of the extrinsic cardiac ANS and a complex interplay exists between these two systems [8]. Sympathetic fibres are largely derived from major autonomic ganglia along the cervical and thoracic-lumbal spinal cord. These ganglia house the cell bodies of most postganglionic sympathetic neurons whose axons form the superior, middle and inferior cardiac nerves, and terminate on the surface of the heart [30]. Parasympathetic innervation predominately originates in the ambiguous nucleus of the medulla oblongata. The parasympathetic fibres are then carried within the vagus nerve and are divided into the superior, middle and inferior branches [31, 32].

The interaction between these two ANS domains are complex, and the characteristic of autonomic influences on the heart is essentially, albeit simplistically put, of an antagonistic nature [33-36]. However, it was Levy in the 1960s [6] that coined the phrase ‘accentuated antagonism’, a term describing the enhanced negative chronotropic effect of vagal stimulation in the presence of background sympathetic activity. Shen and co-workers observed this interaction about four decades later, when they examined that chronic vagal nervous stimulation leads to a significant reduction in sympathetic nerve activity [37].

2.4 Mental stressors and the cardiovascular system

The cardiovascular system (CVS) is one of the most stress-susceptible systems. It has been documented that increases in mental stress elevate BP and haemodynamic reactivity [11]. The sympathetic nervous system (SNS) is the major motor autonomic output pathway activated by sensory perception and from the preceding discussion it is quite clear that the heart itself is richly innervated with sympathetic nerves [33-36]. These nerves innervate the myocardium, coronary arteries and conduit systems
respectively. The predominant innervation of the cardiovascular system is via sympathetic nerve fibres. However, various types of nerve fibres innervate different organs [15]. These sympathetic fibres elicit a vasoconstrictive effect via $\alpha_1$-adrenergic receptors. The cortical and sub-cortical regions of the brain influence systemic vascular resistance via these nerve fibres. In the heart, as well as in active muscle tissue, the vasoconstrictor effects of these nerves are counterbalanced. This counterbalance is due to local metabolic alterations and conditions that promote vasodilation – such as increased oxygen demand – effectively matching supply and demand, blood flow to workload [38]. Therefore, it is likely that alterations to this adrenergic innervation may be involved in the deleterious effects of mental stressors on the heart. The myocardium itself contains adrenergic receptors that transduce the effects of mental stress, justifying the hypothesis that sympathetic hyperactivity due to acute stress may directly relate to the development of cardiomyopathy [38].

The $\beta_1$- and $\beta_2$-adrenergic receptors are the predominant receptors in the myocardium itself and their stimulation during mental stress is a result of local (endogenous) and exogenous catecholamine release [7, 38]. The CNS and peripheral sympatho-adrenal effector systems play a prominent role in mental stress responses. Therefore, it is of utmost importance to evaluate cardiac innervation and its key role in order to create a comprehensive model of mental stress effects on the heart.

**Chronic and Acute mental stress**

Before attempting such a comprehensive model, a temporal distinction between chronic and acute stress is necessary to comprehend their influence on the cardiovascular system. Chronic stress is long standing and exerts an influence over a long period of time. Such stress includes persistent conditions related to an individual’s occupation, emotional taxation, social relationships and communal environment [39]. Contrarily, acute stress involves transient changes resulting from abrupt onset, brief
duration and aversive challenging environmental events – as experienced during laboratory stress testing. Research regarding acute stress may contribute to the understanding of clinical cardiac events such as myocardial ischemia and coronary artery events, which clinically manifest as ST elevation myocardial infarction, non-ST elevation myocardial infarction, unstable angina and sudden cardiac death, as discussed in greater detail in section 5.2.

**The Stroop Colour-Word Conflict Test**

An example of an acute mental stress test utilized in this study, is the colour-word conflict test, also known as the Stroop test [40]. The Stroop test requires an individual to identify the colours of colour-word cards in contrasting ink colours under time pressure. The elapsed time due to the reaction to colours caused by the presence of conflicting word stimuli is then taken as a measure of interference of word stimuli when naming colours [40]. Such a reaction to conflicting colour-word stimulation also elicits a specific cardiovascular response [2]. This response is greatly dependent on an individual’s perception of the task at hand. If the Stroop test is perceived as too challenging, therefore as a threat, the individual would most likely present an elevation in BP accompanied by increased peripheral resistance and decreased cardiac output discussed in detail in the sections to follow). This type of haemodynamic reaction has been identified as an α-adrenergic vascular response [2, 11]. However, if the Stroop test is perceived to be easily achievable and positively challenging, subjects present an elevation in BP and cardiac output and a decrease in peripheral resistance. The latter reaction is defined as a central cardiac β-adrenergic response [2, 11]. These reactions and their presentation by individuals will be discussed in the following sections.
Acute mental stress-induced modifications in cardiovascular haemodynamics

During application of an acute mental stressor the ultimate cardiovascular result of this stimulation is an increase in cardiac contractility [15]. Furthermore, the sympatho-vagal balance shifts towards greater sympathetic activation, resulting in an increased heart rate. Consequently, the myocardial metabolic oxygen demand increases [41]. ANS augmentation during mental stress may result in neuro-hormonal release, inducing detrimental effects on haemodynamic reactivity, cardiac stress, conduction and contractility [38]. Haemodynamic changes in response to mental stress may include changes in blood pressure, cardiac output, stroke volume and cardiac loading conditions [38, 41]. However, it is important to note that these reactions always largely depend on an individual’s perception of the applied stress as threatening (the individual displays lack of control), challenging (in control of situation) or neutral.

Cardiac output (CO) is defined as the quantity of blood expelled into the aorta by the left ventricle each minute [41]. It is deemed as one of the most important factors to be taken into consideration when investigating blood pressure changes. According to the Frank-Starling law [42] the heart will automatically adjust the amount of blood it ejects according to the amount of blood it receives [41]. This law implies that peripheral factors are the main contributors to changes in CO under normal conditions. However, when a stressor is not successfully managed, the heart becomes the limiting factor. As the metabolic demand of peripheral tissues increases, due to stress application, heart rate (HR) and stroke volume (SV) also adjust accordingly – HR and SV being the main determinants of CO (Figure 2.3). Sympathetic stimulation affects both the cardiac muscle and systemic circulation by increasing cardiac contractility and HR as well as increasing the mean systemic filling pressure (increases the resistance to venous return). Therefore, if sympatho-vagal balance is not successfully maintained,
detrimental changes in the factors determining CO may occur both acutely and eventually persist chronically [2].

The SV is the amount of blood ejected by the left ventricle during one contraction. Metabolic demand, as well as changes in the pre-load (the amount of stretch due to blood filling the ventricle) and afterload (resistance against which the ventricle must pump the blood to eject the SV), may also alter SV. An excessive pre-load and/or afterload alter cardiac contractility and therefore SV [41]. Any variable that detrimentally influences the contractile capabilities of the heart (i.e. left ventricular hypertrophy, ischemia and infarction) will lead to a decreased SV and the metabolic demand will not be met [41].

The rate of contraction or HR is mainly affected by direct sympathetic innervation [38]. The ANS is the main contributing factor to increase the HR and contractile strength of the ventricles [41]. Therefore, if any unfavourable modifications were to occur, with regards to sympatho-vagal modulation, a systemic maladaptation will ensue. The factors that control CO, SV and HR are summarized in Figure 2.3.
Figure 2.3: The various factors that influence the components of cardiac output. Where: LVH, left ventricular hypertrophy.

*Studies regarding acute mental stress-induced modifications in haemodynamics*

It is quite clear that the ANS and alterations to or by it may play an essential role in the development of certain pathologies and target organ damage.

The SNS plays a central role in the pathogenesis of primary hypertension and also in certain forms of secondary hypertension [38]. The pathological role of neuro-adrenergic factors is well established in the development of hypertension, although it is a disease of multifactorial aetiology [38]. Multiple studies have confirmed this claim by assessing the adrenergic drive, either directly (evaluating blood levels of circulating neurotransmitters) or by considering vagal (parasympathetic) and sympathetic frequency elements [43, 44].

A hyperkinetic circulatory state is evident during the early stages of hypertension, which is mediated by an increased adrenergic drive and reduced parasympathetic
function [45]. These reciprocal alterations in autonomic cardiovascular control have been verified by several studies [38, 45-47]. In borderline hypertensive individuals, intravenous administration of atropine (an antagonist to the parasympathetic neurotransmitter acetylcholine) produces increases in HR and CO to a lesser extent than that observed in pure normotensive age-matched controls [46]. This modification demonstrates the impairment in vagal HR control. However, it is not limited to parasympathetic function; it affects sympathetic cardiovascular control as well. Additional evidence is provided by micro-neurography, where an increased central sympathetic outflow was shown to be present in borderline hypertensive subjects [47]. However, borderline hypertension may additionally involve other abnormalities in the haemodynamic state, metabolic profile and haemorheological condition [38]. The majority of these abnormalities are triggered and reinforced by autonomic alterations, specifically by sympathetic overdrive – particularly in the case of increased metabolic demand, such as during mental stress.

Human and experimental studies on hypertensive animals indicate a progressive potentiation of SNS drive. Marked increases in sympathetic nerve traffic and clear-cut sympathetic activation were associated with elevated blood pressure values, suggesting that adrenergic neural factors not only contribute to the development of a hypertensive state, but also to the progression thereof [47, 48].

Increased sympathetic cardiovascular influences not only favour increases in blood pressure, but also promote hypertension-related target organ damage [38, 47, 48]. Left ventricular hypertrophy is associated with a significant increase in sympathetic nerve traffic when compared with uncomplicated hypertensive states and this was also true for left ventricular dysfunction, congestive heart failure and CAD [49-51].

The manner by which mental stress influences cardiovascular haemodynamics has been investigated in numerous studies [11, 52]. Generally, these investigations
suggest that cardiovascular haemodynamic variability, in response to mental stress, is shared both in healthy individuals and those suffering from CAD. The Psychophysiological Investigations of Myocardial Ischemia Study (PIMI), conducted in healthy individuals, indicated that mental stress tasks (Stroop-test as well as public speaking) resulted in elevated HR and BP, with a calculated rate-pressure product increase of 30%-45% [53]. More recent results imply the presence of two major response patterns to mental stress in healthy subjects [11, 54]. Healthy participants primarily exhibit an increase in cardiac output, stroke volume and an alleviated total peripheral resistance [11]. Contrarily, others present an increase in blood pressure and peripheral resistance [2, 11]. The latter pattern is also observed in CAD patients. The magnitude of haemodynamic responses to mental stress may be attributed to myocardial ischemia as well as autonomic arousal [53].

The Transition in Health during Urbanization in South Africa (THUSA) study showed that Africans living in an urban environment exhibited an α-adrenergic vascular response when exposed to the hand-grip test [11]. However, the rural-dwelling Africans presented a β-adrenergic response to a similar task [11].

In the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, a very intriguing discovery was made with regard to specific differences in haemodynamic response profiles between ethnicities. These findings confirmed those from the THUSA study. In a South African target population including Caucasian and urban Africans, the latter group presented a higher prevalence of hypertension [2, 3, 10, 11]. During mental stress testing (Stroop testing), the African group mainly exhibited increased peripheral resistance, BP, as well as HR and decreased CO as well as SV, accompanied by depressed HR variability. This profile was defined as an α-adrenergic vascular response [2]. Their Caucasian counterparts predominantly presented elevated CO, HR, SV and blood pressure accompanied by alleviated peripheral resistance – this profile is also accepted as a central cardiac β-adrenergic
response. Addressing the previously mentioned aspect of autonomic arousal, investigators found that when α-adrenergic vascular responsiveness prevails, a dysregulation or desensitization of the β-adrenergic receptors may occur [10]. The assembly of increased blood pressure, heart rate and depressed heart rate variability may reveal possible diminished β-adrenergic responsiveness [2]. Additional explanations included that α-adrenergic hyperactivity might be due to poor ventricular performance, as observed in Africans [2]. Ischemia was also more prevalent in the African group, possibly attributed to sympatho-vagal dysregulation. SABPA indicated that detrimental vascular modifications may contribute to this prevalence of ischemia in Africans [2, 10].

The PIMI study indicates that haemodynamic increases associate with increased plasma epinephrine levels during acute mental stress, presumably mediated via the cognitive stress and effector systems discussed in previous sections. Other investigations suggested that increases in peripheral vascular resistance associate with transient left ventricular dysfunction during acute mental stress. An exaggerated haemodynamic response has also been linked to myocardial ischemia in subjects presenting with a high CAD risk [52, 53]. This response may be linked to increased sympathetic activity [11].

It is quite clear that these haemodynamic factors are influenced by a multitude of variables, finally giving rise to the observed reactions (Figure 2.3). Considering the aforementioned discussion, increased cardiovascular risk may therefore be observed in those experiencing greater stress in conjunction with sympathetic hyperactivity and an α-adrenergic vascular response. However, aside from haemodynamic alterations, it is unclear how other tangible markers of cardiac stress, such as levels of cardiac troponin T and N-terminal pro-brain natriuretic peptide, may be affected and/or are modified during acute mental stress.
2.5 Cardiac troponin T (cTnT)

Cardiac Troponin T (cTnT) forms part of the cardiac troponin complex. Troponin itself consists of three sub-units, troponin T, C and I, with each of these units contributing to force generation during contraction. cTnT specifically binds to tropomyosin interlocking troponin and tropomyosin [55] and resulting in the formation of a troponin-tropomyosin complex (Figure 2.4).

![Figure 2.4](image)

**Figure 2.4:** An illustration of the troponin-tropomyosin complex in cardiac tissue. Where: Ca$^{2+}$, calcium ions; cTn, cardiac troponin. (Image adapted from Messer et al, 2016).

In the cytosol, cTnT is found both in free and protein-bound forms. The unbound or free pool of cTnT is the main source of cTnT released in the early stages of myocardial injury [56]. The cTnT that is protein-bound is released as the myofibrils degrade, the end result being irreversible myocardial damage.

Investigations regarding cTnT usually pertain to its properties as a diagnostic marker and/or therapeutic agent [56, 57]. Recently, it has become abundantly clear that cTnT elevation may also be present in several other conditions affecting cardiomyocyte
integrity, such as congestive heart failure, stable CAD and atrial fibrillation [56]. cTnT’s release from cardiomyocytes has been linked to an increase in cardiomyocyte wall permeability, myocyte apoptosis and necrosis [56]. cTnT is a specific biomarker of myocardial injury and has a well-established role with regard to CVD prognosis in patients suffering from critical illness, stroke, CAD or pulmonary embolism [58-60]. In a healthy reference population the upper limit for cTnT levels in the circulation is <0.01 ng/mL – usually undetectable by typical analytical procedures [57]. cTnT has also been identified as a potential biomarker for CVD risk in the general population [56]. This approach has arisen due to the introduction of higher sensitivity troponin assays [61]. Elevated levels of cTnT are defined as a cTnT level exceeding the 99th percentile value of a healthy reference population [61].

Recent population-based studies in which cTnT levels were associated with adverse events include the Dallas Heart study [56], a study conducted in China [62] and the Atherosclerosis Risk in Communities study [63]. Data on all-cause mortality were provided in 66% of the studies reporting clinical outcomes pertaining to increased cTnT levels’ relation to increased cardiovascular-related mortality in these populations studied [64]. Reported results demonstrated a significant relationship between cTnT concentration increase (per unit cTnT) and increasing risk of cardiovascular outcomes – establishing cTnT as a continuous variable [56, 63]. The association of elevated cTnT with a poor outcome is consistent with the findings in specific clinical populations, such as hospitalised patients with renal disease and CAD that did not have acute cardiac symptoms [63, 65]. However, the aforementioned studies extended this result to demonstrate the significance of elevated cTnT in asymptomatic individuals from a general population. In individuals who did not meet diagnostic criteria for myocardial infarction, increased high sensitivity cTnT was associated with a greater incidence of myocardial infarction, structural and functional heart diseases (i.e. diastolic dysfunction), cardiovascular mortality and all-cause mortality. Hence, elevated levels
of cTnT can be considered one of the most proximal sentinel markers of heart disease [56, 65, 66]. The mechanisms of cTnT elevation in apparently healthy individuals are not fully understood [63]. The widespread prevalence of detectable cTnT levels in healthy populations appears to contradict the traditional assumption that cTnT release into the systematic circulation only occurs in the presence of clinically relevant myocardial necrosis [63]. There are several possible theories that might explain this occurrence. It is possible that sub-clinical plaque rupture with minimal concurrent necrosis may lead to cTnT release, even in the absence of clinical symptoms. Alternatively, isolated ischemia may be sufficient to facilitate cTnT release from the myocyte cytosol, without the incidence of infarction. Be it the former or latter, either instance involves some kind of myocardial injury, albeit permanent or temporary, causing a cTnT elevation and adverse cardiovascular outcomes in individuals. A more recent discovery implied elevated levels of cTnT during mentally strenuous circumstances, suggesting a relation to the SNS [66].

**Cardiac troponin T and acute mental stress**

In recent studies conducted by Lazzarino and co-workers [66, 67], the authors identified an intriguing association between acute mental stress and increased cTnT levels in a healthy population. Both investigations showed positive associations between elevations in cTnT levels and acute mental stress responsivity. Allostasis is a convenient context within which to examine the association between acute mental stress and cardiovascular health, as discussed in section 1 (p 3-4). The role or neural control systems during acute mental stress exposure may inform the scientific community of a brain-heart link and its association with CV health.

Damage to brain centres (such as the hypothalamus) that affect autonomic function may lead to autonomic dysregulation, which results in sympathetic hyperactivity and neurogenic myocardial injury [68]. It has been hypothesised that the right insular area
plays a significant role in cTnT release – not by instigation but via direct release by this brain area. This implies a more direct link between SNS and cTnT activity. Unfortunately, research in this area of cTnT regulation and reactivity is currently lacking. In other words, associations between cTnT and cardiovascular haemodynamic reactivity are yet to be discovered. However, cTnT has recently been positively associated with another marker of cardiac stress, function and condition of cardiomyocytes, specifically N-terminal pro-brain natriuretic peptide (NT-proBNP) (Section 7.2).

### 2.6 N-terminal pro-Brain (B-type) Natriuretic Peptide (NT-proBNP)

B-type natriuretic peptide (BNP) is a ringed peptide secreted by the heart and brain to regulate fluid balance and blood pressure [69]. BNP is stored in membrane granules in the ventricles in an inactive pre-hormone form (proBNP) [69]. The proBNP is released in response to ventricular volume expansion and/or pressure overload, and its N-terminal rapidly enzymatically cleaved to obtain biologically active BNP [30, 70]. All natriuretic peptides are synthesized as pre-hormones and subsequently cleaved by multiple enzymes to become biologically active (Figure 2.5). Physiologically, BNP regulates the water and electrolyte balance ensuring sustained normal blood pressure. It does this by inhibiting the renin-angiotensin-aldosterone system as well as the SNS, modifying vascular function and acting directly on regulatory brain sites [70]. Additionally, BNP also contributes to veno-dilation though inhibition of vascular smooth-cell contraction (Figure 2.6).
Figure 2.5: Illustration presenting the enzymatic activation of NT-proBNP to BNP. Where: NT-proBNP, N-terminal pro-brain natriuretic peptide; BNP, brain natriuretic peptide; NEP, nor-endopeptidase; DPPIV, dipeptidyl peptidase IV; IDE, insulin degrading enzyme.

Figure 2.6: Diagrammatic depiction of BNP’s influence on physiological haemodynamic variables. Where: BNP, brain natriuretic peptide; RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system.

Therefore, under normal conditions, active BNP would suppress sympathetic activity [30]. Both BNP and NT-proBNP are markers of ventricular and to a lesser extent atrial distension due to increased intra-cardiac pressure. Circulating levels of BNP have also been closely associated with decreased ventricular contractility, dilation of the left ventricle as well as increased ventricular compliance [70-71]. NT-proBNP is traditionally regarded as a reliable biochemical marker of cardiac conditions such as heart failure, arrhythmias, congestive heart failure and cardiac hypertrophy [9, 71].
Reference values for NT-proBNP are highly dependent on age and gender, but on average the normal range for a healthy individual is <100pg/mL [65]. Recently NT-proBNP has also been identified as a robust prognostic marker for the prediction of cardiovascular disease risk in the general population [70].

In the offspring study from the Framingham population, elevated NT-proBNP levels significantly predicted cardiovascular-based mortality in apparently healthy subjects [72]. Additional investigations in Copenhagen and the USA established increased NT-proBNP to be a more robust cardiac biomarker able to predict cardiovascular mortality and morbidity, independently from conventional clinical risk factors and cardiac structural modifications [73, 74]. Variations in NT-proBNP levels have also been related to alterations in autonomic activity [74].

**NT-proBNP and acute mental stress**

Increased NT-proBNP levels have been associated with or they are present in patients suffering from depression and disrupted autonomic innervation [9, 70]. Increased levels of NT-proBNP were also observed in an at-risk population during application of an acute mental stressor [75]. In this population, acute mental stress, cardiovascular hyperactivity and cardiac strain were positively associated with elevations in NT-proBNP. However, they could not elucidate the relevance of short-term changes in NT-proBNP levels. Contrary to the latter, lower levels of NT-proBNP during applied mental stress were identified as a possible marker for predicting diastolic dysfunction in apparent healthy individuals [76]. These findings emphasized that NT-proBNP has a cardio-protective effect in healthy individuals and suggested that lower levels of NT-proBNP during acute mental stress application may contribute to diastolic dysfunction. Therefore, governing under the premise that lower NT-proBNP implies less protective effects during increased cardiac volume load, this study implies greater risk with lower
levels of NT-proBNP. However, no thought has been given to the possibility of decreased NT-proBNP sensitivity caused by an acute applied mental stressor.

Concerning ethnical differences, the Dallas heart study found that African Americans had significantly lower NT-proBNP baseline levels compared to Caucasians and Hispanics and, therefore, African Americans could be at greater risk of developing CVD [77]. The aforementioned discrepancies emphasize the lack of data regarding the relationship between acute stress-induced changes in autonomic activity and NT-proBNP levels.

2.7 The novel relationship between cTnT and NT-proBNP

In a recent investigation conducted as part of the SABPA study, a positive relationship existed between baseline cTnT and NT-proBNP in a cross-sectional survey [78]. Increased levels of both cTnT and NT-proBNP were observed in Africans – the latter supported by multiple studies verifying elevated levels of NT-proBNP in Africans compared to Caucasian individuals [2, 78, 79]. Elevated levels of cTnT and NT-proBNP in the African group might be attributed to sympathetic hyperactivity, specifically in individuals chronically exposed to demanding environmental situations [78]. This phenomenon suggests a link between these markers of cardiac stress and sympathetic activity. However, tangible evidence regarding this link is lacking. Investigating the reactivity of cTnT and NT-proBNP during an applied stressor might not only elucidate this relationship, but also their relationship to previously discussed haemodynamic markers pertaining to CVD risk. cTnT and NT-proBNP reactivity might also be associated with specific response profiles (α- or β-adrenergic responses). Such a reactivity-based relationship may also exist between these biochemical markers and alterations in cardiac electrical activity during experimental stress exposure. Such a relationship should be investigated, especially taking into account
that any structural alterations that influence the role of these biochemical markers may most definitely imply modifications in electrical conduction throughout the myocardium.

Associations have recently been established between NT-proBNP elevation and prolongation of the electrocardiographic parameter, the HR corrected QT interval (QTc) [80]. Physiologically it is logical that increases in NT-proBNP levels will relate to a prolonged QTc, as increased myocyte stress is one of the main stimuli for NT-proBNP secretion. Therefore, it is possible that an indirect link between QTc prolongation and cTnT may exist, due to NT-proBNP’s relationship with both.

2.8 The corrected QT interval (QTc)

The electrophysiological events that occur during impulse generation and conduction are reflected by electrical skin surface recordings as measured via an electrocardiogram (ECG). Every heartbeat originates as electrical excitation generated in the sino-atrial node and is rapidly conducted throughout the atria. On electrical surface recordings, such as an ECG, the QT interval represents the flow of an electrical current through the ventricles, or rather the duration of a ventricular action potential (Figure 2.7) [81, 82].
The duration of the QT interval, at any given moment in any individual, is primarily determined by the HR, or more exactly the preceding cycle length [15, 81]. Due to HR's influence on QT duration, correction for HR is required for investigations pertaining to repolarization duration [15]. A multitude of HR correcting formulae has been developed so as to compare QT duration between individuals irrespective of gender, ethnicity or age. The universally accepted method for correction is application of Bazett's formula \((\frac{QT}{RR})^{1/2}\) [15]. Aside from the aforementioned variables, a broad set of factors exist that may influence the duration of the QT interval, be it physiological, genetic or pathophysiological [15].

**Variables influencing QT duration**

In general, women tend to have a longer (410 ms ± 10-20 ms) QTc than men [83]. QTc also displays significant diurnal variation and dependence. An average QTc
prolongation of 13 ms has been observed during sleep, relating to either increased vagal tone, reduced sympathetic activity or a combination of the two [15, 84, 85].

Cardiac as well as non-cardiac drugs may also alter the QTc interval, either directly (influencing the repolarization currents) or indirectly via multiple drug interactions. Excessive alcohol consumption and alcoholic liver disease have also been associated with increased QTc duration and these alterations were shown to be HR independent [86, 87].

Disturbances in electrolytes, such as hypomagnesaemia, hypokalaemia and hypocalcaemia also lead to QTc prolongation [88-90]. The aforementioned imbalances in addition to hypoglycaemia in type 1 diabetic patients also lead to QTc prolongation, especially when autonomic neuropathy is present [90]. Therefore, any condition that may influence the contractile abilities of cardiac tissue will influence electrical conduction and thus the duration of the QT interval. It is important to note that little research has been done on the effect of psychosocial factors on the QTc interval.

**The QTc, autonomic activity and acute mental stress**

The QTc interval reflects autonomic modulation of ventricular electrical activity [91]. A positive linear relationship exists between QTc interval and sympathetic tone; therefore QTc interval alterations may potentially be a measure of cardiac sympathetic tone or decreased vagal modulation [92]. The QTc interval represents the flow of electrical current through the ventricles; from depolarization of the ventricles (start of the Q-wave) to the repolarization of the ventricles (denoted by the T wave) [91]. The entire process of ventricular repolarization is complex and varies in duration from site to site and even from beat to beat [91]. Until recently, no effort has been made to determine which physiological mechanisms may give rise to or alter QTc interval during acute mental stress. An increase in QTc interval prolongation was evident during infusions of isoproterenol, an intervention that clearly increases β-adrenergic stimulation or elicits a
central cardiac response pattern [93]. According to Berger [91], QTc interval alterations are not a direct measure of ventricular sympathetic activity, but it is indicative of the status of sympathetic tone. Prolongation of the QTc is regarded as a marker of imbalanced distribution of SNS activity on the heart [91]. This relation may be due to the fact that when multiple variables influence a specific physiological parameter, the correlation between this physiological parameter and any one input variable is greatest when that specific input variable’s strength exceeds that of the others.

Aside from the HR, the ANS may directly (cellular level) or indirectly (modulating the HR) be responsible for alterations in the QTc interval [15]. The role of the ANS was demonstrated by QTc prolongation observed during sleep, independent of HR, due to circadian alterations in the sympatho-vagal balance [94]. As stated in the previous paragraph, the QTc of diabetic patients suffering from autonomic neuropathy is prolonged. The QTc interval is also increased in patients with primary autonomic failure due to utter autonomic failure and/or multiple systems atrophy [95, 96]. Chronic, as well as acute mental stressors stimulate neuroendocrine and cardiovascular responses. Lethal arrhythmias may be triggered if neural transmissions to the heart are altered during stress-induced ANS activation [97]. This rationale is supported by multiple epidemiological investigations that confirmed the relationship between acute mental stress and cardiac morbidity and mortality in at-risk individuals [2, 66, 67]. Additionally, structural alterations such as pre-clinical ventricular hypertrophy or myocardial ischemia may also influence the electrical conduction and therefore the QTc duration in the heart. Increased QTc reactivity is associated with end-organ damage such as transient ischemic events, as well as pre-clinical and progressive left ventricular hypertrophy (LVH) [10, 30]. Increased variation in the QTc interval also identifies individuals with a greater risk of arrhythmia development [15, 30]. It was also recently shown that an increased QTc interval is predictive of cardiovascular mortality in at-risk patients [98, 99].
However, investigations relating to acute mental stress-induced QTc alterations remain a highly speculated subject. Published reports provide conflicting data on the effect of acute mental stress on the QTc duration. Some report QT interval shortening during ‘stressful interviews’ as measured via Holter monitoring [97]. Conversely, other laboratory results observed QT interval prolongation during alarm calls [100]. It is important to note that in these studies no HR correction was calculated. However, the degree to which such alterations in the QTc interval occurs due to acute mental stress has yet to be determined.

Alterations in sympathetic activity of the heart can be observed by applying an acute laboratory stressor that elicits an acute cardiovascular response. The electrical activity of the heart changes in a similar manner, regardless of whether the induced stressor is physical or psychological [101, 102]. Sympathetic influences on cardiac electrophysiology and structure also modulate general myocardial function [8].

2.9 The combined assessment of acute electrical and cardiac stress reactivity

Coping disability has been related to SNS α-adrenergic vascular responsiveness, hypertension prevalence and silent ischemic events indicative of reduced oxygen supply to the heart, especially in Africans experiencing greater psychosocial stress in comparison with Caucasians [2, 3, 10, 103]. Therefore, increased QTc dispersion may be accompanied by α-adrenergic vascular reactivity independent of the presence of LVH, or early structural alterations, especially in at-risk individuals (such as those presenting cardio-metabolic vulnerability) [104]. Evaluating early possible structural alterations via acute changes in electrical and previously discussed biochemical markers may assist in the assessment of pre-clinical target organ damage in these individuals.
QTc interval variations, due to an acute mental stressor, may therefore provide an immediate *indication* of possible autonomic alterations, whereas the cardiac stress and biomarkers may indicate possible long-term variations in autonomic function due to chronic stress exposure. Bearing this statement in mind, perception (sensory input as well as perception based on previous experience, genetic factors and coping mechanisms) of an applied mental stressor activates the hypothalamus which in turn leads to the activation of the SNS. This activation of the SNS has both adrenal and peripheral consequences [30, 105], both of which will have a positive inotropic and chronotropic effect. α-adrenergic hyperactivity may increase total peripheral resistance: therefore increase cardiac afterload, inducing a chronic increase in preload [2, 30, 41]. An increase in pre-load will infer greater cardiomyocyte stretch and an elevation in NT-proBNP, since an increased pre-load is one of the main stimulants of NT-proBNP release [106]. Increases in NT-proBNP may be accompanied by elevations in cTnT, due to a positive relationship that has been observed between these markers [78]. Additional support for cTnT elevation might be due to the increase in afterload. As the afterload increases, the SV decreases (as pressure and resistance increase) and such a decrease in SV implies a decrease in myocardial relaxation (QTc prolongation) as well as an increased myocyte oxygen demand [80]. Due to insufficient oxygen supply (pressure increase and impaired coronary flow), ischemic events may ensue, leading to additional cTnT release. It is possible that individuals with a high cTnT and NT-proBNP reactivity may also be hyper-reactive to mental stress in their daily life, supporting the notion that acute alterations may lead to chronic maladaptation [107]. This additionally implies increased myocardial ischemia and CAD risk. Chronic elevated levels of NT-proBNP have been associated with cardiac dysfunction and may occur due to increased cTnT levels, thereby acting as a protective homeostatic buffer [108]. As inferred, this entire process may provoke an eventual increase in preload due to a chronic increase in afterload, exacerbated by the
increase in peripheral responsiveness [2, 10, 11]. These proposed series of events are depicted in Figure 2.8.
Figure 2.8: Proposed alterations in cardiac stress and electrical reactivity and their relation to target organ damage and cardiovascular risk.

Where: SNS, sympathetic nervous system; TPR, total peripheral resistance; SV, stroke volume; ESV, end-systolic volume; QTc, corrected QT interval; NT-proBNP, N-terminal pro-brain natriuretic peptide; cTnT, cardiac troponin T; ↑, increase; ↓, decrease
Due to the increased psychosocial stress exhibited by urban Africans [2, 11, 107, 109, 110], it is of utmost importance to successfully identify systemic pathologies and cardiovascular anomalies: thereby effectively reducing CVD-related mortality in the South African population. Currently, no ethnic-comparative data regarding these markers are available in a South African cohort. It is therefore crucial that markers such as QTc interval reactivity, NT-proBNP and cTnT, which can be measured with relative ease and noteworthy accuracy, be utilized as tools for the early identification of individuals with increased risk for CVD based mortality.

2.10 General objectives

The main objective of this study is to provide ethnic-comparative data regarding cTnT, NT-proBNP (indicators of cardiac stress) and QTc stress reactivity in an attempt to identify pre-clinical risks for end-organ damage.

Thus, the main objectives are to:

- Determine the relationship between cTnT, NT-proBNP, QTc and blood pressure reactivity in response to the Stroop test in a South African bi-ethnic population, as well as possible differences in these relationships.
- Establish associations between cTnT, NT-proBNP, QTc and blood pressure reactivity with specific adrenergic (α or β) response profiles; and
- Verify the possible cardiovascular risks (such as increased RaVL amplitude and increased biomarker levels) pertaining to specific response profiles.

2.11 Questions arising from the literature

- What will the transient changes, due to acute stress application, be in these markers and how will these changes differ between ethnicities?
• Will acute stress reactivity of cTnT, NT-proBNP and QTc relate to a specific
  haemodynamic responsivity profile?
• What might the potential clinical implication be of transient changes in cTnT,
  NT-proBNP and QTc?

2.12 Hypotheses

We therefore hypothesise that:

• Blood pressure, QTc and cardiac stress responses will differ between
  ethnicities.
• α-adrenergic blood pressure responses will be associated with increased QTc
  prolongation and cardiac stress levels in our African cohort; and
• α-adrenergic blood pressure, QTc and cardiac stress (cTnT and NT-proBNP)
  reactivity will be indicative of pre-clinical cardiac structural and loading
  alterations (measured as possible elevations in NT-proBNP and cTnT levels as
  well as increased RaVL amplitude), specifically in our African cohort.
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Acknowledgements

Acknowledgements should be made only to those who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions.

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Title: Electrical and cardiac stress reactivity associations with pre-clinical target organ damage: The SABPA study

Short title: Stress reactivity and CVD

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The present study was partially funded by the National Research Foundation, South African Medical Research Council, ROCHE Diagnostics, North-West University (Potchefstroom Campus), North-West Department of Education South Africa as well as the Metabolic Syndrome institute, France.

Disclaimers: NONE

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Abstract

**Background and Aims**: Acute mental stress influences autonomic responsivity of the heart and is recognized as a risk factor for cardiovascular disease (CVD). However, the manner in which cardiac stress markers, i.e. cardiac troponin T (cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels, change in response to acute mental stress, and whether these levels relate to specific vascular and-cardiac responses, remains unclear.

**Methods**: Africans (n=193) and Caucasians (n=195) from the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, were included in our analysis. The Stroop Colour-Word Conflict test was administered for 1 minute whilst Finapres® beat-to-beat BP and 10-lead ECG responses were obtained. Blood samples for cTnT, NT-proBNP and cardio-metabolic markers were obtained at baseline and 10 min post-stress application.

**Results**: CVD markers changed detrimentally in Africans who exhibited greater vascular, cTnT and NT-proBNP responses to acute mental stress compared to that of Caucasians. There were inverse associations of stroke volume with ∆DBP ($\beta$=-0.31; 95%CI -0.38 to -0.20; $p<0.001$), ∆cTnT ($\beta$=-0.13; CI -0.22 to -0.01; $p$=0.009) and ∆QTc ($\beta$=-0.25; CI -0.35 to -0.15; $p<0.001$) in Africans. ROC analyses revealed that acute increases (7.67pg/mL) in and resting values (3.5pg/mL) of cTnT were associated with 24 hour diastolic hypertension [specificity/sensitivity 65%/69%; AUC 0.63 (95% CI, 0.55, 0.70)]. The R wave of the aVL lead predicted stress induced increased cTnT [Odds ratio of 11 (95%CI)] in both ethnicities.

**Conclusions**: Cardio-metabolic vulnerability was accompanied by acute cardiac stress and α-adrenergic responsivity in Africans. Ultimately, this reaction may increase the risk for coronary artery disease.
**Key words:** Stressor reactivity; ethnicity; South Africa; Cardiac Troponin T; NT-proBNP; CVD

**Abbreviations:** $\Delta$, reactivity change; BP, blood pressure; CAD, coronary artery disease; CO, cardiac output; cTnT, cardiac troponin T; CVD, cardiovascular disease; CWC; Colour-Word Conflict test; ECG-LVH, electrocardiogram left ventricular hypertrophy; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEF, left ventricular ejection fraction; QTc, corrected QT interval; SV, stroke volume.
Condensed Abstract

Cardiac stress markers, cardiac troponin T (cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels, may change in response to acute mental stress, and might relate to specific vascular and-cardiac responses. The Colour-Word-Conflict test was administered for 1min whilst Finapres® haemodynamic and ECG responses were obtained. Blood samples for all cardio-metabolic markers were obtained. CVD markers changed detrimentally in Africans who exhibited greater vascular, cTnT and NT-proBNP responses to acute mental stress compared to Caucasians. Cardio-metabolic vulnerability, accompanied by acute cardiac stress and α-adrenergic responsivity may increase the risk for coronary artery disease due to the effects of excessive volume loading.
3.1 Introduction

The ability of people to react and adapt to a specific threat or challenge in the environment is governed by a psychophysiological stress response [1]. The resulting physiological adaptations differ between individuals but relate to ethnicity, gender, age, personality and previous experiences [1].

Autonomic control of the heart is an essential pathophysiological pathway that links cardiovascular risk factors with the development of cardiovascular disease (CVD) [2]. Mental stress alters the autonomic responses of the heart and is a recognized risk factor for CVD in both chronic and acute conditions [3]. Malan et al. demonstrated that urban-dwelling Africans tend to have an increased hypertension prevalence and exhibit α-adrenergic vascular responses, while their Caucasian counterparts predominantly present a central cardiac, β-adrenergic response accompanied by essentially normal blood pressure (BP) values [4, 5]. Increased cardiovascular risk may therefore be observed in those experiencing greater stress in conjunction with α-adrenergic vascular responses.

Cardiac Troponin T (cTnT) is associated with a high risk of cardiovascular mortality in the general population [6]. cTnT forms part of the cardiac troponin complex and its release from cardiomyocytes may be linked to an increase in cardiomyocyte wall permeability [7]. Elevated cTnT, recognised as a reliable marker of CVD risk, links mental stress with pre-clinical cardiac and vascular complications [8, 9].

In combination with cTnT, N-terminal pro-brain natriuretic peptide (NT-proBNP) may be a marker of pre-clinical structural alterations [6, 10]. NT-proBNP is a cardiac neurohormone secreted by the myocardium in response to increased cardiac volume and/or stress [6]. It was also shown that increased NT-proBNP levels associate with symptoms of heart failure, arrhythmias, cardiac hypertrophy, depression and disrupted autonomic innervation [6, 11]. Several discrepancies were found during application of
a mental stressor. If the population being studied is healthy, lower levels were observed predicting diastolic dysfunction [12] as opposed to higher levels in a high-risk cohort [13].

Therefore other markers, such as electrocardiographic (ECG) QT interval and cardiovascular reactivity, were utilized in recent epidemiological studies as measures of cardiac autonomic control and predictors of CVD risk, due to their simplicity and comparable accuracy [5, 14-17]. A positive linear relationship exists between corrected QT (QTc) interval and sympathetic tone; therefore QTc interval alterations may potentially be a measure of cardiac sympathetic tone [18].

Currently, no-ethnic comparative data integrating BP and cardiac stress marker responses are available in South Africa. Hence we hypothesised that BP, QTc and cardiac stress responses will differ between ethnicities. Secondly, α-adrenergic BP responses will be associated with increased QTc prolongation and cardiac stress levels in a South African bi-ethnic cohort. Lastly, associations between α-adrenergic BP responses, QTc and cardiac stress (cTnT and NT-proBNP) will be indicative of pre-clinical structural and loading alterations.
3.2 Methods

This sub-study forms part of a cross-sectional population cohort study being the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study. The SABPA study was conducted between late summer and late autumn of 2008 and then again in 2009, as to avoid seasonal variations [19].

Research participants

The study sample comprised urban-dwelling African and Caucasian, male and female teachers (n=409) from the North-West Province, South Africa, aged between 20 and 65 years [19]. The purpose of selecting teachers exclusively was to obtain a sample with similar socio-economic status from a similar working environment; although cultural differences could not be excluded. Exclusion criteria comprised: α-, β-blocker and psychotropic substance users (which include anti-depressants), vaccination or blood donation within 3 months prior to the investigation, tympanum temperatures >37.5°C and pregnant or lactating women. Additional exclusion criteria for this sub-study included atrial fibrillation, a history of myocardial infarction, presence of electrocardiographic left ventricular hypertrophy (ECG-LVH) and ventricular ectopic episodes. The study sample finally included 388 participants.

Ethical considerations

Ethical approval, for this sub-study, was obtained from the Health Research Ethics Committee (HREC) of the North-West University, Potchefstroom Campus (number NWU-00036-07-S6) and written informed consent had been obtained from all volunteers prior to participation. All procedures adhered to the applicable institutional guidelines and terms, as stated by the Declaration of Helsinki of 1975.
**General procedure of investigation**

Between 07h00 and 08h00, during four working days of the week, four participants, daily, were each fitted with the Cardiotens CE120® for 24 hour ambulatory BP measurements (24H ABPM), as well as an Actical® accelerometer to attain physical activity recordings. The participants subsequently continued with their normal daily activities, reporting any peculiarities such as nausea, headaches, visual disturbances, palpitations, fainting, physical activity and stress on the issued 24 hour diary cards. At 16h30, participants were transported to the NWU Metabolic Unit Research Facility to stay overnight and were, upon arrival, familiarized with the experimental setup and received pre-counselling regarding HIV/AIDS.

Participants were advised to go to bed at 22h00, fasting overnight. The mentioned apparatus were removed after the last programmed BP reading had been recorded at 06h00 the following day, followed by anthropometric measurements. After a resting period of 30 minute participants remained in a semi-recumbent position after which blood sampling commenced. Participants enjoyed breakfast and individual confidential feedback and referrals were made regarding immediately available clinical measures.

**Cardiovascular measurements**

*Blood pressure measurements:* Ambulatory BP was measured with the Cardiotens CE120® (Meditech, Budapest, Hungary), validated by the British Hypertension Society (BHS), which was fitted with a suitable cuff size, to the non-dominant arm of each participant. The device was programmed to ideally measure BP and ECG variability in 30 minute intervals during the day (08h00-22h00), and 60 minute intervals during the night (22h00-06h00) [20]. The data was subsequently analysed by means of the CardioVisions 1.19 Personal Edition Software (Meditech®). The successful inflation rates were 72.60% and 84.86% for the African and Caucasian participants respectively.
According to ESH guidelines; hypertension is classified as an ambulatory SBP ≥ 130mmHg and DBP ≥ 80mmHg [21].

The Finapres (Finapres Measurement Systems©, Amsterdam, Netherlands), validated for stroke volume relative changes, was utilized to continuously assess beat-to-beat BP changes throughout psychophysiological testing [22]. An integrated age-dependent aortic flow curve was calculated from the surface area beneath the pressure/volume curve using the Beat-Scope version 1.1a software package. This calculation determined cardiac output (CO), stroke volume (SV), total peripheral resistance (TPR) as well as arterial compliance (Windkessel compliance (C\textsubscript{wk})) of the small and large arteries [22]. Participants were in a semi-recumbent position for beat-tot-beat BP and ECG mental stress testing. Resting beat-tot-beat BP and 10-lead ECG measurements were obtained for 5 minutes followed by venous blood sampling. The average of the last 2 minutes of the resting recordings and the average of the last 15 seconds of the stressor recordings were obtained. The cardiovascular reactivity of each participant was calculated as the percentage change from the resting value.

Electrocardiographic measurements: The corrected resting and stressor QT-interval values (QTc) were obtained via a 10-lead electrocardiogram (ECG) (Norav NHH-1200© ECG) (NORAV Medical LTD OC 1200, Israel, Software version 5.030) and were calculated according to the formula ([QT/RR]\textsuperscript{1/2}). The 10-lead electrocardiogram (ECG) was performed while the participants were in a semi-recumbent position for 30 minutes. The formula (Cornell product, [RaVL + SV3] \times QRS duration) was utilised to determine the resting ECG-LVH (Norav NHH-1200© ECG) (NORAV Medical LTD OC 1200, Israel, Software version 5.030). The following cut-off points were used to determine a prolonged QTc interval: ≥ 450 ms (male), ≥ 470 ms (female); Cornell product of ≥ 244 mV.ms [23, 24].
**Mental stress testing**

Figure 3.1 illustrates the stress protocol. An additional resting period of 5-10 min ensured stabilizing BP to resting values before administering psychophysiological stress task for 1 min, specifically the Stroop Colour-Word Conflict (Stroop) test [25]. Throughout mental stress testing, beat-to-beat BP and ECG responses were obtained. The Stroop test requires participants to identify the colours of colour-word cards in contrasting ink colours under time pressure [25]. Participants received a monetary incentive in accordance with their performance and for motivation on completion of the Stroop task. Another blood sample was obtained 10 minutes post-stress.

![Diagram](image)

**Figure 3.1**: SABPA beat-to-beat blood pressure measurement, blood sampling and mental stress testing

**Lifestyle variables**

Body mass and height were measured by two qualified Level II anthropometrists in triplicate to calculate body mass index (BMI) and body surface area (BSA), using the Mostellar formula [26]. The inter- and intra-observer variability was less than 10%. The
Actical® (Montréal, Québec) was used to determine total energy expenditure (TEE), which was calculated in calories per 24 hour considering the resting metabolic rate. Alcohol consumption was determined using gamma glutamyl transferase (γGT) as a biochemical marker [27, 28]. Habitual smoking was determined by assessing the nicotine metabolite, serum cotinine values [29, 30].

**Biochemical measurements**

Fasting serum, sodium fluoride (NaF) glucose samples were obtained from the antebrachial vein branches of each participant's dominant arm with a sterile winged infusion set by a registered nurse. Blood samples were dealt with according to the standardised protocol. Cotinine values were determined by means of a homogeneous immunoassay with a Modular Roche automated (Switzerland). γGT and C-reactive protein (CRP) were analysed with the Konelab™20i (ThermoScientific, Vantaa, Finland). Whole blood glycated haemoglobin (HbA₁c) was determined via a turbidometric immunoassay (Integra 400®, Roche, Switzerland). Each 1% change in HbA₁c relates to a 2mmol/L change in plasma glucose. Total cholesterol and high-density lipoproteins (HDL) were determined via the timed end-point method (Unicel DxC 800®, Beckman & Coulter, Germany). The total cholesterol/HDL cholesterol ratio was subsequently calculated. NT-proBNP and cTNT were measured via an electrochemiluminescence method on the e411 (Roche®, Basel, Switzerland) apparatus. Inter batch variability was 4.6% and intra-batch variability 4.2%. The diagnostic accuracy of single baseline measurement of the Elecsys® Troponin T high-sensitivity assay was determined by Zhelev et al. [31]. In total 73 data points were identified as being lower than 3, both during rest and the Stroop test, and were substituted using the method of Croghan and Egeghy [32] for lower than detectable values.
Statistical analyses

Data analyses were completed using the computer software package Statistica® version 13.0 (Statsoft Inc., Tulsa, USA, 2015). Kolmogorov-Smirnov tests assessed normality of all variables. γGT, CRP and HbA_{1c} were logarithmically transformed and exponentials of these variables were used in correlation models. Descriptive t-tests illustrated ethnic characteristics. Chi-square (X²) statistics were used to determine proportions and prevalence. A priori covariates included age, gender, body surface area (BSA), cotinine, cGGT, total energy expenditure (TEE), HbA_{1c}, total cholesterol/HDL cholesterol ratio and Windkessel compliance (C_{wk}) [21]. Single one-way ANCOVAs determined ethnic x gender differences for all cardiovascular risk markers, independent of a priori co-variates. Two-way ANCOVA analyses determined the least square mean difference in reactivity markers between ethnic groups, independent of the a priori covariates. Multi-variative linear regression analyses determined associations between mental stress responses in several models. The dependent variables included percentage changes (Δ) for SV, cTnT and NT-proBNP. Independent variables included ΔDBP, ΔCO, ΔLVEF, ΔQTc and a priori covariates age, gender, BSA, cotinine, γGT, TEE HbA_{1c} and total cholesterol/HDL cholesterol ration. ΔcTnT and ΔNT-proBNP were added as independent variables with regard to ΔSV and individually in analyses where ΔcTnT and ΔNT-proBNP were the dependent variables respectively. ΔSV, ΔCO and ΔLVEF were never added simultaneously into the same model so as to avoid co-linearity. For all the aforementioned analyses, significance was set at \( p \leq 0.05 \) (two-tailed) and the F to enter was fixed at 2.5. Non-parametric receiver-operating characteristics (ROC) analyses were performed with SPSS software version 22 (SPSS Inc., Illinois, USA) to determine an optimal ethnic-specific cTnT cut-point value predicting 24 hour diastolic hypertension (24H DBP HT) for the maximum of the Youden index (J) (sensitivity + specificity – 1). Odds ratios (OR) were calculated for several models to establish the probability of increased CVD...
risk as presented by an increased RaVL amplitude, in the detectable cTnT category resting and stressor values, for the total group of each ethnicity.
3.3 Results

Significant differences existed between ethnic groups independent of a priori covariates for ∆QTc %, [p=0.010]; ∆NT-proBNP % [p=0.009]; ∆DBP % [p= 0.013]; ∆cTnT % [p<0.001]. These findings allowed stratification into specific ethnic groups.

Table 3.1 shows that higher risk of cardiovascular vulnerability was observed in Africans (p=0.05). They specifically displayed higher levels of alcohol consumption (γGT), inflammatory levels, glycated haemoglobin, although lower total cholesterol:HDL ratio, BSA and physical activity levels were also evident in the African group. Compared to Caucasians, Africans also presented an increased RaVL amplitude, ECG-LVH prevalence and a greater average of ischemic events. The reactivity pattern differed overall between the African and Caucasian groups.

Figure 3.2 shows that acute mental stress responses in Africans DBP (p=0.002), accompanied by a lesser increase in CO (p<0.001) and a decrease in SV (p=0.001). Their Caucasian counterparts indicated a greater decrease regarding LVEF (p=0.001). Pertaining to cardiac stress markers, Africans exhibited greater increases in cTnT (p<0.001) and NT-proBNP (p<0.001) reactivity, compared to those of Caucasians.

Table 3.2 generally describes inverse associations of ∆SV with ∆DBP, ∆cTnT and ∆QTc in Africans (β=-0.31; 95% confidence interval (CI) -0.38 to -0.20; p<0.001), (β=-0.13; CI -0.22 to -0.03; p=0.009) and (β=-0.25; CI -0.35 to -0.15; p<0.001) respectively, while only ∆SV, ∆DBP and ∆QTc were associated in Caucasians. ∆NT-proBNP associated positively with ∆cTnT in both ethnicities. However, the extent of this association was far greater in the Caucasian group (β=0.55; CI 0.42 to 0.68; p<0.001).

A ROC cTnT cut-point of 4.16 pg/mL predicted 24H DBP HT with sensitivity/specificity of 61%/67% [AUC 0.65 (95% CI 0.58, 0.74)] in Africans. In the Caucasian group, cTnT cut-points, indicative of 24H DBP HT, was 7.03 pg/mL with sensitivity/specificity of 45%/85% [AUC 0.67 (95% CI 0.59, 0.75)]. When ROC analyses were performed to
determine cTnT cut-points during acute mental stress (scTnT), Africans presented a scTnT cut-point of 4.19 pg/mL [specificity/sensitivity 65%/69%; AUC 0.63 (95% CI, 0.55, 0.70)] approximately similar to cut-points calculated at resting cTnT values. In the Caucasian group a much lower scTnT cut-point of 3.24 pg/mL [specificity/sensitivity 61%/77%; AUC 0.67 (95% CI, 0.58, 0.75)] was obtained. Figures 3.3.1 and 3.3.2 illustrate these cut-points in Africans and Caucasians during rest and acute mental stress respectively.

Figure 3.4, illustrates that both ethnicities were reported to show increased RaVL amplitude associated with increased levels of scTnT. In the total group a greater RaVL amplitude was associated with an OR of ± 11 for increases in scTnT during acute mental stress application (Africans OR= 10.54; Caucasians OR=11.05).
Table 3.1: Baseline and reactivity characteristics between ethnicities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Africans (N=193)</th>
<th>Caucasians (N=195)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>44.17 ± 8.11</td>
<td>45.04 ± 10.86</td>
<td>0.372</td>
</tr>
<tr>
<td>Gender, N</td>
<td>100 (male)</td>
<td>92 (male)</td>
<td>0.363</td>
</tr>
<tr>
<td></td>
<td>93 (female)</td>
<td>103 (female)</td>
<td></td>
</tr>
<tr>
<td>Body Surface Area (BSA) (m²)</td>
<td>1.92 ± 0.23</td>
<td>2.00 ± 0.28</td>
<td>0.003</td>
</tr>
<tr>
<td>Body Mass Index (BMI) (kg/m²)</td>
<td>30.19 ± 7.05</td>
<td>27.64 ± 6.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity (kcal/day)</td>
<td>2599.29 (2163.75, 3122.80)</td>
<td>2942.37 (2384.84, 3508.75)</td>
<td>0.002</td>
</tr>
<tr>
<td>Serum cotinine (ng/ml)</td>
<td>27.11 (18.60, 36.00)</td>
<td>20.62 (10.45, 31.00)</td>
<td>0.340</td>
</tr>
<tr>
<td>γGT (U/L)</td>
<td>41.11 (27.67, 72.99)</td>
<td>18.00 (12.00, 28.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.86 (2.00, 9.60)</td>
<td>1.60 (0.99, 3.80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.09 ± 1.21</td>
<td>5.50 ± 0.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/L)</td>
<td>1.44 ± 1.29</td>
<td>1.18 ± 0.75</td>
<td>0.0168</td>
</tr>
<tr>
<td>Total cholesterol: HDL</td>
<td>4.50 ± 2.07</td>
<td>4.97 ± 1.58</td>
<td>0.013</td>
</tr>
<tr>
<td>Cardiovascular measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting SBP (mmHg)</td>
<td>137 (128, 152)</td>
<td>132 (124, 143)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP CWC (mmHg)</td>
<td>162 ± 24</td>
<td>151 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resting DBP</td>
<td>80 (75, 87)</td>
<td>76 (71, 81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------------------------------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>DBP CWC (mmHg)</td>
<td>94 ± 13</td>
<td>85 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resting Stroke volume (mL)</td>
<td>96.58 (85.50, 117.33)</td>
<td>95 (81.30, 113.47)</td>
<td>0.152</td>
</tr>
<tr>
<td>Stroke volume CWC (ml)</td>
<td>91.5 ± 23.25</td>
<td>94.04 ± 23.56</td>
<td>0.297</td>
</tr>
<tr>
<td>Resting Cardiac output (L/min)</td>
<td>6.87 ± 1.91</td>
<td>6.42 ± 1.95</td>
<td>0.023</td>
</tr>
<tr>
<td>Cardiac output CWC (L/min)</td>
<td>7.82 ± 2.12</td>
<td>8.16 ± 2.45</td>
<td>0.147</td>
</tr>
<tr>
<td>Resting LVEF (s)</td>
<td>0.32 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEF CWC (s)</td>
<td>0.31 ± 0.02</td>
<td>0.31 ± 0.02</td>
<td>0.145</td>
</tr>
<tr>
<td>Resting cTnT (pg/mL)</td>
<td>4.18 (2.97, 5.53)</td>
<td>4.81 (3.16, 6.81)</td>
<td>0.704</td>
</tr>
<tr>
<td>cTnT CWC (pg/mL)</td>
<td>6.41 (5.06, 7.76)</td>
<td>4.02 (2.02, 6.02)</td>
<td>0.085</td>
</tr>
<tr>
<td>Resting NT-proBNP (pg/mL)</td>
<td>28.88 (16.74, 51.79)</td>
<td>34.98 (20.40, 55.49)</td>
<td>0.828</td>
</tr>
<tr>
<td>NT-proBNP CWC (pg/ml)</td>
<td>49.99 (37.25, 72.90)</td>
<td>49.38 (34.88, 69.97)</td>
<td>0.901</td>
</tr>
<tr>
<td>Resting QTc (ms)</td>
<td>386 (362, 402)</td>
<td>392 (374, 414)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>QTc CWC (ms)</td>
<td>437 (405, 468)</td>
<td>438 (406, 469)</td>
<td>0.659</td>
</tr>
<tr>
<td>Resting RR rest (ms)</td>
<td>915 (903, 927)</td>
<td>947 (935, 960)</td>
<td>0.092</td>
</tr>
<tr>
<td>RR CWC (ms)</td>
<td>706 (697, 716)</td>
<td>716 (706, 727)</td>
<td>0.929</td>
</tr>
<tr>
<td>Resting HR (b/min)</td>
<td>68 (60, 72)</td>
<td>65 (58, 69)</td>
<td>0.037</td>
</tr>
<tr>
<td>HR CWC (b/min)</td>
<td>88 (82, 96)</td>
<td>87 (80, 91)</td>
<td>0.981</td>
</tr>
<tr>
<td>RaVL (mV)</td>
<td>0.35 ± 0.29</td>
<td>0.20 ± 0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 hour Silent (ms)</td>
<td>3 (2, 14)</td>
<td>3 (1, 8)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Hypertensive, N (%)</td>
<td>Diabetic, N (%)</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
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</tr>
<tr>
<td>N</td>
<td>67 (34.72)</td>
<td>9 (4.15)</td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>22 (24.72)</td>
<td>2 (1.03)</td>
<td></td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>0.0381</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data presented as median and interquartile ranges. Data expressed as arrhythmic mean ± SE. Where: N, number of participants; %, percentage change. CRP, C-reactive protein; HbA1c, glycated haemoglobin; γGT, gamma glutamyl transferase; SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output; LVEF, left ventricular ejection fraction; CWC, colour-word conflict test; ECG-LVH, Cornell Product >244 mV.ms; ECG-QTc, Electrocardiogram corrected QT interval; NT-proBNP, N-terminal pro-brain natriuretic peptide; RaVL, Right arm VL lead; cTnT, Troponin T; QT-interval ([QT/RR]^{1/2}).
Figure 3.2: Comparing reactivity (%) markers between ethnicities, independent of *a priori* covariates.

*Represents $p<0.001$. Data expressed as an arithmetic mean ($\pm$ SE). *A priori* covariates included age, gender, body surface area, physical activity, cotinine, gamma glutamyl transferase, HbA1c, total cholesterol:HDL ratio and Windkessel compliance ($C_{wk}$). Where SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output; LVEF, left ventricular ejection fraction; cTnT, cardiac troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide; QTc, Corrected QT-interval ($([QT/RR]^{1/2})$).
Table 3.2: Forward stepwise regression analyses depicting associations between electrical and cardiac stress reactivity markers in different ethnicities.

<table>
<thead>
<tr>
<th>Africans (N=193)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta$SV (%)</td>
<td>$\Delta$cTnT (%)</td>
<td>$\Delta$NT-proBNP (%)</td>
</tr>
<tr>
<td></td>
<td>$\beta$ (95% CI)</td>
<td>$\beta$ (95% CI)</td>
<td>$\beta$ (95% CI)</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.68</td>
<td>0.14</td>
<td>0.32</td>
</tr>
<tr>
<td>Gender</td>
<td>-</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>$\Delta$DBP (%)</td>
<td>-0.31 (-0.38, -0.20)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>$p&lt;0.001$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$CO (%)</td>
<td>-</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>$\Delta$SV (%)</td>
<td>-</td>
<td>-0.15 (-0.49, -0.06)</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>$p=0.012$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$LVEF (%)</td>
<td>-</td>
<td>-0.15 (-0.31, -0.004)</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>$p=0.045$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$NT-proBNP (%)</td>
<td>ns</td>
<td>0.25 (0.10, 0.40)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$p=0.002$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$cTnT (%)</td>
<td>-0.13 (-0.22, -0.03)</td>
<td>-</td>
<td>0.25 (0.10, 0.40)</td>
</tr>
<tr>
<td></td>
<td>$p=0.009$</td>
<td></td>
<td>$p=0.002$</td>
</tr>
<tr>
<td>$\Delta$QTc (%)</td>
<td>-0.25 (-0.35, -0.15)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>$p&lt;0.001$</td>
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<table>
<thead>
<tr>
<th>Caucasians (N=195)</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta$SV (%)</td>
<td>$\Delta$cTnT (%)</td>
<td>$\Delta$NT-proBNP (%)</td>
</tr>
<tr>
<td></td>
<td>$\beta$ (95% CI)</td>
<td>$\beta$ (95% CI)</td>
<td>$\beta$ (95% CI)</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.66</td>
<td>0.33</td>
<td>0.56</td>
</tr>
<tr>
<td>Gender</td>
<td>-</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>$\Delta$DBP (%)</td>
<td>-0.12 (-0.18, -0.02)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>$p=0.013$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in Parameter</td>
<td>% Change</td>
<td>CI</td>
<td>p-value</td>
</tr>
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<tr>
<td>ΔCO (%)</td>
<td>-</td>
<td>ns</td>
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<tr>
<td>ΔSV (%)</td>
<td>-</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>ΔLVEF (%)</td>
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<tr>
<td>ΔNT-proBNP (%)</td>
<td>ns</td>
<td>0.55 (0.42, 0.68)</td>
<td>&lt;0.001</td>
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<tr>
<td>ΔcTnT (%)</td>
<td>ns</td>
<td>-</td>
<td>0.55 (0.42, 0.68)</td>
</tr>
<tr>
<td>ΔQTC (%)</td>
<td>-0.20 (-0.30, -0.11)</td>
<td>ns</td>
<td>ns</td>
</tr>
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All analyses were adjusted for age, gender, body surface area (BSA), physical activity (TEE), cotinine, C-reactive protein, cholesterol, glycated haemoglobin glucose (HbA1c) and Windkessel compliance (Cwk). Abbreviations: ns, not significant; CI, confidence interval; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; CO, cardiac output; SV, stroke volume; NT-proBNP: N-terminal pro-brain natriuretic peptide; cTnT; cardiac troponin T; QTc, Corrected QT-interval ([QT/RR]^{1/2}); %, percentage; Δ, reactivity change.
**Figure 3.3.1**: ROC curves depicting the cTnT cut-points for 24H DBP HT in Africans and Caucasians respectively, under resting conditions. AUC 0.65 (95% CI 0.58, 0.74) in Africans [sensitivity/specificity 61%/67%]. AUC 0.67 (95% CI 0.59, 0.75) for the Caucasian group [sensitivity/specificity 45%/85%].

Where AUC, area under the curve; 24H DBP HT, 24 hour diastolic blood pressure hypertension; CI, confidence interval of 95%; cTnT, cardiac troponin T.

**Figure 3.3.2**: ROC curves depicting the cTnT cut-points for 24H DBP HT in Africans and Caucasians respectively, during stressor application. AUC 0.63 (95% CI, 0.55, 0.70) in the African group [specificity/sensitivity 65%/69%]. In the Caucasian group, AUC 0.67 (95% CI, 0.58, 0.75) with [specificity/sensitivity 61%/77%].

Where AUC, area under the curve; 24H DBP HT, 24 hour diastolic blood pressure hypertension; CI, confidence interval of 95%; cTnT, cardiac troponin T.
Figure 3.4: Probability of increased RaVL amplitude in Africans and Caucasians with above detectable limits of cTnT during mental stress. Data depicted as odds ratios for a detectable cTnT level (>3ng/L) (95% CI). Strength of modelled relationship depicted as Nagelkerke$^2$ values ($R^2$).
3.4 Discussion

Overall novel findings revealed significant associations in both ethnicities between markers of cardiac structural changes and markers pertaining to the loading conditions and cardiac stress experienced during mental stress. Cardio-metabolic vulnerability was accompanied by acute cardiac stress, QTc prolongation and α-adrenergic responsivity in the African cohort. Ultimately, this may increase the Africans' risk for coronary artery disease due to volume loading effects. Additionally, BP, QTc and cardiac stress responses were associated with pre-clinical structural and loading alterations, or CVD risk, as exhibited by an increased RaVL amplitude and prevalence of 24H DBP HT.

Reactivity differences between Africans and Caucasians

This study supports prior evidence indicating a vulnerable cardiovascular stress response pattern in Africans when compared with that of Caucasians [4, 5, 19]. Resting values for cardiovascular markers were relatively similar between ethnicities. However, the degree to which these values changed during application of a stressor, differed significantly. The findings also demonstrate that a positive association exists between cTnT and NT-proBNP reactivity in both ethnic groups. However, SV relates negatively with cTnT as well as QTc reactivity, especially in the African group.

During application of a mental stressor, the sympathetic nervous system (SNS) is activated. Due to the dominance of α-adrenergic activity in Africans [4], again observed here, this increase in SNS activity will result in increases in DBP, TPR, cardiac contractile force and decreased SV. SNS hyperactivity may also be accompanied by the elevated levels of cTnT, as this biomarker’s secretion is linked to SNS activity in the insula region of the brain with the result that additional cTnT release is directly regulated by the brain [35].
An increase in TPR is further supported by decreased SV, due to the negative association of afterload with SV [36]. As SV is mainly determined by cardiac preload, afterload and contractility [12, 34], it may emphasize increases in cTnT followed by NT-proBNP when an α-adrenergic response pattern is prompted. It has been shown that increased levels of NT-proBNP are positively associated with increased preload in individuals with high volume load [33, 34]. Decreased SV is also associated with an increase in relaxation time and repolarization of the ventricles, as is evident from an increase in QTc duration [37]. Therefore delayed relaxation and an increased oxygen need may be accompanied by increased levels of cTnT and increased duration of the R wave in the aVL lead of the ECG, as we revealed in both ethnic groups with an odds ratio of approximately 11.

Previous findings in our African cohort showed that increased resting cTnT levels were positively associated with increased levels of NT-proBNP [38] and support our own findings, where acute mental stress induced increases in both markers. Greater cardiac stress and cardiomyocyte stretching, induced NT-proBNP increases and this has been identified as a determinant of cardiac preload [34]. SNS hyperactivity, supported by increased NT-proBNP reactivity, also implies an increased QTc duration during acute mental stress and a greater ventricular contractile force [37]. According to Berger [39], QTc interval alterations are not a direct measure of ventricular sympathetic activity, but is indicative of the status of sympathetic tone. It would be presumptive to imply that SNS hyperactivity is solely responsible for this trend when parasympathetic withdrawal may be involved. However, the observed relation may be due to the fact that when multiple variables influence a specific physiological parameter, the correlation between this physiological parameter and any one input variable is greatest when that specific input variable’s strength exceeds that of the others.
In the Caucasian group, who mainly presented a β-adrenergic cardiac response pattern, the cardiac stress and QTc reactivity markers were weakly associated. However, a strong positive association between NT-proBNP and cTnT was evident in this group. Lower levels of NT-proBNP are present during acute stress, which may imply an increased sensitivity to NT-proBNP in Caucasians eliciting a β-adrenergic response, compared to Africans who may experience attenuated TPR and cardiac preload [40]. The slight decrease in SV may be explained by an increased heart rate [41] due to the dominating β-adrenergic response elicited by this group [4]. Therefore, an α-adrenergic profile might indicate greater cardiac stress in a cohort that displays cardio-metabolic vulnerability, as supported by other SABPA sub-studies [4, 19].

**Pre-clinical risks associated with increased reactivity levels of cTnT**

During application of the Stroop test, results in the Africans indicated that considerable increases in cTnT predicted increased probability of CVD prevalence, as indicated by an increased RaVL amplitude. Although the Caucasian group did not present with an increase in cTnT levels during acute mental stress, detectable cTnT levels were still associated with increases in the RaVL amplitude. Each 0.1 mV increase in the R wave voltage in lead aVL is associated with a 9% higher risk of CVD [42]. This may be indicative of delayed conduction due to decreased relaxation and structural alterations, which may further support increased NT-proBNP and QTc reactivity in the African group. However, even though Caucasians elicited a different adrenergic response, cTnT was still predictive of increased RaVL amplitude. This might indicate an overall novel association between RaVL and cTnT during acute mental stress. The slight discrepancy between ethnicities might be due to the combination of cardio-metabolic vulnerability and the α-adrenergic profile exhibited by Africans, compared to the β-adrenergic response elicited by Caucasians.
Additionally, ROC analyses revealed that cTnT cut-points far below values which are normally considered clinically relevant, predicted 24H DBP HT in both ethnicities. The decrease in ∆SV and the negative association of ∆QTc, ∆ cTnT and ∆NT-proBNP with ∆SV – especially in the African group – may possibly represent stress-induced alterations in the loading conditions of the heart [12]. The cTnT levels predicting DBP HT further support this assumption, as a decrease in SV is physiologically associated with a decrease in DBP [36], and not an increase, as presented by the African group. The aforementioned relation is typical of an α-adrenergic, vascular response profile. This irregular association may suggest inadequate blood supply to and from the heart during acute mental stress, implying increased coronary artery disease risk.

It is possible that individuals with a high cTnT and NT-proBNP responsivity may also be hyper-reactive to mental stress in their daily life [43]. cTnT elevation indicates pre-clinical cardiac and vascular complications [9]. This notion was additionally supported by the increased number of 24 hour ischemic events existent in the African group, as cTnT and NT-proBNP levels may be chronically increased in overly challenged individuals. Chronic elevated levels of NT-proBNP have been associated with cardiac dysfunction and may occur due to increased cTnT levels acting as a protective homeostatic buffer [44]. It is also evident that the cTnT cut-point predicting 24H DBP HT in the African group was far lower than that of their Caucasian counterparts. This may suggest sensitization of hyper-responsiveness in cTnT receptors of the myocardium or a hypo-responsiveness of beta receptors if the stressor is perceived as a threat [4]. The lower cTnT cut-points in Africans accompanied greater increases in DBP or an α-adrenergic responsiveness, inducing prompter myocardial ischemic events, suggestive of hyper-responsiveness in the African group. In the Caucasian group, which presented a more β-adrenergic response profile, however, a lower level of cTnT associated with DBP HT during mental stress possibly infers an increased myocardial sensitivity to reduced oxygen supply and SNS stimulation. This lower cTnT
tendency during mental stress application may have a lower secretion of NT-proBNP as the cohort appears healthier. We partially accept both hypotheses, since α-adrenergic BP responses were associated with cardiac stress and QTc levels indicative of pre-clinical structural and loading alterations, exclusively in the African cohort. We have also discovered the possibility that lower levels of NT-proBNP during acute mental stress may accompany a β-adrenergic cardiac response in this group.

Recommendations and limitations

Due to the cross-sectional design of the study, we cannot infer causality. The conclusions made also only pertain to two specific ethnic and socio-economic groups from South Africa. Hence, it is recommended that all procedures should be repeated in a prospective study, which includes different ethnic populations from various demographics, so as to determine the validity of our conclusions, especially those concerning Africans. Additional markers of SNS activity should also be included to verify the hypothesis of SNS hyperactivity and CVD risk in this population.

Conclusions

Cardiac stress and QTc reactivity were independently associated with increased pre-clinical risk of mechanistic and structural alterations, specifically in the SABPA African cohort. Mental stress-induced reactions may prompt both acute and chronic alterations in the loading and mechanistic conditions of the heart. In cases where α-adrenergic reactivity and cardio-metabolic vulnerability are predominant, such as in this African group, modifications in the aforementioned conditions may be detrimental, as presented by an increased DBP HT, CVD, ischemia and coronary artery disease risk. Novel findings also reveal the possibility that a β-adrenergic response to acute mental stress may be accompanied by lower levels of NT-proBNP. Alternatively, increased cardiac stress and QTc reactivity, associated with α-adrenergic responsivity, may
contribute to early sensitization and damage to the myocardium as well as signs of CVD, especially in an at-risk population.

Acknowledgements

The author acknowledges all who assisted in obtaining the data and blood samples. The present study was partially funded by the National Research Foundation, South African Medical Research Council, ROCHE Diagnostics, North-West University (Potchefstroom Campus), North-West Department of Education South Africa as well as the Metabolic Syndrome institute, France. Any opinion, findings and conclusions or recommendations expressed in this material are those of the author(s) and therefore the funding bodies do not accept any liability in regard thereto.
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Chapter 4

General conclusions

Limitations and Recommendations
4.1 Introduction

A brief summary of the main findings from the article manuscript – as presented in Chapter 3 – follows, including a concise discussion of the main aims, results and conclusion of the study. We will also acknowledge chance and confounding factors, the limitations and strengths of the study design as well as propose recommendations to negate the detrimental influences of such limitations in future research pertaining to cardiac stress’ and electrical reactivity’s association with target organ damage.

4.2 Summary of the main findings and comparison with the current literature

The research focus and article manuscript reported, is entitled:

**Electrical and cardiac stress reactivity associations with pre-clinical target organ damage: The SABPA study**

The aim of this study was to investigate the cardiovascular risk accompanied by specific haemodynamic, cardiac stress and electrical reactivity patterns in a South African bi-ethnic cohort. This general aim was warranted since previous studies have identified significant ethnic differences relating to blood pressure and stress hormone reactivity during application of a mental stressor [1-4]. However, no attempt has been made to assess possible differences in cardiac stress and electrical reactivity between ethnicities and their possible relation to target organ damage. Relevant literature pertaining to such ethnic-specific reactivity investigations is exceptionally limited.

Due to the ethnic variability regarding stress reactivity and physiological adaptation we therefore hypothesised (as stated in Chapter 2) that:

1) BP, QTc and cardiac stress reactivity will differ between our two South African ethnic groups
2) α-adrenergic BP responses will be associated with a prolongation of the QTc and increased cardiac stress levels, specifically in the SABPA African cohort, and that

3) associations between α-adrenergic BP responses, QTc and cardiac stress (cTnT and NT-proBNP) reactivity will be indicative of pre-clinical cardiac loading and structural alterations.

The first hypothesis was confirmed, as we found that an increased cardiovascular vulnerability was observed in the African group and that moreover such a vulnerability profile was accompanied by acute cardiac stress, QTc prolongation and typical α-adrenergic responsivity. This corroborates previous findings according to which Africans displayed an α-adrenergic vascular reactivity profile in response to the hand-grip test as well as to acute mental stress testing [1-4]. Although the resting values of the assessed cardiovascular markers were quite similar between our African and Caucasian groups, the extent to which these values changed during acute stress application differed significantly, emphasizing the difference in reactivity profile (α- or β-adrenergic) between ethnicities.

Second hypothesis: Our findings also demonstrated that a positive association existed between cTnT and NT-proBNP reactivity in both ethnic groups. This verifies previous findings from our group, that baseline cTnT and NT-proBNP levels were associated with each other [5]. However, stroke volume (SV) associates negatively with cTnT as well as QTc reactivity, especially in the African group. An increase in TPR is also supported by decreased SV, due to the negative association of afterload with SV [6]. As SV is mainly determined by cardiac preload, afterload and contractility, it may emphasize increases in cTnT followed by NT-proBNP, as well as QTc prolongation [7], when an α-adrenergic response pattern is prompted. α-adrenergic BP responses were associated with increased cTnT and NT-proBNP reactivity during acute mental stress
application; thus, providing ample evidence to accept and confirm our second hypothesis, namely that increased cardiac stress (NT-proBNP and cTnT) and electrical reactivity associates with an α-adrenergic BP response.

Third hypothesis: During application of the Stroop test, results in the Africans indicated that considerable increases in cTnT predicted increased probability of CVD prevalence, as indicated by an increased RaVL amplitude. Increases in the R wave of the aVL lead have been associated with increased CVD risk [9]. Elevated cTnT levels are also associated with an increased risk for cardiac-related mortality [10]. Although the Caucasian group did not show an increase in cTnT levels during acute mental stress, detectable cTnT levels were still associated with increases in the RaVL amplitude. Additionally, ROC analyses revealed that cTnT cut-points far below values which are normally considered clinically relevant, predicted 24 hour diastolic blood pressure hypertension (24H DBP HT) in both ethnicities. The decrease in ΔSV and the negative association of ΔQTc, Δ cTnT and ΔNT-proBNP with ΔSV – especially in the African group – may possibly depict stress-induced alterations in the loading conditions of the heart [11]. Lazzarino and co-workers [12, 13] showed that elevated levels of cTnT were associated with increased acute mental stress. This corroborates with our findings, according to which increased cTnT reactivity during acute mental stress testing accompanied cardiovascular vulnerability, α-adrenergic responses and possible increased coronary artery disease risk. Elevated levels of cTnT have been identified as a marker of increased CVD risk as well as pre-clinical cardiac and vascular complications [10, 14]. This notion was additionally supported by the increased number of 24 hour ischemic events found in the African group, as cTnT and NT-proBNP levels may be chronically increased in overly challenged individuals [15]. However, this is the first study to investigate cTnT’s reactivity changes and its related risk. These observations support our third hypothesis.
4.3 Chance and Confounders

**Chance**

When observing the previously discussed assumed credible results, one should take into account the possibility of chance factors that might influence the validity of these results. Despite rigorous efforts and control measures, stepwise regression analyses indicate that five percent of all correlations might be due to chance. Weaknesses in this sub-study might concern methodological issues, which may influence the credibility of our results. The size of the investigated population might have been inadequate, as the division of the entire SABPA population according to ethnic groups, although still comparable, led to smaller sample sizes and decreased statistical power.

**Confounders**

Age, body surface area, gender, glycated haemoglobin, gamma glutamyl transferase, cotinine, physical activity, C-reactive protein, total cholesterol: HDL cholesterol ratio and Windkessel compliance were adjusted for in all regression analyses and analyses of co-variance to ensure credibility of results [11]. However, adjusting for these variables might lead to an over-or underestimation of the associations between cardiac stress, blood pressure and QTc reactivity markers, because potential mediation effects were not accounted for. Therefore only essential co-variates, specifically pertaining to each investigated dependent variable, were adjusted for. Additional care was taken to ensure that no variables that physiologically influence one another were added in the same model, thereby successfully avoiding co-linearity.

**Strengths**

- The SABPA-study target population presented individuals of both investigated ethnicities equally and also ensured equal distribution of gender and socio-economic status.
• The average age of the study population was relatively young (± 45 years). This allowed us to investigate acute mental stress induced reactivity differences between ethnicities from the similar working environment. It is important to note that reactivity time modifies with age, but the reactivity profile or pattern remains the same [16].

• All measurements were conducted in an extremely controlled scientific environment, the Metabolic Research Unit, ensuring utmost environmental stability such as temperature, humidity and environment exposure.

• All measurements were conducted between February and May of 2008 and 2009, so as to avoid any seasonal variations.

• A precise protocol was followed with regard to all measurements, analyses and sample management.

• During reactivity-stress testing, ample time was allowed for baseline measurement and blood samples prior to acute stress application.

• Blood samples were immediately stored at -80°C to ensure sample stability and integrity.

• Exclusion of participants with a history of atrial fibrillation, stroke, myocardial infarction, ECG-left ventricular hypertrophy and ectopic ventricular episodes ensured a study sample free from apparent atherothrombotic CVD.

• Additional adjustments were also made for ejection fraction, especially where NT-proBNP was concerned.

• Due to the exclusion criteria of the SABPA study, our study population comprised generally healthy participants.

**Limitations**

• The total study sample was quite small after additional exclusion criteria were implemented (N=388). However, the necessary statistical power was still reached.
• The conclusions made in this study only pertain to two ethnic and socio-economic groups from a specific demographic area in South Africa – a country rich in ethnic and cultural diversity. Therefore these results cannot be extrapolated to the entire South African population.

• Due to the cross-sectional design of our study, we could not claim causal inferences.

• Only blood pressure, two markers of cardiac stress and one electrocardiography marker were used to measure reactivity differences in this bi-ethnic South African cohort. We can therefore not conclude that SNS hyperactivity or dysregulation is at play, because no direct markers of SNS activity were evaluated (eg. catecholamines and/or heart rate variability).

• No renal markers were assessed (e.g. glomular filtration rate or creatinine excretion), especially where analyses of NT-proBNP were concerned.

4.4 Discussion of the main findings
Overall an increased cardiovascular vulnerability was observed in the African group and this vulnerability profile was accompanied by acute cardiac stress, QTc prolongation and α-adrenergic responsivity [1-4]. Although the resting values of the assessed cardiovascular markers were quite similar between our African and Caucasian groups, the extent to which these values changed during acute stress application differed significantly.

The sympathetic nervous system (SNS) is activated during acute mental stress application. Due to the dominance of α-adrenergic activity in Africans [4], again observed here, this increase in SNS activity will result in increases in DBP, total peripheral resistance (TPR), cardiac contractile force and decreased SV. SNS hyperactivity may also be accompanied by the elevated levels of cTnT, as this biomarker’s secretion is linked to SNS activity in the insula region of the brain with the
result that additional cTnT release is directly regulated by the brain [8]. This SNS hyperactivity in our African cohort is accompanied by increases in DBP, TPR, cardiac contractile force and decreased SV. This increase in TPR is further supported by a decreased SV. SV is mainly determined by cardiac contractility, preload and afterload, emphasizing the increases in cTnT, NT-proBNP and QTc prolongation when an α-adrenergic response pattern is elicited. Elevated levels of cTnT are linked to SNS activity in the insular area of the brain, and SNS hyperactivity may imply additional cTnT release directly from the brain [6, 8]. The delay in relaxation of the ventricles, implying increased repolarization time of the ventricles involves an increased oxygen need which may further support increased levels of cTnT and increased duration of the RaVL amplitude [10], in both ethnicities, as revealed with an odds ratio of approximately 11.

This increased RaVL amplitude indicates an increased risk for cardiovascular disease (CVD) predicted by elevated levels of cTnT. Although the Caucasian group did not present an increase in cTnT during mental stress, detectable levels of cTnT were still associated with an increased RaVL amplitude [11, 12]. This might indicate an overall novel association between RaVL and cTnT levels during acute mental stress. The slight discrepancy between our two ethnic groups might be due to the combination of the α-adrenergic profile and cardio-metabolic vulnerability exhibited by the Africans, when compared with the β-adrenergic response elicited by Caucasians.

ROC analyses also revealed cTnT levels far below general clinically relevant values, predicting 24H DBP HT in both ethnicities. This analysis was particularly significant in the Africans, possibly depicting stress-induced alterations in the loading conditions of the heart. cTnT levels predicting 24H DBP HT further support the assumption that a decrease in SV is accompanied by a decrease in DBP, but not an increase in the latter as presented by the African group. This inappropriate relation is typical of an α-adrenergic response profile and may suggest inadequate blood supply to and from the
heart during acute mental stress, indicating an increased risk for coronary artery disease [8]. This might also suggest a decreased myocardial sensitivity to cTnT during acute stress in the African group exclusively.

We also illustrated that increased cTnT reactivity and NT-proBNP reactivity were positively associated in both ethnicities, which supports previous findings from our group. Individuals with high cTnT and NT-proBNP responsivity may also be hyper-reactive to mental stress in their daily life [15]. This opinion was additionally supported by the increased number of 24H ischemic events displayed by the African group. Therefore, NT-proBNP and cTnT levels may be chronically increased in overly challenged individuals. The lower cTnT cut-point predicting 24H DBP HT in Africans was far lower than that of their Caucasian counterparts, suggesting sensitization or hyper-responsiveness of the cTnT receptors in the myocardium, or hypo-responsiveness of the β-receptors if the stressor is perceived as a threat.

The cardiac stress and QTc reactivity markers were weakly associated with a β-adrenergic response, a pattern mainly presented by the Caucasian group. Decreased levels of NT-proBNP were present during acute mental stress, which may indicate an increased sensitivity to NT-proBNP in Caucasians presenting a β-adrenergic response [1-4, 14]. Therefore, an α-adrenergic response pattern might indicate greater cardiac stress in a cohort that displays cardio-metabolic vulnerability, as supported by other SABPA sub-studies.

Therefore cardiac stress, electrical and blood pressure reactivity profiles exhibited by Africans and Caucasians differed significantly and α-adrenergic blood pressure response profile was associated with increased cardiac stress and QTc reactivity, indicative of pre-clinical structural and loading alterations, in our African cohort exclusively. Additionally, the possibility of lower NT-proBNP levels accompanying a β-adrenergic profile during acute mental stress was revealed. Alternatively, increased
cardiac stress and QTc reactivity may contribute to early sensitization and damage to the myocardium as well as signs of CVD, especially in an at-risk population [1, 17].

4.5 Conclusion

Cardiac stress and QTc reactivity were independently associated with increased pre-clinical risk of mechanistic, loading and structural alterations, specifically in the SABPA African cohort. Mental stress induced reactions may prompt both acute and chronic changes, especially in the loading conditions of the heart. In situations where α-adrenergic reactivity and cardio-metabolic vulnerability predominate, such as in this African group, such modifications may be detrimental – as exhibited by an increased DBP HT, CVD, ischemia and coronary artery disease risk. Novel findings also reveal the possibility of specific cardiovascular reactivity profiles (α-or β-adrenergic responses) may be accompanied by increased or decreased levels of NT-proBNP, respectively. Overall, cardiac stress and QTc reactivity, specifically associated with an α-adrenergic reactivity profile, may contribute to the early sensitization of and damage to the myocardium as well as early signs of CVD.

4.6 Recommendations for future studies

- Conduction of a prospective study to investigate whether these markers might elucidate a causal mechanism in this study population.
- All procedures can be repeated in a larger population-based study that includes different ethnic groups from various demographics, specifically to determine the external validity of our conclusions, and especially those concerning Africans.
- Additional markers of SNS activity (eg. catecholamines and heart rate variability) should be included to verify the hypothesis that SNS hyperactivity is present in an at-risk population.
Acute physical stress modifications may also be investigated to clarify the role of the sympatho-vagal balance in CVD progression, specifically among various ethnicities.
References


management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology. *J Hypertens* 2013;31:1281–1357.
Appendix

Ethical approval (SABPA study)
Extension of ethical approval (SABPA study)
Ethical approval (Sub-study)
Patient informed consent form
Complete author Instructions *Journal Hypertension*
Copy of SABPA Ethical Approval

Dr L. 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: NWU-30835-07.1.56

Approval date: 12 November 2007

Expiry date: 11 November 2012

Special conditions of the approval (if any): None

General conditions:

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

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

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

Yours sincerely,

Prof M M J Lowes

(chair NWU Ethics Committee)
To whom it may concern

31 August 2012

Dear Prof./Dr./Mr./Ms.

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 study till 2017 has been approved.

Kind regards.

[Signature]

[Name]
Prof. H.H. Yorster
Chair person
ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by Health Research Ethics Committee (HREC) on 15/07/2016 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: Electrical and cardiac stress reactivity is associated with pre-clinical target organ damage: the SABPA study  
Study Leader/Supervisor: Prof L Malan  
Student: A Wentzel

Ethics number: NWU - 0005416 A1

Application Type: Single study

Commencement date: 2016-07-15

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 deviation 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: 2016.07.19

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Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)
PART 1

PRINCIPAL RESEARCHER: Dr Leoné Malan, Subject Group Physiology
PROJECT LEADER: Dr. Leoné Malan, Subject Group Physiology

Associate Researcher(s): The postdoctoral fellow involved in this trial is Dr. Szabolcs Péter. Other persons assisting in the study are Dr. Hugo W. Huisman, Prof. Johannes M. van Rooyen, Prof. Nico T. Malan, Dr Ron Schutte, Mrs. Carla M.T. Fourie, Mrs. Tina Scholtz (Cardiovascular research group, Physiology), Prof. Salomé Kruger & Dr. Ramoteme Mamabolo, (Physical activity), Proff. Hans de Ridder (Anthropometry), Marié Wissing (Psychology), Linda Brand & Brian Harvey (Pharmacology), Kobus Mentz (Education), Francois van der Westhuizen (Biochemistry), Hester Klopfer (Nursing), Nancy Frasure-Smith & Francois Lespérance (Psychology, Canada), Alaa Alkerwi (Epidemiology, Luxembourg), Yackoob Seedat (ECG, Kwazulu Natal), Paul Rheeder (Sonar, Pretoria Univeristy), Drs. Johan Potgieter & Michael Temane & Mr Thumi Khumalo (Psychology), Mrs Gedina de Wet (Nursing).

This Participant Information and Consent Form is 7 pages long. Please make sure you have all the pages.

Your Consent
You are invited to take part voluntarily in this research project.

This participant information document contains detailed information about the research project which has been explained to you verbally. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part.

Please read this Participant Information Form carefully. Feel free to ask questions about any information in the document. You may also wish to discuss the project with a relative or friend or your local health worker. Feel free to do this.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Participant Information and Consent Form to keep as a record.

What is the study about?
The aim of this project is to have an impact on the eventual prevention and treatment of lifestyle diseases in Africans from South Africa. New knowledge regarding the relationship between higher nervous system activity implicating cardiovascular, metabolic and psychological well-being will improve understanding and change strategies at the roots of
treatment and prevention of lifestyle diseases.

Our research has shown that lifestyle diseases in urbanised Africans present higher obesity levels, high blood pressure or hypertension prevalence rates and the experiencing of more stress. This pattern is enhanced during psychosocial stress/urbanisation in participants with a specific coping style.
Hence the planned SABPA project, which is the first study in South Africa where coping and direct markers of nervous system activity in Africans will be measured.

**Purpose of study**

The purpose of this study is to investigate biological markers associated with higher sympathetic nervous system activity in urbanised teachers with a specific coping style.

To investigate the relationship between blood pressure, inflammation, obesity, stress and coping in more detail we are going to perform this study in 400 men and women from the North-West province, aged 25-60 years. A comprehensive assessment of the cardiovascular and nervous systems by means of non-invasive painless techniques will be performed and a blood sample will be taken by an experienced research doctor and nurse to determine your blood sugar, cardiovascular, inflammation and stress hormone levels amongst other health markers.

**Procedures**

All measurements are performed in the Metabolic Unit (lipid clinic) of the University. A researcher has explained the entire procedure in detail and while you are reading this information document you have time to ask questions and to have clarified matters. If you are fine with the explained procedure you are requested to sign a *consent form (at the end of this document). Remember all personal data will be handled with care and remain confidential.

*By consenting to participate in this study, you consent to the storage and later analysis and testing of your stored blood samples for the purposes noted above. Your blood will also be tested for preliminary results on HIV status, since your HIV status may directly influence the main purposes of this study. If you would like to know what your HIV-status is, we will provide it. If tested positive we will refer you to your doctor and he/she will perform the necessary tests which will allow you to apply for chronic medication benefit. Also, the blood cells from your donated blood sample will be used to investigate the molecular genetics of higher nervous system activity and type 2 diabetes in order to enable pre-symptomatic diagnosis of hypertension and diabetes in the long term.*

**Why was I chosen?** Teachers are exposed to changing curricula and disciplinary problems whilst living in an urbanised environment adding to higher stress experiencing and nervous system activity.

**How was I chosen?**

*Inclusion criteria:*

Phase I: 200 black Africans aged 25-60 years (male=100, female = 100)  
Phase II: 200 white Africans (n = male, 100 = female) aged 25-60 years.

*Exclusion criteria: pregnancy, lactation, any acute/chronic medication (e.g. high blood pressure, TB/tuberculosis, high sugar/diabetes, arthritis, anti-clotting/stroke factors, epilepsy/mental diseases or being treated for it as well being addicted to the medicine). You cannot be included if you have been vaccinated in the previous 3 months and if you are a regular blood donor.*

**What will be expected of me?**

You, as participant will be screened once by a registered nurse to be eligible complying to the inclusion criteria. The following procedures will be followed:

- Recruitment, screening and informed sessions with all participants will be done two months prior to the study (October - November 2007, Phase I, and November, 2008, Phase II) and informed consent forms will be signed.
- After selection of all participants, the details of the project will be discussed with you in English or your home language, i.e. what the exact objectives of the study are, what procedures will be taken and what will be expected from each of you (e.g. overnight stay, resting blood pressure procedures
and fasting urine and blood samples are required, importance of complying with the correct sampling methods, incentives). You will be given the opportunity to ask questions.

- Data collection for each participant will involve two days (15min in the morning and 2½ hours in the evening) on Day I; and 2 hours on Day II:

**DAY I**

- On day I at 07:00, the blood pressure apparatus, which will measure your blood pressure and heart function as well as a physical activity meter will be applied to your arm and waist at your school and you can then resume your normal daily activities. In the afternoon you must complete the Neethling Brain Instrument questionnaire which measures thought processes of the brain.

- At the end of Day I (± 16:30) you will be transported from your schools to overnight in the Metabolic Unit Research Facility of the North-West University. This unit is a research unit for human studies and equipped with 10 well furnished bedrooms, a kitchen, two bathrooms and a television room. Each of you will be subjected to the following procedures:
  - At the end of Day I between ± 17:15 and 18:00 you will be welcomed and each of you will receive your own private bedroom.
  - The procedures, which will be done, will be explained again and each of you will then complete a general socio-demographic health questionnaire. Afterwards you will receive dinner.
  - After dinner, psychological questionnaires will be completed under supervision of registered education specialists and psychologists. Completion of questionnaires will take approximately 40 min, including a break of 20 minutes with coffee/tea and biscuits. This will be you last meal for Day I as you must be fasting on Day II for obtaining good results.
  - Thereafter, you can relax and watch television or socialise with your c-participants. It will be wise to go to bed not later than 22:00 as the blood pressure apparatus will take measurements every hour during the night and it can be tiring.

**DAY II**

- At 06:45 on Day II the AMBP will be removed and an urine sample collected. Once this has been done you will be directed to the anthropometric station where your weight, height and body circumferences will be measured.

- The next station involves the blood pressure measurement station. Whilst in a sitting position your blood pressure will be taken in duplicate with the sphygmomanometer (the same as used at clinics) with a resting period of 5 minutes in between. Our registered research doctor/nurse will take a fasting saliva sample as well as a blood sample of 45ml from a vein in your dominant arm. The infusion set will be left in your arm to lessen the effect of inserting a needle again for blood sampling after exposure to the two stressors. A small amount of diluted heparin will be left in the infusion set in your arm to prevent clotting.

Next the cardiovascular measurements will follow consisting of three separate procedures:

- The 1st measurement involves an ECG apparatus, which measures heart function, with 12 leads, which will be placed into position on your rib cage/front part of the body.

- The 2nd measurements are non-invasive and will be done by means of the Finometer device which also involves the assessment of heart functioning such as pulse (beats per minute), stroke volume (blood volume ejected by the heart per beat), cardiac output (blood volume ejected by the heart per minute), total peripheral resistance (resistance against the blood flow created by small arteries), central resistance (resistance against which the heart has to work while ejecting the blood into the aorta) as well as the elasticity of your large arteries (compliance). For this procedure a blood pressure cuff will be placed around your left arm and middle finger which will be inflated and stepwise deflated. You will not have
more discomfort than during a common blood pressure measurement. This will take about 5 minutes.

- The stressor application procedure follows: You will now be exposed to a stressor for 1 minute whilst your blood pressure and ECG will still be taken. After exposure a saliva and blood sample (45ml) will be taken. After 10 minutes another saliva sample will be taken. Then the stressor application procedure will be repeated with the second stressor.

- At another station your 3rd measurement includes the assessment of pulse wave velocity, i.e. how fast your blood travels through your arteries. This measurement gives us an indication about how stiff your vessel walls are. The stiffer your vessel wall is the faster the blood travels from one point of your body to another. These painless measurements will require two technicians using blunt probes (tonometer) putting light pressure on the neck and on the foot to measure the velocity of the pulse waves. This takes only a few minutes. An ultrasound device will be taken of your arteries in the neck with a blunt probe to indicate the intrinsic thickness of your arteries which contributres to high blood pressure.

The two stressors you will be exposed to for one minute include:

1. The Colour-Word-Conflict Chart (applied for 1 minute) is written in various colours. You must say or select the ink colour rather than the name of the colour spelled out by the word. A sliding scale with monetary incentives (maximum of R55.00) will be given if you can complete reading the chart.

2. The Cold Pressor Test (Foot) (applied for 1 minute): Immersion of your foot up to the wrist in ice water (4 degrees Celcius). As the cold can make you hold your breath you must quietly count to yourself during cold exposure to breath more rythmic.

> You have reached the end of the sampling phase.

> Thank you for your participation! You now will have the opportunity to shower and a take away breakfast will be given.

> Immediate feedback on your HIV/AIDS status, obesity, blood pressure and blood glucose/sugar values will be given. HIV/AIDS post-test counselling will be arranged if you are tested positive.

> You are now transported back to your school and after one week you will receive your Neethling Brain Instrument and 24-hour blood pressure reports.

**Possible Risks**

The measurements performed in our study will include only non-invasive techniques that are not expected to reveal any risks but might cause little discomfort. The taking of blood samples is an invasive procedure with a minimal risk of bleeding. Thus the procedure may cause only a few seconds of light discomfort. All tests will be performed by experienced research nurses of our department. There may be additional unforeseen or unknown risks.

**Precautions to protect the participant**

The Metabolic Unit facility of the NWU is fully equipped, and in case of an emergency which could not be handled by the registered nurse, the supervising medical doctor Emile Kotzé will be contacted. Dr. Kotzé was notified before the study commenced that this study will be taking place, and that there is a slight possibility that he may be contacted. Supporting medical treatment care facilities will be at hand anytime if needed.

**Other Treatments Whilst on Study**

It is important to tell the research staff about any treatments or medications you may be taking, including non-prescription medications, vitamins or herbal remedies during your participation in the study.
Incentives

1. All teachers will receive feedback on their health profile and if necessary references will be given to physicians/clinics/hospitals.
2. Printout feedback on 24 hour blood pressure monitoring report (normally costing R637.60), sonar of the artery (R1200.00), resting ECG (R600.00) and other variables (R500.00). Your benefit of participation is a comprehensive assessment of the cardiovascular and metabolic condition including investigation of blood pressure, inflammatory status and psychological well-being. These examinations will help us to assess the degree of vascular impairment of the arteries and to predict your risk of possible cardiovascular events such as heart attacks and stroke. The results may assist your doctor in decision making for further treatment or for instituting preventive measures. Our study will also contribute to the identification of possible factors leading to high blood pressure. As 24 hour ambulatory blood pressure monitoring is required for the diagnosis of hypertension, medical aids insist on this method of diagnosis to qualify for chronic medication. Additional testing could also reveal illnesses of a chronic nature and would serve as a motivation to qualify for chronic medication, such as metabolic syndrome, anti-inflammatory and cholesterol-lowering drugs.
3. Monetary incentive on completion of the colour word conflict chart (± R55.00).
4. Dinner and breakfast (± R24.00).
5. Neethling Brain Instrument profiles done by registered user of the Whole Brain (normally costing ± R350.00).
6. Coping skills workshop will be arranged on request.

Privacy, Confidentiality and Disclosure of Information

By consenting to participate in this study, you consent to the storage and later analysis and testing of your stored blood samples for purposes noted above. Your blood samples will be discarded immediately after analysis. All information provided by you and the results of tests will be treated in the strictest confidence, and will only be used for the purpose of this research project. It will only be disclosed with your permission, except as required by law. The results of your medical tests will be labelled only with a code number, and will be stored separately from any identifying information. When the results are analysed we will be looking for differences between groups of people, not at the results of individuals. No information that could identify any person taking part in the study will be revealed when the results are reported.

Participation is Voluntary

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the North-West University.

Before you make your decision, a member of the research team will be available so that you can ask any questions you have about the research project. You can ask for any information you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team before you withdraw.
Ethical Guidelines
This project will be carried out according to Ethical Guidelines of the Helsinki declaration from 2000, with additional notes in 2002. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethical aspects of this research project have been approved by the Human Research Ethics Committee of North-West University Potchefstroom.

Further Information or Any Problems
If you require further information or if you have any problems concerning this project, you can contact the principal researcher or the other researchers responsible for this project.

Dr Leoné Malan (018-299 2438) Signature:
Sr. Chrissie Lessing (018-299 2480) Project Leader: Dr Leoné Malan

PART 2

To the subject signing the consent as in part 3 of this document

You are invited to participate in a research project as described in paragraph 2 of Part 1 of this document. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project: Participation in this project is voluntary.

1. It is possible that you personally will not derive any benefit from participation in this project, although the knowledge obtained from the results may be beneficial to other people.

2. You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.

3. The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.

4. We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.

5. We require that you indemnify the University from any liability due to detrimental effects of treatment by University staff or students or other subjects to yourself or anybody else. We also require indemnity from liability of the University regarding any treatment to yourself or another person due to participation in this project, as explained in Part 1. Lastly it is required to abandon any claim against the University regarding treatment of yourself or another person due to participation in this project as described in Part 1.

6. If you are married, it is required that your spouse abandon any claims that he/she could have against the University regarding treatment or death of yourself due to the project explained in Part 1.
PART 3
Consent

Title of the project:
“THE SABPA STUDY (SYMPATHETIC ACTIVITY AND AMBULATORY BLOOD PRESSURE IN AFRICANS)”.

I, the undersigned ................................................................. (full names) read/listened to the information on the project in PART 1 and PART 2 of this document and I declare that I understand the information. I had the opportunity to discuss aspects of the project with the project leader and I declare that I participate in the project as a volunteer. I hereby give my consent to be a subject in this project.

(Signature of the subject)

Signed at ................................................................. on ................................................. 2008/9

Witnesses

1. .................................................................

2. .................................................................

Signed at ................................................................. on ................................................. 2008/9
Guidance for Authors on the Preparation and Submission of Manuscripts to Journal of Hypertension

These instructions comply with those formulated by the International Committee of Medical Journal Editors. For further details, authors should consult the following article: International Committee of Medical Journal Editors, "Uniform Requirements for Manuscripts Submitted to Biomedical Journals". The complete document appears at www.icmje.org.

The Journal is a member of the Committee on Publication Ethics (COPE) which aims to define best practice in the ethics of scientific publishing. COPE has established a number of guidelines including a Code of Conduct, and created flow charts that help editor's process cases of suspected misconduct (www.publicationethics.org).

Appeals on editorial decisions should be sent to the Editor. Complaints related to how your paper was processed during peer-review and not resolved by the Editor, should be referred to the person named as publisher in "About the Journal" under "Journal Info" contacts (http://journals.lww.com/jhypertension/) or if unsatisfied to COPE (www.publicationethics.org).

Submitted articles undergo a preliminary review by the editor. Some articles may be returned to authors without further consideration. Those being considered for publication will undergo further assessment and peer-review by the editor and those invited to do so from the board and reviewer pool.

Scope
The Journal of Hypertension publishes papers reporting original clinical and experimental research which are of a high standard and which contribute to the advancement of knowledge in the field of hypertension. The Journal publishes full papers and reviews or editorials (normally by invitation). Authors who submit papers to the Journal must document that all persons acknowledged have seen and approved the mention of their name in the paper.

Points to consider before submission
Redundant or duplicate publication
Submissions are accepted on the understanding that they have not been published in their current form or a substantially similar form (in print or electronically, including on a web site), that they have not been accepted for publication elsewhere, and they are not under consideration by another publication.

Conflicts of interest
Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. All relevant
conflicts of interest and sources of funding should be included on the title page of the manuscript with the heading "Conflicts of Interest and Source of Funding:”. For example:

Conflicts of Interest and Source of Funding: A has received honoraria from Company Z. B is currently receiving a grant (#12345) from Organization Y, and is on the speaker’s bureau for Organization X – the CME organizers for Company A. For the remaining authors none were declared.

Copyright: In addition, each author must complete and submit the journal's copyright transfer agreement, which includes a section on the disclosure of potential conflicts of interest based on the recommendations of the International Committee of Medical Journal Editors, “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” (www.icmje.org/update.html).

A copy of the form is made available to the submitting author within the Editorial Manager submission process. Co-authors will automatically receive an Email with instructions on completing the form upon submission.

Permissions to reproduce previously published material
Authors should include with their submission copies of written permission to reproduce material published elsewhere (such as illustrations) from the copyright holder. Authors are responsible for paying any fees to reproduce material.

Patient consent forms
Patients have a right to privacy that should not be infringed without informed consent. Identifying details (written or photographic) should be omitted if they are not essential, but patient data should never be altered or falsified in an attempt to attain anonymity. Complete anonymity is difficult to achieve, and a consent form should be obtained if there is any doubt. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. When informed consent has been obtained it should be indicated in the published article.

Ethics committee approval
All authors must sign a declaration that the research was conducted within the guidelines below and under the terms of all relevant local legislation. (Such a statement is included in the model submission letter on the journal’s web site.) The Editors reserve the right to judge the appropriateness of the use and treatment of humans or animals in experiments for publication in the journal.

Human experiments: All work must be conducted in accordance with the Declaration of Helsinki. Papers describing experimental work on human participants which carries a risk of harm must include (1) a statement that the experiments were conducted with the understanding and the consent of each participant, and (2) a statement that the responsible ethical committee has approved the experiments.

Animal experiments: In papers describing experiments on living animals, include (1) a full description of any anaesthetic and surgical procedure used, and (2) evidence that all possible steps were taken to avoid animals’ suffering at each stage of the experiment.

Experiments on isolated tissues: Indicate precisely how you obtained the donor tissue.
Systematic Reviews and Meta-analysis
Authors should follow the PRISMA guidelines (www.prisma-statement.org) on reporting items for systematic reviews and meta-analyses. Such reviews often serve as a basis for many health policy decisions and direction for further research, and following these guidelines will assist in improving the quality of reports available.

Clinical Trials and Behavioural and Public Health Evaluations
Authors reporting results of randomised controlled trials should include with their submission a complete checklist from the CONSORT statement (www.consort-statement.org). For behavioural and public health evaluations involving non-randomised designs, authors should include with their submission a complete checklist from the TREND statement (www.cdc.gov/trendstatement/).

Registration of clinical trials: As a condition for publication of a clinical trial in the Journal, registration of the trial in a public registry is required. The editor does not advocate one particular registry but require that the registry utilised meet the criteria set out in the statement of policy of the ICMJE (www.icmje.org).

Authorship
All authors must sign the letter accompanying their submission to confirm that they have read and approved the paper, that they have met the criteria for authorship as established by the International Committee of Medical Journal Editors, that they believe that the paper represents honest work, and that they are able to verify the validity of the results reported. In addition to those from the ICMJE the International Society for Medical Publication Professionals, ISMPP (www.ismpp.org) have produced some useful guidelines on authorship of studies sponsored by companies: Good Publication Practice (GPP2) (www.ismpp.org/initiatives/gpp2.html).

Compliance with NIH and Other Research Funding Agency Accessibility Requirements
A number of research funding agencies now require or request authors to submit the post-print (the article after peer review and acceptance but not the final published article) to a repository that is accessible online by all without charge. As a service to our authors, LWW will identify to the National Library of Medicine (NLM) articles that require deposit and will transmit the post-print of an article based on research funded in whole or in part by the National Institutes of Health, Wellcome Trust, Howard Hughes Medical Institute, or other funding agencies to PubMed Central. The revised Copyright Transfer Agreement provides the mechanism.

Copyright assignment
Papers are accepted for publication on the understanding that exclusive copyright in the paper is assigned to the Publisher. Authors are asked to submit a signed copyright assignment form with their submission. They may use material from their paper in other works published by them after seeking formal permission.

Submissions
Authors are strongly encouraged to submit their manuscripts through the web-based tracking system at http://www.editorialmanager.com/jh. Signed author forms may be included in the submission as a 'supporting document' or mailed to the journal office. Authors should submit the text of the paper as a word-processed document, and not as a PDF. The site contains instructions and advice on how to use the system. Authors should
NOT in addition then post a hard copy submission to the editorial office, unless you are supplying artwork, letters or files that cannot be submitted electronically, or have been instructed to do so by the editorial office. Include the following where appropriate: subject consent forms; transfer of copyright form; permission to reproduce previously published material; checklist. Editor address: Alberto Zanchetti, The Editor, Journal of Hypertension, Centro di Fisiologia Clinica e Ipertensione, University of Milan, Ospedale Maggiore, Via F. Sforza 35, 20122, Milan; tel: 39 02 5518 4606, fax: 39 02 503 20480, email: j.hypertension@centroipertensione.191.it

Margins should be not less than 3 cm. Double spacing should be used throughout the manuscript, which should include the following sections, each starting on a separate page: title page, abstract and keywords, text, acknowledgements, references, individual tables and captions. Pages should be numbered consecutively, beginning with the title page, and the page number should be placed in the top right hand corner of each page. Abbreviations should be defined on their first appearance in the text; those not accepted by international bodies should be avoided.

Please note that as a new feature of the Journal of Hypertension, published articles will be followed by a short summary of strengths and weaknesses prepared by each of the reviewers.

Presentation of Papers
Title Page
The title page should carry the

- full title of the paper, consisting of no more than 20 words (only common abbreviations should be used if absolutely necessary); titles should be clear and brief, conveying the message of the paper
- a brief short title, which will be used as running head (consisting of not more than 40 characters, including spaces)
- all authors’ names: the full first name, middle initial(s) and last (family name) name of each author should appear; if the work is to be attributed to a department or institution, its full name and location should be included. The last (family name) must appear in CAPITAL letters. Persons listed as authors should be those who substantially contributed to the study’s conception, design, and performance
- the affiliations of all the authors; when authors are affiliated to more than one institution, their names should be connected using a, b, c, etc. These letters should follow the surname but precede the address; they should be used for all addresses
- information about previous presentations of the whole or part of the work presented in the article
- the sources of any support, for all authors, for the work in the form of grants, equipment, drugs, or any combination of these
- Disclose funding received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).
- a statement on potential conflicts of interest: if authors have financial interests relevant to the research or constituting a conflict of interest, these must be stated. If not applicable, state NONE disclaimers, if any
the name and address of the author responsible for correspondence concerning the manuscript, and the name and address of the author to whom requests for reprints should be made. If reprints are not to be made available, a statement to this effect should be included. The peer-review process as well as publication will be delayed if you do not provide up to date telephone and fax numbers, and E-mail address, if available.

- word count: please list full word count (including references, but not tables and legends)
- number of tables
- number of figures
- number of supplementary digital content files

Authors are encouraged to submit colour and non-colour versions of illustrative figures, should the editor choose to publish gratis the colour version online only. Colour images should be prepared to the standards indicated in the section below on illustrations, and take into account that colour and non-colour versions need to be interpretable by the reader. Please ensure that the different versions of the illustrations are labeled for easy identification.

Authors are also encouraged to submit supplementary digital content that may include figures, tables, a PowerPoint slide deck, audio or videos. Material submitted should not duplicate what is in the paper but contain extra material that a reader would find useful to access, but not critical for interpretation of the study. Audio or video should be no longer than 5 minutes in length. Please consult the Supplementary Digital Content section below for further advice.

Abstracts
The second page should carry a structured abstract of no more than 250 words. The abstract should state the Objective(s) of the study or investigation, basic Methods (selection of study subjects or laboratory animals; observational and analytical methods), main Results (giving specific data and their statistical significance, if possible), and the principal Conclusions. It should emphasise new and important aspects of the study or observations.

Review articles and case reports should include an unstructured summary of no more than 150 words.

Condensed Abstracts
A condensed abstract will be published in the ‘forthcoming contents’ section of the issue preceding the published article. This should be supplied with the submission, and should consist of no more that 100 words, this abstract should briefly summarise the main findings of your study.

Key Words
The abstract should be followed by a list of 3–10 keywords or short phrases which will assist the cross-indexing of the article and which may be published. When possible, the terms used should be from the Medical Subject Headings list of the Index Medicus (http://www.nlm.nih.gov/mesh/meshhome.html).

Abbreviations and symbols
Use only standard abbreviations. Avoid abbreviations in the title and abstract. A short list
of non-standard abbreviation definitions that may not be familiar to readers should be included in a separate mandatory document submitted with your paper.

Text
Full papers of an experimental or observational nature may be divided into sections headed Introduction, Methods (including ethical and statistical information), Results and Discussion (including a conclusion), although reviews may require a different format.

Acknowledgements
Acknowledgements should be made only to those who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions.

References
References should be numbered consecutively in the order in which they first appear in the text. They should be assigned Arabic numerals, which should be given in brackets, e.g. [17]. References should include the names of all authors when seven or fewer; when eight or more, list only the first six names and add et al. References should also include full title and source information. Journal names should be abbreviated as MEDLINE (www.nlm.nih.gov/tsd/serials/lji.html).

Articles in journals

More than seven authors:

Supplements:
Dean RT, Wilcox I. Possible atherogenic effects of hypoxia during sleep apnea. Sleep 1993; 16 (suppl 8):S15–S21.

Letter/Abstract:


Books
Book:
Katz AM, Konstam MA. Heart Failure. Pathophysiology, Molecular Biology, and Clinical Management. Philadelphia: Lippincott Williams & Wilkins; 2008

Chapter in a book:
Personal communications and unpublished work should not feature in the reference list but should appear in parentheses in the text. Unpublished work accepted for publication but not yet released should be included in the reference list with the words ‘in press’ in parentheses beside the name of the journal concerned. References must be verified by the author(s) against the original documents.

**Tables**
Each table should be typed on a separate page in double spacing. Tables should not be submitted as photographs. Each table should be assigned an Arabic numeral, e.g. (Table 3) and a brief title. Vertical rules should not be used. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all non-standard abbreviations that are used in each table. Identify statistical measures of variations, such as standard deviation and standard error of the mean.

Be sure that each table is cited in the text. If you use data from another published or unpublished source, obtain permission and acknowledge the source fully.

**Illustrations**

**A) Creating Digital Artwork**

1. Learn about the publication requirements for Digital Artwork: [http://links.lww.com/ES/A42](http://links.lww.com/ES/A42)
2. Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).
3. Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

**B) Digital Artwork Guideline Checklist**

Here are the basics to have in place before submitting your digital artwork:

- Artwork should be saved as TIFF, EPS, or MS Office (DOC, PPT, XLS) files. High resolution PDF files are also acceptable.
- Crop out any white or black space surrounding the image.
- Diagrams, drawings, graphs, and other line art must be vector or saved at a resolution of at least 1200 dpi. If created in an MS Office program, send the native (DOC, PPT, XLS) file.
- Photographs, radiographs and other halftone images must be saved at a resolution of at least 300 dpi.
- Photographs and radiographs with text must be saved as postscript or at a resolution of at least 600 dpi.
- Each figure must be saved and submitted as a separate file. Figures should not be embedded in the manuscript text file.

**Remember:**

- Cite figures consecutively in your manuscript.
- Number figures in the figure legend in the order in which they are discussed.
• Upload figures consecutively to the Editorial Manager web site and enter figure numbers consecutively in the Description field when uploading the files.
• Photomicrographs must have internal scale markers.
• If photographs of people are used, their identities must be obscured or the picture must be accompanied by written consent to use the photograph.
• If a figure has been published before, the original source must be acknowledged and written permission from the copyright holder for both print and electronic formats should be submitted with the material. Permission is required regardless of authorship or publisher, except for documents in the public domain.
• Figures may be reduced, cropped or deleted at the discretion of the editor.
• Colour illustrations for reproduction in print are acceptable but authors will be expected to cover the extra reproduction costs (for current charges, contact the publisher).

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Captions should be typed in double spacing, beginning on a separate page. Each one should have an Arabic numeral corresponding to the illustration to which it refers. Internal scales should be explained and staining methods for photomicrographs should be identified.

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