Comparing autonomic and cardiovascular responses in African and Caucasian men:

The SABPA Study

Aletta Sophia Uys

20030223

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Promoter:	Prof L Malan
Co-promoter:	Prof JM van Rooyen
Assistant promoters:	Prof HS Steyn
	Prof Dr T Ziemssen
	Dr M Reimann

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"I can do all this through Him who gives me strength" Philippians 4:13

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Title: Comparing autonomic and cardiovascular responses in African and Caucasian men: The SABPA Study.

SUMMARY

Motivation

Hypertension is a pertinent health problem for urban black African men (hereafter referred to as African). Sympathetic hyperactivity and a dominant α-adrenergic response pattern have both been implicated as contributing factors to their poor cardiovascular health. In addition to the deleterious effect of neurogenic hypertension on target organs, sympathetic hyperactivity may promote the accelerated progression of left ventricular hypertrophy and structural vascular disease.

Aim

The overarching aim of this study is to scrutinize autonomic control of the cardiovascular system in a cohort of urban African and Caucasian men during a mental challenge. Associations were investigated between potential sympatho-vagal imbalance, blood pressure and target organ damage markers to determine cardiovascular risk in ethnic male groups.

Methodology

The SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study involved the participation of 200 male teachers (99 African and 101 Caucasian) in the Kenneth Kaunda Education District of the North-West Province, South Africa. Of the participant group, HIV-infected (13 African) and clinically confirmed diabetics (1 Caucasian and 6 African men) were excluded from further analyses. Stratification was based on ethnicity and further as indicated through statistical interaction effects. Cardiovascular and autonomic responses were assessed during rest and on stressor exposure (cold pressor test

and Stroop colour-word conflict test). Autonomic measures included baroreceptor sensitivity (BRS), 3-methoxy-4-hydroxy-phenylglycol (MHPG) and nitric oxide metabolite (NOx) levels. Cardiovascular variables consisted of blood pressure, cardiac output, stroke volume, total peripheral resistance, heart rate, arterial compliance and ST-segment from the 12-lead electrocardiogram. Markers of target organ damage included the Cornell product (indication of left ventricular hypertrophy) and carotid intima-media thickness as indication of structural vascular disease. Means and proportions were compared by means of standard t-test and Chi-square test, respectively. Significant differences of mean cardiovascular and autonomic measures between ethnic male groups were also determined through analysis of covariance. Uni- and multivariate regression analyses were employed to demonstrate associations between target organ damage, cardiovascular and autonomic markers.

Results and conclusion of each manuscript

To assess autonomic nervous system and cardiovascular function as well as target organ damage, we clearly focussed on responses where our participants were challenged. Markers of autonomic responses assessed were baroreceptor sensitivity, 3-methoxy-4-hydroxy-phenylglycol and nitric oxide metabolites.

- The first manuscript (Chapter 2) focused on left ventricular hypertrophy as marker of target organ damage, blood pressure and baroreceptor sensitivity as marker of autonomic function. The objective was to determine whether BRS was significantly lower in African men than in the Caucasian men. Furthermore, the possible association between attenuation of BRS and increased levels of ambulatory blood pressure as well as left ventricular hypertrophy was investigated in these population groups. Results revealed that the African men had significantly lower BRS stress responses. This attenuated BRS profile was coupled with dominant α-adrenergic response patterns, which was associated with an elevation of ambulatory blood pressure. BRS attenuation (rest and stress response) was not associated with left ventricular hypertrophy. It was concluded that lower BRS, especially during stress, may pose a significant health threat

for urban African men regarding the development or promotion of α-adrenergic-driven hypertension and higher cardiovascular disease risk.

- The aim of the second sub-study (Chapter 3) was to investigate possible associations between structural vascular disease (carotid intima-media thickness as marker), autonomic function (MHPG as marker) and nocturnal blood pressure in the African and Caucasian men. Results showed a higher prevalence of nocturnal hypertension in the African men, with night-time blood pressure significantly higher compared to the Caucasian men. In the African and Caucasian men, carotid intima-media thickness was linearly predicted by nocturnal systolic and diastolic blood pressure respectively. In conclusion, no associations were demonstrated between MHPG and carotid intima-media thickness or between MHPG and nocturnal blood pressure. Elevated nocturnal blood pressure evidently seems to promote structural vascular disease in this cohort of urban African and Caucasian men.
- The aim of the third manuscript presented in Chapter 4, was to investigate bio-availability of NO during mental challenge (autonomic function marker) and the possible association with structural vascular disease (carotid intima-media thickness as marker). In the African men, an attenuated NOx response was demonstrated to the Stroop colour-word conflict test. After stratification into high and low NOx response groups, in the African men with a low NOx response enhanced α-adrenergic with significant ST-segment depression responses was demonstrated indicating reduced myocardial oxygen supply during mental stressor exposure. Only in the African men, a ST-segment depression was significantly associated with structural vascular disease. It was concluded that the African men demonstrated a vulnerable cardiovascular profile. In this cohort of African men, the significant association between structural vascular disease and myocardial ischemia may particularly indicate a possible higher risk for future cardiovascular events.

General conclusion

Through the assessment of autonomic and cardiovascular responses a possible higher cardiovascular risk was demonstrated in the African men. In this cohort sympathetic hyperactivity was evident, coupled with dominant vascular response patterns and reduced myocardial oxygen supply during mental stress exposure. Based on these findings, this population group's risk for accelerated target organ damage, as well as for future cardiovascular events, appear significantly higher than those of the Caucasian male cohort.

Keywords: Autonomic responses, cardiovascular function, hypertension, target organ damage.

AFRIKAANSE TITEL: Vergelyking van outonome en kardiovaskulêre response in Afrikane en Kaukasiër-mans: Die SABPA-Studie

OPSOMMING

Motivering

Hipertensie is ’n pertinente gesondheidsprobleem vir verstedelikte swart Afrikaan mans (voortaan hierna verwys as Afrikane – met inbegrip van mans). Beide simpatiese hiperaktiwiteit en ’n dominante α-adrenerge responspatroon is al geïmpliseer as bydraende faktore tot hulle swak kardiovaskulêre gesondheid. Benewens die nadelige effek van neurogeniese hipertensie op teikenorgane, bevorder simpatiese hiperaktiwiteit die versnelde ontwikkeling van linkerventrikulêre hipertrofie, strukturele vaskulêre siekte sowel as koronêre arteriële siekte.

Doelstelling

Die oorkoepelende doelstelling van hierdie studie was die bestudering van outonome beheer van die kardiovaskulêre sisteem in ’n kohort van verstedelikte Afrikane en Kaukasiër mans tydens ’n mentale uitdaging. Assosiasies is ondersoek tussen die potensiële simpato-vagale wanbalans, bloeddruk en teikenorgaan skademerkers om die moontlikheid van kardiovaskulêre risiko in die manlike etniese groepe vas te stel.

Metodologie

Die SABPA- (simpatiese aktiwiteit en ambulatoriese bloeddruk in Afrikane) studie het 200 manlike onderwysers (99 Afrikane en 101 Kaukasiër-mans) in die Kenneth Kaunda Onderwys-distrik van die Noordwes Provinsie, Suid-Afrika, as deelnemers betrek. Van die deelnemer-groep is HIV-geïnfekteerdes (13 Afrikane) en klinies bevestigde diabete (1 Kaukasiër en 6 Afrikane mans) uitgesluit van verdere analises. Stratifisering is gebaseer op

etnisiteit en verder soos aangedui deur statistiese-interaksie-effekte. Kardiovaskulêre en outonome response is geëvalueer vir rus sowel as stressor-blootstelling (kouepressor-toets en Stroop kleur-woord konfliktoets). Outonome metings het baroreseptorsensitiwiteit (BRS), 3-metoksie-4-hidroksie-fenielglikol (MHPG) en nitro-oksiedmetaboliet- (NOx) vlakke ingesluit. Kardiovaskulêre veranderlikes het bestaan uit bloeddruk, kardiale omset, slagvolume, totale perifere weerstand, harttempo, arteriële meegewendheid en die ST-segment van die elektrokardiogram. Merkers van teikenorgaanskade het die Cornell-produk (aanduiding van linkerventrikulêre hipertrofie) en karotiese intimamedia-dikte as aanduiding van strukturele vaskulêre siekte ingesluit. Gemiddelde en proporsies is deur standaard *t*-toetse en Chi-kwadraattoetse onderskeidelik vergelyk. Betekenisvolle verskille in gemiddeldes van kardiovaskulêre en outonome metings is deur kovariansie-analises bepaal. Enkel- en multiveranderlike regressie-analises is aangewend om assosiasies te demonstreer tussen teikenorgaanskade en kardiovaskulêre en outonome merkers.

Resultate en gevolgtrekkings van onderskeie manuskripte

Om die outonome senuweestelsel en kardiovaskulêre funksie sowel as teikenorgaanskade te assesseer het ons duidelik gefokus op response waar ons deelnemers uitgedaag is. Merkers van outonome response wat geassesseer is, is BRS, 3-metoksie-4-hidroksie-fenielglikol en nitro-oksied metaboliete.

- Die eerste manuskrip (Hoofstuk 2) het gefokus of linkerventrikulêre hipertrofie as merker van teikenorgaanskade, bloeddruk en BRS as merker van outonome funksie. Die doel was om vas te stel of baroreseptorsensitiwiteit betekenisvol laer in die Afrikane was as in die Kaukasiër mans. Verder is die moontlike assosiasie tussen verlaging van baroreseptorsensitiwiteit en verhoogde vlakke van ambulatoriese bloeddruk sowel as linkerventrikulêre hipertrofie in hierdie populasiegroepe ondersoek. Resultate het aangetoon dat die Afrikane betekenisvol laer baroreseptorsensitiwiteit stresresponse getoon het. Hierdie verlaagde baroreseptorsensitiwiteitsprofiel was gekoppel aan dominante α-adrenerge responspatrone wat geassosieer was met verhoging van

ambulatoriese BP. Baroreseptorsensitiwiteitsverlaging (rustend en stresresponse) was nie geassosieer met linkerventrikulêre hipertrofie nie. Daar is tot die gevolgtrekking gekom dat laer baroreseptorsensitiwiteit, veral tydens stres 'n betekenisvolle gesondheidsrisiko vir verstedelikte Afrikane inhou rakende die ontwikkeling of bevordering van α-adrenerggedrewe hipertensie en hoër kardiovaskulêre risiko.

- Die doel van die tweede substudie (Hoofstuk 3) was om moontlike assosiasies te ondersoek tussen die strukturele vaskulêre siekte, outonome funksie (MHPG as merker) en nagtelike bloeddruk in die Afrikane en Kaukasiër mans. Resultate het aangedui dat daar 'n hoër voorkoms van nagtelike hipertensie in die Afrikane voorkom, met nagtelike bloeddruk betekenisvol hoër as by die Kaukasiër mans. In die Afrikane en Kaukasiër mans was karotiese intimamedia-dikte liniêr deur nagtelike sistoliese en diastoliese bloeddruk onderskeidelik voorspel. Die gevolgtrekking was dat geen assosiasies bewys is tussen MHPG en karotiese intimamedia-dikte of tussen MHPG en nagtelike bloeddruk nie. Verhoogde nagtelike bloeddruk bevorder klaarblyklik strukturele vaskulêre siekte in hierdie kohort van verstedelikte Afrikane en Kaukasiër-mans.
- Die doel van die derde manuskrip aangebied in Hoofstuk 4 was om die biobeskikbaarheid van NO tydens mentale uitdaging (outonomefunksie-merker) te ondersoek asook moontlike assosiasies met strukturele vaskulêre siekte (karotiese intima-media dikte as merker). By die Afrikane is verlaagde NOx response getoon met die Stroop kleur-woord konfliktoets. Na stratifisering in hoë en lae NOx responsgroepe, is daar in die Afrikane saam met die lae NOx respons 'n verhoogde α-adrenerge respons met betekenisvolle ST-segment depressie getoon wat dui op verlaagde miokardiale suurstofvoorsiening tydens mentale stressor-blootstelling. Slegs in die Afrikane mans was die ST-segment depressie betekenisvol geassosieer met strukturele vaskulêre siekte. Daar is tot die gevolgtrekking gekom dat die Afrikane mans 'n kwesbare kardiovaskulêre profiel toon. In hierdie kohort van Afrikane mans kan die betekenisvolle assosiasie tussen strukturele vaskulêre siekte en miokardiale ischemie veral dui op 'n moontlike hoër risiko vir toekomstige kardiovaskulêre gebeurtenisse.

Algemene gevolgtrekking

Deur die evaluering van outonome en kardiovaskulêre response in ’n kohort van Afrikane en Kaukasiër mans, is ’n betekenisvolle hoër kardiovaskulêre risiko aangedui vir die Afrikane mans. In hierdie kohort was simpatiese hiperaktiwiteit duidelik, gekoppel aan dominante vaskulêre responspatrone en verlaagde miokardiale suurstofvoorsiening tydens mentale stressor-blootstelling. Gebaseer op hierdie bevindinge is hierdie populasiegroep se risiko vir versnelde teikenorgaanskade sowel as vir toekomstige kardiovaskulêre gebeurtenisse oënskynlik betekenisvol hoër as dié van die Kaukasiër manlike kohort.

Sleutelwoorde: Outonome respons, kardiovaskulêre funksie, hipertensie, teikenorgaan-skade.

PREFACE

This thesis is presented in the article-format, consisting of peer-reviewed published or submitted articles. This format is approved, supported and defined by the North-West University guidelines for postgraduate PhD level studies. The first chapter of this thesis is a detailed literature overview, aside from the appropriate literature backgrounds discussed in each of the manuscripts. Chapters 2, 3 and 4 comprise the respective manuscripts in the form of original research articles. All articles were submitted for publication in peer reviewed journals. The promoter, co-promoter and assistant promoters were included as co-authors in each paper. The first author was responsible for initiation and all parts of this thesis, including literature searches, data mining and statistical analyses, the interpretation of results and writing of the research papers. All co-authors gave their consent that the research articles may form part of this thesis. References included in this thesis are according to the Vancouver format.

The first article was submitted to *Blood Pressure* journal (published), the second to *Heart, Lung and Circulation* (in rebuttal) and the third to *Journal of Hypertension* (submitted). Relevant references are given at the end of each chapter according to author's instructions of each specific journal where papers were submitted.

STATEMENT BY THE AUTHORS

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

Me AS Uys	Responsible for proposal of study, literature searches, design and planning of research articles and thesis, statistical analyses, interpretation of results and writing of entire thesis.
Prof L Malan	**Promoter.** Guidance, intellectual input. Supervised the initial planning of thesis and design, collection of data and writing of thesis. Project leader of the SABPA study.
Prof JM van Rooyen	**Co-promoter.** Intellectual input, data collection and assessment of content of thesis.
Prof HS Steyn	**Assistant Promoter.** Intellectual input and assessment of validity of statistical methods followed.
Prof Dr T Ziemssen	**Assistant Promoter.** Intellectual input in writing of research papers.
Dr M Reimann	**Assistant Promoter.** Intellectual input in writing of thesis.

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

I hereby declare that I approved the afore-mentioned manuscripts and that my role in this study, as stated above, is representative of my actual contribution and that I hereby give my consent that these manuscripts may be published as part of the PhD thesis of Aletta S Uys.

Prof L Malan

Prof JM van Rooyen

Prof HS Steyn

Dr M Reimann

Prof Dr T Ziemssen

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LIST OF ABBREVIATIONS

α – Alpha

β – Beta

Δ – Relative change

µmol/l – Micromole per litre

γ-GT – Gamma-glutamyl transferase

ABPM – Ambulatory blood pressure

ADH – Alcohol dehydrogenase

AIDS – Acquired Immune Deficiency Syndrome

AMS – Artery measurement system

ANCOVA – Analysis of covariance

ATP – Adenosine triphosphate

BMI – Body mass index

BP – Blood pressure

bpm – Beats per minute

BRS – Baroreceptor sensitivity

BSA – Body surface area

°C – Degrees Celsius

cAMP – cyclic adenosine monophosphate

C_w – Windkessel arterial compliance

CIMT – Carotid intima-media thickness

CIMTf – Carotid intima-media thickness of the far wall

cm – Centimetre

CO – Cardiac output

COMT – Catechol-O-methyltransferase

CRP – C-reactive protein

DBP – Diastolic blood pressure

DHPG – 3,4-dihydroxy-phenylglycol

ECG – Electrocardiogram

ECLA – Electrochemiluminescence immunoassay

ESH – European Society of Hypertension

Et al. – Et alia “and others”

FMS – Finapres Medical Systems

h – Hours

HIV – Human immunodeficiency virus

kcal – Kilocalories

kg – Kilogram

kg/m^2 – Kilograms per meter squared

HPLC – High performance liquid chromatography

ISAK – International society for the advancement of kinanthropometry

L/min – Litre per minute

LVH – Left ventricular hypertrophy

m^2 – Square metre

MAO – Monoamine oxidase

MD – Medical doctor

MHPG – 3-methoxy-4-hydroxy-phenylglycol

MHz – Mega Hertz

min – Minute

mg/L – Milligrams per litre

ml/mmHg – Millilitre per millimetre Mercury

mm – Millimetre

mmHg – Millimetre Mercury

mmHg/ml/s – Millimetre Mercury per millilitre per second

mmol/L – Millimole per Litre

MN – Metanephrines

ms/mmHg – Milliseconds per millimetre Mercury

mV – Millivolt

mV.ms – Millivolt by millisecond

n – Number of

ng/ml – Nanogram per millilitre

NMN – Normetanephrines

NO – Nitric oxide

NOx – Nitric oxide metabolite

NWU – North-West University

p – Probability

pmol/l – Picomole per litre

r – Correlation coefficient

R^2 – Relative predictive power of a model

RN – Registered nurse

SABPA – Sympathetic activity and Ambulatory Blood Pressure in Africans

SBP – Systolic blood pressure

SD – Standard deviation

SE – Standard error

SMAC – Sequential Multiple Analyser Computer

SV – Stroke volume

TPR – Total peripheral resistance

U/l – Units per litre

USA – United States of America

VMA – Vanillylmandelic acid

CHAPTER 1

LITERATURE STUDY, AIM AND HYPOTHESES

1. INTRODUCTION

Over 200 years ago, it was postulated that the muscle fibres of blood vessel walls may be under neural control.[1] Since then, research has shown that the heart as well as smooth muscle of the vascular walls are richly innervated with nerve fibres of the autonomic nervous system.[1]

The autonomic nervous system consists of two major pathways, the sympathetic and parasympathetic nervous system.[2,3] The reciprocal functioning of these two pathways allows the maintenance of homeostatic conditions, by continuously responding to internal and external stimuli (e.g. exercise, stressor exposure and orthostatic changes).[4,5] A concept, namely the sympatho-vagal balance, is often referred to when one system is activated and the other is inhibited.[6] Regarding the cardiovascular system, increased sympathetic stimulation and subsequent increase in catecholamine secretion leads to elevation in heart rate and contractile force as well as an increase in blood pressure.[7] Parasympathetic stimulation results in a slower heart beat and a decrease in blood pressure.[7] Activity of sympathetic and parasympathetic activity regarding cardiovascular control must be kept in check by a reflex arch known as the baroreceptor reflex.[8] This reflex arch appropriately adapts autonomic activity in response to cardiovascular changes.[8]

The autonomic nervous system not only plays a vital role in regulating instantaneous cardiovascular responses to stimuli,[6,9] it also regulates the circadian pattern the cardiovascular system follows.[8] During the day, the sympathetic nervous system dominates and appropriately responds to everyday stimuli (fight-or-flight response) by adapting catecholamine secretion appropriately. At night during sleep the dominating influence of the parasympathetic nervous system sets the body in its "*rest and digest"* mode by decreasing heart rate and blood pressure to much lower levels compared to that of daytime.[10]

Although this regulatory interaction may sound simple, it is far from it. The autonomic nervous system's involvement in cardiovascular control entails a complex and dynamic relationship of various messengers, receptors and effector cells.[11] This relationship varies from appropriate cardiovascular control by the baroreceptor reflex arch,[12] catecholamine secretion[9] as well as bio-availability of the powerful vasodilator, nitric oxide.[13] In the event of dysfunction of any of these components, pathological cardiovascular changes are promoted.[1] This literature review will focus on various aspects of autonomic control of the cardiovascular system (baroreceptor sensitivity, catecholamine secretion and nitric oxide bio-availability). The pathology associated with cardiovascular autonomic dysregulation will particularly be assessed and what this may entail for urban African men.

2. AN OVERVIEW OF AUTONOMIC CONTROL OF CARDIOVASCULAR FUNCTION

2.1. Central nervous system control

Although the autonomic nervous system is responsible for cardiovascular control, its activity is regulated by other sections of the central nervous system including the medulla oblongata, hypothalamus and cortical regions.[14] Located within the medulla are the cell bodies of both sympathetic and parasympathetic afferent nerves. The hypothalamus is responsible for integration and coordination of medullary activity and higher cortical regions are responsible for adapting autonomic control associated with emotion or fear, e.g. blushing or increased heart rate and other.[14] Peripheral receptors located throughout the body send sensory information to specific regions of the brain and autonomic activity is adjusted to alter cardiovascular function accordingly.[15]

2.2. The sympathetic nervous system and its role in cardiovascular control

Neurons that are responsible for the sympathetic (or adrenergic) control of the cardiovascular system originate in the medulla oblongata, their axons (preganglionic fibres) progress down the spinal cord and exit between segments T-1 and L-2 (from the thoracic and lumbar regions).[2,16] Some of the preganglionic sympathetic nerves enter paravertebral ganglia and travel within the ganglia to synapse with postganglionic fibres, which innervate with the heart and blood vessels. Other preganglionic sympathetic nerves, generally from the lower thoracic and upper lumbar regions, synapse in pre-vertebral ganglia and synapse with postganglionic fibres. These fibres primarily innervate the vasculature (Figure 1).[15] Additionally, a collection of postganglionic sympathetic nerves never develop axons. Jointly, they form the endocrine glands called the adrenal medullae.[3]

Within the terminal endings of the sympathetic nerves, norepinephrine, the main neurotransmitter, and epinephrine are synthesized and stored in vesicles (Figure 2).[17] Epinephrine and norepinephrine are collectively called catecholamines. Upon adrenergic stimulation, the storage vesicles release their contents into the synaptic gap. Located on the external cell membrane of the effector cells (heart and blood vessels) are specific areas where the catecholamines bind, called adrenergic receptors. These receptors are divided into two subtypes, alpha (α) and beta (β) which in turn are divided into types one and two.[2]

In response to sympathetic stimulation, e.g. in the event of stress exposure, the adrenal medullae additionally releases epinephrine and norepinephrine into the circulation. The additive effect of the secreted catecholamines is relatively slower compared to the effect of direct sympathetic stimulation of the vasculature and the heart.[3]

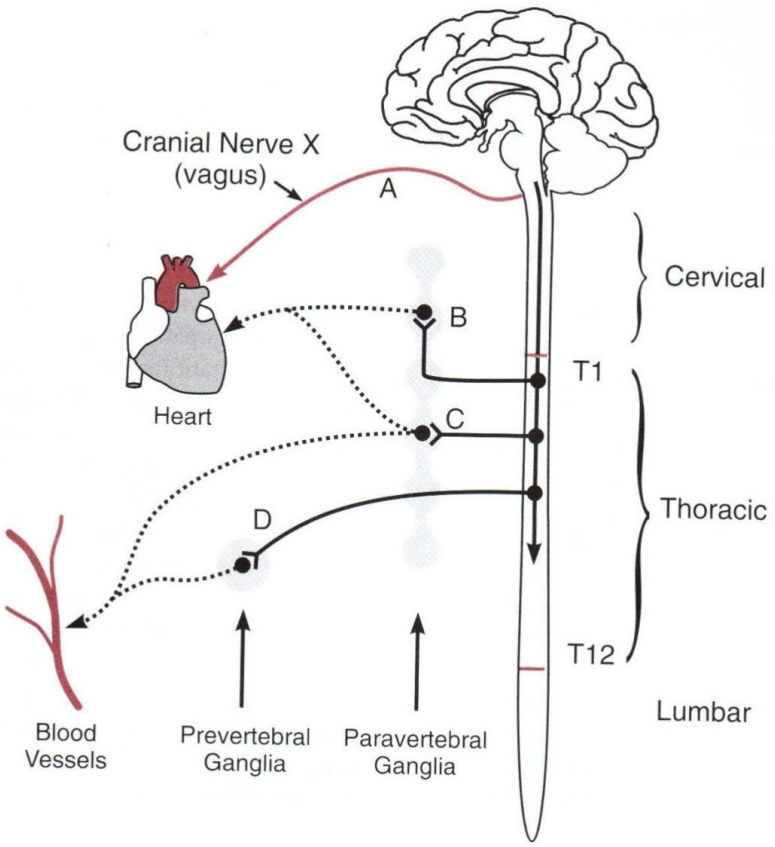

Figure 1: Innervation of sympathetic and parasympathetic nerves of the heart and blood vessels. Preganglionic fibres of the tenth cranial nerve (parasympathetic) represented by the solid red line (A), travel to the heart. Some of the preganglionic fibres that arise from thoracic and lumbar regions enter paravertebral ganglia to synapse above (B) or below entry level, others at their level of entry (C). The dotted black line represents the cervical ganglia which innervate the heart and the thoracic ganglia of which the latter innervate blood vessels as well as the heart. Preganglionic fibres originating from the upper lumbar and lower thoracic regions synapse with prevertebral ganglia (D), which travel to the vasculature. Excerpt from "*Klabunde.2005*"[15]

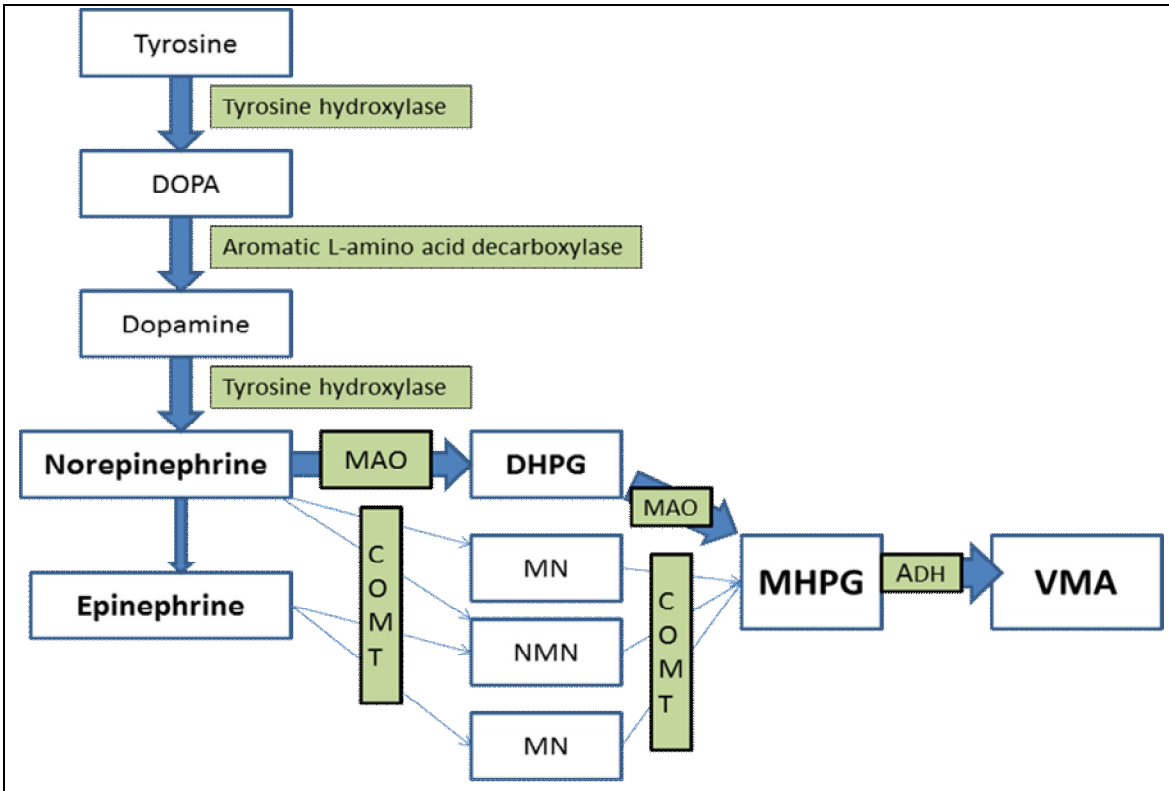

Figure 2: Diagram illustrating the path from catecholamine synthesis to elimination. The major metabolic pathway is indicated by the thick blue line. Abbreviations: MAO, monoamine oxidase; DHPG, 3,4-dihydroxy-phynylglycol; COMT, catechol-O-methyltransferase; MN, metanephrines; NMN, normetanephrines; MHPG, 3-methoxy-4-hydroxy-phenylglycol; ADH, alcohol dehydrogenase; VMA, vanillylmandelic acid. Adapted from "*Currie et al., 2012"* and "*Eisenhofer et al., 2004"* [17,18]

2.2.1. Adrenergic control of the heart

Located within the heart (myocardium as well as conduction system) are β_1-adrenergic receptors.[16] Stimulation of the receptors located in the Sino-atrial node increases activity of the enzyme adenylate cyclase, which stimulates the G_s-protein (stimulatory G protein). This protein in turn increases the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), increasing the rate of spontaneous pacemaking.[9,15,19] As a result, the heart rate increases. Within the myocardium, cAMP increases the amount of open calcium channels, promoting the greater influx of calcium into the myocardial cells. The

release of calcium from the sarcoplasmic reticulum stores are increased as well. This results in a significant increase of the heart's contractile force.[20] Sympathetic stimulation therefore increases heart beats per minute as well as the force of each contraction.[7]

2.2.2. Adrenergic control of the vasculature

Sympathetic stimulation is the key role player of blood pressure regulation.[8,21] There are two types of adrenergic receptors involved in the control of the diameter of arteries and arterioles.[2] Stimulation of the α-adrenergic receptor type 1 results in vasoconstriction whilst stimulation of α-adrenergic receptor type 2 results in vasodilation. However, a predominantly vasoconstrictory effect is achieved by binding of norepinephrine to α_1-adrenergic receptors on the vascular smooth muscle.[8] Vasoconstriction is accomplished by activation of another G-protein (G_h-protein) which increases the activity of the enzyme, phospholipase C. This enzyme splits the compound phosphatidylinositol into IP_3 (Inositol Triphosphate) and DAG (Diacylglycerol). IP_3 increases the release of calcium from sarcoplasmic reticulum stores, resulting in contraction of vascular smooth muscle and reduction of vascular diameter.[8] Pressure within the blood vessel increases as a result.

Opposing the vasoconstrictory effect of α-adrenergic stimulation is the vasodilatory effect of β_2-adrenergic receptor stimulation.[16] Binding of epinephrine and norepinephrine to this receptor increases the activity of the calcium pump of the sarcoplasmic reticulum, thereby enhancing the reuptake of calcium from cytoplasm.[15] Interestingly, the overall effect of sympathetic stimulation of the vasculature results in vasoconstriction.[8] It has been theorized that this is due to a possible larger density of α_1-adrenergic receptors and that the receptors are located closer to the site of norepinephrine release.[8]

It should also be noted that sympathetic stimulation of the vasculature never ceases. Instead, due to continuous tonic sympathetic stimulation, blood vessels are continuously in a

partial state of constriction to maintain vascular tone, which varies depending on the rate of sympathetic stimulation.[22]

2.2.3. Catecholamine elimination

After being released, catecholamines are eliminated by various pathways. There are three routes of elimination, which entails diffusion into the circulation, destruction by tissue enzymes, and elimination through re-uptake by the adrenergic nerve endings.[18] The latter is the main route of catecholamine metabolism.[9] Neuronal and extra-neuronal metabolisation of catecholamines is depicted in Figure 2. Through the various routes of elimination, the major metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG) is formed and converted mainly in the liver (by oxidation) to the end-product of epinephrine and norepinephrine metabolism, vanillylmandelic acid (VMA) which is excreted in the urine.[18] MHPG is often measured experimentally in plasma and saliva as indication of sympathetic activity.[23-25]

2.2.4. Adrenergic stress response patterns

The sympathetic system enables the body to respond almost instantaneously during daily life to internal and external stimuli, often referred to as the "*fight or flight*" reaction.[10] There are two major types of response patterns, α- or β-adrenergic, that may occur in the event of stress exposure or a mixture of the two. An α-adrenergic response pattern, which is characterised by increased total peripheral resistance, diastolic blood pressure as well as a decrease in arterial compliance.[8] A dominant β-adrenergic response pattern entails an increase in systolic blood pressure, heart rate and cardiac output.[8] Acute laboratory stress exposure has been used by researchers for years with the aim to mimic everyday life situations and observe the cardiovascular responses that are stimulated.[26] Some stressors are used to evoke a dominant α-adrenergic response and others a dominant β-adrenergic response.[27]

A popular laboratory stressor used to evoke a dominant α-adrenergic response is the cold pressor test.[27] This stressor entails the immersion of a hand or foot in icy water.[28] It has been postulated that if individuals display an exaggerated vascular response to the cold pressor test, they are likely to develop hypertension.[28] Another mental stress test often used, is the colour-word conflict test. Successive series of five colour words written in incongruent colours are shown in random order. The person exposed to this test is then required to recognize and verbally confirm the colour of a given word within a certain time limit.[29] This laboratory stressor has been noted to elicit a mixed α-β adrenergic response.[27]

However, contradictory to the above-mentioned, an alternative theory has been developed. Kamarck et al. [29] and Lovallo et al.[30] both noted that an individual is more likely to have a dominant adrenergic type response in general, rather than the response depending on the stressor. Therefore they are likely to have a "vascular" (α) or "cardiac" (β) response pattern, regardless of the stressor. However, it also depends on lifestyle factors. When urban dwelling Africans are exposed to stress, an α-adrenergic response pattern dominates.[31] However, in Africans residing in a rural environment, stressor exposure rather elicits a central β-adrenergic response pattern.[31] This shift of a central to a peripheral response pattern may be due to the adaptation of individuals, residing in an urban environment, to westernized habits. These habits include smoking, excessive alcohol use, unhealthy diets (processed foods, high salt intake) and lower physical activity.[32,33]

2.3. The parasympathetic nervous system and its role in cardiovascular control

Preganglionic parasympathetic nerve fibres (also referred to as cholinergic fibres) originate within the medulla oblongata. Their cell bodies are found in collective regions called dorsal vagal nucleus and nucleus ambiguous (collectively the cardio-inhibitory centre).[15] Efferent fibres leave the medulla oblongata through cranial nerves III, IX and X and innervate the

heart and vasculature.[2,16,34] Within postganglionic cholinergic fibres, the neurotransmitter acetylcholine is synthesized and stored within vesicles.[34] When stimulated, acetylcholine is released and binds to a specific site located on the cell membrane of the effector cell, called a muscarinic receptor.[3,20]

2.3.1. Cholinergic control of the heart

Upon cholinergic stimulation, acetylcholine is released from parasympathetic terminal nerve endings at specific sites of the heart.[15] Occupation of sino-atrial node muscarinic receptors stimulates the α_i component of the G-protein, opening the G-dependent potassium channels.[35] Permeability for potassium is increased, promoting the outward flow of potassium which results in slower spontaneous action potential generation (pacemaking). Furthermore, binding of acetylcholine to muscarinic receptors of myocytes stimulates the inhibitory G-protein, decreasing the activity of the enzyme adenylate cyclase. The formation of cAMP is hampered, reducing the entry of calcium into cytoplasm and thus the contractile force of the heart.[8,14]

2.3.2. Cholinergic control of the vasculature

The direct vascular innervation by parasympathetic nerves is to a much lesser extent than sympathetic innervation.[12] In the vasculature receiving parasympathetic stimulation, e.g. the coronary circulation,[36] acetylcholine binds to muscarinic receptors located on the vascular endothelium, stimulating the release of the vasodilating agent, nitric oxide (NO).[8] Nitric oxide is synthesized from the semi-essential amino acid, L-arginine.[37] This process is catalized by the enzyme, nitric oxide synthase (NOS), which has three isoforms. The isoforms include endothelial NOS (eNOS), neuronal NOS (nNOS) and immunological NOS (iNOS).[37] In the vascular smooth muscle, NO stimulates the enzyme guanylate cyclase, in turn increasing the formation of vasodilatory cyclic guanosine monophosphate.[8]

2.3.3. Elimination of acetylcholine

After its release into the synaptic space, acetylcholine will continue to stimulate the cholinergic receptors until it is metabolised. This is achieved by the enzyme, acetylcholinesterase, splitting acetylcholine into its metabolites choline and acetate.[10] Choline is reabsorbed by the presynaptic terminal, where it is used to synthesize asetylcholine.[3]

2.4. Regulating adrenergic and cholinergic control of cardiovascular function – the baroreceptor reflex

Arterial pressure is continuously monitored by a powerful central operating system.[38] This negative feedback system, known as the baroreceptor reflex, prevents blood pressure from increasing too high or decreasing too low, by promptly adjusting the autonomic stimulation of the cardiovascular system.[38] The sensitivity of the baroreceptor reflex system is often used to evaluate the efficacy of autonomic control of the cardiovascular system.[12]

Baroreceptors are mainly found in the aorta and carotid arteries, with major density in the aortic arch and carotid bifurcations.[2] These mechanoreceptors respond to stretching of the arterial walls. In the event of arterial pressure elevation, the receptors respond by increasing their rate of action potential generation.[7] The largest increase in firing rate of baroreceptors occurs at a specific mean arterial pressure value at about 100 mmHg, known as the set point.[39]

Action potentials generated in baroreceptors, which are located in the carotid bifurcation, travel along the carotid sinus nerve, joining the glossopharyngeal nerves before entering the medulla oblangata. From aortic baroreceptors, afferent fibres travel along the vagus nerves travelling to the medulla oblangata.[14] Signals are particularly conveyed to the nucleus tractus

solitarius and relayed to higher brain structures. This includes the hypothalamus, where integration of signals takes place.[40] The blood vessels and heart therefore are the effectors of the reflex arch. In response to an increased rate of signals received from the baroreceptors, tonic sympathetic stimulation of the cardiovascular system is inhibited whilst activity of cardiac parasympathetic nerves is augmented.[14] Reflex bradycardia (reduced heart rate) as well as a reduction in heart contractile force, total peripheral resistance and venous return occurs as a result. These changes collectively result in blood pressure reduction.[12]

In the event of a decrease in blood pressure, the rate of signals received by the medulla oblangata from the baroreceptors lessens. In response, the medulla oblangata induces increased adrenergic stimulation and subsequently an attenuation of vagal stimulation of the cardiovascular system.[15] The result is an increase in heart rate (tachycardia), increased contractile force as well as elevation of total peripheral resistance and venous return, all contributing to an increase of arterial pressure.[12]

2.5. Relationship between autonomic cardiovascular control and the endothelium

The autonomic control of cardiovascular responses cannot be studied without taking into account the important role of the endothelium.[13] The endothelium, a single cellular layer forming the inner lining of blood vessels maintains the balance between the effects of sympathetic and parasympathetic stimulation of the vasculature.[13] During rest, continuous blood flow through the vascular system causes friction against blood vessel lining, which is called shear stress.[20] This friction stimulates the endothelium to release NO which counteracts the vasoconstrictive effect of tonic sympathetic stimulation.[41] In the event of increased sympathetic stimulation, an augmentation of shear stress occurs as a result of vasoconstriction and subsequent increase in blood flow.[42] This stimulates greater endothelial

release of NO, promoting vasodilation by inhibiting central and peripheral sympathetic activity.[43]

Located on endothelial cells are α_2- as well as β_2-adrenergic receptors. Sympathetic stimulation of α_2-receptors results in endothelial release of NO, thereby counteracting the vasoconstrictive effect of vascular smooth muscle α_1-adrenergic receptor stimulation.[44] The function of endothelial β-adrenergic receptors is still quite unclear, although it has been postulated that they also have a role in promoting vasodilation through NO release.[44] It is important to note that the endothelial layer must be intact for it to elicit a vasodilatory effect through NO release.[50] In the event of endothelial damage, NO bio-availability is reduced significantly. Vasoconstriction is particularly promoted, decreasing vascular diameter and resulting in reduced blood supply to the specific affected area.[13]

3. AUTONOMIC AND CARDIOVASCULAR DYSFUNCTION

3.1. The neurogenic component of hypertension

The focus to this point has been on how the autonomic nervous system achieves its goal in controlling cardiovascular function to meet the demands of everyday life. With the ever increasing prevalence of cardiovascular disease, the possible contribution of autonomic cardiovascular dysregulation cannot be ignored. It comes as no surprise that indeed, a significant association was established between cardiovascular disease, particularly hypertension and sympatho-vagal imbalance.[46] This imbalance is characterised by sympathetic hyperactivity (demonstrated through elevated norepinephrine spill-over and increased rate of sympathetic nerve firing) as well as decreased parasympathetic activity.[47-50]

To date it is still unclear what initially causes the sympatho-vagal imbalance.[7] Growing evidence indicates chronic exposure to psychosocial stress as a possible culprit.[51] However, not only the exposure, but also the response elicited need to be taken into account. Exaggerated cardiovascular responses have significantly been linked to the development of hypertension.[31,52] Light et al.[53] demonstrated that exaggerated cardiovascular reactivity particularly promoted by high levels of stress exposure during everyday life predicted elevation of blood pressure over 10 years.

By observing an individual's response pattern to an acute, controlled physical or mental stressor, an indication may be given of the complex physiological mechanisms involved in cardiovascular disease development.[54,55] As previously mentioned, certain individuals are prone to have a vascular (α) type response whilst others exhibit a predominant cardiac (β) response pattern.[25,49] In hypertensive individuals, an exaggerated vascular response, coupled with reduced β-adrenergic response, has proven to promote chronic blood pressure elevation.[56,57]

3.1.1. Reduced baroreceptor sensitivity

Another underlying neurogenic mechanism for hypertension development is the reduction of baroreceptor sensitivity (BRS).[21] The question may be raised as to why? The answer is multifaceted. The emphasis, however, falls on the attenuation of BRS as manifestation of sympathetic hyperactivity as well as reduced parasympathetic control.[11,58,59]

In an attempt to continuously compensate for exaggerated vascular responses, the baroreceptors are overstimulated, resulting in desensitization.[2] With frequently occurring episodes of blood pressure elevation coupled with exaggerated cardiovascular responses,

hypertension development is accelerated.[60] It has also been indicated that if blood pressure levels remain elevated for a prolonged period, the firing rate of the baroreceptors decrease gradually. Eventually it reaches the initial set point firing rate,[14,61] hereby contributing to maintaining higher blood pressure.[11] If blood pressure levels remain high, subsequent remodelling of arterial walls and increase in stiffness occurs. This further impairs the baroreceptors' ability to stretch and therefore their ability to respond to elevation of blood pressure.[11,62] Sympathetic tone is sustained and parasympathetic inhibition impaired, markedly contributing to a hypertensive state.[63]

3.1.2. Nocturnal hypertension

Autonomic dysregulation of cardiovascular function is not only apparent during stress but also impairs the circadian rhythm of cardiovascular function.[64] This impairment is characterised by a shift from parasympathetic to sympathetic predominance during sleep,[65] promoting nocturnal blood pressure and heart rate elevation.[66] According to the European Society of Hypertension, nocturnal hypertension is defined as systolic and/or diastolic blood pressure above120 mmHg and 75 mmHg respectively.[67]

Abnormal nocturnal blood pressure is regarded as a significant risk factor for cardiovascular morbidity and mortality[68] by promoting target organ damage and future cardiovascular events.[69,70] Sympathetic hyperactivity in itself promotes structural vascular disease by increasing inflammatory responses as well as growth factor secretion. This eventually leads to vascular wall thickening and formation of atherosclerotic plaques.[13]

3.2. Target organ damage development

3.2.1. Atherosclerosis

Atherosclerosis is often referred to as a neurogenic phenomenon,[51] with sympathetic hyperactivity particularly contributing to the promotion of cardiovascular disease.[71] Sympathetic hyperactivity has a significantly detrimental influence on the endothelium.[13] With exaggerated stress-induced blood pressure responses resulting in excessive shear stress, the endothelial layer is eventually damaged[72-74] resulting in impaired NO release and eventually vasoconstriction.[75]

Endothelial dysfunction is considered one of the most pertinent contributing factors of atherogenesis.[75] Endothelial nitric oxide normally inhibits atherosclerotic processes by reducing leukocyte adhesion and platelet aggregation.[76] In the event of endothelial damage, disrupted NO bioavailability leads to increased adhesion and migration of leukocytes into vascular wall promoting inflammation, lipid incorporation and vascular disease development is promoted.[77,78] Growth factors are also released which stimulate smooth muscle cell proliferation and subsequently an increase in vascular wall thickness, inevitably resulting in full blown atherosclerosis.[78,79]

Endothelial dysfunction of the coronary circulation[80] leads to impaired coronary vasodilation in response to shear stress.[45] During sympathetic stimulation brought about by daily life occurrences, e.g. mental stress and exercise vasoconstriction is exaggerated at sites where the endothelium is damaged (Figure 3).[74] When cardiac work increases (as during physical activity) impaired vasodilation and exaggerated vasoconstriction elicits myocardial ischemia.[81,82] This is particularly indicated by an ST-segment depression on Holter ECG.[54,83] These plethora of events manifest inevitably as coronary artery disease[84] increasing the risk markedly for future cardiovascular events.[83]

3.2.2. Left ventricular hypertrophy

A growing body of evidence has indicated that sympathetic hyperactivity contributes to acceleration of left ventricular hypertrophy development.[85] Greenwood et al. [86] demonstrated the presence of greater sympathetic outflow results in an accelerated development of left ventricular hypertrophy in patients with hypertension. The sympathetically mediated increase in peripheral resistance and resultant arterial stiffening, leads to pulse wave augmentation which in turn increases cardiac afterload.[87] Thus, for a specific stroke volume, a higher more myocardial force is required to eject blood into the aorta. Over time cardiac myocytes undergo hypertrophic changes and become unable to relax. Diastolic dysfunction commences.[88]

Regardless of the additive effects of sympathetic hyperactivity to hypertension, adrenergic overdrive independently contributes to left ventricular hypertrophy.[86] Studies in animal models[89,90] and in humans have shown increased norepinephrine levels induce an increase of cardiac myocyte size (Figure 3).[86,91] The relationship between sympatho-vagal dysregulation and both left ventricular hypertrophy as well as diastolic dysfunction has particularly been demonstrated by reduced BRS.[92,93]

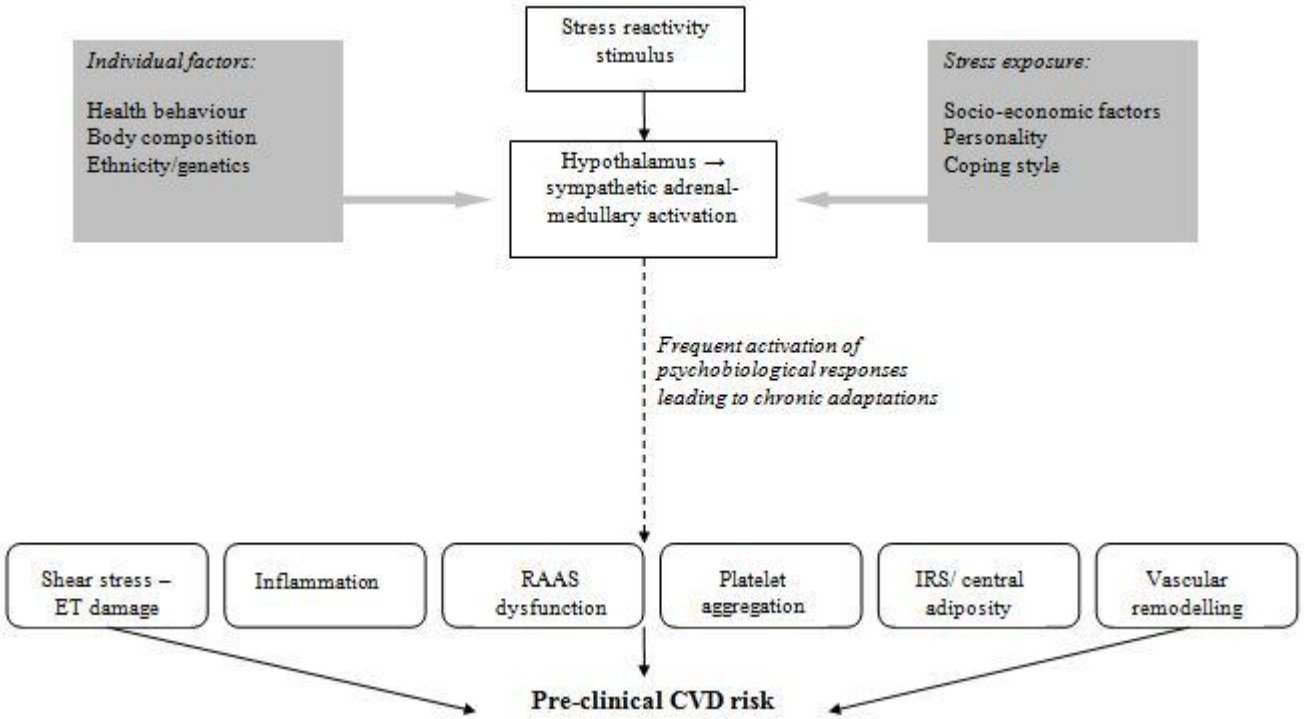

Figure 3: A proposed model of stress reactivity and cardiovascular disease. The mechanisms leading to pre-clinical risk may not act independently of one another, but to some extent be caused or influenced by each other. Abbreviations; ET, endothelial; RAAS, the renin-angiotensin-aldosterone system; IRS, insulin resistance; CVD, cardiovascular disease Excerpt from "*Hamer & Malan, 2010*"[32]

4. AUTONOMIC AND CARDIOVASCULAR DYSFUNCTION CONCERNING AFRICANS

The prevalence of developing cardiovascular diseases is ever growing globally.[94] It is predicted that by 2030 ischemic heart disease and stroke will be the major cause of mortality worldwide.[95] In sub-Saharan Africa the incidence of cardiovascular disease has been predicted to double by 2020.[96] A significantly higher prevalence of hypertension has been documented by the NHANES study in African Americans when compared to their Caucasian counterparts.[97] In this population group, especially men, research has implicated a high exposure to psychosocial stress[98] in turn resulting in sympathetic hyperactivity with an exaggerated vascular resistance response pattern.[99]

Regarding South Africa, hypertension in particular has also been indicated as a significant health issue for the urban dwelling black African community, especially men. [100-102] The same pattern has been implicated in cardiovascular disease development as is the case with African Americans. Sympathetic hyperactivity's involvement as possible contributing factor has also been postulated in this population group.[31,103,104] Studies have also indicated that Africans have a propensity of a dominant vascular response pattern when exposed to stressors,[31,54,104,105] increasing this population group's risk for cardiovascular disease.[100]

An additional alarming observation is that black population groups tend to have a high prevalence of nocturnal hypertension.[106] This may also be the case for urban Africans, based on the suggested sympatho-vagal imbalance,[66] rendering them even more vulnerable to target organ damage[85] and future cardiovascular events.[68]

The suggested sympatho-vagal imbalance in Africans, especially urban African men, paints a grim picture for their cardiovascular health. A limited number of studies have, however, attempted to assess different components of autonomic control of the cardiovascular system and related responses. It is particularly important to establish whether this suggested pattern of dysregulation does exist in this population group.

5. AIMS

5.1. Overarching aim

The main aim of this thesis was to examine and compare a cohort of African and Caucasian men regarding different aspects of autonomic control of the cardiovascular system including resting and mental stress responses. When the proposed presence of a sympatho-vagal imbalance is established in one of or both the groups, possible associations with bio-availability of NO, blood pressure elevation and target organ damage markers will be examined to determine possible cardiovascular risk.

5.2. Detailed aims

5.2.1. Chapter 2 – Baroreceptor sensitivity, cardiovascular responses and ECG left ventricular hypertrophy in men: The SABPA study

The aims of this research article were:

- To determine whether BRS is significantly lower in a cohort of urban African men than in their Caucasian counterparts.
- To determine whether attenuated BRS (during rest and stressor exposure) is significantly associated with blood pressure elevation as well as left ventricular hypertrophy in these population groups.

5.2.2. Chapter 3 – Nocturnal blood pressure, 3-methoxy-4-hydroxy-phenylglycol and carotid intima-media thickness: The SABPA study

The aim of this research article was:

- To assess differences between urban African and Caucasian men in terms of
    - Sympathetic activity with salivary 3-methoxy-4-hydroxy-phenylglycol as marker of sympathetic activity (resting responses were observed only as correlations were observed with nocturnal blood pressure, therefore a resting state).
    - Nocturnal blood pressure.
    - Structural vascular disease with carotid intima-media thickness as marker.

5.2.3. Chapter 4 – Nitric oxide, cardiovascular function and structural vascular disease in men: The SABPA study

The aim of this research article was:

- To explore the relationship between NO metabolite responses resting as well as to a mental stressor, cardiovascular function and structural vascular disease in a cohort of African and Caucasian men.

6. HYPOTHESES

6.1. Overarching hypothesis

The overarching hypothesis of this thesis was that the cohort of urban African men will demonstrate a disrupted sympatho-vagal balance, characterized by sympathetic hyperactivity and/or attenuated parasympathetic activity. This imbalance will be coupled by a dominant vascular resting and stressor response pattern, in other words a dominant

α-adrenergic response pattern). The above-mentioned will be associated with a deleterious cardiovascular profile and markers of target organ damage (Figure 4).

6.2. Detailed hypotheses

6.2.1. Chapter 2 – Baroreceptor sensitivity (BRS), cardiovascular responses and ECG left ventricular hypertrophy in men: The SABPA study

In this article it was hypothesized that:

- African men will have significantly lower BRS (during rest and stressor exposure) than the Caucasian men.
- In the African men, attenuated BRS will be associated with ambulatory blood pressure.
- In the African men, attenuated BRS will be associated with ECG left ventricular hypertrophy.

6.2.2. Chapter 3 – Nocturnal blood pressure, 3-methoxy-4-hydroxy-phenylglycol (MHPG) and carotid intima-media thickness: The SABPA study

In this article it was hypothesized that:

- The African men will display significantly higher nocturnal blood pressure values than the Caucasian men.
- MHPG will be significantly higher in the African men compared to their Caucasian counterparts.
- MHPG and nocturnal blood pressure will be associated with structural vascular disease in nocturnal hypertensive African men.

6.2.3. Chapter 4 – Nitric oxide, cardiovascular function and structural vascular disease in men: The SABPA study

In this article it was hypothesized that:

- African men will have attenuated NO metabolite resting and stressor responses compared to the Caucasian men.
- Attenuated NO responses will be associated with a detrimental cardiovascular profile and structural vascular disease in African men.

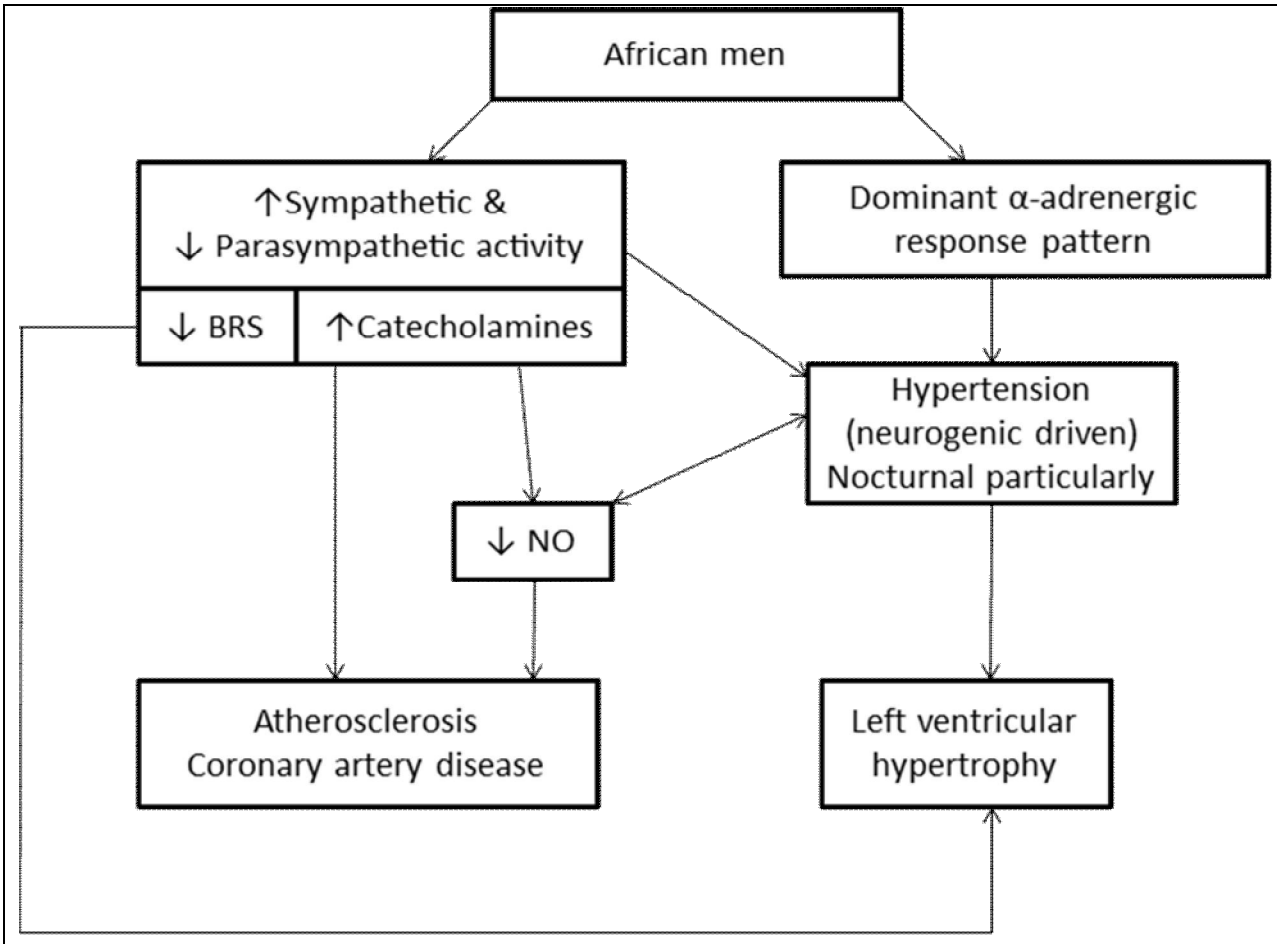

Figure 4: Hypothesized influence of a sympatho-vagal imbalance and dominant vascular (α-adrenergic) response pattern on the cardiovascular profile in a cohort of African men. *Symbols*: ↑, increase; ↓, decrease; α, alpha. *Abbreviations*: BRS, baroreceptor sensitivity; NO, nitric oxide.

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CHAPTER 2
RESEARCH ARTICLE 1

Blood Pressure, 2011; 20: 355–361

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ORIGINAL ARTICLE

Baroreceptor sensitivity, cardiovascular responses and ECG left ventricular hypertrophy in men: The SABPA study

LISA VAN LILL[1], LEONÉ MALAN[1], JOHANNES VAN ROOYEN[1], FAANS STEYN[2], MANJA REIMANN[3] & TJALF ZIEMSSEN[3]

[1]*Hypertension in Africa Research Team (HART), School for Physiology, Nutrition and Consumer Science Sciences,* [2]*Statistical Consultation Service, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, 2520, South Africa,* [3]*Department of Neurology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Germany*

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Abstract

Aim. Research has shown a significant relationship between hypertension and attenuated baroreceptor sensitivity (BRS), which in turn reflects alterations of autonomic control of the cardiovascular system. The objective of this study was to compare BRS of African and Caucasian men and determine possible associations with blood pressure and left ventricular hypertrophy. *Materials and methods*. Participants included African (N=82) and Caucasian (N=100) male teachers, aged between 20 and 65 years, recruited in the North-West Province, South Africa. Ambulatory blood pressure monitoring was conducted for a 22-23 hour period and, thereafter, cardiovascular parameters were recorded with a Finometer and 12-lead ECG during rest and while challenging the cardiovascular system with the cold pressor and Stroop colour-word conflict tests. Spontaneous BRS was calculated as well as the Cornell product [marker of left ventricular hypertrophy (LVH)]. *Results.* The African men had significantly lower BRS stress responses. Attenuated BRS coupled with an α-adrenergic response pattern predicted elevation of blood pressure in the African men. BRS reduction did not prove to be a significant predictor of LVH. *Conclusion.* Lower BRS, especially during stress, may pose a significant health threat for African men regarding earlier development or promotion of α-adrenergic-driven hypertension and greater risk for cardiovascular disease.

Keywords: *Baroreceptor sensitivity, cardiovascular responses, left ventricular hypertrophy, African, Caucasian.*

Introduction

The evaluation of baroreceptor sensitivity (BRS) has proven to be of great value in the assessment of autonomic control of the cardiovascular system (1) and changes of the sensitivity may indicate autonomic imbalance (2-4). This imbalance may be characterized by an increase of sympathetic activity, a decrease of parasympathetic activity or possibly a combination of both (1,5). Various researchers have found a significant relationship between BRS attenuation and chronic blood pressure elevation in hypertensive (6), borderline hypertensive (7-8) as well as normotensive subjects (2). Furthermore, high blood pressure, prolonged sympathetic hyperactivity and blunted BRS poses a significant threat to much faster development of target organ damage including left ventricular hypertrophy (LVH) (1,9). It is established that hypertension is one of the most pertinent health problems for urban black African men (10-13) and sympathetic hyperactivity has been implicated as a possible significant contributor (14). Whether a decrease of BRS contributes to hypertension in black African men, is unknown. Furthermore, other research indicated a lack of any significant differences in BRS where black participants (African American as well as West-Africans) were compared to Caucasians (15-17). Whether this is also the case in this population group is uncertain.

The aim of this study is, therefore, to determine if BRS is significantly lower in African men in comparison to their Caucasian counterparts and whether attenuation of BRS predicts an elevation of ambulatory blood pressure as well as LVH in these population groups.

Materials and Methods

Study design and population

The SABPA (**S**ympathetic **A**ctivity and **A**mbulatory **B**lood **P**ressure in **A**fricans) study was conducted in the North-West Province, South African during the same timeframe in 2008 and 2009 (to avoid seasonal changes). This target population comparative study included 82 black African (hereafter referred to as African) and 100 Caucasian male teachers. All participants worked as teachers for the Department of Education in the Dr Kenneth Kaunda Education District of the North-West Province. The reason for this selection was to obtain a homogenous sample from a similar socio-economic class. Participants were aged between 20 and 65 years and exclusion from the study was based on the following criteria: ear temperature >37.5°C, use of alpha and beta blockers, vaccinated or blood donors in the previous 3 months, as well as diabetic medication users (1 Caucasian and 6 African men) and clinically confirmed HIV positive status (13 African men).

All participants signed an informed consent form. The study complied with all applicable regulations, in particular, the Helsinki Declaration of 1975 (as revised in 1993) for investigation of human participants (18). The Ethics Review Board of the North-West University approved the study (project number: NWU-00036-07-S6).

Procedure

Ambulatory blood pressure measurements (ABPM) were conducted during working days. Between 0700 and 0800, participants were fitted with a British Hypertension Society validated apparatus (Meditech CE120® Cardiotens, Budapest, Hungary). Blood pressure cuffs of appropriate size were attached to the non-dominant arm. The ABPM apparatuses were programmed to measure blood pressure (oscillometrically) in 30 minute intervals during the day (0800-2200) and 60 minute intervals during the night (2200-0600) according to a preset program (19). The successful mean inflation rate for the 22h-23h ABPM period was

82.7% (±3.8%) in African and 94.6% (±3.7%) in Caucasian men respectively. The office blood pressure of each participant was also obtained. Actical® accelerometers (Montréal, Québec) were also applied to measure physical activity (in kilocalories) over the ABPM recording period, taking resting metabolic rate into account (20). Participants were asked to continue with their usual daily activities and complete ambulatory diary cards, reporting any abnormalities associated with cardiovascular disease such as headache, visual disturbances, nausea and fatigue (21). Participants spent the night at the Metabolic Unit Research Facility of the North-West University (consisting of ten bedrooms, two bathrooms, one kitchen, one dining room and one television room). Upon arriving, participants were introduced to the experimental setup to lessen anticipation stress (22). Each participant received HIV/AIDS pre-counselling from a registered nurse.

The following morning after the last ABPM at 0600 the ABPM and Actical® apparatuses were removed. Following anthropometric measurements, participants were in a semi-recumbent position for two hours. A resting 12-lead electrocardiograph activity of six cardiac cycles (Norav NHH1200® Kiryat Bialik, Israel) as well as 5 minute continuous measurement of cardiovascular variables was recorded (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). Hereafter, blood samples were collected from the participant's right arm brachial vein branches with a sterile winged infusion set. A rest period of 5-10 minutes was allowed before participants were exposed to two stressors in counterbalanced design. The duration of each stressor was one minute with a recovery period of 30 minutes between stressor applications. The stressors were the Stroop colour-word conflict test, which elicits a mixed α-β-adrenergic responsiveness and the Cold pressor test (foot immersed in icy water at ± 4˚C up to the ankle), which induces dominant α-adrenergic responsiveness (23). An increase of total peripheral resistance (TPR) and diastolic blood pressure (DBP) as well as a decrease of arterial compliance is indicative of a dominant α-adrenergic response pattern (24). A β-adrenergic response pattern is characterized by an increase of heart rate, systolic blood pressure (SBP) and cardiac output

(CO) (24). ECG and cardiovascular measurements were obtained during mental stress testing.

After completion of all procedures, participants were thanked for their participation, received a monetary incentive for the Colour-word conflict stressor according to their performance, enjoyed a breakfast and received post counselling for HIV/AIDS. After freshening up, participants were transported back to their workplace and received feedback on their health profile within one week.

Anthropometric measurements

The stretched stature of each participant was measured to the nearest 0.1 cm using a stadiometer. A KRUPS scale was used to determine the mass of each participant to the nearest 0.1 kg. The listed measurements were done in triplicate and body mass index (body mass/height^2) (BMI) calculated (25). Waist circumference was measured to the nearest 0.1 cm.

Questionnaires

Participants completed a general health questionnaire (indicating blood pressure, diabetic and statin-containing medication use) as well as a physical activity questionnaire. The Berlin questionnaire for risk assessment for sleep apnea (26) was also completed where categorization into low or high risk was based on various criteria including snoring, sleepiness during the day, BMI and blood pressure status.

Cardiovascular measurements

ABPM data was downloaded into a database using the CardioVisions 1.9 Personal Edition. Stressor responses across tasks were aggregated (27) and used to calculate reactivity using the formula: %=[(Xstressor – Xresting)/ Xresting] x 100). Finometer measurements were processed with Beatscope 1.1 software (FMS, Finapres Medical Systems, Amsterdam, The

Netherlands) to obtain SBP, DBP, CO, TPR and Windkessel arterial compliance (C_w) (28). Using software also developed by FMS, spontaneous BRS was calculated with the validated cross-correlation BRS method (29). This method computes BRS through time-domain analysis of spontaneous blood pressure and heart interval variability (29).

Biochemical measurements

Fasting blood samples were handled and prepared according to standardized procedures. HIV status was determined with an antibody test First Response Kit (Premier Medical Corporation LTD, Daman, India) and Confirmatory Pareekshak test (Bhat Bio-tech India (P) LTD, Bangalore, India). In serum, gamma-glutamyl transferase (γ-GT) (indicative of alcohol consumption) levels were determined, by making use of the Konelab TM 20i Sequential Multiple Analyzer Computer (SMAC) (ThermoScientific, Vantaa, Finland). Through homogeneous immunoassay of the serum samples, cotinine levels (indicative of smoking) and electrochemiluminescence immunoassay (ECLA), estrogen, were measured with the Roche Modular system (Roche, Basil, Switzerland).

ECG left ventricular hypertrophy

Data from the 12-lead ECG was used to determine the gender specific Cornell product as a marker of LVH. The Cornell product is calculated with the formula: (RaVL + SV3 ≥ 2.8 mV in men) * QRS > 244 mV.ms (30).

Statistical analysis

Statistica Version 9.0 (Statsoft Inc., Tulsa, OK, USA, 2009) was used for database management and statistical analysis. All variables used for analysis were normally distributed, determined by the Shapiro-Wilkes analysis. Means and proportions were compared by a standard t-test and the chi-square test, respectively. A one-way analysis of covariance (ANCOVA) using least square means was performed on cardiovascular and left ventricular hypertrophy variables to show significant differences between groups. Covariates

included age, γ-GT, cotinine, physical activity, estrogen, body surface area (for TPR indicated as TPR index and CO indicated as CO index) (31) and for cardiovascular responses, resting values were added as covariates. Due to the sensitivity of BRS for age as well as C_w, it was additionally included as covariate for BRS (6,29,32). Linear regression analysis, using the forward stepwise method determined associations between ambulatory BP (models 1 and 2) and the Cornell product (models 3 and 4) (dependent variables) and cardiovascular variables as well as apnea risk (independent variables). Results were regarded as statistically significant when $p \leq 0.05$.

Results

The characteristics of each group are described in Table I. The mean BMI of both African and Caucasian men fell within the overweight category (BMI 25-30 kg/m^2) (33). The African men had higher estrogen levels which were still within normal ranges (<184 pmol/l) (34), whereas their γ-GT levels were also higher compared to Caucasian men and exceeded the normal bounds of 0-65 u/l (35). In comparison to the Caucasian men, the R wave voltage in lead aVL of the African men was significantly higher.

In Figure 1, the African men demonstrated statistically significant higher ambulatory BP which was also within hypertensive ranges according to ESH guidelines (ambulatory SBP > 125 mm Hg and ambulatory DBP > 80 mm Hg) (21). They also had higher CO, heart rate and Cornell product in comparison to their Caucasian counterparts. The Cornell product values of both groups were still below the cutoff for LVH of 244 mV.ms (30). The African men also showed increased 24 hour DBP and α-adrenergic responses (decreased C_w and CO) during stressor exposure coupled to greater decrease of BRS in comparison to the Caucasian men (Figure 2).

Table I: Characteristics of participants as indicated through univariate analysis

	African men (N=82)	Caucasian men (N=100)	p
Age (years)	43.22 ± 8.08	44.96 ± 11.08	0.205
Body mass (kg)	80.72 ± 18.19	95.32 ± 17.65	<0.001*
Height (cm)	170.76 ± 6.21	181.17 ± 6.48	<0.001*
Body mass index (kg/m^2)	27.61 ± 5.79	29.03 ± 5.20	0.067
Waist circumference (cm)	93.51 ± 15.56	101.51 ± 14.44	0.001
Body surface area (m^2)	1.94 ± 0.23	2.18 ± 0.21	<0.001*
Berlin Sleep apnea high risk	29 (35.37%)	39 (39.00%)	0.897
Estrogen (pmol/l)	94.89 ± 34.74	73.46 ± 36.73	<0.001*
Office Systolic blood pressure (mm Hg)	147 ± 21	139 ± 17	0.001*
Office Diastolic blood pressure (mm Hg)	100 ± 13	90 ± 10	<0.001*
Hypertensive[#]	66 (80.49%)	56 (56.00%)	<0.001*
RaVL (mV)	0.38 ± 0.31	0.26 ± 0.20	0.002*
Lifestyle variables			
Cotinine (ng/ml)	35.10 ± 65.24	30.89 ± 96.69	0.718
Gamma-glutamyl transferase (u/l)	84.99 ± 92.15	34.72 ± 29.51	<0.001*
Physical activity (kcal)	2714.85 ± 800.12	3674.35 ± 2059.15	<0.001*
Medication use			

Blood pressure medication	19 (23.17%)	9 (9.00%)	0.039*
Statin medication	1 (0.99%)	6 (5.94%)	0.054*

Mean ± Standard deviation; p values ≤ 0.05 regarded as statistically significant*. Participants classified as hypertensive according to office blood pressure

(systolic blood pressure >140 and/or diastolic blood pressure >90)[#](21)

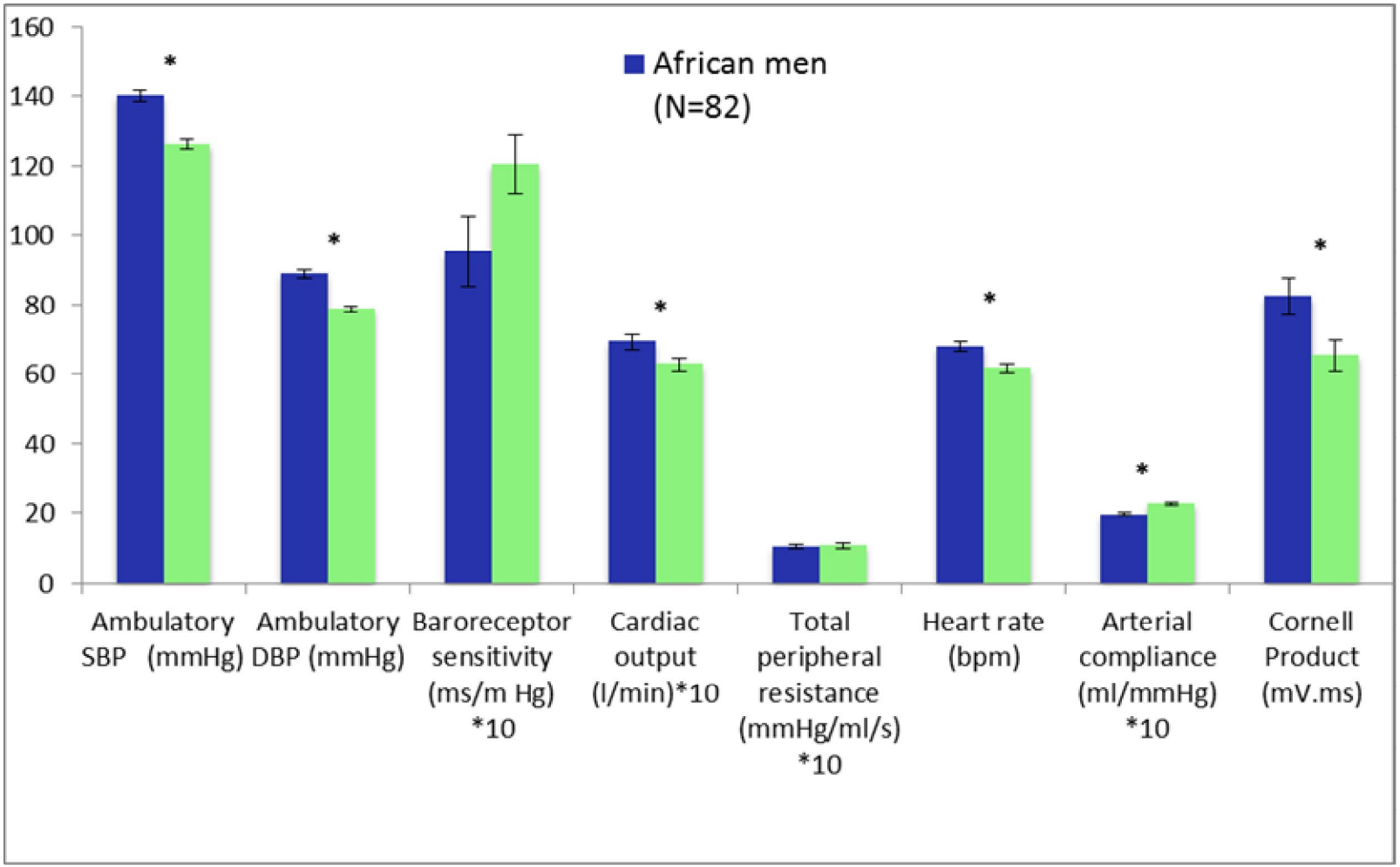

Figure 1: A comparison of resting cardiovascular and left ventricular hypertrophy variables between African and Caucasian men (adjusted for age, BSA, physical activity, cotinine, gamma-glutamyl transferase, arterial compliance additional for baroreceptor sensitivity); *significant differences ($p<0.05$).

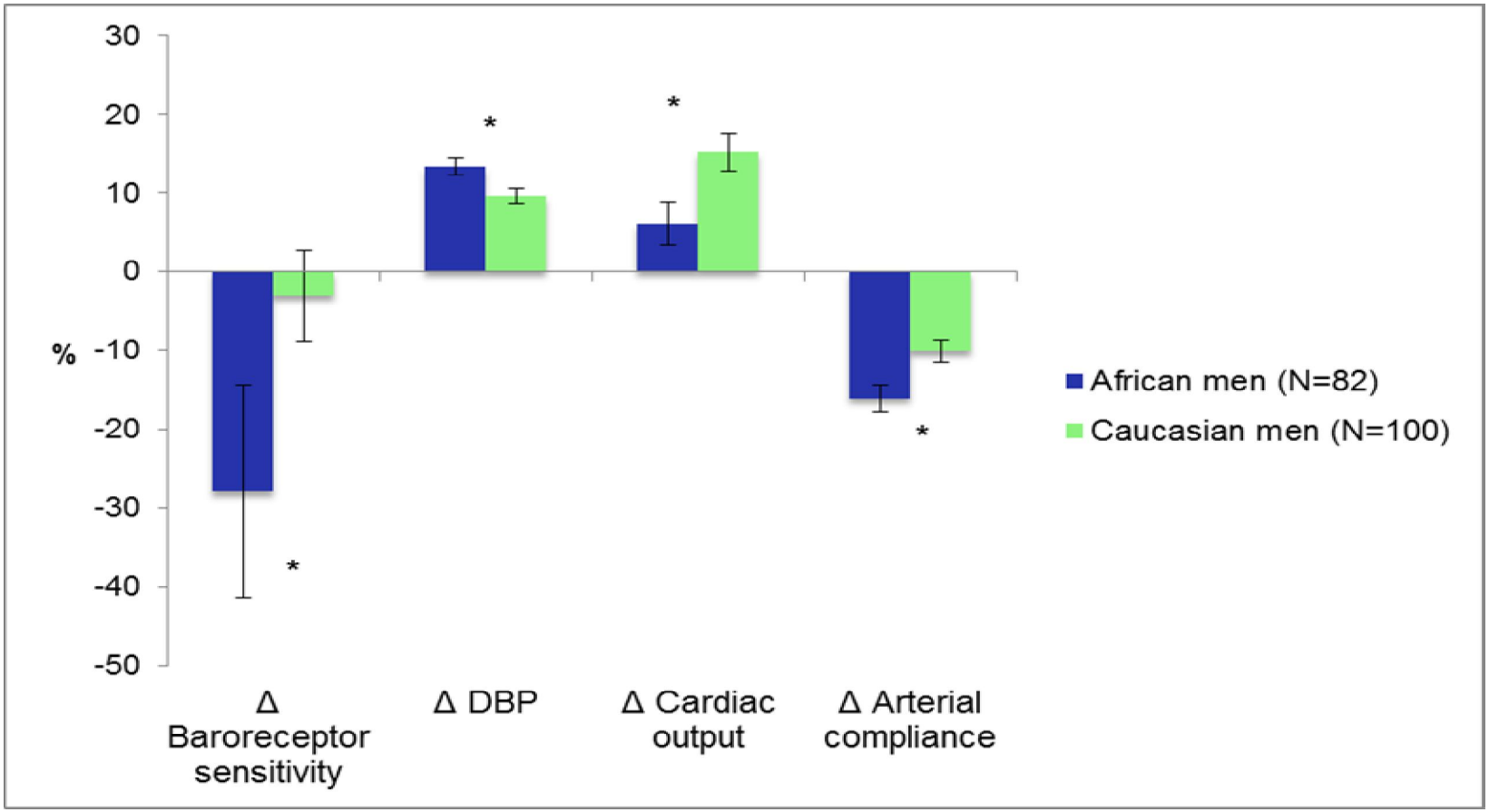

Figure 2 A comparison of the aggregated cardiovascular stress responses between African and Caucasian men (adjusted for age, BSA, physical activity, cotinine, gamma-glutamyl transferase, arterial compliance additional for BRS and resting cardiovascular variables); *significant differences ($p<0.05$).

Predictors of BP and ECG LVH

In Table II, model 1 (resting BP) attenuated resting BRS predicted ambulatory SBP whilst resting α-adrenergic responses i.e. C_w predicted ambulatory SBP and DBP, respectively in Africans. In Caucasians, the Berlin sleep apnea risk, resting β-adrenergic responses (decreased TPR index and increased CO) predicted ambulatory DBP and SBP respectively. In Table II, model 2 (BP %Δ) α-adrenergic responses (decreased C_w) predicted ambulatory BP in both ethnic groups. Additionally an α-adrenergic response, TPR index, was also associated with ambulatory DBP in African men. In Table III, model 3, only resting α-adrenergic responses, C_w predicted ECG LVH in African men.

Sensitivity analyses

Sensitivity analysis performed for usage of hypertension medication did not affect the outcome of our results. Additional analysis adjusting for height and weight was similar when adjusting for BSA.

Table II Independent associations of cardiovascular variables with ambulatory blood pressure in African and Caucasian men

	African men (N=82)				Caucasian men (N=100)			
	Ambulatory SBP (mm Hg)		Ambulatory DBP (mm Hg)		Ambulatory SBP (mm Hg)		Ambulatory DBP (mm Hg)	
Model 1 (Resting values):								
Adjusted R^2	**0.59**		**0.58**		**0.47**		**0.45**	
	β ± 95% CI	**p**	**β ± 95% CI**	**p**	**β ± SE**	**p**	**β ± SE**	**p**
Berlin Sleep apnea high risk	----	----	----	----	----	----	0.33 ± 0.18	<0.001
Arterial compliance (ml/mm Hg)	-0.84 ± 0.25	<0.001	-0.84 ± 0.24	<0.001	-1.01 ± 0.31	<0.001	-0.84 ± 0.27	<0.001
Cardiac output (l/min)	----	----	----	----	----	----	0.33 ± 0.22	0.004
Total peripheral resistance (mm Hg/ml/s)	----	----	----	----	-0.24 ± 0.18	0.010	----	----
Baroreceptor sensitivity (ms/mm Hg)	-0.24 ± 0.18	0.011	----	----	----	----	----	----
Model 2 (Stressor application):								
Adjusted R^2	**0.68**		**0.70**		**0.56**		**0.49**	
	β ± 95% CI	**p**	**β ± 95% CI**	**p**	**β ± 95% CI**	**p**	**β ± 95% CI**	**p**
Δ Arterial compliance (%)	-0.31 ± 0.14	<0.001	-0.21 ± 0.16	0.008	-0.33 ± 0.18	<0.001	-0.31 ± 0.20	<0.001
Δ Total peripheral resistance (%)	----	----	0.18 ± 0.14	0.013	----	----	----	----

F to enter 2.5;

Model 1: Ambulatory SBP and Ambulatory DBP resting: Adjusted for age, TPR and CO indexes, physical activity, Gamma-glutamyl transferase, cotinine, estrogen and arterial compliance (for baroreceptor sensitivity);

Model 2: Ambulatory SBP and Ambulatory DBP responses: Adjusted for age, TPR and CO indexes, physical activity, Gamma-glutamyl transferase, cotinine, estrogen, arterial compliance (for baroreceptor sensitivity) and for all resting cardiovascular values;

Table III Independent associations of cardiovascular variables with the Cornell product in African and Caucasian men

	African men (N=82)		Caucasian men (N=100)	
	Cornell product (mV.ms)		**Cornell product (mV.ms)**	
Model 3 (Resting values):				
Adjusted R^2	0.21		0.04	
	β ± 95% CI	**p**	**β ± 95% CI**	**p**
Arterial compliance (ml/mm Hg)	-0.69 ± 0.18	<0.001	----	----
Model 4 (Stressor application):				
Adjusted R^2	**----**		**----**	
	β ± 95% CI	**p**	**β ± 95% CI**	**p**
	----	**----**	**----**	**----**

F to enter 2.5;

Model 3: Ambulatory SBP and Ambulatory DBP resting: Adjusted for age, TPR and CO indexes, physical activity, Gamma-glutamyl transferase, cotinine, estrogen and arterial compliance (for baroreceptor sensitivity);

Model 4: Ambulatory SBP and Ambulatory DBP responses: Adjusted for age, TPR and CO indexes, physical activity, Gamma-glutamyl transferase, cotinine, estrogen, arterial compliance (for baroreceptor sensitivity) and for all resting cardiovascular values;

Discussion

The aim of the study was to determine if BRS is significantly lower in African men when compared to their Caucasian counterparts and whether attenuation of BRS predicts an elevation of ambulatory BP as well as LVH in these population groups. Our main findings revealed that resting attenuated BRS and decreased arterial compliance predicted the elevation of ambulatory blood pressure, although only decreased arterial compliance predicted LVH in African men. The BP profile of the Caucasian men was stronger associated with increased obesity, sleep apnea and related decrease in arterial compliance.

Despite different algorithms of BRS determination, the lack of ethnic differences in resting BRS in our study is in agreement with previous findings in African-Americans (15-16) and West-Africans (17). However, further results in our participant group showed greater decreases in BRS responses to mental stress in the African men.

Findings of this study also support the notion that reduced BRS contributes to chronic blood pressure elevation (3). This significant association was only present in African men who showed a dominant α-adrenergic sympathetic response pattern (24) and a reduced BRS during stressor application in contrast to the Caucasian men. These findings are contradictory to those of Parmer and co-workers (15) where similar abnormalities in autonomic control of the cardiovascular system, including attenuated BRS and α-adrenergic driven blood pressure elevation, were found for black (African-American) and Caucasian participants. The notion that an imbalance of sympathetic and parasympathetic activity is accompanied by blunted BRS and specifically may contribute to blood pressure elevation was nonetheless supported (4-5).

In contrast to other studies (1,9), our findings did not indicate lower BRS as a possible predictor for LVH, although the Cornell product of the African men was significantly higher. It can be speculated that this result may be due to the fact that a large part of the participant

group was still in their forties and represent a normal population. The LVH index average was still well under the cut-off point, which could mask a possible trend. However, significant associations between blunted BRS and increased risk for cardiovascular disease were demonstrated in other studies, especially in the presence of elevated blood pressure (36-38). Our data could also support findings by Verdeccia et al (39) who determined that the risk for cardiovascular disease increases by 9% for each 0.1 mV higher value of the R wave voltage in lead aVL. Accordingly, the risk for cardiovascular disease in African men would be 10.8% greater in comparison to the Caucasian men and the risk could be even potentiated by a reduced BRS (40,41).

Although BRS did not predict LVH, results of this study may still have further clinical implications. It has been indicated that before morphological changes of the left ventricle can be detected, functional changes may already be present (42). Several studies have found significantly lower BRS in patients with diastolic dysfunction (9,42,43). This is further aggravated by chronically elevated sympathetic activity (43,44) especially elevated heart rate (45). If we consider these results it is imperative to further explore both functional and structural changes e.g., the catecholamine, nitric oxide as well as echocardiography profiles in the African men.

A limitation of our study is that no direct marker of sympathetic nervous system activity was used. An advantage however is the use of golden standard blood pressure measurements ensuring high reliability results. As it was a cross sectional study we could not test or deduce causal factors or the causal reverse of reduced BRS driving chronic elevated blood pressure.

In conclusion, lower BRS, especially during stress, as demonstrated in our male African group may contribute to the development or promotion of α-adrenergic response driven

hypertension. If this is coupled to higher RaVL and Cornell product values, it may increase the risk for future cardiovascular events (46).

Acknowledgements

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CHAPTER 3
RESEARCH ARTICLE 2

Nocturnal blood pressure, 3-methoxy-4-hydroxyphenylglycol and carotid intima-media thickness: the SABPA study

Aletta S Uys (MSc)[1]; Leoné Malan (RN, PhD)[1]; Johannes M van Rooyen (DSc)[1]; Hendrik S Steyn (DSc)[2]; Tjalf Ziemssen (MD)[3]; Manja Reimann (PhD)[3]

[1]Hypertension in Africa Research Team (HART); North-West University, Potchefstroom Campus, South Africa.

2.Statistical Consultation Service, North-West University, Potchefstroom Campus, Potchefstroom, South Africa.

[3]Department of Neurology, University Hospital Carl Gustav Carus, Dresden University of Technology.

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INSTRUCTIONS FOR AUTHORS

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- Financial support for the project should be acknowledged or ‘no external financial support’ declared.
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Abstract

Background: Research demonstrated a significant relationship between elevated nocturnal blood pressure and sympathetic hyperactivity. The study aimed to investigate possible associations between the norepinephrine metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), nocturnal BP and carotid intima-media thickness (CIMT) in urban African and Caucasian men.

Methods: The study included 82 African and 100 Caucasian male teachers, aged 33-56 years, recruited in the North-West Province, South Africa. Ambulatory BP and fasting saliva and blood samples were collected. B-mode ultrasound images were obtained to determine CIMT.

Results: Despite higher usage of anti-hypertensive medication ($p=0.039$), a large number of the African men were nocturnal hypertensives (75, 61%). The nocturnal systolic blood pressure (SBP) ($p<0.001$), diastolic blood pressure (DBP) ($p<0.001$) and heart rate ($p<0.001$) of the African men were higher. After stratifying groups into only nocturnal hypertensives the trend was the same (SBP $p<0.001$; DBP $p<0.001$; heart rate $p=0.058$). In the African and Caucasian men, CIMT was associated with SBP ($\beta=0.33$, $p<0.001$) and DBP ($\beta=0.24$, $p=0.016$) respectively, but not MHPG.

Conclusion: No associations were firstly demonstrated between MHPG as sympathetic activity marker and CIMT or secondly, between MHPG and nocturnal blood pressure. Findings of elevated nocturnal BP evidently seem to promote structural vascular disease in urban African and Caucasian men.

Keywords: Nocturnal blood pressure, 3-methoxy-4-hydroxyphenylglycol, carotid intima-media thickness, African, Caucasian

Introduction

High blood pressure has been established as a major health risk for urban black South Africans, especially men [1-3]. Compared to Caucasians, various black ethnic groups especially displayed significantly higher nocturnal blood pressure [4]. The abnormally high blood pressure during the night may be attributed to sympathetic hyperactivity [5, 6] with elevated nocturnal norepinephrine secretion [7].

Impairment in blood pressure decreases during sleep has been indicated as a predictor of target organ damage [8]. Furthermore, sympathetic hyperactivity and specifically elevated norepinephrine levels have been identified as possible promoting factors of structural vascular disease development and progression [9, 10]. Hence it is conceivable that individuals with nocturnal hypertension may have a higher prevalence of subclinical atherosclerosis [11]. Elevated nocturnal blood pressure may therefore be a significant cardiovascular health threat. No existing data could be found reporting on nocturnal blood pressure, autonomic activity and subclinical atherosclerosis in urban black South African males who have a high cardiovascular risk [1].

Numerous studies have obtained the major metabolite of norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG) as measure of increased sympathetic nervous system activity [12-15]. Much speculation exists pertaining to saliva versus plasma MHPG sampling to indicate increased sympathetic activity. However, salivary MHPG has been closely related to plasma levels [13, 16].

To our knowledge, no other research has been published focussing on nocturnal hypertension, sympathetic activity and structural vascular disease in urban African and Caucasian men. Hence this is an original approach, specifically aimed at assessing urban African and Caucasian men in terms of sympathetic activity by using MHPG as marker,

nocturnal blood pressure and common carotid intima media thickness (CIMT) as marker of structural vascular disease.

Methods

Study design and population

In 2008 and 2009 the **S**ympathetic **A**ctivity and **A**mbulatory **B**lood **P**ressure in **A**fricans (SABPA) study was conducted in the North-West Province, South Africa. This target population comparative study was performed in the same timeframe to avoid seasonal changes and included 82 black African (hereafter referred to as African) and 100 Caucasian men (aged between 33 and 56 years). The participants were all employed as teachers in the Dr Kenneth Kaunda Education District of the North-West Province. The selection of participants was to obtain a homogenous sample from a similar socio-economic class. Exclusion from the study was based on the following criteria: use of alpha or beta blockers, ear temperature > 37.5°C, vaccinated or blood donors in 3 months prior to the study, clinically HIV positive status and diabetic medication users.

The study was approved by the Ethics Review Board of the North-West University (project number: NWU-00036-07-S6) and complied with all stipulations of the Helsinki Declaration (1975, revised 2004) for investigation of human participants [17]. All participants signed an informed consent form.

Procedure

Ambulatory blood pressure measurements (ABPM) were performed during working days. Participants were fitted with a British Hypertension Society validated ABPM apparatus (Meditech CE120® Cardiotens, Budapest, Hungary) as well as a two-lead electrocardiogram (ECG) between 0700 and 0800. Appropriate sized blood pressure cuffs were attached to participants' non-dominant arm. ABPM were obtained by the apparatus according to a pre-set program in 30 minute intervals during the day (0800-2200) and 60 minute intervals

during the night (2200-0600) [18], and sequential recording of the ECG strips were obtained every 5 minutes for 20 seconds. A mean successful inflation rate was 82.7% (±3.8%) in the African and 94.6% (±3.7%) in the Caucasian men respectively for the ~23 hour ABPM period. Participants' physical activity was measured for the ABPM period with Actical® accelerometers (Montréal, Québec) where resting metabolic rate was taken into account [19]. Participants were requested to continue with their usual daily routine and completed an ABPM diary card reporting any abnormalities including headache, visual disturbances, faint feeling or fatigue. Hereafter, participants spent the night at the Metabolic Unit research facility of the North-West University (consisting of 10 bedrooms, two bathrooms, a living room and kitchen). After their arrival at the Metabolic Unit, participants were introduced to the experimental setup to lessen anticipation stress [20] and HIV/AIDS pre-counselling was done by a registered nurse. Afterwards, participants ate a standardized dinner and enjoyed last beverages (coffee or tea and two biscuits) at 20:30 and remained fasting until measurements were completed the following morning. Participants retired to bed at 22:00.

ABPM and Actical® apparatuses were removed the following morning (after the last ABPM measurement at 06:00). Participants' anthropometric measurements were obtained and thereafter remained in a semi-recumbent position for two hours. Two Riva-Rocci/Korotkoff blood pressure measurements were obtained with a resting phase of 3 minutes in between. Measurements were obtained from participants' non-dominant arms. The second measurement was used for further statistical analysis. Resting saliva samples were collected with a Sarstedt salivette® (Sarstedt AG & Co, Nümbrecht, Germany). Blood sampling followed which was performed with a sterile winged infusion set from the participant's right arm brachial vein branches.

To determine levels of structural vascular disease, the carotid intima-media thickness (CIMT) of participants was obtained. A high resolution image of the left and right common carotid artery, carotid bulb and internal carotid arterial segments were obtained from at least two

optimal angles with the Sonosite Micromaxx ultrasound system (SonoSite Inc., Bothell, WA, USA) and 6-13 MHz linear array transducer. Following the Rudy Meijer protocol [21], images were digitised and imported into the Artery Measurement System (AMS) II Version 1.139 automated software (Gothenberg, Sweden). From an image of good quality, a 10 mm segment was chosen for CIMT calculation. Structural vascular disease is indicated by a CIMT of 0.9 mm or higher [22].

Anthropometric measurements

Participants' mass was determined to the nearest 0.1 kg with a digital scale and the stretched stature was determined to the nearest 0.1 cm using a stadiometer. All anthropometric measurements were performed in triplicate by registered level II anthropometrists according to standardized procedures. Body surface area was calculated based on the Mosteller formula [23].

Biochemical measurements

Fasting blood samples were handled and prepared according to standardized procedures. Gamma-glutamyl transferase (γ-GT) as an indicator of alcohol intake, as well as C-reactive protein (CRP) was determined from serum samples with the Konelab TM 20i Sequential Multiple Analyzer Computer (SMAC). Cotinine levels, indicative of smoking, were measured through homogeneous immunoassay with the Roche Modular system (Roche, Basil, Switzerland).

From saliva samples, norepinephrine metabolite (MHPG) levels were determined through HPLC (connected to an electrochemical detector) method [24]. The inter- and intra-assay coefficient of variation was less van 10%.

Statistical analysis

Database management and statistical analyses were performed with Statistica Version 10.0 (Statsoft Inc., Tulsa, OK, USA). Our power calculation was based on the largest standard deviation of 24 hour blood pressure which indicated that a group consisting of 50 participants is enough to indicate significant differences in biological profiles [25]. Normality of the distributions of all variables was determined by means of the Shapiro-Wilk's test. Student t-test and chi-square tests were respectively used for comparing means and proportions. A single two-way analysis of covariance (ANCOVA) was applied to assess interaction between the main effects (ethnicity X nocturnal hypertension). Subsequent one-way ANCOVA analyses followed where biochemical and cardiovascular measurements of African and Caucasian men were compared using least square means tests. Covariates were age, body surface area, physical activity, smoking and alcohol use. Multiple linear regression analyses determined possible associations of CIMT (dependent variable) with nocturnal heart rate, nocturnal blood pressure and MHPG (independent variables). Covariates entered were age, body surface area, physical activity, smoking, alcohol use, CRP, total cholesterol and clinically measured blood pressure. P-values of all analyses refer to two-sided hypothesis, and values equal to or smaller than 0.05 were regarded as significant.

Results

A significant interaction for ethnicity x nocturnal hypertension was indicated for CIMT ($F(1,191)=3.80$, $p=0.052$) with a marginal significant interaction for MHPG ($F(192)=3.06$, $p=0.082$). Table 1 summarizes the characteristics of the African and Caucasian men. The African men had lower body surface area ($p<0.001$) and physical activity ($p<0.001$), whereas their CRP ($p<0.001$) and γ-GT levels were higher ($p<0.001$). Their γ-GT was also above the normal range of 0-65 u/l [26]. Both African and Caucasian men demonstrated similar MHPG levels. The African men had higher nocturnal systolic ($p<0.001$) and diastolic blood pressure ($p<0.001$). Based on ESH guidelines it was regarded as hypertensive (nocturnal SBP >120 mmHg and/or nocturnal DBP>70 mmHg) [22]. This was also the case for their daytime

systolic ($p<0.001$) and diastolic blood pressure ($p<0.001$) (daytime SBP > 135 mmHg and/or daytime DBP > 85 mmHg) [22]. No significant difference was indicated between the African and Caucasian men regarding CIMTf ($p=0.300$).

Table 1 - Characteristics of African and Caucasian male participants as indicated through descriptive statistics

	African men (N=82)	Caucasian men (N=100)	P-value
Age (years)	43.22 ± 8.08	44.96 ± 11.08	0.205
Body surface area (m^2)	**1.94 ± 0.23**	**2.18 ± 0.21**	**<0.001**
Nocturnal hypertensives[a]	**62 (75.61%)**	**39 (39.00%)**	**<0.001**
Ambulatory hypertensives[b]	**66 (80.49%)**	**56 (56.00%)**	**<0.001**
Biochemical measurements			
MHPG (ng/ml)	10.52 ± 11.90	11.05 ± 13.68	0.771
Cholesterol (mmol/l)	**4.74 ± 1.17**	**5.58 ± 1.20**	**<0.001**
High sensitivity C-reactive protein (mg/l)	**5.24 ± 8.25**	**2.40 ± 2.15**	**<0.001**
Cardiovascular measurements			
Day systolic blood pressure (mmHg)	**142.76 ± 15.88**	**133.69 ± 10.65**	**<0.001**
Day diastolic blood pressure (mmHg)	**93.13 ± 10.76**	**84.98 ± 8.30**	**<0.001**
Day heart rate (beats per minute)	**84.61 ± 12.33**	**77.03 ± 12.36**	**<0.001**
Nocturnal systolic blood pressure (mmHg)	**129.02 ± 18.11**	**116.89 ± 11.57**	**<0.001**
Nocturnal diastolic blood pressure (mmHg)	**78.77 ± 12.45**	**68.55 ± 8.28**	**<0.001**

Nocturnal heart rate (beats per minute)	**70.65 ± 11.40**	**62.68 ± 11.03**	**<0.001**
Carotid intima media thickness (mm)	0.70 ± 0.16	0.68 ± 0.15	0.300
Lifestyle variables			
Cotinine (ng/ml)	35.10 ± 65.24	30.89 ± 96.69	0.718
Gamma-glutamyl transferase (u/l)	**84.99 ± 92.15**	**34.72 ± 29.51**	**<0.001**
Physical activity (kcal)	**2713.21 ± 803.98**	**3674.35 ± 2059.15**	**<0.001**
Medication use			
Blood pressure medication n(%)	**19 (23.17%)**	**9 (9.00%)**	**0.039**
Statin medication n(%)	**1 (1.22%)**	**6 (6.00%)**	**0.056**

Mean ± Standard deviation or number of participants (%); p values ≤ 0.05 regarded as statistically significant using t-tests or Chi-square tests.

Note: MHPG, 3-methoxy-4-hydroxyphenylglycol.

[a]Nocturnal hypertensive classification based on nocturnal blood pressure measurement according to ESH systolic blood pressure >120 mmHg and/or diastolic blood pressure >70 mmHg; [b]Ambulatory hypertensive classification based on ABPM measurement according to ESH systolic blood pressure >125 mmHg and/or diastolic blood pressure >80 mmHg.[14]

In contrast to a significantly higher usage of anti-hypertensive medication, the African men ($p=0.035$) also had a significantly higher prevalence of nocturnal hypertension ($p<0.001$). Compared to their Caucasian counterparts, the African men also displayed significantly higher daytime ($p<0.001$) and nocturnal heart rates ($p<0.001$).

In the African ($r=0.23$, $p=0.002$) and Caucasian men ($r=0.23$, $p=0.001$), positive associations were indicated between nocturnal SBP and CIMT (Figure 1). In the Caucasian men, nocturnal DBP correlated with CIMT ($r=0.20$, $p=0.004$). The African men displayed a positive association between nocturnal heart rate and MHPG ($r=0.28$, $p<0.001$), whilst a negative correlation was indicated between nocturnal heart rate and MHPG in the Caucasian men ($r=-0.17$; $p=0.015$) (Figure 2).

No associations existed in nocturnal normotensive men with CIMT; therefore analyses were continued within ethnic nocturnal hypertensives only. After adjusting for confounding factors (Table 2), the African men with nocturnal hypertension still had higher daytime SBP ($p<0.001$) and DBP ($p=0.003$) as well as nocturnal SBP ($p<0.001$) and DBP ($p<0.001$). Their MHPG levels were comparable to those of the Caucasians ($p=0.807$) but their nocturnal heart rates tended to be higher ($p=0.058$). The African nocturnal hypertensive men also displayed lower total cholesterol ($p=0.018$) and higher CRP levels ($p=0.025$).

In Table 3, nocturnal SBP ($\beta=0.33$, $p<0.001$) and nocturnal DBP ($\beta=0.24$, $p=0.016$) were the only significant cardiovascular predictors of CIMT in the African and Caucasian nocturnal hypertensive men, respectively.

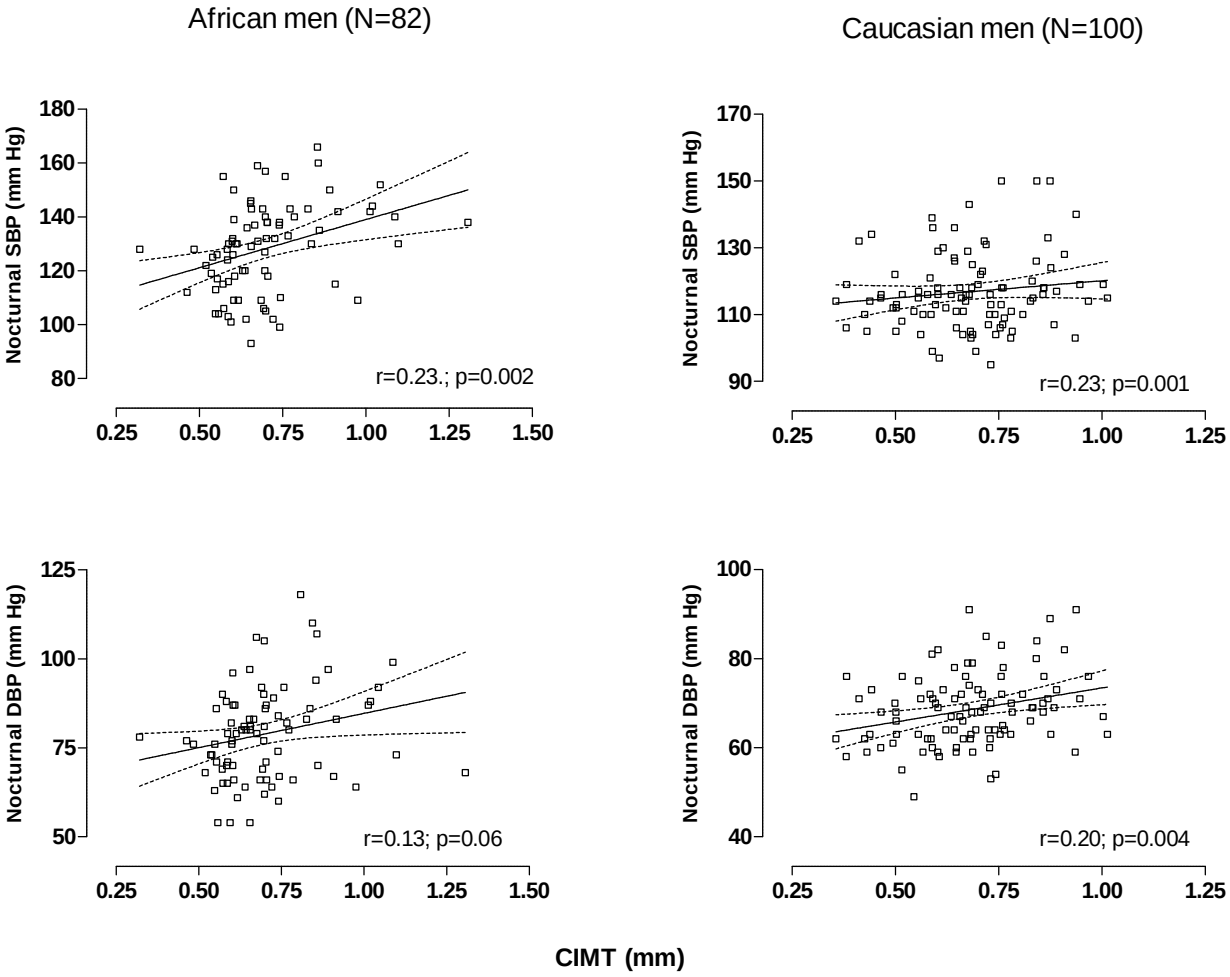

Figure 1 Associations between nocturnal blood pressure and carotid intima-media thickness in African and Caucasian men; Adjusted for age, body surface area, physical activity, cotinine, γ-GT, cholesterol and CRP. Note: SBP, systolic blood pressure; DBP, diastolic blood pressure; CIMT, carotid intima-media thickness.

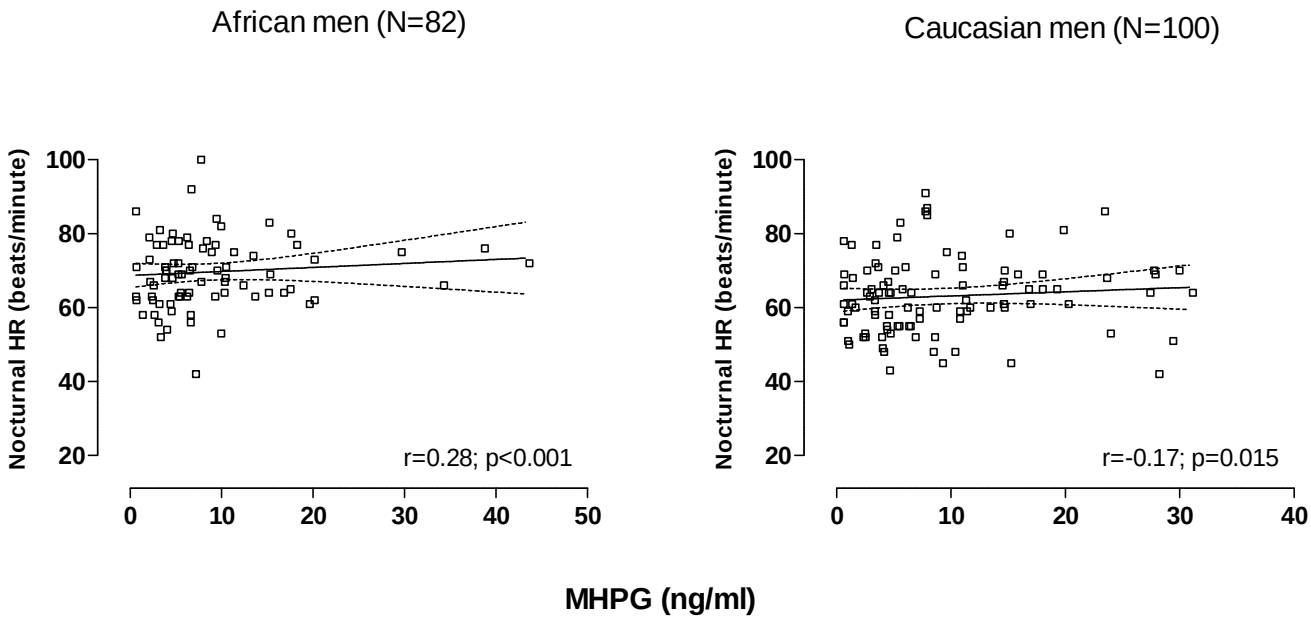

Figure 2 Associations between nocturnal heart rate and MHPG in African and Caucasian men; Adjusted for age, body surface area, physical activity, cotinine, γ-GT, cholesterol and CRP. Note: HR, heart rate; MHPG, 3-methoxy-4-hydroxyphenylglycol.

Table 2 Adjusted results for African and Caucasian nocturnal hypertensive men

	Nocturnal hypertensive African men (N=62)	**Nocturnal hypertensive Caucasian men (N=39)**	
			P-value
Biochemical measurements			
MHPG (ng/ml)	10.63 ± 1.79	11.47 ± 2.63	0.807
Cholesterol (mmol/l)	**4.82 ± 0.15**	**5.51 ± 0.22**	**0.018**
High sensitivity C-reactive protein (mg/l)	**5.94 ± 0.83**	**2.32 ± 1.23**	**0.025**
Cardiovascular measurements			
Day systolic blood pressure (mmHg)	**147 ± 1.19**	**137 ± 1.77**	**<0.001**
Day diastolic blood pressure (mmHg)	**93 ± 0.94**	**88 ± 1.40**	**0.003**
Day heart rate (beats per minute)	84 ± 1.05	82 ± 1.55	0.432
Nocturnal systolic blood pressure (mmHg)	**137 ± 1.65**	**124 ± 2.43**	**<0.001**
Nocturnal diastolic blood pressure (mmHg)	**84 ± 1.10**	**75 ± 1.62**	**<0.001**
Nocturnal heart rate (beats per minute)	***71 ± 1.34***	***66 ± 1.99***	***0.058***
Carotid intima media thickness (mm)*	0.72 ± 0.02	0.70 ± 0.03	0.573

Mean ± Standard error; p values ≤ 0.05 regarded as statistically significant using one-way ANCOVA; adjusted for confounding factors age, body surface area, physical activity, cotinine and γ-GT.*Additionally adjusted for C-reactive protein and cholesterol.

Table 3 - Independent associations of cardiovascular variables measurements with carotid intima-media thickness (dependent variable) in African and Caucasian nocturnal hypertensive men

	African nocturnal hypertensive men (N=62)		**Caucasian nocturnal hypertensive men (N=39)**	
Adjusted R^2	**0.33**		**0.24**	
	β ± 95% CI	**p**	**β ± 95% CI**	**p**
Nocturnal systolic blood pressure (mmHg)	**0.29 ± 0.17**	**<0.001**	**----**	**----**
Nocturnal diastolic blood pressure (mmHg)	**----**	**----**	**0.28 ± 0.22**	**0.016**

F to enter 2.5; Included in model as independent variables: Nocturnal systolic and diastolic blood pressure, heart rate and MHPG. Co-variates included age, body surface area, physical activity, cotinine, γ-GT, cholesterol, C-reactive protein and clinically measured blood pressure.

Discussion

Our study was aimed at assessing possible associations between sympathetic hyperactivity (represented by salivary MHPG), nocturnal blood pressure and CIMT (marker of structural vascular disease) in urban African and Caucasian men. The main finding was that nocturnal blood pressure proved to be a significant predictor of CIMT in both urban African and Caucasian nocturnal hypertensive men. Contradictory to literature, no significant link could be established between salivary MHPG levels and nocturnal blood pressure or between MHPG and CIMT in either of the groups.

Preliminary results of this study were in accordance with others who found significantly higher nocturnal blood pressure in various black ethnic groups when compared to Caucasians [4]. This remained the case even after groups were stratified into nocturnal hypertensive ranges. Further investigation was thus warranted to determine whether high night-time blood pressure may neurogenically be promoted but limited to African men.

Significant associations were previously established by other research between increases in blood pressure during the night, and sympathetic hyperactivity as well as subsequent norepinephrine secretion elevations [5-7]. We have demonstrated sympathetic hyperactivity pertaining to baroreceptor sensitivity (BRS) and cardiovascular reactivity in this particular urban African and Caucasian cohort [27]. Our findings revealed that an attenuated BRS was a significant predictor of increased ambulatory blood pressure. In the current study, we expected the African men to have higher MHPG levels due to sympathetic hyperactivity associated with higher nocturnal blood pressure. It was, however, not the case. The positive association between nocturnal heart rate and MHPG in the African men, though, still support the notion of attenuated BRS in the African men [27], while in the Caucasian men, results may indicate that baroreceptors adequately counteracted increased sympathetic activity in hypertension by reducing heart rate through an increase in vagal stimulation [28].

Thus, if the urban African men display possible sympathetic hyperactivity, why is there no significant difference in MHPG levels when compared to their Caucasian counterparts? A possible explanation was put forward by Eisenhofer et al. [29]. He proposed that in the presence of sympathetic hyperactivity and subsequent elevated norepinephrine spill-over, a reduction in catecholamine stores occur. This down-regulation of storage thus counteracts elevated extracellular concentrations as well as secretion and neuronal turnover. Norepinephrine metabolite levels may therefore be within normal ranges whilst still in the presence of sympathetic hyperactivity.

Research demonstrated deleterious effects of increased sympathetic activity and elevated nocturnal blood pressure on the endothelium. Inflammatory responses and growth factor secretion is promoted, eventually leading to vascular wall thickening and formation of atherosclerotic plaques [9]. In African and Caucasian men with nocturnal hypertension, the night-time SBP and DBP were significant predictors of structural vascular disease. These findings were independent of clinically measured blood pressure. However, no significant association could be established between MHPG and CIMT. This result may also be due to the possible down-regulation of norepinephrine storage masking a possible association. Furthermore, it should be noted that the CIMT was not within pathological ranges (≥0.9 mm) and the participant group is still fairly young (middle-aged). It is known that deleterious processes caused by sympathetic hyperactivity on the endothelium prelude structural vascular disease [9]. Therefore it is suggested that endothelial function should be investigated pertaining to the possible effects of sympathetic hyperactivity in this group (as seen in the blood pressure and inflammatory levels).

The overarching objective of the SABPA study was evaluating ambulatory blood pressure and sympathetic function in a cohort of African participants. This sub-study added further value regarding policy, practice and research by highlighting the importance of not only observing clinical blood pressure or ambulatory mean blood pressure in patients, but also

paying particular attention to assessing blood pressure measured during the night. Nocturnal blood pressure, compared to other blood pressure values, seems to be a more sensitive predictor of cardiovascular disease outcomes [30]. A few studies demonstrated improvement in cardiovascular outcomes if night-time blood pressure is lowered [31, 32]. Taking into account the high prevalence of nocturnal hypertension in our cohort of African men, prospective studies possibly need to implement treatment regimens targeting nocturnal blood pressure.

The limitations of our study should be noted. Salivary MHPG, as an indirect marker for sympathetic activity, was used and not supported by other direct markers of sympathetic activity such as muscle sympathetic activity or norepinephrine spill-over rates. Furthermore, the sample size of the nocturnal hypertensive Caucasian men was small, possibly reducing statistical power. As this was a cross-sectional study, we could not infer causality. Apart from this, the study was well designed and made use of gold standard measurements which ensured highly reliable results.

In conclusion, no associations were firstly demonstrated between MHPG as sympathetic activity marker and CIMT or secondly, between MHPG and nocturnal blood pressure. Elevated nocturnal BP evidently seems to promote structural vascular disease in urban African and Caucasian men. Due to the large number of African men who had nocturnal blood pressure within hypertensive ranges, they can be regarded as a high-risk group. Further research is, however, needed to confirm results found by use of actual norepinephrine values.

Acknowledgements

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Disclosure

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CHAPTER 4
RESEARCH ARTICLE 3

Nitric oxide, cardiovascular function and structural vascular disease in a South African male cohort: the SABPA study

Aletta S. Uys[1]; Leoné Malan [1]; Johannes M van Rooyen[1]; Hendrik S. Steyn[2]; Manja Reimann[3],Tjalf Ziemssen[3];

[1]Hypertension in Africa Research Team (HART); North-West University, Potchefstroom Campus, South Africa.

[2]Statistical Consultation Services, North-West University, Potchefstroom Campus, Potchefstroom, South Africa.

[3]Department of Neurology, University Hospital Carl Gustav Carus, Dresden University of Technology

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Keywords

After the abstract a list of 3-10 keywords or short phrases should be supplied.

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Full papers should be divided into sections headed Introduction, Methods (including ethical and statistical information), Results and Discussion (including a conclusion).

Acknowledgements

Acknowledgements should be made to those who have made a substantial contribution to the study.

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.

Articles in journals

Zhou M-S, Schulman IH, Raij L. Vascular inflammation, insulin resistance, and endothelial dysfunction in salt-sensitive hypertension: role of nuclear factor kappa B activation. J Hypertension 2010; 28:527–535

More than seven authors:

Grassi G, Vailati S, Bertinieri G, Seravalle G, Stella ML, Dell'Oro R, et al. Heart rate as a marker of sympathetic activity. J Hypertens 1998; 16:1635–1639.

Book:

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

Chapter in a book:

Wakhloo AK. Carotid artery revascularization. In: Kandarpa K (editor). Peripheral Vascular Interventions. Philadelphia: Lippincott Williams & Wilkins; 2008. pp. 137–153.

Abstract

Background: Nitric oxide (NO) plays a pivotal role in blood pressure homeostasis. Chronically elevated blood pressure has been associated with impaired NO mediated vasodilation and risk for structural vascular disease. Hence the aim of this study was to determine whether significant associations exist regarding NO metabolite (NOx) responses, cardiovascular function and structural vascular disease in a cohort of African and Caucasian men.

Methods: The Sympathetic activity and Ambulatory Blood Pressure in Africans study included 81 African and 94 Caucasian male teachers. Males were stratified via median splits into low and high NOx ethnic groups. Ambulatory blood pressure (ABPM), ECG monitoring and ultrasound carotid intima-media thickness (far wall) images were obtained. Cardiovascular measurements and fasting blood for NOx responses were measured during rest and on challenging the cardiovascular system with the Stroop colour word conflict test.

Results: The African men displayed significantly higher resting NOx as well as a higher number of 24h silent ischemic events than their Caucasian counterparts. Only low NOx African men displayed enhanced α-adrenergic and ST depression acute mental stress responses. In this group the 24h silent ischemic events was associated with carotid intima-media thickness (Adjusted R^2= 0.47; β=0.25 (0.13;0.41); p=0.057).

Conclusion: African men demonstrated a vulnerable cardiovascular profile. Novel findings revealed α-adrenergic-driven BP responses and less NO bio-availability. Furthermore, the association between ambulatory myocardial ischemia and carotid intima-media thickness in this group emphasized their risk for future cardiovascular events.

Keywords: Nitric oxide, cardiovascular function, myocardial ischemia, structural vascular disease

Introduction

Hypertension is a challenging health problem in South Africa [1], specifically for urban black African men [2,3]. Chronic psychosocial stress due to everyday life demands if residing in an urban environment has been established as a contributing factor to the poor cardiovascular profile of this population group [3-5]. Research has established that psychosocial stress and subsequent chronically elevated blood pressure is associated with impaired NO mediated vasodilation [6]. Reduced bioactivity of NO particularly reduces coronary artery dilation [7]. Reduced NO bioavailability during acute and chronic mental stress may contribute to a higher prevalence of myocardial ischemic responses in African men, making them vulnerable to structural vascular disease. In concert with a pro-atherogenic environment where leukocyte adhesion and platelet activation is no longer reduced [8] the risk for coronary artery disease is significantly increased [9].

Cardillo et al. [10] indicated, a possible link between a reduced sensitivity of the vascular smooth muscle for NO and reduced vasodilation during mental stress in African Americans. This positive association may play a significant role in the higher prevalence of hypertension amongst blacks. Whether this relationship also applies to urban black South African men is not known.

Taking into account the poor cardiovascular profile urban African men may be a highly susceptible group for structural vascular disease, particularly with an increased risk for coronary artery disease [9] as well as future cardiovascular events [11]. The aim of this study was to explore the relationship between NO metabolite (NOx) responses to a mental stressor, cardiovascular function and structural vascular disease in a cohort of African and Caucasian men.

Methods

Study population

The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study was conducted in February – May in 2008 and 2009 as a target population comparative study. Our sub-study included 81 African and 94 Caucasian male teachers, working in the Dr Kenneth Kaunda Education district of the North West Province (South Africa). This participant selection was to obtain a homogenous sample from similar socio-economic class. Exclusion criteria were ear temperature >37.5°C, vaccinated or blood donors in previous 3 months, HIV positive status, clinically diagnosed diabetes mellitus and use of alpha and beta blockers or statin medication.

The study protocol complied with institutional guidelines as well as those of the Declaration of Helsinki (as revised in 2004) [12] for investigation in human subjects. It was approved by the Ethics Review Board of the North-West University (Project number: NWU-00036-07-S6).

Clinical assessment procedure

Ambulatory blood pressure measurements (ABPM) were conducted during working days of the week. Each morning at 07:00 – 08:00 participants were fitted with a British Hypertension Society validated Cardiotens CE12® apparatus (Meditech CE120®, Budapest, Hungary) which measured 24-hour blood pressure and two-channel ECG. Cuffs were applied to the non-dominant arms. ABPM was obtained oscillometrically in 30 minute intervals during the day (08:00 – 22:00) and in 60-minute intervals during the night (22:00 – 06:00) [13]. The mean successful inflation rate was 75% (±9.8%) for the African and 85% (±9.1%) for the Caucasian men. Ambulatory ECG was recorded in 5 minute intervals for 20 seconds. Participants were also fitted with Actical® accelerometers (Montréal, Québec) over the ambulatory period to measure physical activity with resting metabolic rate taken into account [14]. Participants were asked to continue with their normal daily routine, recording in an ambulatory diary card, any abnormalities experienced such as headache, nausea, dizziness,

visual disturbances and fatigue. At 16:30, participants were transported to the Metabolic Unit Research Facility of the North-West University for an overnight stay. On arrival they were introduced to the experimental setup to lessen anticipation stress [15] which included a brief introduction to the Stroop colour-word conflict test. Participants completed general health questionnaires on their medical history and prescribed medications. After having received a standardized dinner, participants were requested to go to bed at 22:00, fasting overnight.

The following morning at 06:00 (after the last blood pressure measurement) the ABPM and Actical® apparatuses were disconnected. Following anthropometric measurements, participants remained in a semi-recumbent position for two hours for a series of cardiovascular measurements. A resting 12-lead ECG was recorded for six cardiac cycles as well as a five-minute reading of beat-to-beat measurement of cardiovascular variables using the Finometer which is validated for relative changes (Finapres Medical Systems, Amsterdam, The Netherlands). Hereafter, venous blood samples were obtained by a registered nurse, using a sterile winged infusion set with a diluted heparin block (0.5ml heparin:9ml normal saline). A rest period was allowed for 5 - 10 minutes before participants were exposed to the Stroop Colour-Word conflict test, known to evoke a mixed α-β adrenergic response at the heart and vasculature [16]. Successive series of five colour words written in incongruent colours on a cardboard were shown to the participant in a random order. The participants were instructed to recognize and verbally confirm the colour of a given word within a certain time limit. A monetary incentive was given as motivation on completing the test. The stressor was applied for one minute which was followed by blood sample collection after flushing of infusion tubes (5 - 10 minutes after stress exposure) and a rest period of 30 minutes. A dominant α-adrenergic response pattern is characterized by an increase in total peripheral vascular resistance (TPR) and diastolic blood pressure (DBP) as well as a decrease of arterial compliance (C_w) [17]. An increase in heart rate, systolic blood pressure (SBP) and cardiac output are all characteristic of a dominant β-adrenergic response pattern [16]. Beat-to-beat cardiovascular (Finometer) and 12-lead ECG

measurements were also obtained during stressor application. The last 15 seconds of the ECG and cardiovascular measurements obtained during stress exposure were used for data analysis.

To determine the presence of structural vascular disease, the carotid intima-media thickness of the far wall (CIMTf) was measured with a SonoSite Micromaxx ultrasound system (SonoSite, Bothell, WA, USA) and a 6-13 MHz linear array transducer. A high resolution image of the left and right common carotid artery was obtained from two optimal angles [18]. Images were digitised and imported into the Artery Measurement System (AMS) II Version 1.139 automated software (Gothenberg, Sweden). From an image of good quality, the far wall of a 10 mm segment was chosen for CIMTf analysis. CIMTf of ≥ 0.9 mm indicates structural vascular disease [19] and a CIMTf of ≥ 0.75 mm indicates an increased stroke risk [20].

Anthropometric measurements

Registered anthropometrists obtained anthropometric measurements in triplicate according to standards of the International Society of the Advancement of Kinanthropometry (ISAK) [21]. Body mass was measured to the nearest 0.1 kg with a digital scale and height was measured to the nearest 0.1 cm with a stadiometer. The body surface area (BSA) was calculated using the Mosteller formula [22].

Cardiovascular measurements

ABPM data was downloaded into a database and processed using the CardioVisions 1.15.2 software. Before analysis, ABPM and ECG data is automatically cleaned by the CardioVisions 1.15.2 software to remove noise. From the two-channel ECG recording, ischemic events were recorded according to the 1-1-1 rule which entails a horizontal or descending ST-segment depression by one mm; duration of the ST-segment episode of at least one minute and a one minute interval from previous episodes [23]. ST-segment

abnormalities defined as deviation of greater than one millimeter were assessed from the 12-lead ECG reading. Depression of the ST-segment is an indication of myocardial ischemia [24]. Finometer measurements were processed with Beatscope 1.1 software (Finapres Medical Systems, Amsterdam, The Netherlands) to obtain SBP, DBP, heart rate, cardiac output (CO), stroke volume (SV), total peripheral resistance (TPR) and Windkessel arterial compliance [25].

Biochemical analyses

Fasting serum and plasma samples were dealt with according to standardized procedures and stored at -80°C. From serum samples, gamma-glutamyl transferase (γ-GT), high sensitivity C-reactive protein (CRP) and total cholesterol were determined with the Konelab TM 20i Sequential Multiple Analyser Computer (SMAC) (Thermo Scientific, Vantaa, Finland). Cotinine levels were determined by homogenous immunoassay with the Roche Modular System (Roche, Basil, Switzerland). Levels of γ-GT and cotinine were used to indicate alcohol abuse [26,27] and of smoking status [28]. The concentration of nitric oxide metabolites (NOx), the sum of plasma nitrite and reduced nitrate were determined with an R&D systems Inc kit (Parameter™, Catalogue number: KGE001, MN, USA), Universal ELX800 Plate reader and GEN5™ software (BioTek Instruments Inc, Winooski, VT, USA). Intra-assay variability was 5.5%. Inter-assay variability ranged between 8.2% - 13.5%.

Statistical analyses

Stress responses (%) were calculated with the following formula as changes from baseline (delta, ∆): %= (X stressor – X resting)/ X resting) x 100. Data analyses were performed with Statistica Version 10 (Statsoft Inc., 2012). Distribution of data was determined by means of the Shapiro-Wilk's test. Subsequently, logarithmic transformation of CRP and γ-GT was deemed necessary due to skewed distribution. Interaction on the main effects was tested with a single 2 x 2 (ethnic x NOx response) analysis of covariance (ANCOVA). Means and proportions were compared between the two ethnic groups, using Student's t-tests and chi-

square tests, respectively. Cardiovascular measurements of African and Caucasian men were compared by means of one-way ANCOVA, adjusted for age, BSA, cotinine, log γ-GT, physical activity and resting values for responses.

Multiple linear regression analysis, with forward stepwise method was applied to analyse the variance in CIMTf (dependent variable) in ethnic groups as well as in Low and High NOx ethnic groups. Independent variables included NOx and ST-segment responses, age, BSA, cotinine, log γ-GT, physical activity, log CRP, cholesterol and 24 hour mean arterial pressure. Statistical significance was indicated by a two-sided α level of 0.05 or less.

Results

The study population characteristics are depicted in Table 1. The African men demonstrated lower cholesterol ($p<0.001$) and physical activity levels ($p<0.001$) than their Caucasian counterparts. Significantly higher γ-GT levels were found in the African men, which also indicated alcohol abuse (γ-GT ≥ 0.65 u/L) [27]. Despite the higher use of anti-hypertensive medication among the African men ($p=0.024$), higher mean ambulatory blood pressure ($p<0.001$), heart rate ($p<0.001$) and more silent ischemic events ($p=0.001$) were revealed. African men also exhibited higher CRP ($p=0.018$) and NOx levels ($p<0.001$) compared to Caucasian men.

After adjusting for covariates, NOx levels ($p<0.001$), blood pressure ($p<0.001$), heart rate ($p=0.05$) and the number of ischemic events ($p=0.01$) remained higher in the African men than in the Caucasian men (Table 2). CRP levels of the African men were within the risk range for cardiovascular disease development (>3.0 mg/L) [29].

Table 1

Characteristics of the study group

Variable	African men (*n*=81)	Caucasian men (*n*=94)	p-value
Age (years)	42.8 ± 8.5	44.2 ± 11.1	0.34
Body surface area (m^2)	1.9 ± 0.2	2.2 ± 0.2	<0.001
Lifestyle factors			
Physical activity (kcal)	2713.4 ± 821.9	3653.1 ± 2128.6	<0.001
Cotinine (ng/ml)	24.9 ± 48.1	30.7 ± 97.7	0.63
Gamma glutamyl transferase (u/L)	75.4 ± 72.0	33.6 ± 28.5	<0.001
Cholesterol (mmol/L)	4.9 ± 1.2	5.6 ± 1.2	<0.001
Biochemical markers			
C-reactive protein (mg/L)	4.8 ± 7.7	2.4 ± 2.1	0.003
Nitric oxide metabolite (µmol/L)	10.03 ± 11.3	2.2 ± 3.9	<0.001
Cardiovascular variables			
24h SBP (mmHg)	138 ± 17	127 ± 10	<0.001
24h DBP (mmHg)	88 ± 11	79 ± 8	<0.001
24h MAP (mmHg)	105 ± 13	96 ± 8	<0.001
24h Heart rate (beats/minute)	79 ± 11	71 ± 11	<0.001
CIMTf mean (mm)	0.70 ± 0.2	0.68 ± 0.1	0.34
ST segment depression (mm)	-0.02 ± 1.2	-0.13 ± 1.11	0.54
Mean ischemic events (n)	9.5 ± 20.6	1.7 ± 5.4	0.001
Participants with ischemic events n (%)	39 (48%)	34 (36%)	0.11

Hypertension medication n (%)	17 (21%)	6 (6%)	0.024
Hypertensives n (%)	62 (77%)	67 (71%)	0.43

Values indicated as mean ± SD

Abbreviations: 24h denotes 24 hours; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; CIMTf, carotid intima-media thickness in far wall.

Table 2

Comparison of C-reactive protein nitric oxide metabolite and cardiovascular measurements between African and Caucasian men

	African men (N=81)	Caucasian men (N=94)	p-value
Nitric oxide metabolite (µmol/L)	9.7 ± 1.1	2.5 ± 1.0	<0.001
C-reactive protein (mg/L)	5.3 ± 0.7	2.0 ± 0.6	0.003
24h SBP (mmHg)	139 ± 1.6	127 ± 1.5	<0.001
24h DBP (mmHg)	88 ± 1.1	80 ± 1.0	<0.001
24h Heart rate (beats/minute)	80 ± 1.4	73 ± 1.2	0.05
CIMTf mean (mm)[a]	0.70 ± 0.02	0.68 ± 0.2	0.55
ST segment deviation (mm)	0.03 ± 0.15	-0.17 ± 1.14	0.34
Mean ischemic events	9.7 ± 1.9	1.6 ± 1.7	0.01

Data are shown as mean ± standard error; Adjusted for age, body surface area, physical activity, cotinine, log γ-GT. [a]Additionally adjusted for C-reactive protein, cholesterol and 24 hour mean arterial pressure.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; CIMTf, mean carotid intima-media thickness of the far wall.

Mental stress test responses

The Stroop colour-word conflict test evoked lower heart rate (p=0.008) and CO (p=0.027) in the African men (Table 3). This study group also showed a reduced NOx response compared to the Caucasian men (p=0.016).

Table 3

Comparison of NOx and cardiovascular responses to the colour-word conflict test between African and Caucasian men

	African men (N=81)	Caucasian men (N=94)	p-value
Δ NO (%)	-132.5 ± 794.3	2868.3 ± 703.8	0.016
Δ SBP (%)	13.2 ± 1.53	16.0 ± 1.34	0.25
Δ DBP (%)	14.0 ± 1.4	11.9 ± 1.2	0.31
Δ Heart rate (%)	24.9 ± 2.5	34.9 ± 2.2	0.008
Δ Stroke volume (%)	-6.9 ± 2.3	-3.4 ± 2.0	0.31
Δ Cardiac output (%)	16.9 ± 4.0	30.3 ± 3.5	0.027
Δ Total peripheral resistance (%)	18.4 ± 10.5	-0.7 ± 9.2	0.23
Δ Arterial compliance (%)	-17.2 ± 1.9	-12.9 ± 1.6	0.14
Δ ST segment (%)	9.6 ± 49.6	-18.8 ± 43.2	0.71

Data are shown as mean ± standard error;

Abbreviations: NO denotes nitric oxide; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Comparison of high and low NOx response groups

A significant interaction on the main effects (ethnicity x NOx responses) existed for SV ($F(1, 154) = 6.45$; $p=0.012$) and marginal significant interaction for CO responses ($F(1, 154) = 3.64$; $p=0.058$) to the Stroop colour-word conflict test. After a median split of the African and

Caucasian group into low and high NOx response groups, the low response group of the African men was characterized by lower responses of SV (p=0.003) and CO (p=0.007) to mental stress and a significant ST-segment depression (p=0.012) (Figure 1).

Predictors of CIMTf

Depression of the ST-segment was significantly associated with CIMTf (Adjusted R^2 0.32; β= -0.28, 95% CI: -0.49; -0.05) in the African men explaining (Figure 2). Furthermore (Table 4), in the low NOx African men (Adjusted R^2 0.47; β= 0.25, 95% CI: 0.13; 0.41), ambulatory silent ischemia was significantly associated with CIMTf.

Table 4

Independent associations between far wall carotid intima-media thickness and cardiovascular measurements (dependent variable) in African and Caucasian low NOx response groups.

	Low NO response African men (N=38)		Low NO response Caucasian men (N=45)	
Adjusted R^2	**0.47**		**0.37**	
	β (95% CI)	**p**	**β (95% CI)**	**p**
Ischemic events	**0.25 (0.13;0.41)**	**0.057**	**-----**	**-----**

Covariates included age, body surface area, physical activity, cotinine, γ-GT, cholesterol, C-reactive protein and 24 hour mean arterial pressure

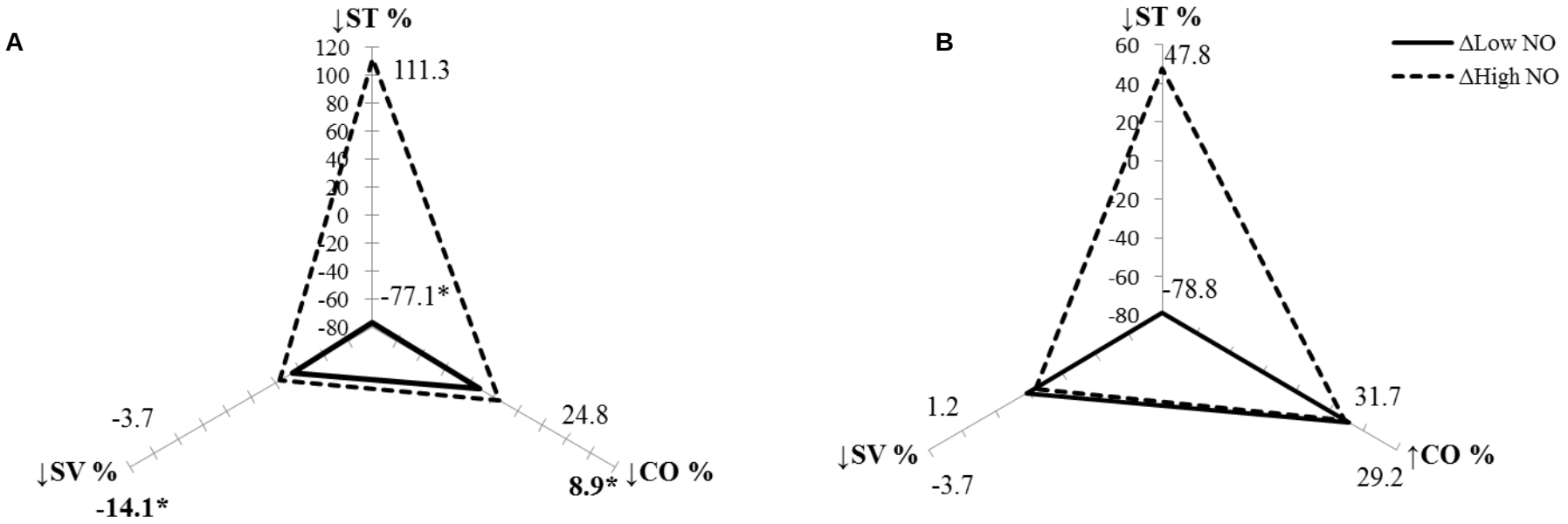

Fig. 1. A comparison of cardiovascular responses between high and low NO response groups of the African (A) and Caucasian (B) men.

Adjusted for age, body surface area, physical activity, γ-GT, cotinine and resting values.

Abbreviations: CO denotes cardiac output; SV, stroke volume; ST, ST-segment depression. *p ≤ 0.05

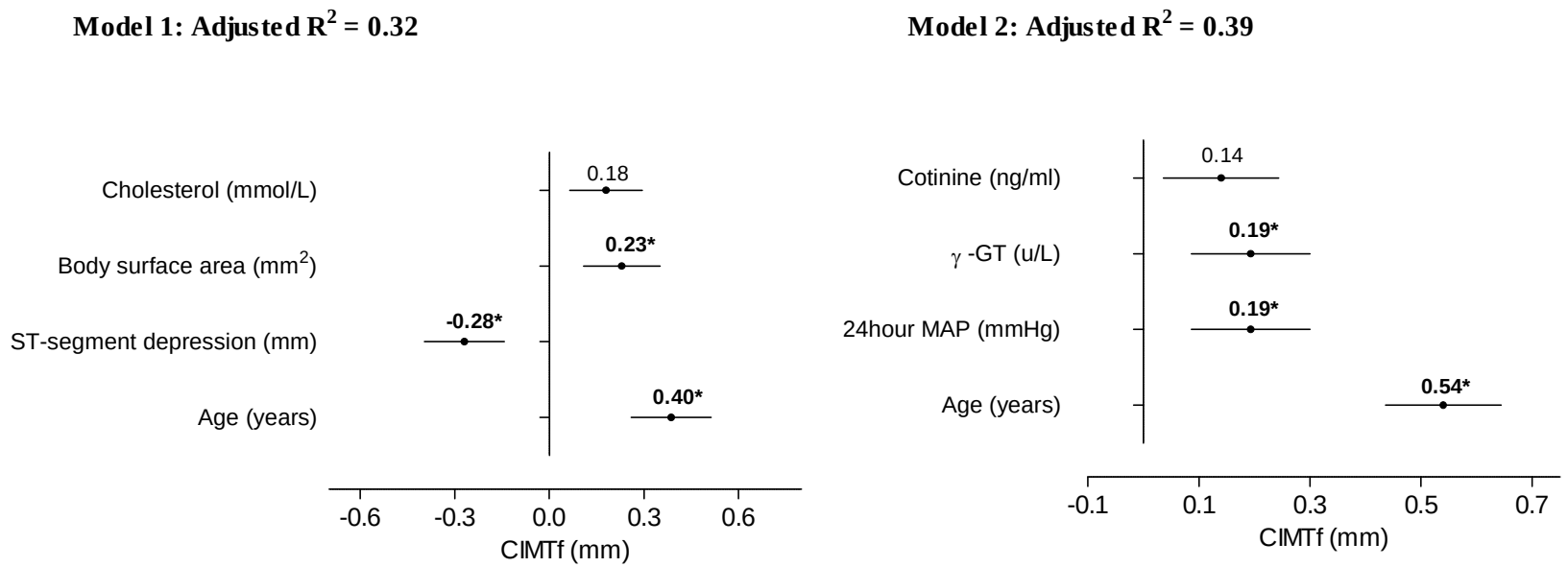

Fig. 2. Associations with CIMTf (mm) using stepwise multiple linear regression in African (model 1) and Caucasian (model 2) men. Values are indicated as standard β (95% Confidence Interval); *p≤0.05. Abbreviations: γ-GT denotes gamma-glutamyl transferase; MAP, mean arterial pressure; CIMTf, carotid intima-media thickness of the farr wall.

Discussion

The main objective of our study was to determine associations between NOx responses, cardiovascular function and structural vascular disease in a cohort of African and Caucasian men. Our findings revealed less NO availability in African men coupled with dominant α-adrenergic and ST depression mental stress responses. In this population group acute mental stress ST segment depression responses were associated with CIMTf. These events were supported by chronic 24h ischemic events being associated with CIMTf as well.

A significant reduction of NO bioavailability is often found in hypertension, probably due to endothelial dysfunction and/or increased oxidative stress [17, 30]. Despite elevated mean blood pressure levels, the African men exhibited higher resting NOx levels than the Caucasians. Cardillo et al. [10] suggested a desensitization of vascular smooth muscle cells to NO, rather than a reduction of bioavailability in African-Americans. This finding is substantiated by our study, where excessive levels of NOx may indicate an attempt of the cardiovascular system to compensate for the elevated blood pressure in the African men.

A previous study indicated that sympathetic hyperactivity was present together with a dominant α-adrenergic response pattern in this specific cohort of African men [31]. The vasoconstrictory profile and the reduced NOx stress response seem to support our assumption of sympathetic predominance and possible attenuation of parasympathetic influence on cardiovascular function in the African men of this study [31]. Intriguingly, the African men with a lower NOx stress response showed significant ST-segment depression during acute mental stress exposure. Conversely, only chronic ambulatory silent ischemic events explained 25% of variance in CIMTf in these men. Hence it appears that these African men tend to have attenuated NOx responses accompanied not only by systemic vasoconstriction but possibly also by myocardial ischemia when exposed to mental stress. It may further imply that if their ambulatory silent ischemic responses are simulated by

everyday environmental stress [4], their future risk for myocardial ischemic events may increase [32].

Previous studies have indicated a link between reduced NO bioavailability, increased vascular inflammation [33] and smooth muscle cell proliferation [34], proving to be a major causal factor in atherogenesis [35, 36]. A specific region affected is the coronary circulation [37]. Quyyumi et al. [38] indicated reduced NO bioavailability in the presence of coronary risk factors. Some of these factors are also highly prevalent in the group of African men, including high blood pressure, chronic stress [2-4], lower physical activity and elevated CRP levels [39,40]. The reduced NO bioavailability supported by low grade inflammatory levels during acute and chronic mental stress may further contribute to the higher prevalence of myocardial ischemic responses in the African men, making them vulnerable to structural vascular disease. Accordingly Drexler [36] also noted that the common presence of coronary atherosclerosis reduced systemic NO bioavailability and inevitably results in myocardial ischemia. Results demonstrated in the African low NOx response groups are in support of the postulated lack of NO sensitivity of vascular smooth muscle as possible promoting factor in increased occurrence of silent ischemia associated with structural vascular disease during everyday life.

Our findings may therefore be of high clinical importance as the reduced NO bioavailability during acute and chronic stress can desensitize smooth muscle for NO. If supported by sympathetic-driven responses it may enhance these effects and increase myocardial ischemic events constituting risk for coronary artery disease in our cohort of African men.

The strengths and limitations of this study must be recognized. Due to the cross-sectional design, it was not possible to examine the causal relationship between disturbed NO bioavailability and structural vascular disease in this group of African men. With stratification of ethnic groups according to NOx responses, the sample size was reduced and could

therefore have decreased statistical power. However, the unique sample of African and Caucasian men of similar social standing and the application of gold standard methods ensure highly reliable results. Further study with longitudinal design is necessary to elucidate possible trends indicated by our findings.

In conclusion, the African men demonstrated a vulnerable cardiovascular profile. Novel findings revealed α-adrenergic-driven BP responses and less NO bio-availability. Furthermore, the association between ambulatory myocardial ischemia and carotid intima-media thickness in this group emphasized their risk for future cardiovascular events.

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CHAPTER 5

GENERAL FINDINGS AND CONCLUSIONS

1. Introduction

A summary is given of the main findings from the three research articles reported in this thesis. The results will be discussed, interpreted, elucidated and compared to the relevant literature reviewed in Chapter 1. Conclusions are subsequently drawn and recommendations made to researchers investigating autonomic and cardiovascular responses in a cohort of urban African and Caucasian men, with the aim of ultimately identifying possible associations between a likely sympatho-vagal imbalance, dominant vascular response pattern and target organ damage markers.

2. Summary of main findings

To assess autonomic nervous system and cardiovascular function as well as target organ damage, we clearly focussed on responses where our participants were challenged. Baroreceptor sensitivity, 3-methoxy-4-hydroxy-phenylglycol and nitric oxide metabolite responses were assessed as markers of autonomic function. Possible associations with target organ damage markers, left ventricular hypertrophy and structural vascular disease were addressed, and the findings of the three studies reported in this thesis were:

2.1. Baroreceptor sensitivity, cardiovascular responses and ECG left ventricular hypertrophy in men: The SABPA study (Chapter 2)

This sub-study aimed to determine whether BRS was significantly lower in African men than in their Caucasian counterparts, and furthermore, whether attenuated BRS was significantly associated with elevation of ambulatory blood pressure as well as left ventricular hypertrophy in these population groups. Significantly lower BRS (during rest and stressor exposure) therefore was hypothesized in the African men. Results substantiated this hypothesis only during stressor exposure. It could thus only be partially accepted.

The second hypothesis put forward was that attenuated BRS will significantly be associated with ambulatory blood pressure elevation in the African men. In this population group a significant association was demonstrated between ambulatory blood pressure elevation and attenuated resting BRS, coupled with a dominant α-adrenergic response pattern during rest and stressor exposure. Hence the second hypothesis is accepted.

The last hypothesis stated that in the African men, attenuated BRS will significantly be associated with ECG left ventricular hypertrophy. This was, however, not the case, since only a decrease in arterial compliance was significantly associated with ECG left ventricular hypertrophy in this cohort of African men. Hence the hypothesis is rejected.

2.2. Nocturnal blood pressure, 3-methoxy-4-hydroxy-phenylglycol and carotid intima-media thickness: the SABPA study (Chapter 3)

The next aim was to assess differences between urban African and Caucasian men in terms of sympathetic activity (with MHPG as marker), nocturnal blood pressure and structural vascular disease using carotid intima-media thickness (CIMT) as marker. It was hypothesized that the African men will display higher nocturnal blood pressure levels. Findings revealed that when comparing the African men with their Caucasian counterparts, the African men demonstrated significantly higher nocturnal blood pressure even after being stratified into nocturnal hypertensive groups. The first hypothesis thus is accepted.

Based on the finding of the previous sub-study in which lower BRS indicated the possibility of sympathetic hyperactivity in the African men we proposed the second hypothesis. It was proposed that significantly higher MHPG levels will be indicated in the African men when

compared to their Caucasian counterparts. This was not the case, substantiating the rejection of the second hypothesis.

The last hypothesis postulated that MHPG as well as nocturnal blood pressure will significantly be associated with structural vascular disease in the nocturnal hypertensive African men, particularly. In both ethnic cohorts, structural vascular disease was associated with only nocturnal blood pressure and not MHPG. Based on these findings, the third hypothesis can only be partially accepted.

2.3. Nitric oxide, cardiovascular function and structural vascular disease in men: the SABPA study (Chapter 4)

The objective of this sub-study was to determine whether significant associations exist between a reduced supply of nitric oxide and target organ damage when the African and Caucasian male participants were challenged.

In the African men, contradictory to what was hypothesized, resting NOx levels were significantly higher than those of Caucasian men. NOx stressor responses were, however, significantly attenuated in the African men. The first hypothesis thus is partially accepted.

The second hypothesis put forward was that attenuated NOx responses in the African men will be associated with a detrimental cardiovascular profile as well as structural vascular disease. Results have indicated an association between reduced NO bioavailability during stress, possible systemic vasoconstriction and myocardial ischemia. However, no strong association was indicated in the low NOx African men between the structural vascular

disease marker, CIMT, and possible myocardial ischemia. Nor was any association further indicated with other cardiovascular variables. Therefore the second hypothesis put forward was only partially accepted.

3. Comparison of findings with the literature

When comparing the results from this study with existing literature (as presented in Chapter 1) it is evident that certain findings confirmed whilst other contradicted those described in the literature. Findings of this study furthermore add to existing knowledge in the literature on autonomic and cardiovascular responses in urban African and Caucasian men.

In South Africa, research has established hypertension as a significant health threat, particularly for urban African men.[1-3] In accord, findings of this study illustrated a significantly higher prevalence of hypertension among the cohort of African men involved, compared to their Caucasian counterparts. As is the case with African Americans, sympathetic hyperactivity particularly has been suggested as contributing factor to their chronically elevated blood pressure levels.[4,5] In preceding research, it has been indicated that Africans are prone to demonstrate a dominant α-adrenergic response pattern when exposed to stressors,[6,7] further increasing their cardiovascular risk.[1,8,9] In support of this notion, this study has illustrated, in the African men, a significant correlation between a dominant vascular (α-adrenergic) response pattern and ambulatory blood pressure elevation as well as ECG left ventricular hypertrophy. Furthermore, this response pattern was also coupled with population groups' cardiovascular health is possibly compromised.

Conversely, their cardiovascular health may imply even more risk, since decrease in BRS, characteristic of sympathetic hyperactivity, as well as attenuated parasympathetic activity

was apparent.[10,11] In hypertension, diminished BRS has specifically been established as a contributing factor in the maintenance of chronically elevated blood pressure levels[11,12] as well as accelerated development of left ventricular hypertrophy.[13,14] No racial differences, however, have been indicated comparing black population groups (African American, West African) and Caucasians.[15-17] For resting responses, our study was in agreement with these findings. However, in response to stress, the African men demonstrated a significantly larger attenuation of BRS than the Caucasian participants, illustrating the possibility of sympathetic hyperactivity in this group. Regarding the promotion of hypertension, in accord with the literature, a significant association was demonstrated in the African men between elevation of ambulatory blood pressure and attenuated BRS. No association was indicated, however, in either of the ethnic groups is this study regarding ECG left ventricular hypertrophy and BRS.

The significant role autonomic regulation plays in the maintenance of the blood pressure circadian rhythm should be emphasized.[14] Abnormally high nocturnal blood pressure values have particularly been linked to sympathetic hyperactivity characterised by elevated norepinephrine spill-over.[18] This phenomenon particularly manifests more often in black population groups compared to Caucasians.[19] This was also the case with the cohort of African men participating in the current study. However, no significant difference could be indicated between the ethnic groups regarding the norepinephrine metabolite (MHPG) levels. A possibility of downregulation of norepinephrine stores, in the presence of a sympathetic hyperactive state, might exist.[20] Therefore MHPG (norepinephrine metabolite) levels may not be elevated.

Atherosclerosis is regarded as a neurogenic phenomenon[21] with sympathetic hyperactivity particularly promoting the atherogenic process.[22] No significant association could,

nevertheless, be indicated between structural vascular disease and MHPG in either of the ethnic groups.

In addition, with exaggerated stress-induced blood pressure responses, as demonstrated in the African men, endothelial damage may be induced through excessive sheer stress. This may result in diminished NO bioavailability, particularly promoting atherogenesis.[23-25].The coronary circulation in particular is affected.[26] Findings of this study particularly demonstrated attenuated NO stress responses and ST-segment depression as a manifestation of myocardial ischemia. Furthermore, in support of the literature, structural vascular disease was correlated with myocardial ischemia in the cohort of African participants,.

Lastly, in accord with the literature, autonomic dysregulation of cardiovascular function[18] in particular may render urban African men particularly vulnerable to target organ damage[27] as well as future cardiovascular events.[28]

4. Chance and confounding

It is imperative that before discussing the main findings of this study, some important confounding factors be addressed.

All statistical results were interpreted from a physiological perspective; therefore not all statistical significance necessarily indicates physiological significance. Furthermore, the possibility of chance should be taken into account. Through forward stepwise regression analysis, it was indicated that one out of twenty significant correlations may be due to chance. A power analysis using standard deviation of sympathetic activity determined that

fifty participants per group would be sufficient to indicate significant trends. However, as this thesis focuses on multiple outcomes drawn from one study, the number of participants included may possibly be inadequate to establish significant trends.

Based on the cross sectional design of the study, it was not possible to examine the causal relationships of autonomic as well as cardiovascular responses and target organ damage. Confounding factors such as diet, physical well-being, HIV and medication use could have influenced results causing over and underestimation of the associations. By adjusting for age, body surface area, physical activity, smoking and alcohol consumption, these possible confounders were addressed. Participants who were HIV infected, had a tympanum temperature of >37.5°C, clinically diagnosed diabetics or taking statin containing medicine were also excluded from analyses.

5. Discussion of main findings

Research has indicated the possibility of sympathetic hyperactivity as a prominent occurrence in urban Africans,[5] significantly contributing to the high prevalence of hypertension.[29-32] The additive effect of a dominant vascular response pattern may increase this population's risk for cardiovascular disease and future cardiovascular events.[1-3,5-7,9,33] Literature in which different methods were applied in African men to increase a high level of interpretation pertaining autonomic as well as cardiovascular responses, is limited. This could establish whether the suggested sympatho-vagal imbalance as well as dominant vascular response pattern can possibly be implicated in the weak cardiovascular profile demonstrated.[1]

The focus of this study was to scrutinize autonomic and cardiovascular responses in a cohort of urban African and Caucasian men. The possibility of the African men having a disturbed sympatho-vagal balance was particularly postulated as well as its association with blood pressure elevation and target organ damage.

It is known that hypertension presents as a significant, prevalent health risk for urban Africans, particularly men.[1,3,5-7] Sympathetic hyperactivity and an exaggerated vascular response pattern have both been suggested as promoting factors of this manifestation of chronic elevation of blood pressure.[5-7] Although findings of this study cannot be extrapolated to the entire urban male African community in South Africa, results indicated the same trend as described above.

The assessment of BRS is an accurate indication of autonomic control of the cardiovascular system.[34] Reduced sensitivity indicates an increase of sympathetic activity and a decrease of parasympathetic activity.[10,35,36] In this study, the African men particularly exhibited attenuated BRS, which thus supports the validity of a suggested sympatho-vagal imbalance postulated in this population group.[5] In hypertension, attenuated BRS particularly manifests as an initiating/promoting factor, accelerating left ventricular hypertrophy development and subsequent diastolic dysfunction. A correlation was only illustrated in the African men between lower BRS and blood pressure. However, a 10.8% larger cardiovascular risk was indicated in this cohort than in their Caucasian counterparts.

A sympatho-vagal imbalance particularly affects the circadian rhythm of blood pressure.[14] With sympathetic hyperactivity found in black population groups[4] as indicated in this cohort of African men as well, nocturnal hypertension manifests particularly often.[19] The

vulnerability to cardiovascular disease and target organ damage may therefore be increased even further, particularly the risk for atherosclerosis.[27] This study's findings particularly support this notion.

The validity of the notion of sympathetic predominance and attenuated parasympathetic influence on cardiovascular function in the African men was reinforced by a reduced NOx stress response. The depletion of NO bioavailability particularly promotes atherogenesis[23] affecting the coronary circulation in particular.[26] This reduction of NO bioavailability was accompanied by myocardial ischemia. In addition, presenting with a pattern of high blood pressure, elevated systemic inflammatory levels and lower physical activity, this population group particularly presents with a high risk for silent coronary artery disease[19,37-39] and future cardiovascular events.

6. Conclusions

To conclude, through assessing autonomic and cardiovascular responses, it has been established that this cohort of urban African men may have a significantly larger cardiovascular risk through sympathetic hyperactivity and coupled dominant vascular responses, particularly during exposure to challenges or psychosocial stress. Based on these findings, this population group's risk for accelerated target organ damage, particularly atherosclerosis and coronary artery disease as well as future cardiovascular events, may significantly be higher compared to those of the Caucasian men involved in this study. The overarching hypothesis of this thesis thus was accepted.

7. Recommendations

The following recommendations are proposed to possibly improve the cardiovascular health of African men:

The following recommendations are proposed for future studies:

- Participant groups should be larger to allow sufficient statistical power for sub-stratification.
- Rather than using an indirect marker, e.g. the Cornell product, left ventricular hypertrophy should be measured through echocardiography.
- Exploration of autonomic and cardiovascular function within a longitudinal design is necessary to substantiate possible trends established in this study.
- Autonomic and cardiovascular responses should be assessed in women as well to indicate whether the trend of a sympatho-vagal imbalance is possibly present in this group as well.
- Micro-albuminuria as marker of endothelial dysfunction should be included in future studies as substantiation of trends indicated through NOx responses.
- To further support the notion of autonomic dysregulation of cardiovascular function, recovery of cardiovascular variables, e.g. heart rate and blood pressure, should additionally be assessed after stressor application.

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I, **Ms Cecilia van der Walt,** hereby confirm that I took care of the **editing** of the **Thesis** of **Ms AS Uys** titled ***Comparing autonomic and cardiovascular responses in African and Caucasian men: The SABPA Study.***

MS CECILIA VAN DER WALT

HED, BA
Plus Language editing and translation at Honours level,
Plus Accreditation with SATI for Afrikaans and translation
Registration number with SATI: 1000228

Email address: ceciliavdw@lantic.net

Cell phone number: 072 616 4943