Neuro-endocrine coping responses in African and Caucasian teachers from the North-West Province: the SABPA study

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Artworks were hand drawn by Truitjie du Plessis, adapted from Inside the Human Body (Singapore: Pepin Press, 2009).

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Summary

TITLE

Neuro-endocrine coping responses in African and Caucasian teachers from the North-West Province: the SABPA study

MOTIVATION

Myocardial infarction, stroke and hypertension (HT) prevalence have escalated in urban Africans. Psychosocial stress and behavioural defensive coping (DefS) have been depicted as important contributory factors in cardiovascular disease (CVD) risk, particularly in urban African men. The specific mechanism by which neuro-endocrine coping responses impact on CVD risk is however, uncertain. Furthermore, studies on the neuro-endocrine stress mediators, cortisol and norepinephrine (NE), have shown variance in affecting cardiovascular health. Conversely, estradiol (E2) has attracted minimal attention in stress and coping research, but is deemed a coping response modulator and may be cardioprotective. However, it is still debatable whether E2 is cardioprotective in men and it is controversial whether reduced or excessive E2 may be beneficial. It is therefore evident that more research is needed to clarify the roles of E2, NE and cortisol in neuro-endocrine coping responses, especially in DefS Africans with elevated CVD risk.

AIMS

The primary aim of this study was to assess the influence of neuro-endocrine coping responses on CVD risk, in an urban South African cohort. The impacts of the neuro-endocrine stress mediators (NE and cortisol) on subclinical vascular and renovascular disease risk, respectively, were to be determined. Additionally, E2 was studied to determine its effects on neuro-endocrine coping responses and CVD risk. Furthermore, the effects of particularly DefS utilisation as regards to the aforementioned were to be determined.

METHODOLOGY

This study is embedded in the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study. Recruited participants included n=200 African and n=209 Caucasian teachers from the North-West Province (Dr Kenneth Kaunda Education District), South Africa, of a similar socioeconomic status. From these participants, n=19 HIV positive, n=12 clinically confirmed diabetic, and n=1 renal impairment cases were excluded from analyses. Additionally, cortisone users (n=2) were excluded from *Manuscripts 2* and *3*, due to its effects on the cortisol values studied. The final participant group consisted of n=168 Africans and n=207 Caucasians. Groups were stratified according to sex, ethnicity and/or coping style, and according to statistical significant interactions between major variables.

The Coping Strategy Indicator questionnaire was used to assess preferred coping responses of each participant; their stress experience was indicated on the ambulatory diary cards. Neuro-endocrine variables included MHPG (3-methoxy-4-hydroxyphenolglycol), cortisol, E2 and the original cortisol-to-E2 ratio. Cardiometabolic variables included waist circumference, cholesterol, glycated haemoglobin, C-reactive protein and blood pressure (BP), while carotid intima-media thickness of the far wall (CIMTf), the albumin-to-creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) indicated target organ damage.

Statistical analysis comprised receiver operating characteristics (ROC), which determined a cut point for exacerbated CVD risk. Means and proportions were determined with standard T-tests, analysis of covariance and Chi-squares. Multiple univariate and multivariate linear regression analyses calculated independent relationships between major variables while odds ratios (OR) determined probability, independent of covariates.

RESULTS AND CONCLUSIONS

The results and conclusions of the three manuscripts prepared for this thesis are as follows:

1. **Defensive coping and subclinical vascular disease risk - Associations with autonomic exhaustion in Africans and Caucasians: The SABPA study**

   In the first manuscript, the impact of NE (MHPG) levels on subclinical vascular disease risk in Africans and Caucasians utilising DefS was determined. Main findings revealed high self-reported stress and DefS scores in urban Africans. In African women this was found in co-occurrence with decreased MHPG levels and increased subclinical vascular disease risk (CIMTf). Lower or possibly down-regulated MHPG also predicted increased CIMTf in African men. African men and women also displayed low-grade inflammation (C-reactive protein >3 mg/l) and a pre-diabetic state (glycated haemoglobin >5.7%). Therefore, the urban Africans presented with higher subclinical vascular disease risk, especially when defensive coping "fails" and sympathetic activity diminishes (possible autonomic exhaustion), probably ensuing sympathetic hyperactivity, NE overload and highly stimulated α_1-adrenergic activity; predisposing to pathology risk.

2. **Defensive coping and renovascular disease risk - Adrenal fatigue in a cohort of Africans and Caucasians: The SABPA study**

   The second manuscript explored the association between urinary cortisol levels and renovascular disease risk in Africans and Caucasians, and the impact of DefS on this risk. Results demonstrated that high cortisol Caucasians were more vulnerable to renovascular disease than their low cortisol counterparts. Conversely, more Africans reported severe stress but displayed lower cortisol concentrations. Increased ACR and decreased eGFR were shown in co-occurence with this decreased cortisol in Africans, especially in the DefS users. Therefore, sustained uncontrollable stress may drain coping abilities and resources in DefS Africans, giving rise to HPA dysfunction and/or adrenal fatigue with subsequently decreased cortisol. Nevertheless, possibly preceding hypercortisolism levels may have facilitated permanent physiological damage; this may contribute to persistent renovascular disease risk in low cortisol DefS Africans.

3. Defensive coping and estradiol - Unravelling neuro-endocrine dysfunction and augmented hypertension risk in Africans compared to Caucasians: The SABPA study

The final manuscript investigated whether urban Africans with higher self-reported stress than Caucasians, would present with increased E2 levels. Furthermore, we aimed to determine whether increased E2 would be associated with HPA hypoactivity and an augmented risk of HT in urban DefS Africans, particularly in men. The main findings revealed increased stress experience, E2 levels and HT risk in Africans compared to Caucasians. The original cortisol-to-E2 ratio was decreased in Africans, particularly in men, and was associated with augmented BP. These findings indicate that HT risk in DefS African men coincides with neuro-endocrine dysfunction and possibly highly stimulated α_1-adrenergic vasoconstrictory responsiveness. In sustained stress, increased E2 may contribute to cardiovascular risk rather than accommodating cardioprotection, particularly in urban DefS African men.

GENERAL CONCLUSION

Neuro-endocrine dysfunction was evident in urban Africans, particularly in men who reported severe stress. Nonetheless, the Africans' coping resources (stress mediators) were inadequate for effective coping. In sustained stress, neuro-endocrine stress mediators (vasoconstrictory properties) may be down-regulated while E2 as coping modulator is up-regulated to enhance its vasodilatory and cardioprotective effects. Nevertheless, excessive E2 may also be detrimental and exacerbates CVD risk, particularly in men. Coping ability may further be impaired with sympatho-adrenal-medullary and HPA suppression, negatively influencing future coping responses of already severely stressed Africans. Increased E2 may therefore augment CVD risk in especially DefS African men, possibly through highly stimulated α_1-adrenergic vasoconstrictive activity. In fact, the observed increase in E2 and

decrease in neuro-endocrine stress mediators were respectively associated with increased risk of HT, subclinical vascular and renovascular disease in urban DefS Africans.

KEY WORDS

Defensive coping; Dissociation; Norepinephrine; Cortisol; Estradiol; Hypertension; Subclinical vascular disease; Renovascular disease; Autonomic exhaustion; Adrenal fatigue.

Opsomming

TITEL

Neuro-endokriene coping reaksies in Afrikaan en Kaukasiër onderwysers van die Noordwes Provinsie: die SABPA studie

MOTIVERING

Hipertensie (HT), miokardiale infarksie en beroerte voorkoms is aan die styg in stedelike Afrikane. Psigososiale stres en defensiewe coping (DefS) is albei al geassosieer met kardiovaskulêre siekte (CVD) risiko, veral in stedelike Afrikaan mans. Daar is egter nog onsekerheid oor die spesifieke meganisme waarop neuro-endokriene coping reaksies ‘n effek uitoefen op CVD risiko. Vorige studies oor neuro-endokriene stres tussengangers, naamlik norepinefrien (NE) en kortisol het verskillende effekte getoon met betrekking tot CVD risiko. Nietemin het estradiol (E2) nog min aandag gekry in stres en coping studies, maar is as coping moduleerder aangewys en dit het ook kardiobeskermende effekte. Nogtans is dit debatteerbaar of E2 beskermend is in mans en dit is onbekend of verhoogde of verlaagde E2 uiteindelik voordelig kan wees. Dit is dus duidelik dat verdere navorsing benodig word om die rolle van E2, NE en kortisol in neuro-endokriene coping reaksies te bepaal, veral in DefS Afrikane met ‘n verhoogde CVD risiko.

DOELSTELLINGS

Die oorhoofse doelstelling vir hierdie studie was om die invloed van neuro-endokriene DefS reaksies op kardiovaskulêre risiko te bepaal, in ‘n Suid Afrikaanse geslagskohort. Die impak van die neuro-endokriene stres tussengangers (NE en kortisol) op subkliniese aterosklerose en renale vaskulêre skade is onderskeidelik bepaal. Daarbenewens is E2 bestudeer om te bepaal watter effek dit het op neuro-endokriene coping reaksies en CVD risiko. Verder is die effekte van spesifiek DefS met betrekking tot die bogenoemde bestudeer.

METODOLOGIE

Hierdie studie vorm deel van die SABPA (Simpatiese aktiwiteit en Ambulatoriese Bloeddruk in Afrikane) studie. Gewerfde deelnemers het n=200 Afrikaan en n=209 Kaukasiër onderwysers, met soortgelyke sosio-ekonomiese status, van die Noordwes Provinsie (Dr Kenneth Kaunda-onderwysdistrik), Suid-Afrika ingelsuit. Van die bogenoemde deelnemers is egter n=19 MIV positiewe, n=12 klinies bevestigde diabete en n=1 deelnemers met nierskade gevalle uitgesluit uit analises. Verder is kortisoon gebruikers (n=2) ook uitgesluit uit analises vir *Artikels 2* en *3*, aangesien dit effekte het op die kortisolvlakke bestudeer in hierin. Die finale deelnemergroep het n=168 Afrikane en n=207 Kaukasiërs ingelsuit. Groepering is behartig volgens etnisiteit, geslag en/of coping, met verdere groeperings bepaal deur statistiese interaksies.

Die Coping Strategie Indikator vraelys is gebruik om te bepaal watter coping strategie elke deelnemer hoofsaaklik gebruik. Ervaring van stres is aangedui op die ambulatoriese bloeddrukkaarte. Neuro-endokriene veranderlikes het MHPG (3-metoksie-4-hidroksiefenielglikol), kortisol, sowel as E2 en die kortisol-tot-E2 verhouding ingesluit. Kardiometaboliese veranderlikes het weer middellyf omtrek, kolesterol, hemoglobien A1c, C-reaktiewe proteïen en bloeddruk (BD) ingesluit. Teikenorgaan skade merkers het bestaan uit karotis intima-media verdikking van die ver wand (CIMTf), die albumien-tot-kreatinien verhouding (ACR), asook die beraamde glomerulêre filtrasie tempo (eGFR).

Statistiese analises het die volgende ingesluit: "*receiver operating characteristics*" (ROC) analises om 'n afsnypunt vir verhoogde CVD risiko te bereken; gemiddelde waardes en proporsies is bereken met standaard T-toetse, analise van kovariansie en Chi-kwadraattoetse. Veelvuldige een- en meerveranderlike lineêre regressie analises het verhoudings tussen die hoofveranderlikes van elke manuskrip bereken. Kans verhoudings is

ten einde bereken om die waarskynlikheid van verhoogde CVD risiko in bepaalde groepe te bereken.

RESULTATE EN GEVOLGTREKKINGS

Hieronder volg die resultate en gevolgtrekkings van elke manuskrip in die proefskrif:

1. **Defensiewe coping en subkliniese aterosklerose risiko – Assosiasies met outonome uitputting in Afrikane en Kaukasiërs: Die SABPA studie**

   In die eerste manuskrip is die impak van NE (MHPG) vlakke bepaal op subkliniese aterosklerose risiko in Afrikane en Kaukasiërs wat van DefS gebruik maak. Hoof bevindinge het aangetoon dat beide gerapporteerde stres en gebruik van DefS hoog was in stedelike Afrikane. In Afrikaan vrouens is hierdie tesame met verlaagde MHPG vlakke en verhoogde subkliniese aterosklerose risiko (CIMTf) verkry. Daarbenewens het verlaagde of moontlik afgereguleerde MHPG vlakke verhoging van CIMTf in Afrikaan mans voorspel. Die Afrikaangroepe het verder ‘n lae-graadse inflammatoriese (CRP >3 mg/l) en prediabetes (glikeerde hemoglobien > 5.7%) toestande getoon. Die Afrikane het dus ‘n verhoogde risiko vir subkliniese aterosklerose getoon, veral wanneer DefS reaksies “misluk” en simpatiese aktiwiteit afneem (moontlike outonome uitputting). Hierdie uitputting kan moontlik voorafgeloop word deur simpatiese hiperaktiwiteit, NE oormaat en verhoogde α_1-adrenergiese aktiwiteit, wat hierdie groep dus vatbaar maak vir patologie.

2. **Defensiewe coping en risiko vir renale vaskulêre skade – Adrenale uitputting in ‘n Afrikaan en Kaukasiër kohort: Die SABPA studie**

   Die tweede manuskrip het die assosiasie tussen urienkortisol vlakke en renale vaskulêre skade in Afrikane en Kaukasiërs bestudeer, asook die impak van DefS op hierdie risiko. Resultate het daarop gedui dat Kaukasiërs wat hoër kortisol vlakke het meer vatbaar is vir renale vaskulêre skade. Aan die ander kant het Afrikane hoër stres gerapporteer, maar hul kortisol vlakke was laer in vergelyking met die van Kaukasiërs. Ter gelyke tyd, is

verhoogde ACR en verlaagde eGFR ook in die Afrikane verkry, veral in diegene wat gebruik maak van DefS. Volgehoue onbeheerbare stres mag dus coping vermoëns en hulpbronne so aantas dat dit HPA disfunksie veroorsaak en/of adrenale uitputting, wat uiteindelik kan lei tot 'n verlaging in kortisol vlakke. Nietemin, sal die moontlik voorafgaande HPA hiperaktiwiteit en gepaardgaande hoë kortisolvlakke permanente fisiologiese skade kan veroorsaak wat dus bydra tot die renale vaskulêre skade risiko in veral lae kortisol DefS Afrikane.

3. **Defensiewe coping en estradiol – Ontrafeling van neuro-endokriene disfunksie en verhoogde hipertensie risiko in Afrikane in vergelyking met Kaukasiërs: Die SABPA studie**

   Die laaste manuskrip het bepaal of stedelike Afrikane, met hoër self-gerapporteerde stres as Kaukasiërs, verhoogde E2 vlakke sal toon. Verder is bepaal of verhoogde E2 geassosieer sou word met HPA hipoaktiwiteit en 'n verhoogde risiko van HT in DefS Afrikane, veral in mans. Resultate het aangetoon dat stres ervaring, E2, en BD verhoog was in stedelike Afrikane teenoor Kaukasiërs. 'n Verlaagde kortisol-tot-E2 verhouding was verkry in assosiasie met verhoogde BD in veral Afrikaan mans, maar nie in Kaukasiërs nie. Hierdie bevindinge toon aan dat HT risiko in DefS Afrikaan mans saamval met neuro-endokriene disfunksie en moontlik hoogs gestimuleerde α_1-adrenergiese vasokonstriktiewe reaktiwiteit. Gedurende onbeheerbare volgehoue stres, kan verhoogde E2 bydra tot kardiovaskulêre risiko eerder as beskerming, in veral stedelike DefS Afrikaan mans.

ALGEMENE GEVOLGTREKKING

Neuro-endokriene disfunksie is opgemerk in Afrikane en veral in mans wat ernstige stres gerapporteer het, maar onvoldoende coping hulpbronne (stres tussengangers) gehad het. Volgehoue stres kan E2 met vasodilatoriese en kardiobeskermende eienskappe, opreguleer.

Nietemin kan oormatige E2 ook skadelik wees en CVD risiko beïnvloed, veral in mans. Daarbenewens kan af-regulering van vasokonstriktiewe neuro-endokriene stres tussengangers ook plaasvind as homeostatiese meganisme. Toekomstige coping vermoë kan egter negatief beïnvloed word deur SAM en HPA onderdrukking in Afrikane wat reeds oorweldig word deur stres. Verhoogde E2 vlakke kan dus kardiovaskulêre risiko verhoog in stedelike DefS Afrikaan mans. Dit kan moontlik toegeskryf word aan verhoogde α_1-adrenergiese vasokonstriktiewe aktiwiteit. Inderdaad is die waargenome afname in neuro-endokriene stres tussengangers en toename in E2 onderskeidelik geassosieer met verhoogde risiko vir subkliniese aterosklerose, renale vaskulêre skade en HT in stedelike DefS Afrikane, veral in mans wat volgehoue stres ervaar.

SLEUTELWOORDE

Defensiewe coping; Dissosiatiewe coping; Norepinefrien; Kortisol; Estradiol; Hipertensie; Subkliniese aterosklerose; Renale vaskulêre skade; Outonome uitputting; Adrenale uitputting.

Preface

This thesis is written in article format and consists of three peer-reviewed published (or submitted for publication) original research manuscripts. A comprehensive overview of the coping literature as well as a concise but critical revision of neuro-endocrine coping responses and cardiovascular disease risk is presented in *Chapter 1*. This chapter also includes the aims and hypotheses, for the entire study and each manuscript separately, followed by references according to the Vancouver style. The three manuscripts can be found in *Chapters 2, 3,* and *4*, entailing abstracts, introductions, methods, results, conclusions, and appropriate referencing formats according to each specific peer-reviewed journal's guidelines. *Chapter 5* consists of the main findings and conclusions, as well as the study limitations and recommendations for future research. Of note, black South Africans are referred to as Africans while white South Africans are referred to as Caucasians, throughout the thesis.

A web-based citation management programme namely RefWorks, was used to finalise all reference lists. All graphs were created with Microsoft® Excel and GraphPad Prism® computer software. Tables and figures were allocated Arabic numerals consecutively in order of appearance and according to the respective chapter of the thesis. The artwork on the first page of every chapter was hand-drawn by Mrs T du Plessis. Descriptions and labels for the artwork were prepared by A de Kock.

All manuscripts have been submitted to peer-reviewed journals for publication. The first article namely; *Defensive coping and subclinical vascular disease risk – Associations with autonomic exhaustion in Africans and Caucasians: the SABPA study*, has been published in the journal *Atherosclerosis* with an impact factor of 3.97. Results of this manuscript were presented at the *SA Heart Congress 2012* and *2013*, with the abstracts published accordingly in the *SA Heart Journal* of the corresponding year.

The second article namely; *Defensive coping and renovascular disease risk – Adrenal fatigue in a cohort of Africans and Caucasians: the SABPA study*, has been published in the journal *Physiology and Behavior*, with an impact factor of 3.03. The third research article *(Defensive coping and estradiol – Unravelling neuro-endocrine dysfunction and exacerbated hypertension risk in a South African cohort: The SABPA study)* has been submitted to *Endocrine,* with an impact factor of *3.88*. This manuscript has been assigned a number *(ENDO-D-15-00888)* and is currently under review.

The promoter and co-promoters agreed on co-authorship in all three manuscripts, and gave consent for the use of these manuscripts as part of the final thesis. Additionally, in *Manuscripts 2* and *3*, a statistical consultant validated all results and was included as co-author for her expertise input. The first author was, however, solely responsible for literature searches, all initial statistical analysis, interpretation of all results, as well as planning and writing of the three manuscripts and the entire thesis. This author also contributed to collection and interpretation of data in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study as well as the Prospective Urban Rural Epidemiology (PURE) study, at the North-West University, Potchefstroom *(see Postgraduate Student Skills)*.

Postgraduate Student Skills 2015

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

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

Prof L Malan
(RN, HED, PhD)
Principal Investigator of SABPA

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Statement by Authors

Herewith follows each author's contributions to the study, manuscripts, and entire thesis:

Mrs A de Kock	*Main author* - Responsible for the initial planning and proposal of the doctoral study and manuscripts, data collection, statistical analysis, interpretation of results, and writing of the manuscripts and entire thesis.
Prof L Malan	*Promoter* - As principal investigator of the SABPA study, aided in the study design and data collection. Supervised the planning and writing of the manuscripts and entire thesis. Provided support, guidance and expertise intellectual input.
Prof M Hamer	*Co-promoter* - Assisted in planning of the manuscripts and provided critical feedback as well as expertise input to the written material.
Prof NT Malan	*Co-promoter* - Assisted in planning of the manuscripts as well as data collection. Provided critical feedback and intellectual input to the written material.
Mrs M Cockeran	*Co-author and Statistician* - Validated statistical analysis and results to ensure accuracy and reliability of data, in both *Manuscripts 2* and *3*.

Herewith is a statement of all co-authors verifying their actual contribution to the study and giving permission that all three manuscripts may form part of the thesis.

"I hereby declare that my role as indicated above is representative of my actual contribution to the study and/or thesis. I approve the manuscripts and give my consent that these manuscripts may be published as part of the thesis for the degree Philosophiae Doctor of Mrs Andrea de Kock."

Prof Leoné Malan

Prof Mark Hamer

Prof Nico T. Malan

Mrs Marike Cockeran

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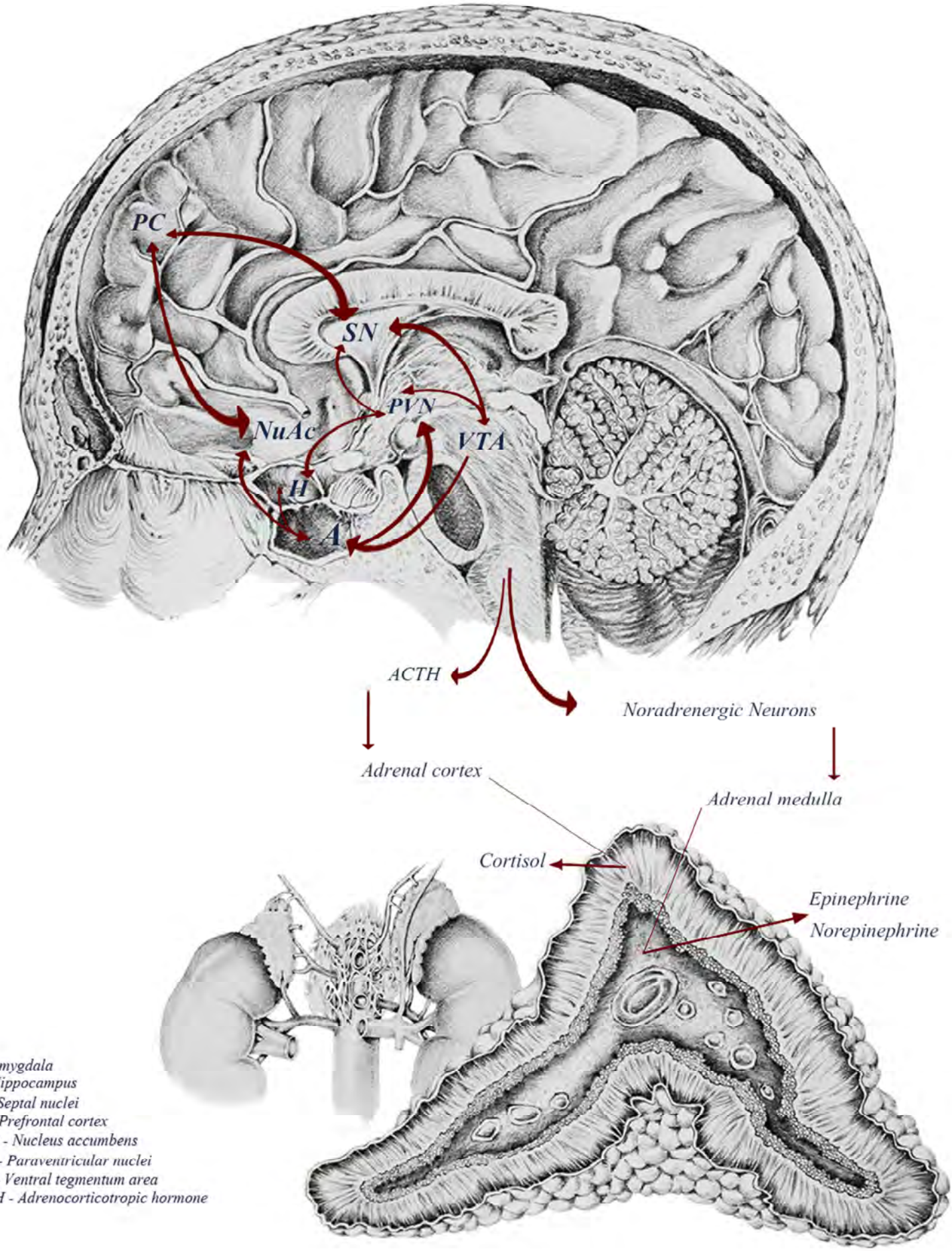
List of Symbols and Abbreviations

α	alpha
β	beta
ɣ	gamma
%	percentage
ABPM	ambulatory blood pressure monitoring
ACE	angiotensin converting enzyme
ACR	albumin-to-creatinine ratio
ACTH	adrenocorticotropic hormone
ADH	antidiuretic hormone
AIDS	Acquired Immune Deficiency Syndrome
ANCOVA	analysis of covariance
BP	blood pressure
bpm	beats per minute
BSA	body surface area
°C	degree Celsius
CBG	corticosteroid binding globulin
CCR	cortisol-to-creatinine ratio
cGGT	serum gamma glutamyl transferase
CI	confidence intervals
CIMT	carotid intima-media thickness
CIMTf	carotid intima-media thickness of the far wall
cm	centimetre
CO	cardiac output
Cort:E2	cortisol-to-estradiol ratio
CRF	corticotrophin releasing factor
CRP	C-reactive protein
CSI	Coping Strategy Indicator
CV	coefficient of variance
CVD	cardiovascular disease
DefS	defensive coping
DBP	diastolic blood pressure
E2	estradiol
EDTA	ethylenediaminetetraacetic acid
ER	oestrogen receptor

ESC	European Society of Cardiology
ESH	European Society of Hypertension
et al.	*et alia* (and others)
eGFR	estimated glomerular filtration rate
GFR	glomerular filtration rate
h	hour
HART	Hypertension in Africa Research Team
HbA1c	glycated haemoglobin
HDL-C	high-density lipoprotein cholesterol
HIV	Human Immunodeficiency Virus
HPA	hypothalamic-pituitary-adrenal
HR	heart rate
hs-CRP	ultrahigh-sensitivity C-reactive protein
HT	hypertension
i.e.	*id est* (that is)
kcal	kilocalories
kg	kilogram
kg/m^2	kilograms per square metre
HDL-C	high-density lipoprotein cholesterol
HPLC	high performance liquid chromatography
LDL-C	low-density lipoprotein cholesterol
m^2	square metre
MDRD	Modification of Diet in Renal Disease
MHPG	3-methoxy-4-hydroxyphenolglycol
min	minute
ml	millilitre
mg/l	milligrams per litre
mm	millimetre
mmHg	millimetre of mercury
mmol/l	millimole per litre
n	number
ng/ml	nanograms per millilitre
NE	norepinephrine
NWU	North-West University
OR	odds ratio
P	probability

PA	physical activity
pg/ml	picograms per millilitre
pmol/l	picomole per litre
PURE	Prospective Urban Rural Epidemiology
r	correlation coefficient
R^2	relative predictive power of a model
ROC	receiver operating characteristics
SABPA	Sympathetic activity and Ambulatory Blood Pressure in Africans
SAM	sympatho-adrenal-medullary
SBP	systolic blood pressure
SD	standard deviation
SHBG	serum hormone binding globulin
SMAC	sequential multiple analyser computer
SV	stroke volume
TPR	total peripheral resistance
u/l	units per litre
WC	waist circumference
WMA	World Medical Association
χ^2	chi-square

CHAPTER 1



A - Amygdala
H - Hippocampus
SN - Septal nuclei
PC - Prefrontal cortex
NuAc - Nucleus accumbens
PVN - Paraventricular nuclei
VTA - Ventral tegmentum area
ACTH - Adrenocorticotropic hormone

Literature Overview, Aims and Hypotheses

1. INTRODUCTION

The World Health Organization has stated that stress is one of the greatest health problems of this century [1], and in South Africa, urbanisation may be considered a chronic stressor [2]. Furthermore, neuro-endocrine coping responses seem to play an important role in enhanced susceptibility to disease [3-4]. Therefore, not only psychosocial stress, but also the specific coping responses additionally utilised in dealing with stress are strongly related to cardiovascular disease (CVD) [2,5-8].

When a stressor is perceived as easy to deal with and the individual feels in control, a defensive active coping response will be activated along with release of norepinephrine and epinephrine [9]. Upon experiencing increasing angst and/or demands, active coping will shift to a more passive response with a loss of control and release of cortisol [9]. Theoretically, higher stress should be accompanied by higher concentrations of neuro-endocrine mediators for effective coping [3], although this is dependent on perception, coping abilities and the resources of the individual [10]. Contradictory results have been found, particularly regarding chronic stress, which may be due to habituation to stressors and/or neuro-endocrine dysfunction [4,11]. Moreover, in chronic stress, reduced stress mediator levels may still incur pathology, as coping ability may be impaired and homeostasis needs to be attained [12-18].

It is here that estradiol (E2), as a coping response modulator and homeostasis regulator, comes into play [19-22]. This sex steroid might further explain the sexual dimorphism observed in coping responses [23], and CVD [24-26]. Since the stress mediators (norepinephrine, epinephrine, and cortisol) mainly evoke vasoconstrictory responses, E2 may be up-regulated in sustained stress together with enhanced vasodilatory responses, as

a protective mechanism [21-22]. However, disparate findings and sex differences complicate the impact of E2, while literature in this regard, particularly that concerning Africans, is scant. Likewise, insufficient research has been undertaken on norepinephrine in the defensive active coping response as most studies have focussed on cortisol and uncontrollable stress, whilst specific coping responses have excited little interest.

The interplay of neuro-endocrine coping responses, E2 as coping modulator and the stress mediators has not been extensively studied in relation to hypertension and vascular disease markers in South Africans. This study may therefore shed light on the cardiovascular vulnerability in Africans. A comprehensive background on coping (behaviourally and physiologically), together with a condensed but critical revision of the modulator and mediators of the coping responses and the cardiovascular effects of these factors, is presented below.

2. COPING

2.1. What is coping?

Everyone encounters stressors throughout life, but how one copes with stress ultimately influences adaptability and pathology risk [27]. Coping involves primary appraisal (what is the problem and should I worry about it?) and secondary appraisal (do I have resources to do anything about it?) [10,28-29]. The term “coping” has been defined as “cognitive and behavioural efforts to manage specific external or internal demands (and conflicts between them) that are appraised as taxing the resources of a person” [30]. These coping resources can be divided into internal psychological resources (i.e. personality characteristics) and external environmental ones (i.e. social support) [31]. Coping also has behavioural facets (activities to deal with stress) as well as physiological implications (neuro-endocrine activity and cardiovascular reactivity).

2.2. Behavioural coping responses

Coping behaviour involves any activities that aid in adaptation to the stressors or challenges of the environment. This may include unconscious behaviour such as defence mechanisms, as well as catharsis, meditation and/or exercise, together with more negative behaviours that are perceived to lessen stress, such as self-medication with alcohol and tobacco [32-33]. Two major coping strategies that have been described are problem-solving and emotion-focused, respectively encompassing active efforts to alleviate or eliminate stressors and efforts to regulate emotional consequences of stressors [28-29,34]. Individuals usually utilise both coping strategies, but one may be predominant. Problem-solving which is also referred to as the defence response [35], is mostly used for dealing with controllable stress. On the other hand, emotion-focused or defeat coping is used when events are perceived as uncontrollable [34]. Literature has further distinguished between active and avoidance coping strategies: active coping has been described as a response whereby the individual alters the stressor itself or the perception thereof. On the other hand, avoidance coping leads individuals into activities (i.e. alcohol use/abuse) or mental states (i.e. emotional withdrawal), which ultimately prevent them from dealing directly with the stressors [34,36]. These two behavioural coping responses are therefore strongly related to the physiological "fight-or-flight" reaction as will be described in *Section 3*.

2.3. The Coping Strategy Indicator

Amirkhan (1990) analytically developed the Coping Strategy Indicator (CSI) questionnaire *(see Appendix A)*, from deductive and inductive methodologies [37]. The CSI is a 33-item self-report measure of situational coping with three subscales. Each subscale refers to one of three coping strategies: problem-solving, avoidance and seeking social support. A higher score in a subscale determines preferred use of that specific coping strategy *(see Table 1.1)*. The questionnaire is answered on a Likert scale whilst the respondent is keeping a stressful event, within the past 6 months, in mind. This is relevant as recent life changes (in the

preceding 6-month period) are positively associated with cardiovascular events and/or sudden cardiac death [38].

Table 1.1: Cut points for utilisation of the CSI coping strategies [37]

	LOW	AVERAGE (mean)	HIGH
PROBLEM-SOLVING	≤ 21	21.5 - 30.5 (26)	≥ 31
SEEKING SOCIAL SUPPORT	≤ 18	18.5 - 27.5 (23)	≥ 28
AVOIDANCE	≤ 15	15.5 - 22.5 (19)	≥ 23

2.3.1. Problem-solving

The problem-solving strategy or defence response involves an active approach to solve the problem at hand [35]. The stressor is appraised as challenging and accepted as a reality, and the individual focuses on the stressor with effortful commitment, whilst all other activities are suppressed until success is achieved in eliminating or alleviating the stressor [29,31,39-40]. This may involve social support as well, and is associated with overall well-being.

2.3.2. Seeking social support

Seeking social support involves actively seeking comfort, help and advice from others in times of stress [39-41]. This basic need for human contact in stress may also form part of the active problem-solving strategy itself. Previous studies have determined that social support has beneficial effects on the endocrine, immune and cardiovascular systems [42-44]. Even perceived social support, and just knowing that support is available, reduce stress reactivity [44].

2.3.3. Avoidance

The avoidance strategy, a.k.a. passive coping or emotional avoidance, refers to physical and/or psychological withdrawal. This is an escape response that usually sets in when

distress is experienced or when the individual has little or no control over the problem or perceives stress as uncontrollable [31,40]. Avoidance has been associated with both depression and pathology [44-46].

2.4. Coping: Sex and ethnicity

Sexual dimorphism exists for stress coping responses and the related disease risks [23]. E2 with cardioprotective effects might be at the root of these differences, as women have shown more resilience [2]. The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study indicated that African men also have elevated E2 levels in accordance with high problem-solving coping, yet augmented CVD risk [5]. This may indicate an up-regulation of E2 as a homeostatic mechanism in these men. E2 and its effects are discussed further in *Sections 3 & 4*.

Ethnic disparities have also been observed in the prevalence and complications of a variety of medical conditions. Additionally, psychosocial stress is associated with these ethnic diverse pathologies, especially in Africans [2,47]. The impact that a stressor has on the physiological systems is, however, partly dependent on the circumstantial coping responses when dealing with the stressor, as well as genetics, lifestyle and previous experiences [27]. Chong *et al.* (2008) agree that genetic and socioeconomic differences may play roles in the abovementioned ethnic disparities, but add that ethnic variations in coping responses to environmental stressors may also be a factor [48]. Previous studies support this notion, where problem-solving, rather than avoidance coping, was determined as a greater risk for CVD in urban Africans when compared to their Caucasian or rural counterparts: particularly so in African men [47,49-50].

2.5. Coping responses and cardiovascular risk

Recent findings have revealed conflicting results about the different major coping behaviours and health outcomes. Research has shown that problem-focused coping is more related to

resilience and well-being, whilst emotion-focused coping is associated with low resilience [45,51-52]. Van Rhenen *et al.* (2008) and Tucker *et al.* (2009) concur that the active problem-solving strategy is a health promoter [53-54]. Furthermore, Park & Adler (2003) stated that greater use of both the problem-solving and emotion-focused coping responses ultimately facilitates better psychological well-being and physical health [55]. The aforementioned results contrast with previous findings in South Africans [5,45,47,49-50]. Between rural and urban Africans there seems to be a distinct difference in utilisation of coping responses and the extent to which this affects health and disease risk. Rural problem-solving Africans have been found to display more resilience, life satisfaction and adaptable coping responses, than their urban counterparts [45,49-50]. Moreover, the higher degree of social support and/or perceived support in rural regions, in contrast to the waning support systems in urban areas, may be protective against stress [45,56]. This is strongly related to the collectivistic, rather than individualistic, nature of African culture [46,57].

Malan *et al.* (2006) proposed a possible explanation for the pathological effects of problem-solving in urban Africans, as dissociation was observed between their behavioural and physiological coping responses, with apparent "loss of control" [49]. The urban Africans reported high behavioural utilisation of active problem-solving but physiologically revealed augmented avoidance responses, which was found to be in accord with higher cardiometabolic disease risk [49-50]. At play here is the enhanced cardiovascular reactivity that utilisation of coping responses (problem-solving and avoidance) in managing acute stress may cause [44,49]. Moreover, chronic stress experiences, such as the psychosocial stresses of an urban-dwelling lifestyle and ultimately acculturation [56], may exacerbate cardiovascular reactivity to acute stressors [49-50], and predispose to hypertension [58]. Therefore, the problem-solving strategy chiefly acts through a β_1-adrenergic stimulation pattern [59-61]. This will increase blood pressure via catecholamine actions, together with increases in heart rate, stroke volume and cardiac output. Total peripheral resistance usually normalises or decreases with problem-solving [61-62]. The avoidance strategy, on

the other hand, is mediated through α_1-adrenergic reactions and subsequent increases in norepinephrine (NE) and cortisol. Consequently, BP will be raised via vascular mechanisms and through vasoconstriction of skeletal muscles, whilst cardiac output decreases [62]. Therefore, the systemic resistance rises and is associated with the manifestation of hypertension (HT) when chronically increased [5,58]. Rural problem-solving Africans therefore seem to be more apt at coping, as their cardiovascular reactivity did reveal β-adrenergic stimulation together with central cardiac regulation and reduced CVD risk [45,49]. However, urban problem-solving Africans have shown α-adrenergic responses to stress, with enhanced vascular rather than cardiac stimulation and subsequently, more vulnerability to hypertension [2,5,63]. As early as 1997, Saab *et al.* commented that African American men display more peripheral resistance responses (α-adrenergic) to active coping mental stress tasks, in which Caucasians usually exhibit β-adrenergic activity [64]. Remarkably, there has been minimal research focused on this phenomenon since then. Hence, further investigation into the physiological coping responses of Africans in accord with CVD risk, is essential. This topic should also be studied in accord with interrelated factors such as E2, as men have been considered vascular reactors while women are cardiac reactors in stress [65], further complicating the pathology risk.

As urbanisation is on the increase, Africans and especially African men, are increasingly at risk of hypertension [5,66], atherosclerosis [47,67], stroke, left ventricular hypertrophy and silent ischaemia [68], as well as renal impairment [47,67]; coping responses may vastly influence their risk [5,8,47,60].

3. NEURO-ENDOCRINE COPING RESPONSES

3.1. The physiological coping responses (“fight-or-flight”)

The coping responses are regulated by the brain, which interprets sensory and motory information and then determines appropriate behavioural and/or physiological responses to

the specific stressor. Various brain areas, controling emotion, contribute to this regulatory function including the thalamus (receives sensory, auditory and visual information), amygdala (coordinates behavioural, autonomic and endocrine responses), prefrontal cortex (behavioural, emotional and cognitive control), ventral tegmentum area (cognition and reward) and the hypothalamus (primary output regulator for the limbic system) [69]. Please refer to *Figure 1.1* below *(also presented on page 1)* for more information on this regulatory process, as further detail does not fall within the scope of this study.

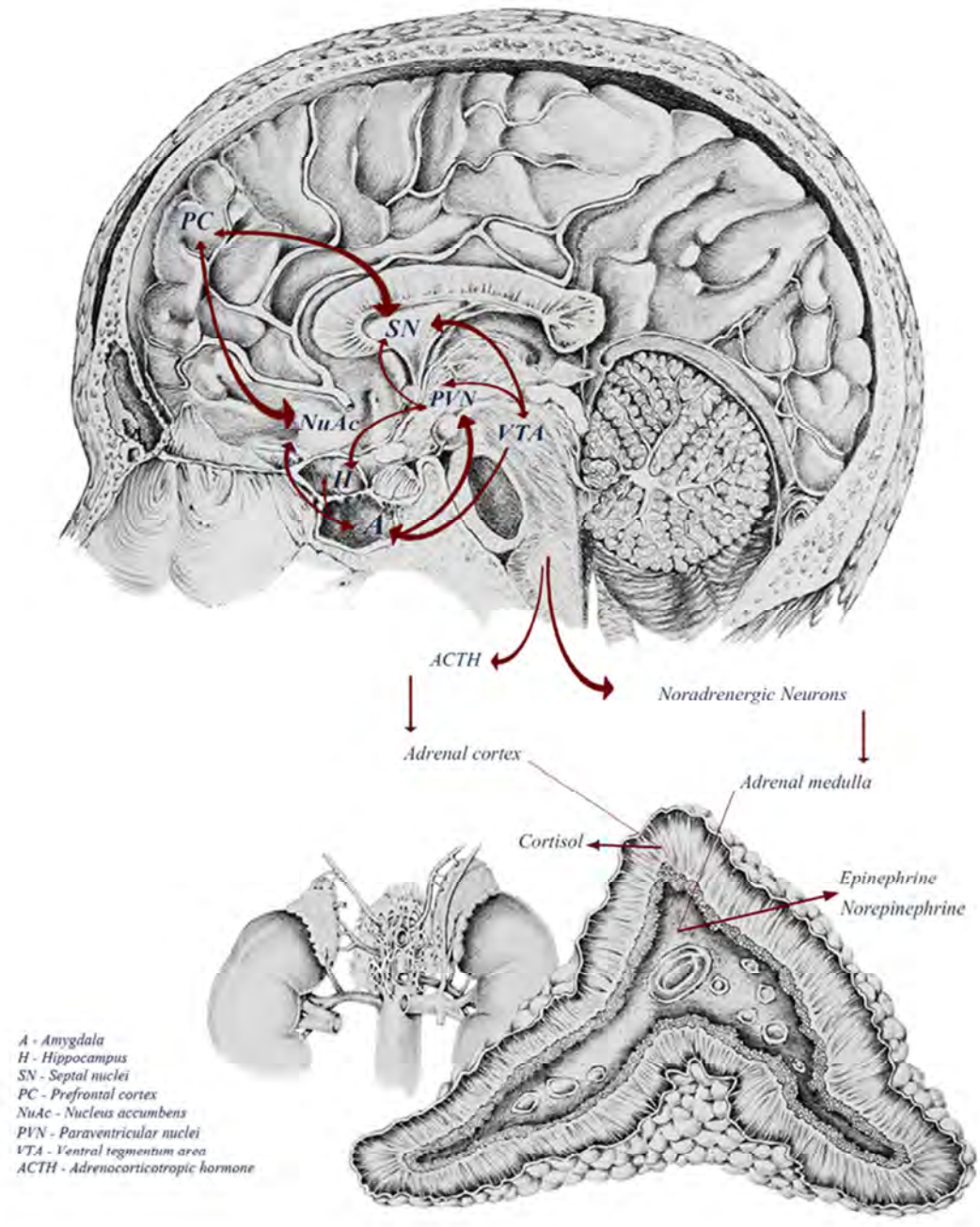


Figure 1.1: Regulation of the neuro-endocrine coping responses by the brain

Ultimately, the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adrenal-medullary (SAM) system, which play pivotal synergistic roles in the physiological "fight-or-flight" neuro-endocrine coping responses [70-71], will be activated. These systems and the stress mediators they stimulate provide usually beneficial, but sometimes detrimental, physiological changes in order to facilitate coping and adaptation [9,11,13,70,72-73] as is next described.

3.1.1. Defensive coping and sympathetic activation

An active and vigilant control seeking response ("fight") can be termed defensive coping (DefS) [74]. When an individual recognises a stressor as challenging and/or threatening, the SAM system will be activated for the first rapid effortful coping response [75]. Various sub-cortical brain areas will be stimulated including the thalamus, amygdala, prefrontal cortex, ventral tegmentum area and hypothalamic nuclei, with subsequent release of two of the major stress mediators, NE and epinephrine [4,70,76]. Both mediators refer to a defence response, but as this study's scope does not pertain to specific novel stressors and/or mental stressor exposure, the focus of this study is basal NE.

Upon release of NE from the adrenal medulla (chromaffin cells) as a hormone or from noradrenergic neurons as a neurotransmitter [77], α_1-, α_2-, and β_1-adrenergic stimulation patterns can be activated by binding to these respective receptors. The distribution and availability of these receptors in tissues as well as the NE concentration will, however, determine the stimulation pattern and NE's effects *(Table 1.2)*. NE levels can be altered by exercise, poor diet, smoking, alcohol abuse, myocardial infarction, essential hypertension and severe renal disease, as well as emotional stress [78] through altered neuronal reuptake and/or removal from the synaptic cleft.

Table 1.2: Catecholamine receptor binding and cardiovascular effects [11,80]

Receptor	Catecholamine Binding	Effect of Binding	Major cardiovascular effect
α_1	Norepinephrine/ Epinephrine	Increased calcium	Vasoconstriction
α_2	Norepinephrine/ Epinephrine	Decreased cyclic AMP	Inhibit NE release: vasodilation
β_1	Norepinephrine/ Epinephrine	Increased cyclic AMP	Increase heart rate & contractility
β_2	Epinephrine	Increased cyclic AMP	Vasodilation

Where: AMP; adenosine monophosphate, NE; norepinephrine.

Ultimately, with SAM stimulation, blood will be supplied to areas where most needed. DefS is therefore accompanied by increases in heart rate, contractility, stroke volume, skin conductance, dilation of bronchioles, blood pressure (BP) as well as increased glucose production for the purpose of rapid effortful coping, whilst non-essential functions, such as immune and gastrointestinal activities, are inhibited [4,11,49,61,72,74]. The DefS response is mostly related to increased cardiovascular β-adrenergic stimulation [49] and well-being [53].

3.1.2. Emotional avoidance and HPA activation

When the stressor is perceived as uncontrollable and/or the DefS response has failed, the hypothalamus and concurrently the entire HPA axis will be activated to facilitate coping [13,73-75,81]. This is a slower coping response, related to “flight”, avoidance, uncontrollability, emotional distress and a defeat reaction (α-adrenergic) [74,82]. It is through the HPA axis that the central nervous and endocrine systems are linked. Upon HPA axis activation, corticotrophin-releasing factor (CRF) will stimulate glucocorticoid (cortisol) release from the adrenal gland [70,75,83-84]. Cortisol will then bind to glucocorticoid receptors and favour α_1-adrenergic cardiovascular responses, since cortisol is permissive to NE’s effects, thus further contributing to vasoconstriction [12,85]. The main function of cortisol is, however, to activate physiological coping processes that aid in homeostasis and survival,

whilst inhibiting long-term functions such as growth, metabolism and immunity. Therefore, cortisol release coincides with increases in BP and respiration, as well as increases in blood perfusion to the brain, heart and muscles, while it regulates lipid and glucose availability for enhanced energy [4,13,83,86].

3.2. Sustained stress

3.2.1. Defensive coping and sympathetic hyperactivity

With successful coping, the vagal system is typically activated to decrease the stress mediator secretions and normalise the autonomic activity, whilst α_2-adrenergic activity stimulates a negative feedback loop to decrease NE levels [87]. However, sustained stress, sleep deprivation or apnoea, sedentary lifestyles, stimulant use or abuse, abdominal obesity, insulin resistance, hypertension and depression, can all cause chronic sympathetic activity with disruption of autonomic homeostasis [71].

Overwhelming or sustained stress may therefore interfere with coping ability, causing distress and hyperactivity of the SAM system, as the body tries to cope with increasing demands [13]. The protective β-adrenergic response will usually normalise, whilst vasoconstrictory α_1-adrenergic activity further augments release of NE through a positive feedback mechanism [79]. By themselves, neither the vagal system nor the negative feedback mechanism of the α_2-adrenergic receptors will be able to reduce the amounts of NE released [14,18]. Concentrations will increase even further and may cumulate in NE overload, causing a resultant hypervigilant DefS state. Indeed, findings have revealed that prolonged SAM activation and/or NE overload may further increase vasoconstriction, alter cardiovascular stress responses and facilitate HT and endothelial dysfunction, as well as atherosclerosis risk [18,58,88].

According to Julius (1994), the stages in the pathophysiology of HT nonetheless differ, regarding sympathetic nervous system activity [89]; the defence response plays a major part herein [90]. In early HT, there will be increased sympathetic but decreased vagal activity, and the resultantly elevated BP may be normalised by autonomic blockade at this stage. On the other hand, in advanced HT, cardiac compliance and β-adrenergic responsiveness diminish, whilst the hypertrophy of resistance vessels will further augment vascular resistance [89]. This is indicative of a shift from DefS (β-adrenergic with higher cardiac output) to emotional avoidance coping (α_1-adrenergic with higher peripheral resistance) responses [5,62]. In addition, this altered BP regulation in advanced HT may be what takes place in urban DefS Africans, which is then depicted as dissociation in coping responses. Ultimately, though, a new set point or cardiac output for essential HT develops [89]. Sympathetic hyperactivity will decrease in the end, as less sympathetic drive is needed to maintain the higher BP at a new set point [91]; this may be observed as a reduction in or as normal NE levels.

3.2.2. Emotional avoidance and HPA hyperactivity

Sustained stress may also result in a prolonged glucocorticoid response with excessive cortisol levels, which might further impair adaptation to stress [81,83]. Chronic stress may hyperactivate the HPA axis, so that the cortisol diurnal decline is flattened [82,92]. This will contribute to hypercortisolism and CVD risk, as the circadian release of cortisol is vital [4,83,92]. In fact, HT is frequently associated with HPA hyperactivity, behavioural loss of control, and hopelessness [93], conclusively implicating emotional avoidance in HT risk [2]. Moreover, cortisol can inhibit endothelial production of vasodilators [85,94] and because Africans display a higher tissue sensitivity to cortisol, this could make them even more vulnerable to the vasoconstrictive effects of cortisol [95]. Stone *et al.* (2001) also concluded that when the normal circadian rhythm of cortisol release is disturbed over a long period, as in sustained stress, the ability to down-regulate cortisol levels may be inhibited, with

resultant robust increases in cortisol [97]. The inhibitory feedback control of cortisol release in the hippocampus may therefore no longer operate functionally as hippocampal steroid receptors may be down-regulated or desensitised [13,84,97].

Huang *et al.* (2013) concur that prolonged production and secretion of cortisol can damage brain structures, especially the hippocampus, which in turn is necessary for the glucocorticoid negative feedback mechanism [65]. This may sequentially enhance stress-induced cortisol overproduction indefinitely, with detrimental effects. Furthermore, in depression (chronic uncontrollable stress), the adrenal and pituitary glands may become enlarged with hypersecretion of cortisol [84,93]. Moreover, depression has been linked to CVD risk, including HT and diabetes [98-99]. Contrastingly, burnout, which is also associated with CVD risk and characterised as vital and emotional exhaustion [97], has been observed along with increased and decreased cortisol levels [100-101]. However, the elevated cortisol in burnout cases has been found together with blunted cortisol stress responses [101]. In this case, free cortisol levels may be high or normal, but habituation and/or adrenal fatigue may have desensitised receptors, resulting in attenuated responses.

3.3. Sustained stress: Autonomic exhaustion and adrenal fatigue

It was previously stated that upon exposure and re-exposure to stress, different reactions are elicited, at first causing great increases in stress mediators which may then be followed by decreased levels. This is probably due to coping failure resulting in autonomic exhaustion and/or adrenal fatigue [13,18,100]. Autonomic exhaustion and adrenal fatigue could, with desensitisation and/or down-regulation of receptors, protect the body from excessive NE and cortisol [72]. Indeed, depression has been associated with reduced NE concentrations [14,102]. However, as mentioned, literature on NE in coping with sustained stress is scant, especially in Africans. Regarding cortisol, findings revealed that after repeated or intense stressor exposure, its release also decreased [15-16,75,103]. The HPA therefore becomes

hyporesponsive to stress, and cortisol down-regulation could follow, possibly indicating adrenal fatigue [13,16,72,75].

Even though the body may then be protected from overexposure to stress mediators, damage might already have occurred and homeostasis may not easily be restored. Prolonged hypercortisolism has been said to cause irreversible damage so that, even when cortisol levels are normalised or reduced, possibly following adrenal fatigue, pathology may still be present [104]. Indeed, hypoactivity of the HPA axis has also been linked to pathology, mortality and morbidity risk [105-107], whether through attenuated glucocorticoid availability or sensitivity [4,108-109]. Previous SABPA findings have also shown attenuated cortisol and NE stress responses in Africans, but in accordance with high-normal resting values of the stress mediators [12,63]. These responses were accompanied by high self-reported severe stress, depressive symptoms and CVD risk [5,12,63]. This possibly indicates the occurrence of autonomic exhaustion and/or adrenal fatigue rather than adaptive successful coping responses [110].

Nonetheless, various factors can alter cortisol levels, such as: psychosocial stress, anticipation of stress, mental stress tasks, time of sampling, method of sampling (serum, urinary, salivary, hair), analysis of samples, age, sex and health profiles of participants, as well as medication use (particularly corticosteroids), despite there still being disagreement in the literature. Thus, flatter diurnal declines have been observed in middle-aged and elderly men and women (50-70 years) in association with augmented CVD risk [81,111-112]. However, this occurrence was also noted in younger adults with a mean age of 40 years [92]. CVD risk was further revealed in association with either reduced cortisol or attenuated cortisol responses in coronary artery disease patients [112], myocardial infarction patients [105], and in SABPA African participants [12,63]. On the other hand, Nijm *et al.* (2007) reported stable profiles in coronary artery disease patients, in association with blunted cortisol responses [112]. Hamer and colleagues (2012) nevertheless ascribed coronary

artery calcium rather to enhanced cortisol responses [113]. Additionally, in coronary heart failure patients, increased serum cortisol levels were associated with increased mortality and risk of future cardiac events [114-115]. Therefore, sustained physiological and psychological stress, in accord with the specific coping responses utilised, may underlie the contradictory associations described, as this has not been previously studied. Additionally, the diverse effects regarding sex steroids needs further clarification.

3.4. Cortisol and Estradiol

Sexual dimorphism has been observed in HPA activity. Accordingly, women exhibit higher basal cortisol and cortisol reactivity than men [23,93]. In rats, this occurrence was further demonstrated through higher adrenocorticotropic hormone (ACTH) and corticosterone levels around the time of ovulation [19]. Additionally, sex steroids are believed to play a major role in the sensitivity of the HPA glucocorticoid negative feedback mechanism [116]. It was indicated that females are resistant to this negative feedback, resulting in prolonged augmentation of cortisol in stress, which may be related to their higher risk of depression [84].

In contrast, anticipation of stress evokes enhanced cortisol responses in men, whilst this anticipatory response is absent in women [76]. An enhanced hypothalamic drive has also been observed in young men, with higher cortisol stress responses compared to young women [19-20,75]. There is also an age-related decrease of this hypothalamic drive in men, resulting in corresponding ACTH responses in elderly men and women [20,75]. Nevertheless, higher cortisol levels and responses were observed in premenopausal compared to postmenopausal women [19,93]. These findings imply that E2 is indeed an important modulator in HPA axis function.

Inconsistent results have been noted, though, regarding the effects of the menstrual cycle on HPA activity [19,100]. Premenopausal women in the luteal phase may show comparable

cortisol levels to men, whilst women in the follicular phase exhibit blunted free cortisol responses [20,100]; therefore increased E2 levels may indeed enhance HPA activity. This was also proven in postmenopausal women treated with E2, who revealed heightened stress-induced HPA responses. Contrastingly, after six weeks of hormone replacement therapy, no alteration in responses was observed, which may have been due to habituation [20]. Another explanation for the differences in cortisol levels over cycles may be variance in cortisol bioavailability. The effects of cortisol binding globulin (CBG) are partly able to explain these differences [19-20,75]. Increased E2 and/or use of oral contraceptives might increase CBG levels. This reduces free biologically active cortisol levels [11,75,117] and may depict down-regulated or reduced cortisol responses. This is of importance as CBG levels can greatly influence studies comparing men and women. With analysis of urinary cortisol though, which is unaffected by CBG, sampling time, fluctuations in the diurnal pattern [3,94,118], and a momentary state of arousal [82], one can better depict effects of neuro-endocrine coping responses.

Therefore, variations in cortisol concentrations seem to be vastly dependent on E2 levels, and as this sex steroid plays a great part in neuro-endocrine coping responses, its role and impact are discussed next.

4. ESTRADIOL AS COPING MODULATOR

4.1. Estradiol

E2 is a sex steroid that is mainly synthesised from cholesterol and is essential in both women and men. In premenopausal women, E2 acts as a circulating hormone, but in postmenopausal women and men, E2 is produced in extragonadal tissues (skin, fat, liver, adrenal, breast, neural and blood vessels) for paracrine or intracrine actions [25,119]. E2 has been said to be cardio-, reno-, and neuroprotective, although variance has been observed between sexes [21-22,120-121], and it can also modulate autonomic activity [4,20,72,93,122]. E2's pleiotropic effects include regulation of the inflammatory response,

lipid profile, insulin sensitivity and diabetes, plus vascular function and atherosclerosis [21,123]. Nonetheless, E2's effects are dependent on its concentration and the specific oestrogen receptor (ER) that it binds to, resulting in either genomic or non-genomic responses [21-22], which are described in the following sections.

4.2. Estradiol levels: Influencing factors and effects

Table 1.3: Normal ranges of estradiol in healthy men and women [126]

		pg/ml	pmol/l
Men		10-30	37-110
Premenopausal women	Follicular phase	14-27	50-100
	Ovulation phase	14-54	50-200
	Luteal phase	19-40	70-150
Postmenopausal women		0-30	0.00-110.13

Various factors may influence E2 levels, including age, sex and menstrual phases *(Table 1.3)*, as well as aromatase and androgen conversion. Regarding age, Shepherd (2001) stated that men aged 60 years and older may have up to three times more available E2 than women of the same age, which might be due to testosterone aromatisation [124]. Therefore, with increasing age, testosterone will decrease together with increases in E2 in men, whilst the opposite is true for women, but both instances are related to heightened CVD risk [21-22,116,125].

Nonetheless, obesity and alcohol consumption also influence E2, whether beneficially or detrimentally. Central obesity has been inversely associated with CBG levels. Reduced plasma CBG in obesity is thus associated with hypercortisolaemia and this may lead to cortisol driven adiposity [127]. However, there also exists sexual dimorphism for adipose tissue distribution, which may further explain differences in CVD risk between men and premenopausal women. Here sex steroids are at play, as E2 promotes gluteo-femoral distribution of fat in women, whilst E2 depletion in menopause [187] and low testosterone in

men [116] promotes central adiposity. Nonetheless, in men, obesity is also associated with increased E2 [129]. This is due to conversion of androgens to oestrogens, which is mainly by aromatase found in adipose tissue [130]. Additionally, obesity is also associated with increased secretion of pro-inflammatory factors from adipocytes [116]. Inflammatory factors contribute to vascular dysfunction through activation of adhesion molecules, vascular inflammation (C-reactive protein) and augmented vasoconstriction [116,131]. Furthermore, increased E2, particularly in abdominally obese men, may stimulate insulin resistance, putting them at higher risk of CVD [116,130]. Therefore, in this instance, augmented and/or excessive E2 is not beneficial.

Alcohol consumption further influences sex steroid levels and a high intake has similar effects to those of obesity on aromatase activity [132], as it will also decrease plasma testosterone, and increase E2. Thus, moderate consumption of alcohol has beneficial effects, especially in postmenopausal women, in replacing E2 levels [133]. Research has also indicated that moderate alcohol consumption in men and women is beneficial for CVD risk, possibly through increased high-density lipoprotein cholesterol (HDL-C) levels [134]. Therefore, moderate alcohol consumption can be regarded as beneficial in elevating E2 levels and preventing CVD. High consumption or abuse, on the other hand, may cause excessive E2 with possible similar effects to those of obesity in vascular dysfunction. This may partly explain the findings of augmented CVD risk in accordance with heightened E2 and alcohol consumption, in SABPA African men [67].

4.3. Oestrogen receptors

E2's effects are mediated by binding to an oestrogen receptor (ER), namely α, β or GPER (formerly known as GP30) [23,135-136]. All the receptors have been found in both sexes [137], but in varying number, locality and activity, even in pre- compared to postmenopausal

women [21,26,138]. Consequently, it is thought that this may explain sexual dimorphism in coping responses [24,139] and CVD risk [21,26].

ERα and ERβ predominantly perform nuclear functions and are probably responsible for E2's chronic effects [21]. These two receptors may differentially regulate gene expression in the same tissues, where ERα has been stated to inhibit and ERβ stimulate nitric oxide (NO) synthase in the vascular smooth muscle tissue of mice [135,140]. This occurrence has also been observed in mice aorta's, with up-regulated gene transcription resultant from ERα activation, but down-regulation with ERβ stimulation [141]. Additionally, ERα has been stated to cause effects in acute and repeated stress responses, whilst it was determined that ERβ played an important role in acute stress responses alone [23]. Therefore, not only the receptors determine E2 effects, but acute and chronic stressors do too.

ERs are further influenced by age, disease and obesity [21,26,138]. This could be related to E2 levels. Augmented E2 has been associated with an increase in number and/or activity of ERα, which might explain pre-menopausal women's lower risk of CVD, as ERα is mostly associated with NO-induced vasodilation [21,25,128]. Long-term treatment with E2 has also been associated with an increase in ERα in endothelial cells, together with a decrease in ERβ [142]. Notwithstanding, both ERα and ERβ have been implicated in cardioprotection in animal models [21,116,137]. However, there are some discrepancies in the literature because some researchers report greater protection from ERα [143], while others report more protection from ERβ activation [26,116].

From research in animals it seems that ERα cannot provide its protective effects if ERβ is absent [116], and ERβ facilitates protection chronically [137]. However, in human studies ERα is deemed more important in cardioprotection than ERβ, and increased ERβ activation appears detrimental [129], which may be related to acute and chronic stress [23]. This is especially evident in men, where obesity plays a role in increased aromatase activity

[21,25,129,144]. However, a lack of ERα has been associated with an increased risk of obesity, together with insulin resistance and impaired glucose homeostasis [26]. In accordance with this finding, Cohen (2008) reported that testosterone usually inhibits ERβ activity, but that decreased testosterone availability via androgen conversion and the related increases in visceral obesity, E2's stimulation of ERβ will escalate, with resultant CVD risk [129].

4.4. Estradiol: Protective or detrimental?

There are significant contradictions in the literature regarding the level of E2 and CVD risk, with some researchers asserting that reduced E2 is detrimental to health [22,120,124,144-149], while others stated that augmented E2 is detrimental to health [24,115,124,128-130,145,149-155]. These inconsistencies can mostly be explained by E2's vasodilatory and vasoconstrictory characteristics, which vary among sexes [21-22,94,144].

4.4.1. Estradiol's cardio- and neuroprotective effects

E2 exhibits a variety of cardiovascular and neuroprotective effects [21,124,135,143]. E2 mostly serves a neuroprotective function through inhibition of apoptosis, maintenance of calcium homeostasis and protection against ischemic injury [156]. E2 as a homeostatic regulator performs important vasodilatory functions too, which may be central in neuroprotection as well, since vasodilation should ensure optimal blood flow to the brain for effective functioning [124,143,149]. This homeostatic control in turn permits adequate adaptive neuro-endocrine coping responses.

The main mechanism of E2 cardioprotection can be summarised as follows: E2 activates vascular ERα and ERβ, which will increase intracellular calcium concentrations via intracellular activities involving kinase signalling (mostly P13K) [21,135]. This in turn activates potassium ion channels, in order to maintain cellular homeostasis [21,25,135]. The

E2-induced cellular activities will enhance production and secretion of vasodilators (mostly NO), whilst inhibiting vasoconstrictors (prostanoids, endothelin-1, angiotensin II) [135], thereby providing protection against vasoconstrictory stress mediator effects.

Table 1.4: Cardiovascular protective effects of estradiol [21,138,144,158-159]

	Functions	Receptor	Research Models
Endothelium	Maintains and repairs via increased bioavailability of: eNOS; Cyclooxegynase 2; P13K expression; HSP; PGI2		Human/ Animals
	Prevents adhesion molecules and apoptosis		Human/ Animals
	Inhibits inflammation	**ERβ**	Animals
	Reduces atherosclerosis (intact endothelium)	**ERα**	Human/ Animals
	Enhances vasodilation and re-endovascularisation		Human/ Animals
Myocardium	Slows cardiac hypertrophy	**ERβ**	Animals
	Permits favourable cardiac remodelling		Animals
	Reduces infarct size	**ERα/ERβ**	Animals
	Improves contractile function		Animals
	Repairs cardiac structure/function after infarction		Animals
	Increases basal coronary blood flow		Human
	Decreases coronary vascular resistance		Human

Where: eNOS; endothelial nitric oxide synthase, HSP; heat shock proteins, PGI2; prostacyclin, ER; oestrogen receptor.

In addition, E2 has anti-inflammatory effects as it inhibits chemokine production [21-22,143], even though this effect may diminish with increasing age. E2 is also deemed an antioxidant because it reduces production of reactive oxygen species (ROS) especially in females [21-22,124,143]. This is implicated in the sexual dimorphism as regards CVD risk, since higher ROS concentrations are directly associated with CVD [21]. Moreover, it was stated that E2 inhibits programmed cardiac cell death, neo-intima formation, expression of adhesion molecules and mitogenic effects, whilst promoting angiogenesis [25,143]. In accordance with these findings, E2 treatment in animal models was associated with cardioprotection [146] through increased atrial natriuretic peptide and heat shock protein production [120].

Additionally, in postmenopausal women, E2 administration has hypotensive effects [148]. Ultimately, E2 should preserve cardiac and endothelial structure and function, whilst protecting against HT and atherosclerosis development, as is evident in *Table 1.4* [21-22,124,143-144].

From the aforementioned characteristics, E2 should promote cardiovascular function and can be deemed cardioprotective, in women at least. In men, physiological levels of E2 can nonetheless also provide cardioprotection. These levels have been said to attain desirable lipid profiles and glucose metabolism, as well as increase HDL-C and decrease low-density lipoprotein cholesterol (LDL-C) [144]. Komesaroff and colleagues (2002) concur that E2 attenuates stress responses, lowers basal and stress-induced BP, and that in men E2 enhances vasodilatory responses [159]. This further infers that E2 may facilitate adaptive and efficient coping *(Section 4.5)*.

4.4.2. Estradiol levels and cardiovascular risk

Notwithstanding the protective effects, highly augmented E2 in men, which may be due to aromatisation of androgens with a related decrease in testosterone, can cause augmented NE-induced vasoconstriction [153]. Additionally, E2 might heighten the affinity of α_1-adrenergic receptors for NE, whilst reducing the affinity of α_2-receptors [79,144], contributing to excessive vasoconstriction and peripheral resistance. It was further stated that E2-induced vasoconstriction in men is mostly facilitated by ERβ stimulation, with a resultant negative impact on the cardiovascular system [129]. Abbott *et al.* (2007) revealed an increase in E2 in elderly men which was associated with risk of stroke [151]. Circulating E2 was further correlated with increases in carotid intima-media thickness in middle-aged Caucasian men [152]. Malan *et al.* (2012) also concluded that increased E2 was associated with higher atherosclerosis and renal impairment risk in Africans [67]. The effect of augmented E2 in men thus seems to be independent of ethnicity, although literature in this

regard, and in particular concerning Africans, is scarce. Contradictorily, an inverse association between E2 and endothelial dysfunction in men has also been indicated [125], whilst still other studies have found no association between E2 and CVD risk [160].

With reference to women, Carr (2003) stated that metabolic syndrome prevalence is increased in postmenopausal compared to premenopausal women. Postmenopausal women also reveal increased carotid intima-media thickness and aortic calcification [128]. Thus, oestrogen deficiency may increase disease risk factors in women. Additionally, results from the Heart and Estrogen/Progestin Replacement Study (HERS) and Women's Health Initiative (WHI) studies revealed that oestrogen replacement therapy might not be cardioprotective in postmenopausal women [146-147]. The cardiovascular protective effects of E2 are therefore not as easily justified in men or postmenopausal women and the related mechanisms are poorly understood. However, a possible reason for the failure of E2 treatment to protect postmenopausal women from CVD risk in these studies was stated to be the onset of therapy [147]. The age of menopause and when hormone replacement therapy commences therefore play important roles in E2's protective effects [149]. It should be noted that the average age of women participating in the WHI study was 63.3 years, while most women were between 60 and 70 years of age, which is far beyond menopause onset [147]. This may have influenced results, as CVD risk may already have been present after years of reduced E2. Hence, E2 replacement therapy might be more beneficial at inception of menopause rather than after years of no hormone treatment.

On the other hand, prominently increased E2 levels could also be detrimental with established pathology [161-162]. Certainly, increased E2 levels have been related to augmented CVD and renal risk in female mice [163], as well as to pathogenesis of diabetic nephropathy in men [164]. The Coronary Drug Project further demonstrated that after myocardial infarction, oestrogen administration was associated with more deaths and recurrent infarction, consequently the project was abandoned [144]. Concurrently, findings

indicate that E2 levels are elevated after myocardial infarction, irrespective of gender [161]. E2 is further related to insulin resistance and hypercoagulability [161], whilst E2 administration may also increase HT and CVD risk [155]. Augmented E2 levels and/or E2 supplementation are therefore considered detrimental and predictive of increased morbidity and mortality, especially in critically ill men and women [125,149-154,161].

4.5. Estradiol and neuro-endocrine coping responses

Sex steroids such as E2 greatly influence stress coping activity, where an increase in E2 usually enhances SAM and HPA activity [19,84]. However, a great deal of contradiction exists in the literature and the modulating effects of E2 need to be addressed. E2 impacts on neurotransmitters including serotonin, dopamine and NE, such as up-regulation of their receptors and their sensitivity, as well as increased re-uptake and synthesis of these neurotransmitters [20,22,124]. Nonetheless, the effects of E2 on the SAM system are complex. E2 has been stated to increase bioavailability of NE (through gene transcription, inhibiting monoamine oxidase, and inhibiting α_2-adrenergic negative feedback), but also to increase the number of adrenergic receptors (thereby decreasing availability, but essentially increasing NE's effects) [124,139,165]. Conversely, it was asserted that E2 may reduce the reactivity of the rat's heart to catecholamines via activation of ERs located on cardiac cells [120]. E2 administration in postmenopausal women also inhibits SAM hyper-reactivity to stress [120]. Moreover, researchers proved that E2 decreases stress-related sympathetic drives, since attenuated cardiovascular responses, together with decreased epinephrine levels, were revealed in postmenopausal women treated acutely with E2 [25]. It is, however, unknown whether the same is true for NE. The contradictory findings of E2's impact are probably related to the specific ER activated, because in rats, ERα can increase but ERβ may decrease production and availability of NE [23].

Additionally, HPA responses to inflammatory stress were significantly reduced in postmenopausal women and ovariectomised rats, receiving physiological doses of E2

replacement therapy [165-166]. Therefore, E2 is also able to inhibit HPA activity. Kirschbaum *et al.* (1999) add that women using oral contraceptives containing ethinyl-E2 revealed blunted cortisol responses to stress [19]. This is in accord with reduced cortisol responses observed in women (between puberty and menopause) compared to age-matched men [167]. Additionally, E2 affects CBG levels, which may decrease the available amount of cortisol after stress [117,168], even though production and release of cortisol may be high or normal. This may account for the seemingly normal cortisol levels observed in SABPA Africans [12], who also revealed high E2 levels [67].

On a related note, E2 increases sex hormone binding globulins (SHBG) which may bind testosterone to a greater extent than E2; this could in turn decrease the bioavailability of testosterone [144]. Moreover, researchers have indicated that individuals who are severely strained or feel a loss-of-control, show decreased testosterone concentrations [3,62]. Swaab and colleagues (2005) add that during depression, testosterone is decreased in men [93]. This is noteworthy since testosterone usually inhibits HPA activity [84,169-170]. Therefore, HPA activity will be highly stimulated when testosterone declines and E2 levels increase in relation. This is further correlated with depression, as higher basal cortisol levels and cortisol awakening responses have been associated with an increased risk of major depressive disorder [171]. These findings also support a greater cortisol-to-testosterone ratio in African men [62], with lower testosterone possibly depicting sustained stress, which may also be true for SABPA African men [67].

The high incidence of abdominal obesity [5,172] and reduced testosterone in SABPA African men [67], may evidence a trend of increased E2 as well as CVD risk, creating concern as they further reveal behavioural and physiological dissociation and inadequate coping responses [5,8]. Interestingly, Carroll *et al.* (2008) implicated blunted sympathetic activity in obesity development [173], which might partly explain the increased E2 and CVD risk in concurrence with blunted neuro-endocrine stress responses in African men.

It is still unclear whether increased E2 merely reflects illness or disease susceptibility as it would be increased homeostatically (enhancing vasodilation), or if it, rather, plays a part in the causal pathway, possibly via modulation of neuro-endocrine coping responses (stimulating vasoconstriction), with impaired cardiovascular function.

5. MOTIVATION FOR THIS SABPA SUB STUDY

This study is essential since CVD is a major cause of mortality and morbidity in South Africa, with about 80 people dying of myocardial infarction or heart failure and an additional 60 people of cerebral infarction, every day [174]. What is more, urban African men seem to be more at risk than their Caucasian, female, and rural counterparts, yet their vulnerability is still poorly understood. Additionally, recent findings have indicated that urban Africans exhibit dissociation in coping responses, blunted NE and cortisol stress responses, depressive symptoms and augmented CVD risk, most of which have been associated with utilisation of DefS. This evoked interest to research probable neuro-endocrine dysfunction (possible autonomic exhaustion or adrenal fatigue) in the Africans' neuro-endocrine coping responses. Furthermore, studies on neuro-endocrine stress mediators (cortisol and NE) have shown variance in affecting cardiovascular health, but both give rise to vasoconstrictory responses. Nevertheless, the significance of a coping-related sex steroid such as E2, has attracted little attention in stress and coping research. E2 may possibly be cardioprotective through its vasodilatory effects. However, it is still debatable whether E2 is cardioprotective in men, and there is controversy over whether reduced or excessive E2 is beneficial. It is therefore clear that more research is needed to clarify the facilitative role of E2 in neuro-endocrine coping responses, especially in urban Africans with augmented CVD risk.

The following questions remain unanswered by the literature:

- Why are urban African men more vulnerable than their female and Caucasian counterparts?

- Why does stress manifest differently (with dissociation in coping responses) in Africans compared to Caucasians?
- Is SAM and/or HPA axis activity (hypo/hyper) associated with target organ damage in Africans and/or Caucasians?
- Is E2 protective (when increased/decreased) in Africans and/or Caucasians, and are there sex differences in its protective impact?
- Does E2 merely reflect cardiovascular illness because it may be up-regulated as a homeostatic agent, or does it actually contribute to CVD risk?

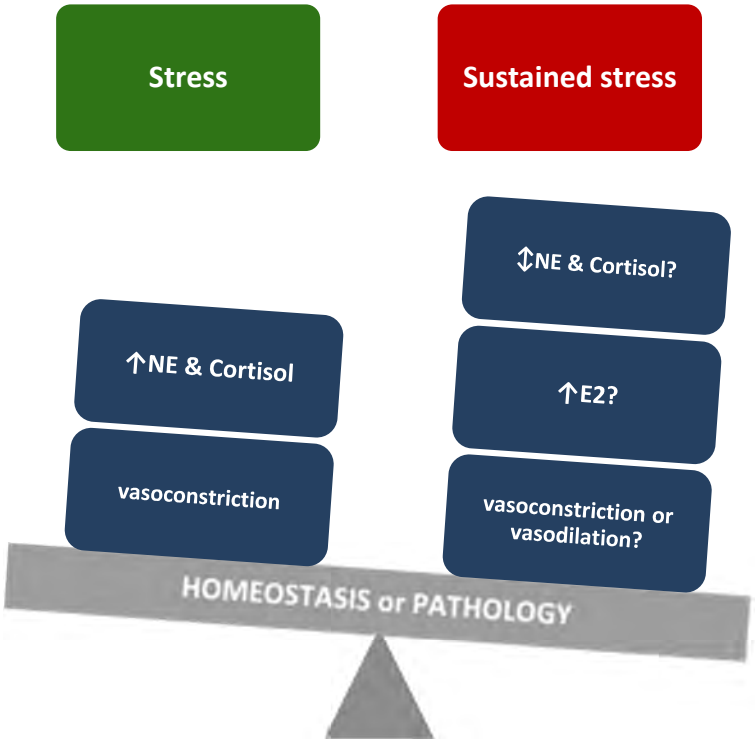


Figure 1.2: Uncertainty regarding the cardiovascular effects of neuro-endocrine coping responses in sustained stress – is E2 cardioprotective?

Where: ↑; increase, NE; norepinephrine, ↕; increase and/or decrease, ?; unknown effects.

6. AIMS

6.1. Main aims of this study

The primary aim of this study was to assess the influence of neuro-endocrine coping responses on CVD risk, in an urban South African cohort. The impact of the neuro-endocrine stress mediators (NE and cortisol) on either subclinical vascular or renovascular disease risk, respectively, was to be determined. Additionally, E2 was studied to determine its effects on neuro-endocrine coping responses and CVD risk. Furthermore, the effects of particularly DefS utilisation during stress were to be determined. The SABPA study was ideal to achieve these aims as it is the sole study in Sub-Saharan Africa which was designed to measure the brain-heart link with behavioural, neuro-endocrine, and cardiovascular differences, in a bi-ethnic sex cohort.

6.2. Detailed aims of each manuscript

6.2.1. Defensive coping and subclinical vascular disease risk - Associations with autonomic exhaustion in Africans and Caucasians: The SABPA study

The aims of the first sub study were to determine whether urban DefS Africans, rather than Caucasians, will demonstrate signs of autonomic exhaustion [lower NE metabolite levels; 3-methoxy-4-hydroxyphenolglycol (MHPG)], and whether decreased MHPG will be associated with increased subclinical vascular disease risk in urban DefS Africans.

6.2.2. Defensive coping and renovascular disease risk - Adrenal fatigue in a cohort of Africans and Caucasians: The SABPA study

The second sub study built on the results of the first published manuscript, aiming to determine whether urban Africans, rather than Caucasians, will demonstrate signs of HPA axis dysfunction (lower cortisol with high stress), and if decreased cortisol will

be associated with increased renovascular disease risk in Africans; and finally, to determine whether the aforementioned risk will be augmented in those utilising DefS.

6.2.3. Defensive coping and estradiol - Unravelling neuro-endocrine dysfunction and augmented hypertension risk in a South African cohort: The SABPA study

The third and final sub study's aims were to determine whether E2 will be increased in urban Africans (with higher self-reported stress) compared to Caucasians. Secondly, if increased E2 will be associated with HPA axis hypoactivity rather than hyperactivity (in an original cortisol-to-E2 ratio); and lastly, whether this neuro-endocrine dysfunction will be associated with hypertension prevalence in urban Africans (especially men), with augmented risk through utilisation of DefS.

7. HYPOTHESES

7.1. Main hypotheses of this study

With consideration of the literature (including recent SABPA findings) and the aims of the study in mind, the following main hypotheses were proposed: urban Africans (especially men) will show more CVD risk than their Caucasian counterparts, with heightened hypertension, subclinical vascular and renovascular disease risk. This increased risk will be associated with neuro-endocrine coping response dysfunction (reduced stress mediator levels), particularly in those utilising DefS. E2 will be associated with neuro-endocrine dysfunction and hypertension risk in urban-dwelling African men, rather than being protective.

7.2. Detailed hypotheses of each manuscript

7.2.1. Defensive coping and subclinical vascular disease risk – Associations with autonomic exhaustion in Africans and Caucasians: The SABPA study

- Urban DefS Africans, rather than Caucasians, will demonstrate signs of autonomic exhaustion, as indicated by reduced NE (MHPG) levels.
- Decreased NE (MHPG) levels will be associated with increased subclinical vascular disease risk in urban DefS Africans.

7.2.2. Defensive coping and renovascular disease risk – Adrenal fatigue in a cohort of Africans and Caucasians: The SABPA study

- Urban Africans will reveal decreased cortisol levels (possible adrenal fatigue), when compared to Caucasians.
- Reduced cortisol levels will be associated with increased renovascular disease risk in urban African men.
- Utilisation of DefS will be associated with augmented renovascular disease risk in urban Africans compared to Caucasians, especially in those with reduced cortisol levels.

7.2.3. Defensive coping and estradiol – Unravelling neuro-endocrine dysfunction and augmented hypertension risk in a South African cohort: The SABPA study

- Urban Africans will reveal increased E2 levels, when compared to Caucasians.
- In urban Africans, increased E2 will be associated with HPA axis hypoactivity rather than hyperactivity (in an original cortisol-to-E2 ratio).
- A decreased cortisol-to-E2 ratio will be associated with hypertension risk in urban Africans (particularly in men), enhanced by utilisation of DefS.

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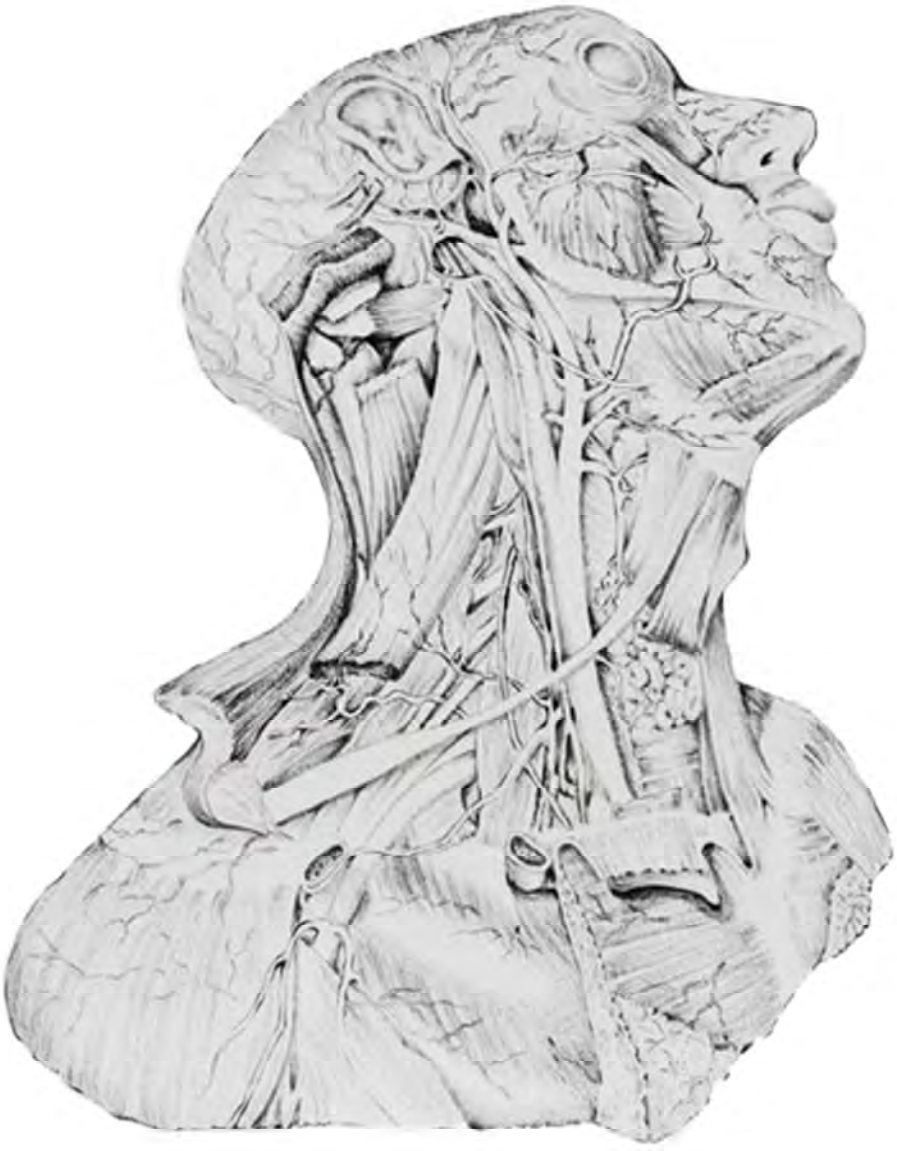
Chapter 2



Manuscript 1

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This journal publishes original basic research articles, regarding atherosclerosis and its complications. This may include studies on factors such as lipoproteins, lipids, inflammation, diabetes, hypertension, arterial and vascular biology and other cardiovascular risk factors.

Manuscripts	– Maximum of 4000 words – Should be divided into sections with headings *(Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, Conclusions, Tables, Artwork)* – Include the source of funding and conflicts of interest if any – Define abbreviations at first use in text
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Tables	– Tables should be provided with legends
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Please note that some of the format requirements were changed to ensure uniformity throughout the thesis.

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Defensive coping and subclinical vascular disease risk – Associations with autonomic exhaustion in Africans and Caucasians: The SABPA study

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ABSTRACT

Objective: The defensive active coping response is a recognised cardiovascular risk factor in Africans, especially in men. It is uncertain whether autonomic dysfunction might be the underlying cause. We therefore investigated associations between salivary MHPG (3-methoxy-4-hydroxyphenolglycol), as a marker of sympathetic activity, and subclinical vascular disease risk in defensive coping Africans and Caucasians.

Methods: The Coping Strategy Indicator questionnaire identified participants who preferably utilise defensive coping. Ambulatory blood pressure was monitored for 24 h and carotid intima–media thickness (CIMT) was determined from ultrasound images, as an indicator of subclinical vascular disease risk. Salivary MHPG was analysed with high performance liquid chromatography.

Results: Defensive active coping Africans ($n = 143$) showed overall poorer health than Caucasians ($n = 148$), with higher self-reported stress, alcohol abuse, hypertension, abdominal obesity, and risk of diabetes ($p \leq 0.05$). African women demonstrated lower levels of MHPG compared with Caucasian women, although no differences in men were found. Furthermore, Africans revealed a trend of increased low grade inflammation and glycated haemoglobin which was associated with increased CIMT. There was an inverse association between MHPG and CIMT [$\beta = -0.22$ (-0.40, -0.03)], in African men with a high risk of subclinical vascular disease ($n = 30$).

Conclusions: Novel findings revealed that defensive active coping Africans are more at risk of subclinical vascular disease, possibly resultant of autonomic exhaustion (decreased MHPG). When defensive coping fails, sympathetic hyperactivity may be followed by autonomic exhaustion and sympatho-adrenal-medullary system desensitisation, resulting in pathology.

1. Introduction

Psychosocial stress has previously been associated with pathology [1,2]. The impact of stress on physiological functioning is, however, partly dependent on the utilised coping responses when dealing with stress, as well as genetics, lifestyle, and previous experiences [3]. Chong et al. concur that ethnic variations in coping responses play an important role in their correspondingly diverse pathologies [4]. And so, preceding studies agree that the defensive active coping response, rather than the emotional avoidance response, is more associated with cardiovascular disease (CVD) in Africans than in Caucasians, and especially in African men [1,2,5]. Additionally, Malan et al. discovered dissociation in the behavioural and physiological defensive coping responses in Africans, with resultant increased CVD risk [2,5]. Defensive active coping can be summarised as a direct approach to actively solve problems and manage stress, with a sense of being in control and an intense focus until the stress is eliminated [6]. Upon stress, the sympatho-adrenal-medullary (SAM) system will be activated for the fight-or-flight response, otherwise known as the physiological defensive active coping response. This stimulates various brain areas i.e. limbic and cortical, and the release of stress hormones, including norepinephrine (NE) [7]. The major metabolite of NE, namely MHPG (3-methoxy-4-hydroxyphenolglycol), is an important biomarker for sympathetic activity, as only a small amount of released NE truly reaches the plasma without being altered or reabsorbed [8,9]. Thus the amount of released NE is represented by plasma MHPG concentrations, which in turn is reflected in salivary

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Abstract

Objective: The defensive coping response is a recognized cardiovascular risk factor in Africans, especially in men. It is uncertain whether autonomic dysfunction might be the underlying cause. We therefore investigated associations between salivary MHPG (3-methoxy-4-hydroxyphenolglycol) as a marker of sympathetic activity and subclinical vascular disease risk in defensive coping Africans and Caucasians.

Methods: The Coping Strategy Indicator questionnaire identified participants who preferably utilise defensive coping. Ambulatory blood pressure was monitored for 24 hours and carotid intima-media thickness (CIMTf) was determined from ultrasound images, as an indicator of subclinical vascular disease risk. Salivary MHPG was analysed with high performance liquid chromatography.

Results: Defensive coping Africans (n=143) showed overall poorer health than Caucasians (n=148) with higher self-reported stress, alcohol abuse, hypertension, abdominal obesity and risk of diabetes ($p \leq 0.05$). African women demonstrated lower levels of MHPG compared with Caucasian women, although no differences were found in men. Furthermore, Africans revealed a trend of increased low grade inflammation and glycated haemoglobin which was associated with increased CIMTf. There was an inverse association between MHPG and CIMTf [β= -0.22 (-0.40, -0.03)], in African men who have a high risk of subclinical vascular disease (n=30).

Conclusions: Main findings revealed that defensive coping Africans are more at risk of subclinical vascular disease, possibly as a result of autonomic exhaustion (decreased MHPG). When defensive coping fails, sympathetic hyperactivity may be followed by autonomic exhaustion and sympatho-adrenal-medullary system desensitisation, resulting in pathology.

Keywords: Coping; ethnicity; norepinephrine metabolite; subclinical vascular disease.

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Psychosocial stress has previously been associated with pathology [1,2]. The impact of stress on physiological functioning is, however, partly dependent on the coping responses utilised when dealing with stress, as well as genetics, lifestyle and previous experiences [3]. Chong *et al.* concur that ethnic variations in coping responses play an important role in the correspondingly ethnic diverse pathologies [4]. Preceding studies agree that the defensive coping (DefS) response, rather than the emotional avoidance response, is more associated with cardiovascular disease (CVD) in Africans than in Caucasians and especially in African men [1,2,5]. Additionally, Malan *et al.* discovered dissociation in the behavioural and physiological coping responses in Africans, with resultant increased CVD risk [2,5]. DefS can be summarised as a direct approach to actively solve problems and manage stress, with a sense of being in control and an intense focus until the stress is eliminated [6]. When stress occurs, the sympatho-adrenal-medullary (SAM) system will be activated for the "fight-or-flight" response, otherwise known as DefS. This stimulates various brain areas, i.e. limbic and cortical, and the release of stress mediators, including norepinephrine (NE) [7]. The major metabolite of NE, namely MHPG (3-methoxy-4-hydroxyphenolglycol), is an important biomarker for sympathetic activity, as only a small amount of released NE truly reaches the plasma without being altered or reabsorbed [8,9]. Thus, the amount of released NE is represented by plasma MHPG concentrations, which in turn are reflected in salivary MHPG levels [9]. Salivary MHPG thus serves well as a biomarker for sympathetic activity as well as psychological stress, as determined from increased post-stress levels [7-10].

To the best of our knowledge, no study has reported on the use of specific coping responses and its relation to autonomic function and cardiovascular risk. However, it is known that intense stress essentially causes sympathetic hyperactivity with persistent increases in NE levels, all of which are detrimental to normal physiological processes. Moreover, the initial hyperactivity may be followed by autonomic exhaustion and decreases in NE [11]. These

alterations in autonomic function are of importance as they have been associated with both depression and CVD [2,12,13]. Additionally, stress also has an influence on the development and progression of sub-clinical atherosclerosis, via structural vascular changes [1,2]. Further research is therefore essential to better understand the concurrent effects of defensive coping and autonomic function in affecting subclinical vascular disease risk.

We hypothesised that urban DefS Africans would demonstrate signs of autonomic exhaustion, as indicated by lower levels of MHPG. We further hypothesised that decreased MHPG would be associated with increased subclinical vascular disease risk in urban DefS Africans.

2. Materials and methods

2.1. Research design and participants

This study is nested in the prospective cohort Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, conducted in 2008 and 2009. The SABPA study population consisted of 198 urban African and 209 urban Caucasian teachers from the Dr Kenneth Kaunda Education district in the North West Province, South Africa. All participants were aged 20 to 63 years and were of a similar socio-economic status. Exclusion criteria included pregnancy, lactation, users of alpha and beta blockers, tympanum temperature ≥37 ℃ and vaccination or blood donation 3 months prior to participation. HIV positive (n=19), diabetic (n=12) and/or renal impairment (n=1) cases were also excluded from the data spreadsheet. The final participant group consisted of n=168 Africans and n=207 Caucasians. Before participation, all procedures were explained prior to participants' informed consent being given and their inclusion in the study. The Ethics Review Board of the North-West University approved all procedures of the SABPA study (NWU-00036-07-56), which were completed following the Declaration of Helsinki guidelines [14].

2.2. Research procedure

On four working days of the week, between 07h00 and 08h00, four participants were each fitted with the Cardiotens CE120® (Meditech, Budapest, Hungary) for ambulatory blood pressure (BP) measurements, as well as the Actical® accelerometers (Montréal, Québec) for physical activity (PA) measurements. The participants thereafter resumed their usual daily activities, reporting any anomalies such as headache, nausea, visual disturbances, fainting, palpitations, PA and any experience of stress, on the diary cards provided. At 16h30 they were transported to the North-West University to stay overnight. Upon arrival, participants were allocated their own rooms and received pre-counselling regarding HIV/AIDS. Afterwards, they were thoroughly briefed on the experimental setup, including the requisites for data sampling to lessen anticipation stress. Participants were to avoid exercising, smoking and consuming alcohol and/or caffeine 8 hours prior to sampling commencement. Furthermore, they were requested to take care with dental hygiene, to prevent bleeding gums, and not to brush their teeth before saliva sampling [15].

Registered clinical psychologists supervised completion of the battery of psychosocial questionnaires with a standardised dinner break at 18h30 in-between. The participants were advised to go to bed at 22h00, fasting overnight. After the last BP recording at 06h00, the Cardiotens CE120® and Actical® were disconnected. Anthropometric measurements followed; thereafter participants were placed in the semi-Fowlers position, head elevated 30-45 degrees. A 10 minute resting period was followed by saliva and blood sampling. Scanning of the carotid intima-media wall was performed according to the Rudi Meijer protocol [16].

2.3. Ambulatory blood pressure monitoring

Preceding publications have described the details regarding the use of the Cardiotens® apparatus for BP monitoring [1]. Hypertension (HT) was classified in accordance with the European Society of Hypertension guidelines as 24 hour BP ≥125/80 mmHg [17].

2.4. Anthropometric measurements

Registered anthropometrists measured PA, waist circumference (WC) and body mass index, according to the SABPA protocol [2]. Body surface area (BSA) was calculated using the Mosteller formula [18,19].

2.5. Subclinical vascular disease indicators

The Sonosite Micromaxx® system (SonoSite Inc., United States) was used to determine structural vascular changes [20]. A 6-13 MHz linear array transducer was used in obtaining ultrasound images of the carotid artery, bulb and internal arterial segments from two angles, on both the right and left sides while in the supine position. The head was contra-lateral to the side being examined with the neck extended. The better of the two angles was stored as an image and the mean carotid intima-media thickness of the far wall (CIMTf) calculated to the nearest 0.1 mm.

2.6. Coping Strategy Indicator questionnaire

The self-reporting Coping Strategy Indicator (CSI), which was developed by Amirkhan and also successfully used in Africans, determined the favoured coping strategy of each participant in specified stressful situations [21,22]. This 33-item questionnaire includes the following 3 coping strategies: problem solving (formerly described as defensive active coping), seeking social support and emotional avoidance [2]. Seeking social support is related to the basic need for human contact and involves actively seeking comfort, advice and help to manage stress [6], while emotional avoidance entails physical and/or psychological withdrawal, accompanied by a feeling of uncontrollability, and is, theoretically, associated more with pathology [23].

A Cronbach's alpha reliability coefficient of between 0.81-0.87 was determined for defensive, 0.61-0.85 for avoidance, and 0.83-0.90 for seeking social support. Values of ≥26 on the

defensive coping scale, as well as ≥19 on the avoidance, and ≥23 on the social support scales were documented as above mean coping responses: from here on referred to as high responses [21]. Only these high coping responders were used in analysis.

2.7. Biochemical analysis

A registered nurse obtained fasting blood samples from the brachial vein branches of the dominant arm with a winged infusion set. Blood samples were handled according to standardised procedures and frozen at -80℃ until analysis. The following analysis were performed with Unicel DXC 800 (Beckman and Coulter, Germany): sodium fluoride glucose and total cholesterol with a timed-end-point method, ultra-high sensitivity C-reactive protein (CRP) with a turbidimetric method, and serum gamma glutamyl transferase (cGGT- alcohol consumption) with an enzyme rate method. Alcohol abuse was determined with cGGT levels >65 u/l (men) and >45 u/l (women) [24]. Cotinine, a metabolite of nicotine, was analysed through homogenous immunoassay with an automated modular system (Roche, Switzerland). Cotinine levels >12 ng/ml verified present smoking status [25]. Glycated haemoglobin (HbA1c) was analysed by turbidometric inhibition immunoassay with the Cobas® Integra 400 (Roche, Switzerland). HbA1c was deemed normal ≤5.7%, pre-diabetic 5.7-6.4%, and diabetic with values >6.4%, according to the American Diabetes Association guidelines [26].

Saliva samples were obtained through chewing a Salivette (Sarstedt Inc., Leicester, UK) cotton swab for 1-2 minutes. This was snap-frozen immediately in liquid nitrogen and stored at -80℃ until analysis with high performance liquid chromatography coupled to an amperometric electrochemical detector. Salivary MHPG was analysed as an indicator of adrenergic activity and psychological stress, as former studies established that salivary MHPG levels reflect both the central and plasma concentrations [9,10]. Inter- and intraday coefficient of variance was less than 10%.

2.8. Statistical analysis

All data analysis were completed with the *Statistica 10* computer software (Statsoft Inc., Tulsa, USA). Results with a $p \leq 0.05$ were considered significant. Kolmogorov-Smirnov tests determined normality and CRP was logarithmically transformed, with the exponential of log CRP depicted in results. T-tests for independent groups calculated means between ethnic groups. χ^2-tests calculated proportions. Least square means analysis were performed independent of *a priori* confounders, including age, BSA, smoking, alcohol consumption and PA. In all analysis pertaining to CIMTf, further adjustments for CRP, cholesterol and BP were considered. Analysis was performed for DefS, avoidance and seeking social support in the respective groups. A three-way analysis of covariance (ANCOVA) determined interactions between the main effects, sex (men and women), ethnicity (African and Caucasian) and each of the three coping responses of the CSI, independent of *a priori* confounders. Only high DefS evidenced significant interactions with the following variables; WC [$F(1,361) = 6.836$, $p=0.01$] and CIMTf [$F(1,354) = 5.658$, $p=0.02$]. Thereafter, our focus remained on the DefS responders [African men (n=60), African women (n=67), Caucasian men (n=83), and Caucasian women (n=81)]. As median splits revealed little varaiance, MHPG was divided into tertiles, to determine the influence of varying levels of MHPG on disease risk in DefS teachers. However, the groups were too small to have adequate statistical power. Supported by the aforementioned significant interaction in the three-way ANCOVA, median splits of CIMTf followed. Further analysis indicated the same trend as the results from the MHPG tertiles, with added power. The CIMTf median split established groups with lower (below median 0.65mm) and higher (equal to and above median 0.65mm) risk of subclinical vascular disease and allowed for improved comparability with the underlying factors thereof, including MHPG. Partial correlations and forward stepwise regression analysis indicated significant associations (adjusted $R^2 \geq 0.30$) between independent variables and subclinical vascular disease risk, independent of lifestyle factors.

3. Results

3.1. Psychosocial variables

More DefS African men self-reported occurrence of severe stress. Overall, DefS Caucasians were more avoidant and utilised social support less than their African counterparts ($p<0.001$).

3.2. Comparison of defensive coping Africans and Caucasians

In *Table 2.1*, Caucasian men were older and more physically active than African men. Total cholesterol was higher in both Caucasian gender groups, in comparison with their respective African counterparts. Caucasian men also recorded greater BSA, whereas African men were more prone to abdominal obesity (102.4 vs. 94.7 cm). Both African sex groups displayed alcohol consumption levels above the cut-points for abuse. Furthermore, the African groups also revealed higher HbA1c (6.28 vs. 5.55% and 5.62 vs. 5.41%) and excessive low-grade inflammation (CRP: 1.81 vs. 1.21 mg/l and 2.63 vs. 1.67 mg/l), together with higher HR and BP ($p\leq0.05$), when compared to their Caucasian counterparts. In addition, African women also displayed a distinctly greater risk of subclinical vascular disease, owing to CIMTf (0.67 vs. 0.60 mm) and HT prevalence ($p=0.01$), but indicated significantly reduced MHPG levels ($p=0.001$).

3.3. Defensive coping men with varying risk of subclinical vascular disease

Figure 2.1 depicts a significant difference in CIMTf, between the lower risk groups of African and Caucasian men (0.60 vs. 0.51 mm). Furthermore, the high risk African men displayed even more vulnerability than their Caucasian counterparts, as observable in their higher CRP and HbA1c levels ($p<0.001$).

Table 2.1: Descriptive characteristics of defensive coping African and Caucasian teachers

	MALE TEACHERS			FEMALE TEACHERS		
	AFRICANS (n=60)	CAUCASIANS (n=83)	p	AFRICANS (n=67)	CAUCASIANS (n=81)	p
CONFOUNDERS						
Age (years)	**42.3 ± 8.46**	**46.5 ± 10.3**	**0.01**	44.9 ± 7.71	46.0 ± 10.4	0.46
Body surface area (m^2)	**1.95 ± 0.24**	**2.18 ± 0.21**	**<0.0001**	1.87 ± 0.21	1.82 ± 0.23	0.14
Physical activity (kcal)	**2773.4 ± 860.2**	**3712.5 ± 2247.0**	**0.003**	2558.1 ± 692.5	2578.2 ± 650.9	0.86
Cotinine (ng/ml)	20.5 ± 42.8	30.0 ± 95.4	0.47	14.6 ± 37.6	7.96 ± 36.3	0.28
ɣ-glutamyl transferase (u/l)	**77.5 ± 79.8**	**30.4 ± 21.4**	**<0.0001**	**48.3 ± 77.8**	**21.0 ± 41.0**	**0.01**
CARDIOVASCULAR RISK MARKERS						
Waist circumference (cm)	**102.4 (100.3, 104.4)**	**94.7 (93.1, 96.4)**	**<0.0001**	**90.4 (88.6, 92.2)**	**85.8 (84.2, 87.4)**	**<0.001**
Cholesterol (mmol/l)	**4.76 (4.43, 5.88)**	**5.60 (5.33, 5.87)**	**<0.001**	**4.40 (4.09, 4.71)**	**5.37 (5.10, 5.65)**	**<0.0001**
Glycated haemoglobin (%)	**6.28 (6.04, 6.53)**	**5.55 (5.35, 5.75)**	**<0.0001**	**5.62 (5.53, 5.71)**	**5.41 (5.33, 5.49)**	**0.001**
C-reactive protein (mg/l)	**1.81 (1.61, 2.00)**	**1.21 (1.05, 1.37)**	**<0.0001**	**2.63 (2.41, 2.84)**	**1.67 (1.48, 1.86)**	**<0.0001**
Salivary MHPG (ng/ml)	11.5 (8.28, 14.6)	10.0 (7.40, 12.8)	0.55	**8.12 (5.30, 10.95)**	**14.7 (12.2, 17.2)**	**0.001**
Mean 24 hour heart rate (bpm)	**79 (76, 82)**	**71 (69, 74)**	**<0.001**	**79 (77, 81)**	**76 (74, 78)**	**0.05**
Ambulatory SBP (mmHg)	**138 (135, 142)**	**127 (124, 129)**	**<0.0001**	**127 (124, 130)**	**121 (118, 123)**	**0.003**
Ambulatory DBP (mmHg)	**89 (86, 91)**	**78 (77, 80)**	**<0.0001**	**78 (76, 80)**	**74 (73, 76)**	**0.01**
Ambulatory hypertension n (%)	45 (75.0)	59 (71.1)	0.60	**34 (50.8)**	**24 (29.6)**	**0.01**
Mean CIMT far wall (mm)	0.68 (0.64, 0.73)	0.69 (0.66, 0.73)	0.84	**0.67 (0.64, 0.70)**	**0.60 (0.58, 0.63)**	**0.001**
PSYCHOSOCIAL VARIABLES						
Avoidance score	**21 (20, 22)**	**25 (24, 26)**	**<0.0001**	**21 (20, 22)**	**25 (24, 26)**	**<0.0001**
Seeking social support score	**26 (24, 27)**	**18 (16, 19)**	**<0.0001**	**26 (25, 28)**	**19 (18, 20)**	**<0.0001**
Defensive coping score	30 (29, 31)	31 (30, 31)	0.36	30 (30, 31)	30 (30, 31)	0.81
24 hour severe stress n (%)	**12 (20.0)**	**4 (4.82)**	**0.004**	13 (19.4)	11 (13.6)	0.34
MEDICATION						
Drugs for hypertension n (%)	10 (16.7)	8 (9.64)	0.21	**14 (20.9)**	**7 (8.64)**	**0.03**
Statins n (%)	1 (1.67)	5 (6.02)	0.20	1 (1.49)	3 (3.70)	0.41

Where: MHPG, 3-methoxy-4-hydroxyphenylglycol; SBP, systolic blood pressure; DBP, diastolic blood pressure; CIMT, carotid intima-media thickness. Data expressed as arithmetic mean ± standard deviation for T-tests, geometric mean (5^{th} and 95^{th} percentile) for ANCOVA's (adjusted for *a priori* confounders, additionally adjusted for C-reactive protein, cholesterol, and blood pressure in CIMT analysis), and percentage of *n* for χ^2-tests. Significant differences depicted in bold.

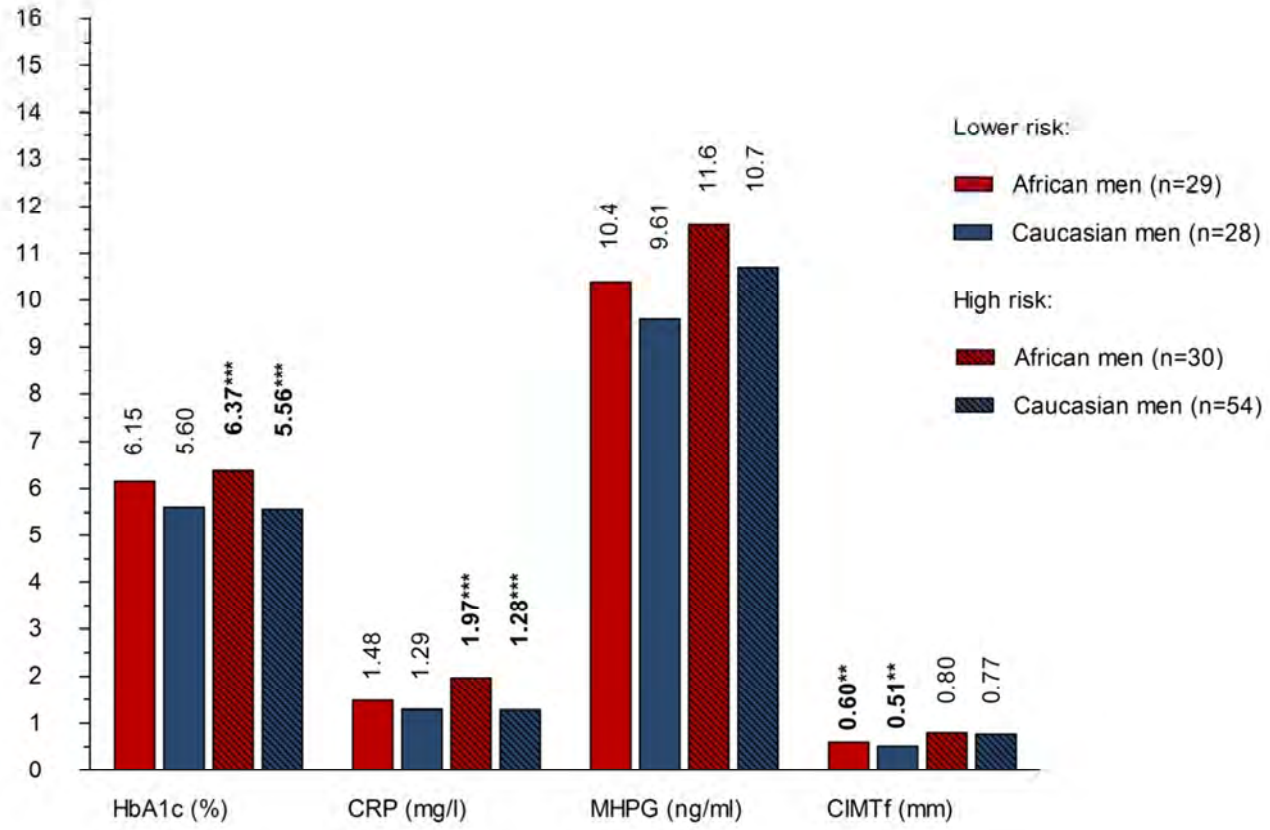

Figure 2.1: Comparing trends in biochemical markers and vascular structure in defensive coping African and Caucasian men

"Lower risk" and "High risk" refers to the subdivision in median splits of CIMT of the far wall. Where: HbA1c, glycated haemoglobin; CRP, C-reactive protein; MHPG, 3-methoxy-4-hydroxyphenolglycol; CIMTf, carotid intima-media thickness of the far wall. Adjusted for *a priori* confounders and additionally for CRP, cholesterol, and blood pressure in CIMT analysis. Significant differences depicted in bold, $p \leq 0.05$*, $p \leq 0.01$**, $p < 0.001$***.

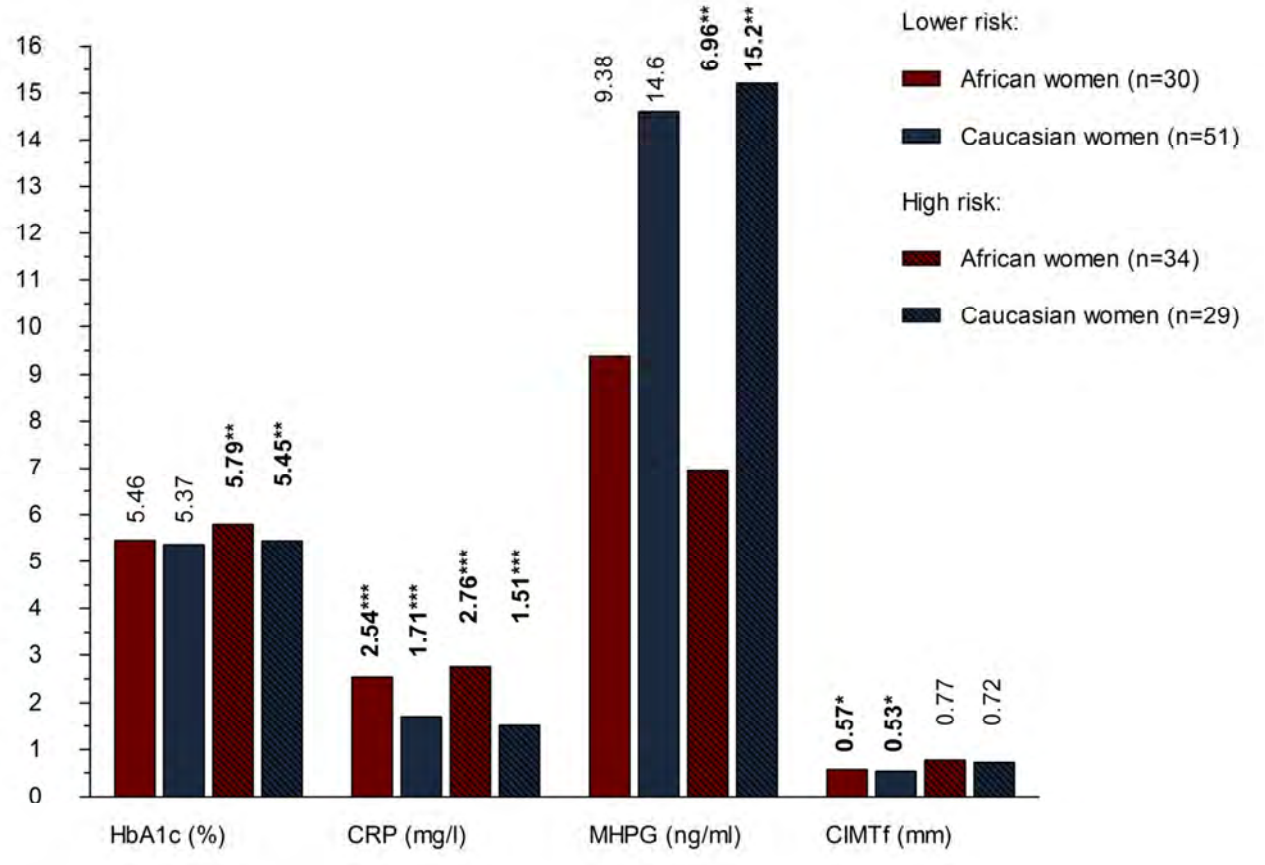

Figure 2.2: Comparing trends in biochemical markers and vascular structure in defensive coping African and Caucasian women

"Lower risk" and "High risk" refers to the subdivision in median splits of CIMTf. Where: HbA1c, glycated haemoglobin; CRP, C-reactive protein; MHPG, 3-methoxy-4-hydroxyphenolglycol; CIMTf, carotid intima-media thickness of the far wall. Adjusted for *a priori* confounders and additionally for CRP, cholesterol, and blood pressure in CIMT analysis. Significant differences depicted in bold, p≤0.05*, p≤0.01**, p<0.001***.

Table 2.2: Regression analysis of CIMTf in defensive coping teachers who have a high risk of subclinical vascular disease

	African men (n=30)		Caucasian men (n=51)		African women (n=34)		Caucasian women (n=29)	
	MODEL 1		MODEL 2		MODEL 3		MODEL 4	
	R^2	β (± 95% CI)	R^2	β (± 95% CI)	R^2	β (± 95% CI)	R^2	β (± 95% CI)
Salivary MHPG (ng/ml)	**0.79**	**-0.22 (-0.40, -0.03)**						
Heart rate (bpm)			**0.35**	**0.37 (0.14, 0.59)**				
Social support					**0.34**	**-0.43 (-0.72, -0.14)**		

Where: CIMTf, carotid intima-media thickness of the far wall; MHPG, 3-methoxy-4-hydroxyphenolglycol. The following covariates were included in Model 1: waist circumference, cholesterol and MHPG, Model 2: heart rate, Model 3: cholesterol and social support, Model 4: systolic and diastolic blood pressure. Analysis adjusted for *a priori* confounders and additionally for blood pressure, cholesterol and C-reactive protein. Only significant results depicted with $p \leq 0.05$ and adjusted $R^2 \geq 0.30$.

3.4. Defensive coping women with varying risk of subclinical vascular disease

In the African women, vastly increased low-grade inflammation was discovered through CRP concentrations of 2.54 and 2.76 mg/l against 1.51 and 1.71 mg/l in the Caucasian women. In the lower risk groups, African women were more prone to subclinical vascular disease than their Caucasian counterparts, indicated by CIMTf values of 0.57 vs. 0.53 mm. Furthermore, high risk African women revealed significantly augmented HbA1c together with reduced MHPG, compared to their Caucasian counterparts.

3.5. Regression analysis indicating associations with CIMTf

In high risk groups, increased CIMTf was associated with reduced MHPG in African men (*Model 1:* ß= -0.22). Augmented heart rate (HR) was associated with increased CIMTf in Caucasian men (*Model 2:* ß=0.35). Additionally, less use of social support in African women was associated with increased CIMTf (*Model 3:* ß= -0.43). No associations were revealed in *Model 4*. The lower risk CIMTf groups were omitted from *Table 2.2* as analysis discovered no significant results.

4. Discussion

Our aim for this study was to determine to what extent salivary MHPG levels contributed to subclinical vascular disease risk in DefS African and Caucasian teachers. The main findings revealed that Africans, rather than Caucasians, have a poorer health and CVD risk profile with elevated low-grade inflammation, HbA1c, HR, BP, and CIMTf, although reduced MHPG levels were only established in African women. Consistent with our hypothesis, we found an inverse association between MHPG and subclinical vascular disease risk in Africans. Overall, these findings indicate that lower MHPG levels may be considered a marker of autonomic exhaustion in Africans utilising DefS.

4.1. Overall health and prognosis

At first glance, the Caucasian men revealed greater BSA than the African men, as was expected from the difference in stature. In contrast, Africans displayed vastly greater WC values. Table 1 depicted that both African gender groups were well above the International Diabetes Federation WC cut points (men: 94 cm, women: 80 cm) [27]. New cut points were, however, recommended by Prinsloo *et al.*, calculated specifically for the SABPA study's African participants (men: 90 cm, women: 98 cm) [28]. This causes concern as abdominal obesity is associated with CVD risk and the African men were almost 14% above the new cut point. What was more, the African men were physically less active than their Caucasian counterparts, which could predispose them to augmented sympathetic activity, ultimately contributing to an increased risk for obesity and CVD [29]. Prior findings concluded that Africans, men in particular, are at higher risk of subclinical vascular disease, even though Caucasians are more prone to dyslipidaemia, as evident in cholesterol levels [1]. Besides increased BP in Africans, this could possibly be explained by previously discovered negative associations between PA and atherosclerosis, as well as inflammatory markers, i.e. CRP [30,31]. Accordingly, our results depicted significant differences between Africans and Caucasians regarding their CRP levels. Furthermore, African women displayed correspondingly increased CIMTf (0.67 vs. 0.60 mm) together with reduced MHPG (8.12 vs. 14.7 ng/ml). Therefore, higher PA can be viewed as a health promoter and might counteract sub-clinical atherosclerosis development and progression in Caucasians, possibly through lowering sympathetic activity [31].

Overall, the Africans revealed poorer health. Furthermore, four times as many African as Caucasian men reported being under severe stress. This corresponds with former studies that concluded Africans exhibit more cardiovascular risk, including but not limited to type 2 diabetes, metabolic syndrome, HT and subclinical atherosclerosis, especially when faced with psychosocial stress [1,2,5,14]. Interestingly, not just stress, but also coping ability/inability, affects health. Consequently, DefS Africans are even more at risk, rendering

a possible underlying cause of pathology, although the mechanism is unfamiliar [1,2]. We propose that dissociative coping responses may play a part.

4.2. Dissociative coping responses and autonomic activity

Previously, Malan *et al.* mentioned dissociation between behavioural and physiological coping strategies of Africans [2,5]. Allegedly, Africans revealed a behavioural DefS response (β-adrenergic), but their physiological responses seemed to coincide more with the emotional avoidance response (α-adrenergic). Upon stress being experienced, secreted NE will activate adrenergic reactions. At first both the α- and β-adrenergic reactions will be stimulated in preparation for coping. Ultimately, blood will be moved into areas where most needed, and increases in HR, stroke volume, BP and energy supplies will follow [32,33]. Generally, during sustained stress, the β-adrenergic reaction will normalise, whilst the α_1-adrenergic reaction will continue to stimulate even more NE release and increase total systemic resistance as a result of vasoconstriction [33]. This is closely related to the early stages of hypertension [34]. In the end, an upsurge in the amount of NE is necessary to cope. However, if the individual is overwhelmed by stress and experiences an inability to cope accordingly, distress will ensue, resulting in hyperactivity of the sympathetic nervous system (SNS) [9,12]. As such, neither the vagal system, nor the negative feedback mechanism of α_2-adrenergic receptors, will be able to reduce the amounts of NE adequately, so that concentrations will increase even further. In relation to the physiological dissociative coping we infer the following autonomic mechanism: the hyperactivity of the SNS, stimulated by overwhelming severe stress (African men: *Table 2.1*), causes an increase in NE. More NE will be available for binding on adrenergic receptors, stimulating NE overload. The DefS Africans demonstrated significantly higher HR and BP *(Table 2.1)* than their Caucasian counterparts (β-adrenergic). A higher prevalence of HT (α-adrenergic) was further observed in African women. Therefore, we can assume that Africans under severe stress will show physiological dissociative coping responses, in accord with sympathetic hyperactivity and NE

overload. But, as homeostasis needs to be attained, this hyperactivity cannot ensue continuously.

4.3. Defensive coping, autonomic dysfunction and cardiovascular risk

It was previously stated that upon stress exposure and re-exposure, different reactions are elicited, at first causing large increases in NE; but after a while the rates will decrease due to exhaustion of the autonomic system [2,11]. Adrenal fatigue may also set in and the SAM system will become non-responsive, with possible desensitisation and/or down-regulation of adrenergic receptors, which is also associated with depression [12,34]. Inevitably, less NE will be released, resulting from decreased α_1-adrenergic binding, but damage may already have occurred. Our results reflected this decrease in sympathetic activity, as a tendency of reduced MHPG in African women. Nevertheless, significantly higher HbA1c and CRP values were reported, in comparison to Caucasian women *(Table 2.1)*. Furthermore, factors recorded in *Table 2.1,* and *Figures 2.1* and *2.2*, revealed that in the African sex groups the HbA1c and CRP were significantly higher than in the respective Caucasian gender groups, especially in the high risk subdivisions, increasing their vulnerability to subclinical vascular disease [35]. However, increased CIMTf together with waning MHPG concentrations (decreased sympathetic activity) were established solely in the African women *(Table 2.1* & *Figure 2.2)*. Nevertheless, reduced MHPG did explain 79% of the variation in subclinical vascular disease risk in African men *(Table 2.2)*. Therefore, decreased sympathetic activity as a result of dysfunctional SAM system activity may explain the particularly high pathology risk in DefS Africans. The aforementioned is cause for great concern as firstly, these Africans seem to experience overwhelming stress and are unable to cope accordingly. Secondly, a tendency to self-treatment with alcohol appears to coincide with this unmanageable stress. And thirdly, the sustained uncontrolled stress of these Africans puts them at higher risk of subclinical vascular disease, due to the reciprocal effects of CRP and HbA1c on subclinical atherosclerosis [35].

4.4. Limitations and recommendations

Due to the cross sectional SABPA study design we cannot infer causality; we therefore propose a follow-up. The role of the immune system should also be analysed, to exclude the effects of cytokines on coping and cardiovascular risk. Repetition of the study with larger groups and 24 hour urinary sampling, for catecholamine and cortisol analyses, is further recommended to support our findings.

5. Conclusions

In conclusion, the Africans seemed to be at higher risk for subclinical vascular disease, especially when DefS "fails" and sympathetic activity diminishes. The declining MHPG concentrations may be due to an inability to cope actively, to such an extent that the SAM system is hyper activated and NE overload persists, with consequential desensitisation and/or down-regulation of the adrenergic receptors. Ultimately, physiological processes are debilitated and may result in pathology such as subclinical vascular disease.

Conflict of interest

None.

Acknowledgements

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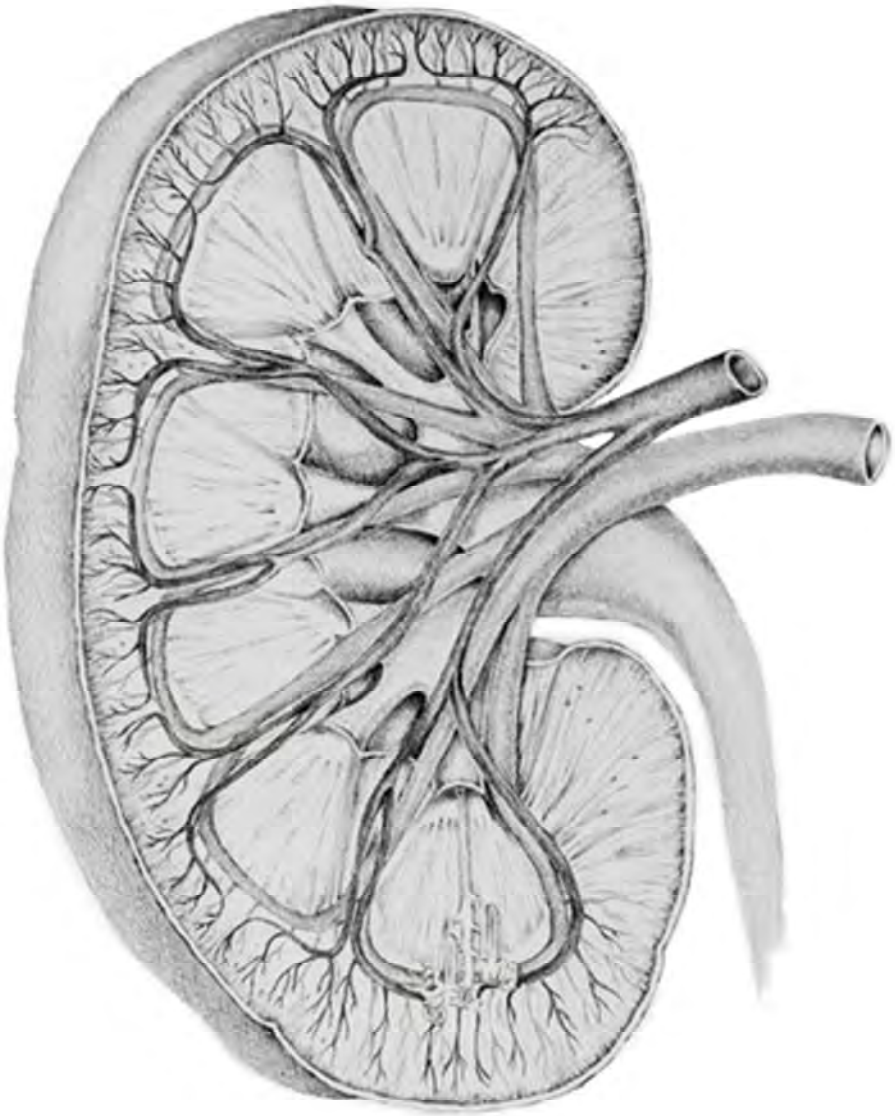
CHAPTER 3



Manuscript 2

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Please note that some of the format requirements were changed to ensure uniformity throughout the thesis.

Defensive coping and renovascular disease risk – Adrenal fatigue in a cohort of Africans and Caucasians: the SABPA study

Running title: Coping, renovascular risk and adrenal fatigue in South Africans

A de Kock, L Malan, M Hamer, M Cockeran, NT Malan

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Defensive coping and renovascular disease risk — Adrenal fatigue in a cohort of Africans and Caucasians: The SABPA study

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HIGHLIGHTS

- Caucasians with high cortisol levels showed increased renovascular risk.
- Africans who self-reported severe stress experience revealed low cortisol levels.
- The low cortisol in Africans was associated with increased renovascular risk.
- Utilisation of defensive coping further enhanced renovascular risk in Africans.
- Overwhelming stress may have resulted in adrenal fatigue and down-regulated cortisol.

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ABSTRACT

Background: Defensive coping is an established cardiovascular risk factor in Africans. Additionally, chronic, excessive or inadequate hypothalamic–pituitary–adrenal axis (HPAA) stress responses could either increase or decrease cortisol responses, which may relate to renal impairment. We scrutinised the relationship between urinary cortisol levels and renovascular disease risk in Africans and Caucasians utilising defensive coping.
Methods: Africans ($n = 168$) and Caucasians ($n = 207$) from the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study were included in our analyses, excluding HIV positive, diabetic, renal impairment, and cortisone users. The Coping Strategy Indicator questionnaire assessed preferred coping responses. Ambulatory blood pressure was recorded together with 8 h fasting blood and urine sampling. Renovascular disease risk markers included the albumin-to-creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR).
Results: The main findings revealed that Caucasians with high cortisol showed augmented renovascular disease risk. Conversely, Africans revealed low cortisol levels whilst 21.84% reported experience of severe stress, possibly depicting HPAA hypoactivity. Additionally, these Africans with low cortisol revealed increased ACR and decreased eGFR, which was further enhanced by defensive coping.
Conclusions: Defensive coping enhanced renovascular risk in Africans, especially in those with lower cortisol, which may be due to HPAA dysfunction and/or adrenal fatigue.

1. Introduction

Excessive release of stress hormones, such as cortisol and norepinephrine, is associated with disease risk. Chronic exposure to these hormones can cause permanent autonomic changes and increases in cardiovascular disease risk [1–4]. Norepinephrine and cortisol are released when the stress response is activated by the sympatho-adrenal–medullary (SAM) and hypothalamic–pituitary–adrenal axis (HPAA) neural systems. These responses are closely associated with the defensive coping response, in which an individual will actively seek ways to solve the problem at hand, with hard work, determination, as well as intense control over the situation and/or by asking others for help [5–6]. The effects of norepinephrine in defensive coping Africans have been explained previously, showing that decreased norepinephrine metabolites are associated with an enhanced risk of subclinical vascular disease in these Africans [2]. This may be due to autonomic

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Abstract

Background: Defensive coping is an established cardiovascular risk factor in Africans. Additionally, chronic, excessive or inadequate hypothalamic-pituitary-adrenal (HPA) axis stress responses could either increase or decrease cortisol responses, which may relate to renal impairment. We therefore scrutinised the relationship between urinary cortisol levels and renovascular disease risk in Africans and Caucasians utilising defensive coping.

Methods: Africans (n=168) and Caucasians (n=207) from the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study were included in our analysis, excluding HIV positive, diabetic, renal impairment, and cortisone users. The Coping Strategy Indicator questionnaire assessed preferred coping responses. Ambulatory blood pressure was recorded together with 8h fasting blood and urine sampling. Renovascular disease risk markers included the albumin-to-creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR).

Results: Main findings revealed that Caucasians with high cortisol showed augmented renovascular disease risk. Conversely, Africans revealed low cortisol levels whilst 21.84% reported experience of severe stress, possibly depicting HPA hypoactivity. Additionally, these Africans with low cortisol displayed increased ACR and decreased eGFR, which was further augmented by defensive coping.

Conclusions: Defensive coping augmented renovascular risk in Africans, especially in those with lower cortisol, which may be due to HPA dysfunction and/or adrenal fatigue.

Keywords: Adrenal fatigue; coping; cortisol; ethnicity; HPA axis; renovascular disease.

1. INTRODUCTION

Excessive release of stress hormones, such as cortisol and norepinephrine, is associated with disease risk. Chronic exposure to these hormones may cause permanent autonomic changes and increases in cardiovascular disease risk [1-4]. Norepinephrine (NE) and cortisol are released when the stress response is activated by the sympatho-adrenal-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axis neural systems. These responses are closely associated with the defensive coping (DefS) response, in which an individual will actively seek ways to solve the problem at hand, by means of hard work, determination, as well as intense control over the situation and/or by asking others for help [5-6]. The effects of norepinephrine in DefS Africans have been explained previously, showing that decreased NE metabolites are associated with an augmented risk of subclinical vascular disease in these Africans [2]. This may be due to autonomic exhaustion subsequent to prolonged sympathetic hyperactivity. It was proposed that desensitisation may occur in the SAM neural system of Africans, but not in Caucasians, with a resultant decrease in the release of NE. However, the same trend of autonomic exhaustion has not been proven for the HPA and the second major stress hormone: cortisol.

Relatively little is known regarding the neuro-endocrine DefS response in Africans, their propensity to renovascular disease and the role of cortisol in this respect. Evidence suggests that renal impairment may be related to autonomic dysfunction [7-8]. According to Zoccali, glomerular hypertension is influenced by sympathetic nervous system hyperactivity [7]. Rosman *et al.* also determined that renal failure patients showed a dysregulated HPA feedback control system [8]. Their study revealed that individuals with moderate or severe renal impairment recorded lower urinary cortisol levels than individuals with no or mild impairment. However, the role of DefS responses and HPA dysfunction has not been investigated with regard to renovascular pathology risk in Africans, while documented data are scarce. We consequently endeavoured to assess the relationship between urinary

cortisol levels and renovascular disease risk in Africans and Caucasians who were utilising DefS.

2. MATERIALS AND METHODS

2.1 Research design and participants

This study is part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study. The study population consisted of urban African (n=200, data sampling in 2008) and Caucasian (n=209, data sampling in 2009) teachers, from the Dr Kenneth Kaunda Education district in the North West Province, South Africa. All participants were of a similar socioeconomic status. Sampling took place from February to May each year, to eliminate seasonal changes. It has been stated that socioeconomic status and how one copes with stress affects cortisol concentrations [9-10]. For this reason, adaptive coping responses such as seeking social support and active problem solving have been associated with lower cortisol levels [10]. Therefore, recruitment of participants with similar socioeconomic status and identification of each participant's favoured coping strategy was essential.

Exclusion criteria included pregnancy, lactation, psychotropic substance use as well as the use of alpha and beta blockers, tympanum temperature >37 ℃ and vaccination or blood donation 3 months prior to participation. Furthermore, HIV positive (n=19), diabetic (n=12), and established renal impairment (n=1) cases, as well as individuals using cortisone medication (n=2), were excluded from analysis. The final participant sample comprised Africans and Caucasians (n=168 & 207, respectively) aged 20-63 years. Prior to their taking part all procedures were explained to participants, before their informed consent was given and their inclusion in the study. The Ethics Review Board of the North-West University approved all procedures of the SABPA study (NWU-00036-07-56), which were completed

following the guidelines from the Declaration of Helsinki. The SABPA study design and methodology are well-described elsewhere [11].

2.2 Research procedure

Each participant was subjected to the experimental setup over a period of 48 hours. On four working days of the week (Monday to Thursday), between 07h00 and 08h00, four participants were each fitted with the Cardiotens CE120® (Meditech, Budapest, Hungary) for ambulatory blood pressure (BP) and heart rate measurements, as well as the Actical® accelerometers (Montréal, Québec) for physical activity measurements. The participants thereafter resumed their usual daily activities, reporting any anomalies such as headache, nausea, visual disturbances, fainting, palpitations, physical effort and any experience of stress, on the diary cards provided. Obese or non-obese cuffs were fitted on the non-dominant arm of each participant for ambulatory BP measurements. A successful inflation rate of 72.60% and 84.64% was recorded for the surveys of 2008 and 2009 respectively. Following the European Society of Hypertension guidelines, hypertension was classified as 24 hour ambulatory BP ≥130/80 mmHg [12].

At 16h30 these participants were transported to the Metabolic Unit of the North-West University to stay overnight. Upon arrival, they were each allocated their own rooms and were meticulously informed about the experimental arrangement, to lessen anticipation stress. Registered clinical psychologists supervised completion of the battery of psychosocial questionnaires with a standardised dinner break at 18h30 in-between. The Coping Strategy Indicator (CSI) questionnaire, developed by Amirkhan (1990), determined the preferred coping strategy of each self-reporting participant, whilst he or she was thinking of a specific stressful situation in the last six months [5]. The following three strategies were included in the CSI: problem solving (formerly described as defensive coping), seeking social support, and emotional avoidance [2,5]. A Cronbach's alpha reliability coefficient of between

0.81 and 0.87 was determined for defensive coping; 0.61-0.85 for emotional avoidance and 0.83-0.90 for seeking social support. Values above the mean of 26 on the DefS scale, as well as 19 on emotional avoidance and 23 on seeking social support, were documented as a high preference for the specific coping response [5]. We focused on DefS as this is associated more with cardiovascular risk in urban Africans [2, 13].

The participants were advised to go to bed at 22h00, fasting overnight and until after measurements on the second day, at approximately 10h00. Eight-hour collected urine samples were obtained from each participant between 05h45 and 06h15 and handled according to standardised procedures for cortisol analysis *(see supplementary material)* [14]. After the last BP recording at 06h00, the Cardiotens CE120® and Actical® were disconnected. Registered anthropometrists took measurements in triplicate with an intra- and inter-observer variability of less than 10%. Waist circumference (WC) was measured perpendicular to the axis of the trunk, at the midpoint between the iliac crest and lower costal border; it indicated augmented disease risk at cut points of 90cm for men and 98cm for women [15]. Body surface area (BSA) was calculated with the Mosteller formula [16-17]. Hereafter participants were placed in the semi-Fowlers position and a 10 minute resting period was followed by blood sampling. A registered nurse obtained fasting blood samples from the brachial vein branches of the dominant arm using a winged infusion set.

Fasting plasma and serum samples were prepared according to standardised procedures and stored at -80 ℃ until analysis *(see supplementary material)*. Cholesterol, γ-glutamyl transferase (cGGT), and ultrahigh-sensitivity C-reactive protein (hs-CRP) were analysed from serum, together with urinary albumin, using two sequential multiple analysers (Konelab 20i; Thermo Scientific, Vantaa, Finland; Unicel DXC 800 – Beckman and Coulter®, Germany). The Elecsys (Roche, Switzerland) determined serum cotinine and urinary cortisol levels by means of homogenous immunoassays, whilst glycated haemoglobin (HbA1c) from EDTA

whole blood and urinary creatinine was analysed with the Cobas Integra 400 (Roche, Switzerland). Estimated glomerular filtration rate (eGFR) as well as the albumin-to-creatinine ratio (ACR) determined chronic kidney disease. The cortisol-to-creatinine ratio (CCR) was calculated to adjust for urine volume and concentration.

2.3 Statistical analysis

Statistical analysis were calculated using *Statistica 12* computer software (Statsoft Inc., Tulsa, USA). Statistical significance was defined as a two-sided α level of 0.05 or less. Kolmogorov-Smirnov tests assessed normality of all variables. Urinary cortisol, hs-CRP, ACR, and eGFR were logarithmically transformed; exponentials of these values were used in correlation models.

Receiver operating characteristic (ROC) analysis were computed by means of SPSS 22 software (SPSS Inc., Illinois, USA) to determine an optimal urinary cortisol cut point which predicted 24h ambulatory hypertension. Thereafter, the cut point was used to stratify the participant sample into low and high urinary cortisol groups. Two-way ANCOVA interaction models between the main effects (Race and ROC urinary cortisol) were computed for renovascular disease risk markers. This determined stratification into ethnic groups with low and high cortisol, for further analysis.

Although statistical analysis did not pertain exclusively to DefS participants, Africans and Caucasians included 74% and 79% of the above mean DefS responders, respectively. Three-way ANCOVAs between main effects (race, sex and the ROC urinary cortisol cut point) and high DefS were calculated, for added significance. It was deemed that most participants preferably utilised this coping response and that the significant physiological trends found were relevant to high use of DefS.

T-tests described ethnic participant characteristics. *A priori* confounders included age, body surface area (BSA), physical activity, cotinine and GGT [12]. Chi-square tests were used to determine prevalence and proportions. Independent T-tests were used to compare renovascular risk markers, firstly in Africans with low and high cortisol levels, and secondly in Caucasians. ANCOVAs determined the least square mean differences between groups independent of *a priori* confounders. Odds ratios (OR) were calculated for several models to determine the probability of increased renovascular disease risk in the low rather than high urinary cortisol category, for the total as well as separate gender groups. Confounders for models included systolic (SBP) and diastolic blood pressure (DBP) and exponentials of ACR and eGFR. The ROC urinary cortisol cut point was used for categorisation together with race and sex. Sensitivity analysis were performed to determine whether utilising DefS further augmented the risk for renovascular disease, after which the above OR models were repeated. Only ACR was included in the results because probable excessive alcohol use impedes proper elucidation of eGFR data.

3. RESULTS

Two-way ANCOVAs revealed interactions for main effects (race and ROC urinary cortisol) and urinary cortisol [F (1, 353), 6.83; p=0.009] and eGFR [F (1,351), 7.67; p=0.006]. Three-way ANCOVAs revealed significance between main effects (race, sex, ROC urinary cortisol) and 24 hour hypertension [F (1,345), 5.66; p=0.02], as well as high DefS (scores above 31 on the CSI) [F (1,345), 8.92; p=0.003]. As statistical power was affected we preferred to use above mean DefS scores (≥26) in stratification, as these have previously been associated with vascular damage. No interactions were revealed either for seeking social support or emotional avoidance with regards to cardiometabolic and/or renovascular risk.

In *Table 3.1*, descriptive statistics demonstrated higher pathology risk in Africans, as evident in possible excessive alcohol abuse (cGGT levels: 61.77 vs. 26.61 U/L), low-grade

inflammation, glycated haemoglobin indicating pre-diabetes levels, as well as decreased high-density lipoprotein cholesterol. Increased renovascular risk was further demonstrated in those Africans with an increased CCR and ACR, together with increased heart rate, BP and hypertension prevalence. Additionally, more Africans used medication for hypertension, whilst more Caucasians used statins. The CSI indicated that Africans and Caucasians similarly utilised the DefS response, whilst Africans were more inclined to make use of social support and Caucasians were more emotionally avoidant. In addition, more Africans self-reported experience of severe stress.

ROC analysis indicated a urinary cortisol cut point of 375.40 nmol/l predicting hypertension with sensitivity/specificity 46%/65% [area under the curve: 0.55 (95% CI: 0.49, 0.61)]. Hereafter, race groups were stratified into low (<375.40 nmol/l) and high (≥375.40 nmol/l) cortisol groups. In *Table 3.2*, Africans and Caucasians had similar scores in their respective low and high cortisol categories for DefS. The highest prevalence of self-reported stress was found in the low cortisol African group (21.84%). A trend of decreased urinary cortisol and increased renovascular disease risk was found in the Africans. The low cortisol Africans revealed significantly decreased eGFR and increased ACR levels, when compared to their high cortisol counterparts. Conversely, those Caucasians with high cortisol levels revealed more susceptibility to renovascular disease. These Caucasians displayed significantly greater BSA values together with a distinctly higher prevalence of hypertension ($p=0.008$), when compared to their low cortisol counterparts.

Table 3.1: Comparing psychosocial stress and disease risk indices in Africans and Caucasians

	AFRICANS (n=168)	**CAUCASIANS (n=207)**	**P**
A PRIORI CONFOUNDERS			
Age (years)	44.05 ± 8.42	45.00 ± 10.88	0.36
Body surface area (m^2)	**1.91 ± 0.23**	**2.00 ± 0.28**	**0.002**
Physical activity (kcal/day)	**2680.73 ± 811.09**	**3104.88 ± 1601.53**	**0.002**
Cotinine (ng/ml)	21.59 ± 52.26	22.93 ± 77.82	0.85
γ-Glutamyl transferase (U/l)	**61.77 ± 72.88**	**26.61 ± 33.66**	**<0.0001**
METABOLIC VARIABLES			
Waist circumference (cm)	**95.74 (94.55, 96.92)**	**90.92 (89.88, 91.96)**	**<0.0001**
C-reactive protein (mg/l)	**8.69 (7.47, 9.90)**	**3.05 (1.97, 4.12)**	**<0.0001**
Glycated haemoglobin (%)	**6.00 (5.89, 6.13)**	**5.49 (5.38, 5.59)**	**<0.0001**
Cholesterol (mmol/l)	**4.55 (4.36, 4.75)**	**5.57 (5.40, 5.74)**	**<0.0001**
High-density lipoprotein cholesterol (mmol/l)	**1.13 (1.08, 1.19)**	**1.22 (1.17, 1.27)**	**0.02**
Metabolic syndrome prevalence n (%)	72 (43.11)	91 (43.96)	0.87
RENOVASCULAR RISK VARIABLES			
Urinary cortisol (nmol/L)	**445.58 (391.68, 499.47)**	**372.43 (325.52, 419.34)**	**0.05**
Cortisol-to-creatinine ratio	**49.27 (44.16, 54.40)**	**36.84 (32.89, 41.29)**	**0.0006**
Glomerular filtration rate (ml/min/1.73m^2)	**111.17 (107.68, 114.66)**	**95.97 (92.89, 99.05)**	**<0.0001**
Albumin-to-creatinine ratio	**1.43 (1.21, 1.66)**	**0.63 (0.42, 0.82)**	**<0.0001**
Microalbuminuria (>20mg/l) n (%)	**15 (8.98)**	**6 (2.90)**	**0.01**
Heart rate (bpm)	**79 (77, 81)**	**74 (73, 76)**	**<0.0001**
Systolic blood pressure (mmHg)	**134 (132, 136)**	**124 (122, 125)**	**<0.0001**
Diastolic blood pressure (mmHg)	**83 (82, 85)**	**77 (75, 78)**	**<0.0001**
Hypertension prevalence n (%)	**81 (48.21)**	**36 (17.39)**	**0.001**
History of kidney disease n (%)	2 (1.19)	5 (2.42)	0.38
PSYCHOSOCIAL VARIABLES			
Defensive coping score	28.15 (27.52, 28.78)	28.75 (28.20, 29.31)	0.17
Above mean defensive coping score n (%)	125 (74.40)	164 (79.23)	0.27
Seeking social support score	**25.49 (24.71, 26.26)**	**18.88 (18.19, 19.56)**	**<0.0001**
Emotional avoidance score	**21.27 (20.54, 22.01)**	**24.01 (23.36, 24.66)**	**<0.0001**
Self-reported severe stress n (%)	**32 (19.05)**	**20 (9.66)**	**0.009**
MEDICATION USE			
Drugs for hypertension n (%)	**57 (33.93)**	**27 (13.04)**	**<0.0001**
Statins n (%)	2 (1.19)	9 (4.35)	0.07

Data expressed as arithmetic mean and standard deviations for T-tests, geometric mean with 5^{th} and 95^{th} percentiles for ANCOVA's (adjusted for *a priori* confounders), and percentage of n for Chi-squares analysis. Metabolic syndrome prevalence calculated from International Diabetes Federation guidelines [28]. Significant differences are depicted in bold, P≤0.05.

Table 3.2: Defensive coping and renovascular disease risk in Africans and Caucasians with low and high cortisol levels

	AFRICANS			CAUCASIANS		
	LOW CORTISOL (n=87)	**HIGH CORTISOL** (n=73)	**P**	**LOW CORTISOL** (n=124)	**HIGH CORTISOL** (n=78)	**P**
A PRIORI CONFOUNDERS						
Age (years)	**45.91 ± 7.61**	**41.30 ± 8.63**	**0.005**	45.85 ± 10.85	44.59 ± 10.25	0.41
Body surface area (m^2)	1.92 ± 0.24	1.90 ± 0.23	0.78	**1.96 ± 0.28**	**2.06 ± 0.28**	**0.01**
Physical activity (kcal/day)	2703.81 ± 712.95	2699.50 ± 944.52	0.97	2950.24 ± 852.49	3359.35 ± 2362.44	0.08
Cotinine (ng/ml)	20.12 ± 57.38	23.92 ± 48.06	0.66	18.97 ± 62.23	30.71 ± 99.47	0.30
ɣ-Glutamyl transferase (U/l)	61.24 ± 63.79	64.14 ± 85.73	0.81	24.37 ± 24.71	29.14 ± 42.61	0.32
RENOVASCULAR RISK MARKERS						
Urinary cortisol (nmol/L)	**213.94 ± 86.70**	**709.38 ± 517.34**	**<0.0001**	**239.83 ± 82.74**	**589.71 ± 214.24**	**<0.0001**
Cortisol-to-creatinine ratio	**32.41 ± 21.15**	**70.03 ± 44.55**	**<0.0001**	**33.35 ± 23.52**	**42.25 ± 23.12**	**0.009**
Albumin-to-creatinine ratio	**1.64 ± 1.67**	**1.10 ± 0.95**	**0.02**	0.75 ± 1.50	0.46 ± 1.11	0.14
Glomerular filtration rate (ml/min/1.73m^2)	**108.22 ± 25.06**	**117.18 ± 30.65**	**0.05**	93.51 ± 17.44	95.97 ± 17.20	0.33
Systolic blood pressure (mmHg)	133 ± 18	131 ± 16	0.46	123 ± 12	126 ± 12	0.08
Diastolic blood pressure (mmHg)	83 ± 12	82 ± 11	0.59	**76 ± 8**	**78 ± 8**	**0.02**
Hypertension prevalence n (%)	56 (64.37)	46 (63.01)	0.86	**51 (41.13)**	**47 (60.26)**	**0.008**
PSYCHOSOCIAL VARIABLES						
Defensive coping score	28.10 ± 3.85	28.00 ± 4.27	0.87	28.65 ± 3.93	29.07 ± 3.58	0.44
Above mean defensive coping n (%)	66 (75.86)	52 (71.23)	0.51	97 (78.23)	64 (82.05)	0.51
Experienced severe stress n (%)	19 (21.84)	12 (16.44)	0.39	14 (11.29)	5 (6.41)	0.25

Data expressed as arithmetic mean and standard deviations for T-tests and percentage of n for Chi-squares analysis. Significant differences are depicted in bold, P≤0.05.

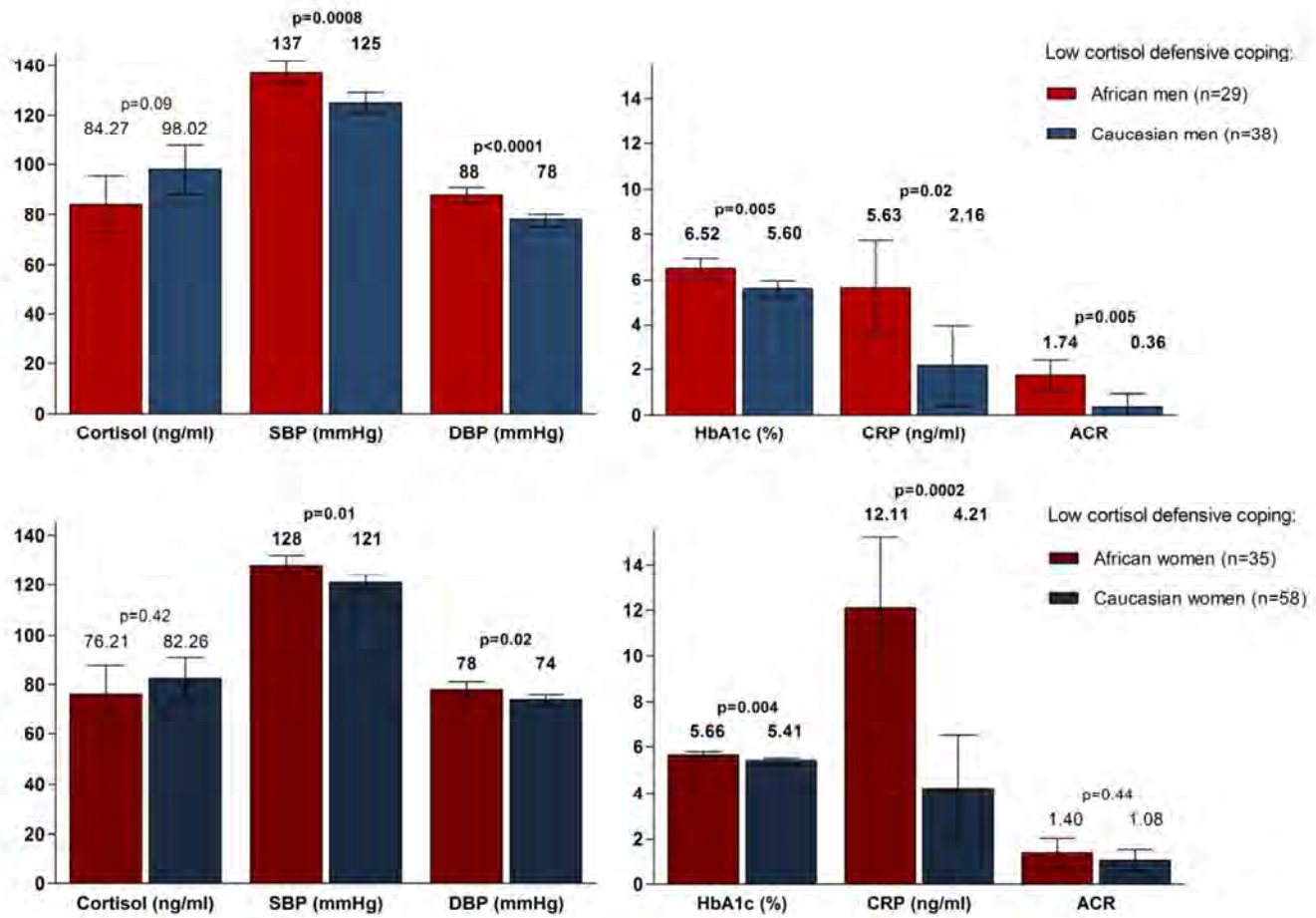

Figure 3.1: Renovascular risk in low cortisol defensive coping Africans and Caucasians

Only above mean defensive coping participants were included from the low cortisol stratification. Data presented as geometric means (95% confidence intervals), statistically significant results depicted in bold. Where: SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin; CRP, C-reactive protein; ACR, albumin-to-creatinine ratio.

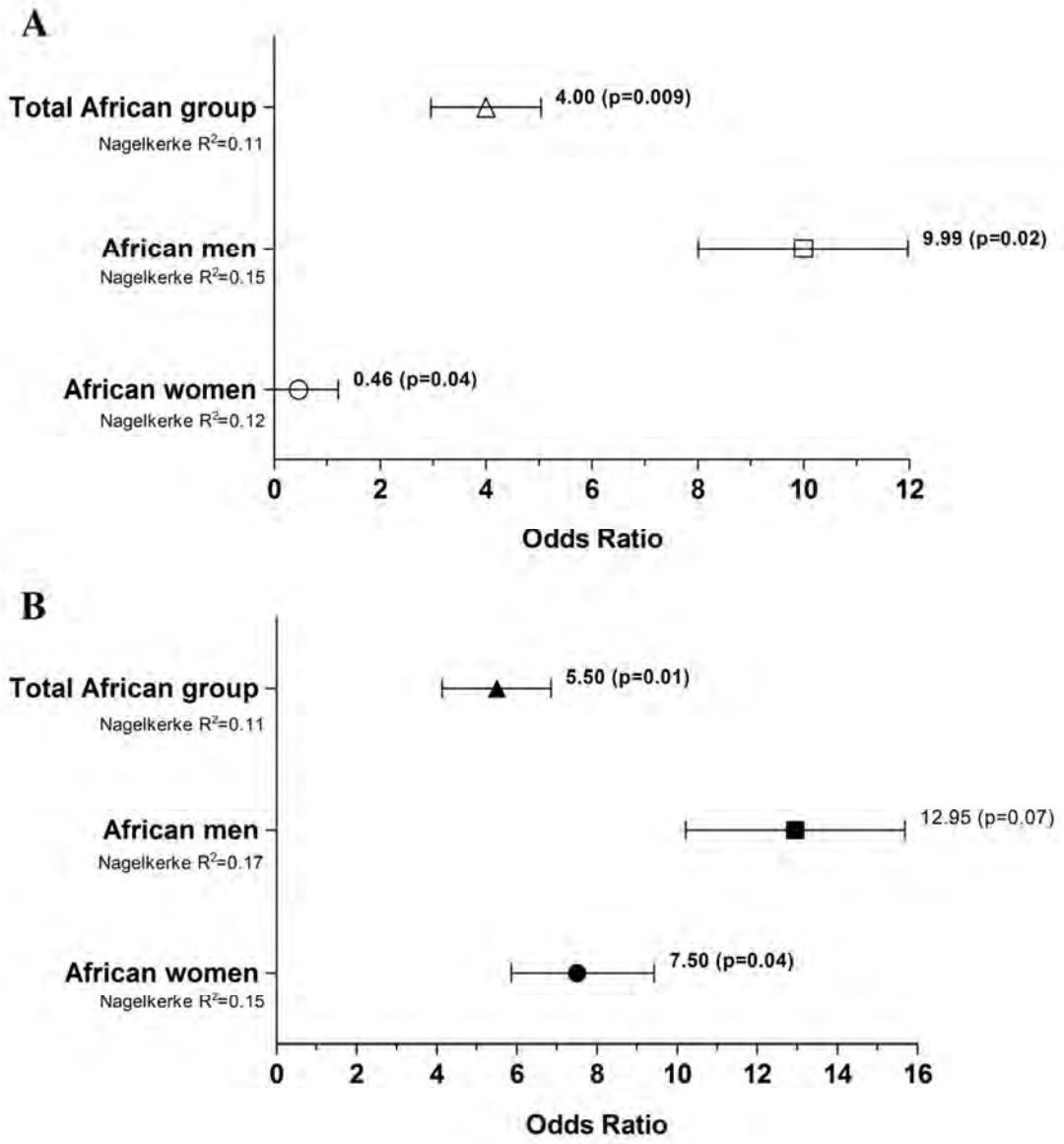

Figure 3.2: Probability of increased ACR in Africans with low rather than high cortisol levels

Where: A, includes all African participants; B, includes only above mean defensive coping African participants. Data presented as odds ratios for an increased albumin-to-creatinine ratio. Strength of the modelled relationship depicted as Nagelkerke R^2 values. Bold values are statistically significant.

In *Figure 3.1*, augmented renovascular risk was evident in the low cortisol DefS African men and women, when compared to their Caucasian counterparts. Their vulnerability to renovascular disease was depicted by heightened blood pressure, as well as increased ACR and glomerulosclerosis risk. Determining renovascular risk, we computed OR. No significant estimates for Caucasian men or women were revealed; only Africans revealed prominent risk in all models. In *Figure 3.2*, in the total African group (section A), low cortisol was associated with a 4.00 times higher risk of increased ACR. Analysis of African men showed an OR of 9.99 for increased ACR together with a slightly higher Nagelkerke R^2. In sensitivity analysis pertaining to DefS participants only (section B), we detected augmented odds ratios with higher Nagelkerke R^2 values. These added significance for renovascular disease risk when utilising DefS. Lower urinary cortisol rather than high cortisol, together with DefS in African men, were associated with increased renovascular disease risk.

4. DISCUSSION

The relationship between urinary cortisol levels and renovascular disease risk was assessed in Africans and Caucasians who were utilising DefS. Overall, findings revealed that: firstly, Africans were more susceptible to renovascular disease than Caucasians. Secondly, renovascular disease risk was more accentuated in DefS Africans with low cortisol levels, possible depicting HPA hypoactivity and/or adrenal fatigue.

4.1 Stress and disease risk in Africans and Caucasians

Africans and Caucasians scored similarly on the DefS scale, but the former seemed to be more prone to pathology. Despite the Africans' reported behavioural control in the face of severe stress, their urinary cortisol levels were not higher compared to their Caucasian counterparts. This may be due to the former's greater use of social support, as seeking social support has been associated with lower cortisol levels [10]. Contrastingly, Africans self-reported much higher severe stress experience when compared to Caucasians; this can

be related to perceived stress, which in turn has been shown to display positive associations with cortisol [18]. Therefore, in considering kidney function, the cortisol-to-creatinine ratio (CCR) was indeed higher than in the Caucasians. Kapoor *et al.* (2012) suggested that a CCR of 15.35 might be indicative of kidney disease risk [19]. As the Africans have a value of 50.06 they seem to be more vulnerable to pathology. In fact, in these participants, this robustly increased CCR was supported by other renovascular disease risk markers such as increased BP and ACR.

In addition, the eGFR were similar in Africans and Caucasians. This may be explained from the probable excessive alcohol consumption in Africans [20], which may be indicated in their greatly increased cGGT concentration of 61.50 U/l. According to Porter, cut points of 65 U/l and 45 U/l are indicative of alcohol abuse in men and women respectively [21]. We hypothesise that this probable excessive alcohol use in Africans may be the reason for their seemingly normal eGFR. Alcohol will increase eGFR by inhibiting the secretion of antidiuretic hormone, further impeding water reabsorption and increasing urine volume [22]. Elevated cGGT levels, as a toxic substance, might also support their heightened BP as well as decreased cholesterol levels [23].

Despite increased cortisol levels which should be suppressing immune responses, augmented low-grade inflammation was evident in the Africans [24-25], suggesting chronic HPA hypoactivity. Accompanying HbA1c concentrations, indicative of pre-diabetes levels [26], further increase their risk of renovascular disease, since increased low-grade inflammation (CRP >3 mg/l) and HbA1c may synergistically increase glomerulosclerosis risk [27]. Moreover, Africans presented with greater WC as well as increased BP and decreased HDL-c concentrations, also suggesting an augmented susceptibility to cardiometabolic disease [28]. In fact, Africans revealed a 43.11% prevalence of the metabolic syndrome.

We suggest that the Africans reporting behavioural control with chronic DefS responses might have physiological loss-of-control, where dysfunctional HPA activity is evident and the inhibitory feedback control of cortisol release no longer operates functionally [24]. Nonetheless, these Africans also indicated probable alcohol abuse; this might augment dysregulation of the HPA [29-30]. Subsequently, the damaging effects of increased cortisol, in prolonged unmanageable stress (when defensive coping "fails") [11], as well as the probable cortisol sensitivity of Africans, may affect end-organ function [2, 24, 31-32]. We were able to positively demonstrate more prominent increased renovascular disease risk in the DefS Africans within the low and high cortisol categories.

4.2 Low cortisol versus high cortisol

In Caucasians, higher DBP and hypertension prevalence was observed in those with high cortisol levels. This trend was, however, accompanied by a significantly greater BSA, when compared to Caucasians with low cortisol levels. Because body size is associated with kidney weight as well as glomerular function, this increased BSA should be beneficial to the high cortisol Caucasians' health [33-34]. However, their increased cortisol may show that they are experiencing more stress than their low cortisol counterparts. In addition, with increased stress, disease risk may therefore be augmented. This can be explained in terms of the HPA coping response system, where increased stress induces increased cortisol release for effective coping, impending other physiological processes until the stress is eliminated [24, 35]. But this stress coping response might increase the high cortisol Caucasian groups' pathology risk, due to enhanced HPA activity, which may be indicated in their significantly increased CCR and DBP levels [2, 6, 19]. Additionally, Caucasians revealed higher cholesterol concentrations while more of them used statin medications, as was expected from their proneness to dyslipidaemia [2]. With cholesterol concentrations exceeding the cut point of 5.20 nmol/l, endothelial damage at the renal vasculature and glomerulosclerosis may have occurred in the Caucasians [36], possibly further impeding

renovascular function in those with high cortisol levels. As such, disease risk in Caucasians was associated with increased cortisol levels; we propose that HPA hyperactivity may be the reason. HPA integrity is essential for adequate coping, while increased cortisol is necessary to determine the appropriate behavioural response to acute stress. However, a glucocorticoid response that is excessive, prolonged or inadequate will impair the individual's adaptation to stress, either by increasing or decreasing HPA activity; this dysfunction is considered a health risk [35, 37]. The HPA may also shift from an over-responsive to a more non-responsive system, which is also referred to as adrenal fatigue [24].

This was evident in the African participants, where decreased cortisol was associated with a higher risk of renovascular disease. Moreover, this decreased cortisol was not associated with less stress, as 21.84% of this African group self-reported experience of severe stress. The HPA thus seems to have become non-responsive or hypoactive to stress in these participants. Possible down-regulation occurred, decreasing sensitivity of the HPA and adrenal fatigue may have set in, with subsequent lower levels of cortisol [24, 32, 38-40]. Thus, with further exposure to stress, the desensitised or habituated HPA defensive coping response may not be able to increase cortisol for effective coping and becomes dysfunctional. However, cumulative effects of low cortisol will be evident in the cortisol diurnal pattern as the integrity of the HPA is essential to an array of physiological systems [35]. Ultimately, dysfunctional HPA activity and decreased cortisol release are associated with renal impairment [8, 41], and our results confirmed this trend, but only in the African participants.

4.3 Low cortisol and renal impairment

Africans in the low cortisol category exhibited an even higher risk for renovascular disease than their high cortisol and Caucasian counterparts. Africans with low cortisol levels revealed odds ratios depicting a four times higher risk of increased ACR than those with high

cortisol. Considering sex differences: African men in the low cortisol category have a ten times higher risk of increased ACR, than those in the high cortisol category. This confirms results from previous studies, where African men revealed attenuated cortisol responses associated with structural wall remodelling [32], as well as poorer health than their female and Caucasian counterparts [2, 13, 32, 42]. From these results, it is evident that African men with low or possible down-regulated cortisol levels reveal more vulnerability to renovascular disease than their high cortisol as well as Caucasian counterparts. Additionally, sensitivity analysis demonstrated even greater odds ratios for increased ACR in those Africans who utilise DefS.

As causality cannot be inferred from the cross-sectional design, we recommend that procedures should be repeated in a prospective study, in larger groups and with 24 hour urinary sampling for added significance.

CONCLUSIONS

In conclusion, the HPA will normally be hyper-activated when loss-of-control is experienced and DefS has failed to eliminate the stressor. However, prolonged or severe stress may alter the HPA to such an extent that cortisol is down-regulated. From our results, it appears that DefS Africans revealed higher renovascular disease risk than their Caucasian counterparts. Indeed, this was especially true for those Africans with lower or down-regulated cortisol levels. We propose that sustained uncontrollable stress may drain coping ability and resources, and subsequently HPA dysfunction and/or adrenal fatigue may ensue. Therefore, in Africans, the lower cortisol is not due to adaptive coping responses such as DefS and seeking social support, but is instead an indication of adrenal fatigue since it was associated with increased renovascular disease risk.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIAL: Biochemical analysis of blood and urine samples with specific reference cut points used

MANUFACTURER	APPARATUS	METHOD	ANALYTE	CUT POINT
Beckman & Coulter, Germany	Unicel DXC800	Timed end-point	**Serum cholesterol**	≥ 5.20mmol/l
Thermo Scientific, Finland	Konelab 20i		**Serum HDL cholesterol**	< 1.03mmol/l(♂)
				< 1.29mmol/l(♀)
		Turbidimetric	**C-reactive protein**	≥ 3mg/l
			Albuminuria	> 20mg/l
		Enzyme-rate	**ɣ-Glutamyl transferase**	> 65u/l(♂)
				> 45u/l(♀)
Roche, Switzerland	Elecsys	Homogenous immunoassay	**Cotinine**	≥ 12 ng/ml
			Urinary cortisol	55.2 - 276 nmol/l
Roche, Switzerland	Cobas Integra	Turbidimetric immunoassay	**Glycated haemoglobin**	> 6.5 %
		Colorimetric	**Urinary creatinine**	0.13 - 0.22 mmol/l
FORMULA			ANALYTE	CUT POINT
Modification of Diet in Renal Disease		eGFR prediction equation	**eGFR**	< 60 ml/min/1.73m^2

Where: HDL; high-density lipoprotein, ADA; American Diabetes Association, eGFR; estimated glomerular filtration rate.

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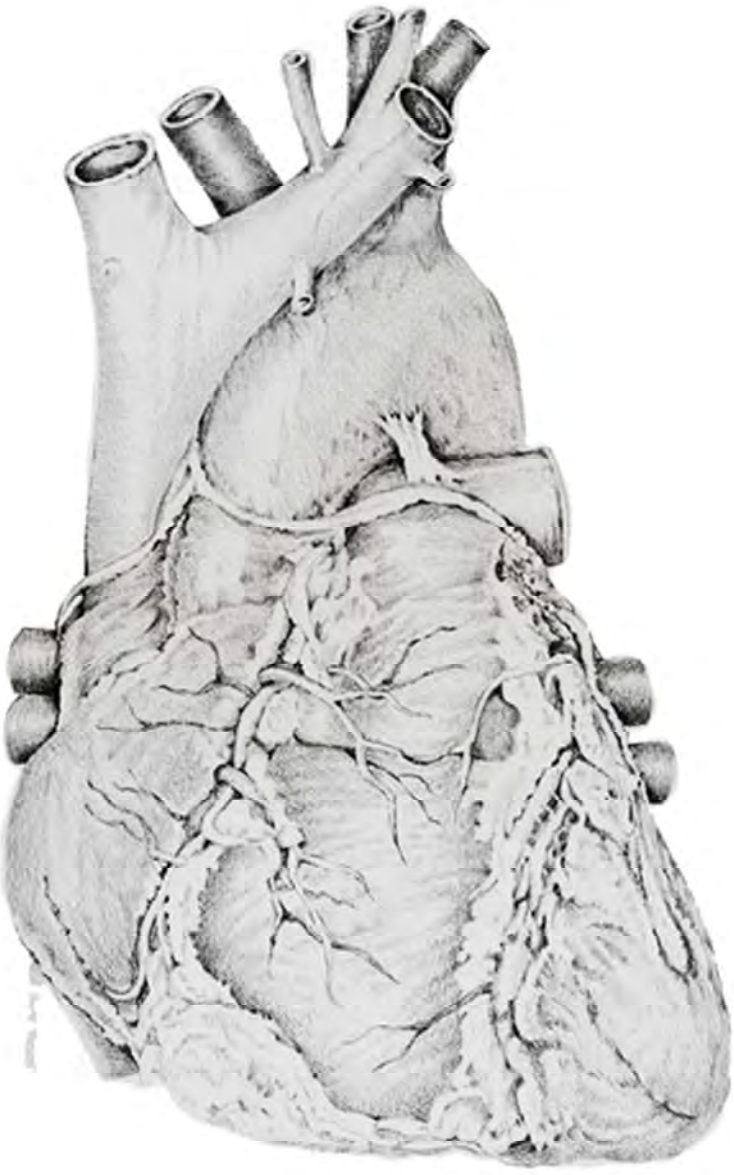
CHAPTER 4



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Manuscripts	– Maximum 4000 words – Should be divided into sections with *headings (Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References Tables, Artwork)* – Include the source of funding and conflicts of interest if any – Define abbreviations at first use in text
Title page	– Include a concise and informative title without abbreviations – Include acknowledgements and funding sources
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References	– Maximum 60 references – Include author(s) name(s), journal title/book title, chapter title/article title, publication year, volume number/book chapter and pagination used, DOI – Provide references with numbers in square brackets – *De Kock, A., Malan, L., Hamer, M., Malan, N.T. Defensive coping and subclinical vascular disease risk – Associations with autonomic exhaustion in Africans and Caucasians: The SABPA study. Atherosclerosis **225**(2), 438-443 (2012). Doi:10.1016/j.atherosclerosis.2012.08.038.*

Please note that some of the format requirements were changed to ensure uniformity throughout the thesis.

Defensive coping and estradiol - Unravelling neuro-endocrine dysfunction and augmented hypertension risk in a South African bi-ethnic cohort: The SABPA study

Running head: Defensive coping, estradiol, neuro-endocrine, hypertension

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ABSTRACT

Purpose: Defensive coping has been associated with neuro-endocrine dysfunction and cardiovascular disease risk. Estradiol has vasodilatory properties with cardio-, reno- and neuroprotective effects. It can however, also induce α_1-adrenergic responsiveness. We endeavoured, by studying a bi-ethnic sex cohort, to determine whether defensive coping would facilitate augmented hypertension risk through neuro-endocrine dysfunction.

Methods: African (n=168) and Caucasian (n=207) men and women (46±9 years) were included. Preferential use of defensive coping was determined from Coping Strategy Indicator questionnaire scores. Ambulatory blood pressure was monitored with the Cardiotens CE120®. Fasting serum estradiol and cortisol were analysed by immunoassay and the original cortisol-to-estradiol ratio was calculated to determine associations with blood pressure.

Results: Higher levels of self-reported psychosocial stress were evident in Africans compared to Caucasians (19.05% vs. 9.66%). Alcohol use and cardiometabolic risk were also higher in Africans. A decreased cortisol-to-estradiol ratio was associated with augmented blood pressure in African men ($p \leq 0.05$), and especially in defensive coping men ($p \leq 0.005$).

Conclusions: A reduced cortisol-to-estradiol ratio was associated with increased blood pressure in defensive coping African men. Defensive coping, possibly via highly activated α_1-adrenergic vasoconstrictory responses, may facilitate neuro-endocrine dysfunction and ultimately increase hypertension risk in African men.

Keywords: Coping; estradiol; dissociation in coping responses; neuro-endocrine dysfunction; adrenal fatigue; hypertension.

1. INTRODUCTION

Stress, and the neuroendocrine coping responses it elicits, is believed to play a crucial role in enhancing susceptibility to disease [1]. In fact, persistently increased blood pressure (BP) is frequently an ensuing pathophysiological state [1-4]. Furthermore, Africans and individuals of African descent record a high prevalence of hypertension (HT) and high HT-related mortality rates via stroke, cardiovascular disease (CVD) and/or renal failure, causing major concern [5-7].

Defensive coping (DefS) is a problem-solving coping response involving an active approach and effortful commitment to eliminate or alleviate stressors, and normally achieves its through β-adrenergic cardiovascular stimulation [6]. During periods of heightened psychosocial stress, the sympatho-adrenal-medullary (SAM) system and hypothalamic-pituitary-adrenal (HPA) axis are activated for rapid and/or long term coping responses, respectively [8-9]. The HPA, in particular, will be activated when situations or stressors are perceived as uncontrollable. When coping responses "fail" to alleviate or eliminate stressors adequately, chronic overwhelming stress may ensue; this may cause dysfunction of the HPA [9]. This chronic stress experience is related to decreased catecholamine reactivity and lowered cortisol levels (autonomic exhaustion and/or adrenal fatigue), which reduce the ability to cope effectively and are accordingly associated with increased CVD risk [9-12]. DefS has certainly been associated with neuro-endocrine dysfunction [decreased rather than increased norepinephrine (NE) and cortisol levels] and is also related to increased HT and target organ damage markers, especially in chronically stressed Africans [6,13-15]. Moreover, Africans may cope in a dissociative style, behaviourally reporting DefS but revealing physiological emotional avoidance responses together with a loss of control [1,6]. While this has further been associated with an increased risk of atherosclerosis and stroke [7,13,15,16], literature regarding Africans is scarce. Nevertheless, they may display higher

tissue cortisol sensitivity [7,17], which might further impact on the vasoconstrictive properties of cortisol as well as NE in coping, to increase hypertension risk [18].

Sex steroids, such as estradiol (E2), are important modulators in the regulation of coping responses and BP, while E2 is also thought to be cardioprotective [19-21]. This function is thought to result mostly from enhanced nitric oxide induced vasodilatory actions [20,21,22,23]. However, controversy exists regarding E2's protective effects, while increased E2 levels have also been linked to increased α_1-adrenergic receptor affinity [21,24] and CVD risk [21,22,24-28].

We intended, firstly, to determine whether E2 will be increased in urban Africans compared to Caucasians. Secondly, if this increased E2 will be associated with HPA axis hypoactivity rather than hyperactivity. And lastly, whether a decreased cortisol-to-E2 ratio (Cort:E2) will be associated with hypertension risk in urban Africans, particularly in men; this risk being augmented by utilisation of DefS.

2. METHODS AND MATERIALS

2.1 Research design and participants

Our study is nested in the SABPA (Sympathetic Activity and Ambulatory Blood Pressure in Africans) prospective cohort study [1]. The participant sample consisted of urban African (n=200) and Caucasian (n=209) teachers from the Dr Kenneth Kaunda Education district, North-West Province, South Africa. Prior to participation, the protocol was explained before informed consent and inclusion in the study took place. The Ethics Review Board of the North-West University approved this study (NWU-00036-07-56), and all procedures were completed according to the Declaration of Helsinki guidelines [29].

Recruited participants were aged between 20 and 63 years, representative of a homogenous sample with respect to education and an urban-dwelling lifestyle. Exclusion criteria were: tympanum temperature >37 ℃, pregnancy and/or lactation, alpha and/or beta blocker use, psychotropic substance use, as well as vaccination and/or blood donation 3 months prior to participation. HIV positive (n=19), diabetic (n=11) and macroalbuminuria (n=1) cases were further excluded from analysis, together with those on cortisone medication (n=3). The final participant sample included n=168 Africans and n=207 Caucasians.

2.2 Research procedure

The research procedure was followed from late summer up untill late autumn of 2008 and 2009 with respect to Africans and Caucasians respectively, avoiding seasonal changes. From Monday to Thursday, between 07h00 and 08h00, four participants were each fitted with a Cardiotens CE120® ambulatory BP apparatus (Meditech, Budapest, Hungary) and Actical® accelerometer (Montréal, Québec). The participants thereafter resumed their usual daily activities and reported any anomalies such as light or severe stress, physical activity, headache, nausea and palpitations on the provided BP diary cards.

The Cardiotens CE120® was fitted with suitable obese or non-obese cuffs on the non-dominant arm of each participant and measured BP every 30 minutes during the day (08h00-22h00), and 60 minutes at night (22h00-06h00) [30]. A successful inflation rate of 72.60% and 84.64% was achieved for 2008 and 2009 respectively. Following the revised guidelines of the European Society of Hypertension/ European Society of Cardiology, we classified 24hour HT as ambulatory BP ≥130/80mmHg [31].

Each day, at 16h30, participants were transported to the North-West University's Metabolic Research Unit to stay overnight. On arrival, each participant was allocated his/her own room and was thoroughly informed about the experimental setup and sampling conditions, to

lessen anticipation stress. Thereafter, registered clinical psychologists supervised the completion of a psychosocial battery of questions, which included the Coping Strategy Indicator (CSI) questionnaire. Participants received a standardised dinner at 18h30 and were advised to go to bed at 22h00, fasting overnight. After the last BP recording at 06h00, the Cardiotens CE120® and Actical® apparatuses were disconnected. Registered anthropometrists measured waist circumference and body surface area (using the Mosteller formula) in triplicate [32], according to standardised procedures, with an inter- and intra-observer variability of less than 10%. The total energy expenditure was further calculated from the Actical® measurements in kcal/day.

Hereafter, participants were placed in a semi-recumbent position; a 10 minute resting period was then followed by blood sampling. A registered nurse obtained fasting blood samples before 09h00, from the antebrachial vein of the dominant arm of each participant, with a winged infusion set. All biochemical samples were handled according to standardised procedures and frozen at -80 ℃ until analysis.

2.3 The Coping Strategy Indicator (CSI)

The CSI was developed by Amirkhan and is a 33-item self-report measure of coping responses with construct, convergent and discriminant validity [33]. It determined each self-reporting participants' favoured coping strategy (problem solving; social support; and avoidance), whilst they were thinking of a specific stressful situation in the preceding 6 months [33]. The problem solving strategy (formerly described as DefS), takes effect when an individual perceives a challenge and responds with effort, intense focus, and commitment, to eliminate the stressor [1,6]. This has been associated with increased CVD and renovascular risk in Africans [1,6,14]. Scores above the mean of 26 for this DefS scale were used to classify participants as having a high preference for this strategy. A Cronbach's alpha reliability coefficient of 0.81-0.87 was determined for DefS.

Seeking social support may facilitate in problem solving or could be utilised as a separate coping strategy. This is usually an active approach and makes use of external resources for comfort, advice and help in coping. Emotional avoidance is the third dimension of the CSI, which is characterised by passive withdrawal, especially when stress is uncontrollable and distress is experienced [6].

2.4 Biochemical analysis

Fasting whole blood samples were analysed for glycated haemoglobin (HbA1c) by the Cobas Integra 400plus (Roche, Switzerland). Serum samples were analysed using two sequential multiple analysers (Konelab 20i, Thermo Scientific, Vantaa, Finland; Unicel DX800, Beckman & Coulter, Germany), for γ-Glutamyl transferase (cGGT), ultrahigh-sensitivity C-reactive protein (hs-CRP), total cholesterol and high-density lipoprotein cholesterol (HDL-C). Hormones including progesterone, E2, and cortisol were analysed with the Elecsys 2010 from Roche (Switzerland). Cort:E2 was calculated from serum data in nmol/l. Inter- and intravariability CV% was less than 10% for all analysis.

2.5 Statistical analysis

Data analysis were completed with the *Statistica 12.5* computer software (Statsoft Inc., Tulsa, USA). Normal distributions of variables were determined with Kolmogorov-Smirnov tests; we logarithmically transformed E2, Cort:E2, and DefS.

Three way analysis of covariance tests (ANCOVAs) determined interactions between main effects (ethnicity, sex and DefS), independent of *a priori* confounders and progesterone. Furthermore, two way ANCOVAs determined interactions of ethnicity and sex with BP and Cort:E2. T-tests for independent groups indicated significant differences in means between ethnic (DefS) groups. Chi-square tests were used to test differences between categorical data.

Least square means analysis from ANCOVAs were performed independent of *a priori* confounders and progesterone. Additionally, adjustments were made for oral contraception (n=24) and hormone replacement therapy (n=4) use, as well as postmenopausal status (n=37), in all analysis pertaining to women.

Partial correlations and forward multiple stepwise regression analysis examined associations between E2 and/or Cort:E2 and BP, adjusting for confounding factors. Sensitivity analysis followed in DefS groups to determine the effects of DefS in the aforementioned analysis. Statistical significance was defined as a two-sided α level of 0.05 or less.

3. RESULTS

A significant interaction was observed between E2 and the main effects; ethnicity, sex and DefS, in three-way ANCOVAs [F (1,357), 3.88, p=0.05]. No significance was discovered as regards emotional avoidance or seeking social support. Further significance was demonstrated in two-way ANCOVAs (ethnicity and sex) for systolic BP [F (1,357), 7.14, p=0.008], and diastolic BP [F (1,357), 9.11, p=0.003], as well as Cort:E2 [F (1,357), 4.62, p=0.03], but not for cortisol or E2 variables alone.

From *Table 4.1*, African participants reported higher stress experience and recorded greater cardio-metabolic risk factors and a higher prevalence of HT, than the Caucasian participants. E2 levels were higher in the DefS Africans compared to their Caucasian counterparts (p=0.02). More Africans used hypertension medications and oral contraception.

Table 4.1: Descriptive characteristics of Africans and Caucasians, with or without defensive coping status

	TOTAL GROUP			DEFENSIVE COPING		
A PRIORI CONFOUNDERS	**AFRICANS** (n=168)	**CAUCASIANS** (n=207)	**P**	**AFRICANS** (n=125)	**CAUCASIANS** (n=164)	**P**
Age (years)	44.05 ± 8.42	45.00 ± 10.88	0.36	**43.62 ± 8.25**	**46.34 ± 10.29**	**0.02**
Body surface area (m^2)	**1.91 ± 0.23**	**2.00 ± 0.28**	**0.002**	**1.90 ± 0.23**	**2.00 ± 0.89**	**0.002**
Physical activity (kcal/day)	**2680.73 ± 811.09**	**3104.89 ± 1601.53**	**0.002**	**2648.77 ± 783.74**	**3152.26 ± 1752.60**	**0.003**
Cotinine (ng/ml)	21.59 ± 52.26	22.93 ± 77.82	0.85	17.70 ± 40.37	19.12 ± 73.13	0.85
ɣ-Glutamyl transferase (u/l)	**61.77 ± 72.88**	**26.61 ± 33.66**	**<0.0001**	**62.28 ± 80.37**	**25.74 ± 32.84**	**<0.0001**
CARDIO-METABOLIC VARIABLES						
Waist circumference (cm)	93.18 ± 15.98	92.85 ± 16.16	0.84	92.46 ± 15.42	92.94 ± 16.20	0.80
C-reactive protein (mg/l)	**8.77 ± 10.68**	**3.09 ± 3.89**	**<0.0001**	**9.11 ± 11.17**	**3.04 ± 3.67**	**<0.0001**
Glycated haemoglobin (%)	**5.99 ± 1.03**	**5.51 ± 0.42**	**<0.0001**	**5.91 ± 0.99**	**5.53 ± 0.41**	**<0.0001**
Cholesterol (mmol/l)	**4.59 ± 1.16**	**5.53 ± 1.28**	**<0.0001**	**4.54 ± 1.09**	**5.47 ± 1.25**	**<0.0001**
HDL cholesterol (mmol/l)	1.16 ± 0.35	1.20 ± 0.41	0.24	1.16 ± 0.37	1.19 ± 0.41	0.48
ENDOCRINE VARIABLES						
Postmenopausal status n (%)	19 (11.31)	18 (8.70)	0.40	15 (12.00)	16 (9.76)	0.54
Progesterone (nmo/l)	5.13 ± 11.52	4.08 ± 10.51	0.36	5.37 ±11.43	4.29 ± 11.35	0.43
Serum estradiol (pmol/l)	203.64 ± 309.55	170.14 ± 273.90	0.27	**216.32 ± 331.71**	**141.17 ± 196.89**	**0.02**
Serum cortisol (nmol/l)	363.66 ± 151.33	383.39 ± 157.92	0.22	363.27 ±154.78	386.20 ±152.84	0.21
Cort:E2	6095.54 ± 8062.45	7359.23 ± 8561.00	0.14	6110.27 ± 8434.08	7724.92 ± 8762.45	0.12

Table 4.1 (continued)

HYPERTENSION RISK						
Hypertension ≥130/80mmHg n (%)	**81 (48.21)**	**36 (17.39)**	**<0.0001**	**57 (45.60)**	**26 (15.85)**	**<0.0001**
Systolic blood pressure (mmHg)	**133 ± 17**	**124 ± 12**	**<0.0001**	**131 ± 16**	**124 ± 11**	**<0.0001**
Diastolic blood pressure (mmHg)	**83 ± 11**	**77 ± 8**	**<0.0001**	**82 ± 10**	**77 ± 8**	**<0.0001**
PSYCHOSOCIAL VARIABLES						
Defensive coping score	28.05 ± 4.05	28.83 ± 3.88	0.06	30.02 ± 2.24	30.42 ± 2.24	0.14
High defensive coping n (%)	125 (74.40)	164 (79.23)	0.27	-	-	-
Experienced severe stress n (%)	**32 (19.05)**	**20 (9.66)**	**0.009**	**24 (19.20)**	**15 (9.15)**	**0.01**
MEDICATION USE n (%)						
Oral contraception	**17 (10.12)**	**7 (3.38)**	**0.008**	11 (8.80)	7 (4.27)	0.11
Hormone replacement therapy	2 (1.19)	2 (0.97)	0.83	0 (0.00)	2 (1.22)	0.22
Hypertension medication	**57 (33.93)**	**27 (13.04)**	**<0.0001**	**39 (31.20)**	**22 (13.41)**	**0.0002**
Statins	2 (1.19)	9 (4.35)	0.07	2 (1.60)	8 (4.88)	0.13
Acetylcholine receptor agonist	3 (1.79)	0 (0.00)	0.05	2 (1.60)	0 (0.00)	0.10
ACE inhibitor	**18 (10.71)**	**5 (2.42)**	**0.0009**	**15 (12.00)**	**4 (2.44)**	**0.001**
Angiotensin II antagonist	1 (0.60)	1 (0.48)	0.88	-	-	-
Angiotensin receptor block	0 (0.00)	2 (0.97)	0.20	0 (0.00)	2 (1.22)	0.22
Diuretics	**21 (12.50)**	**9 (4.35)**	**0.004**	**13 (10.40)**	**6 (3.66)**	**0.02**
Calcium antagonist	**13 (7.74)**	**1 (0.48)**	**0.0002**	**11 (8.80)**	**1 (0.61)**	**0.0005**

Data expressed as mean ± standard deviation for T-tests and percentage of n for Chi-square analysis. Significant differences between ethnic groups depicted in bold, p≤0.05.

Where: HDL, high-density lipoprotein; CRP, C-reactive protein; Cort:E2, cortisol-to-estradiol ratio; ACE, angiotensin converting enzyme.

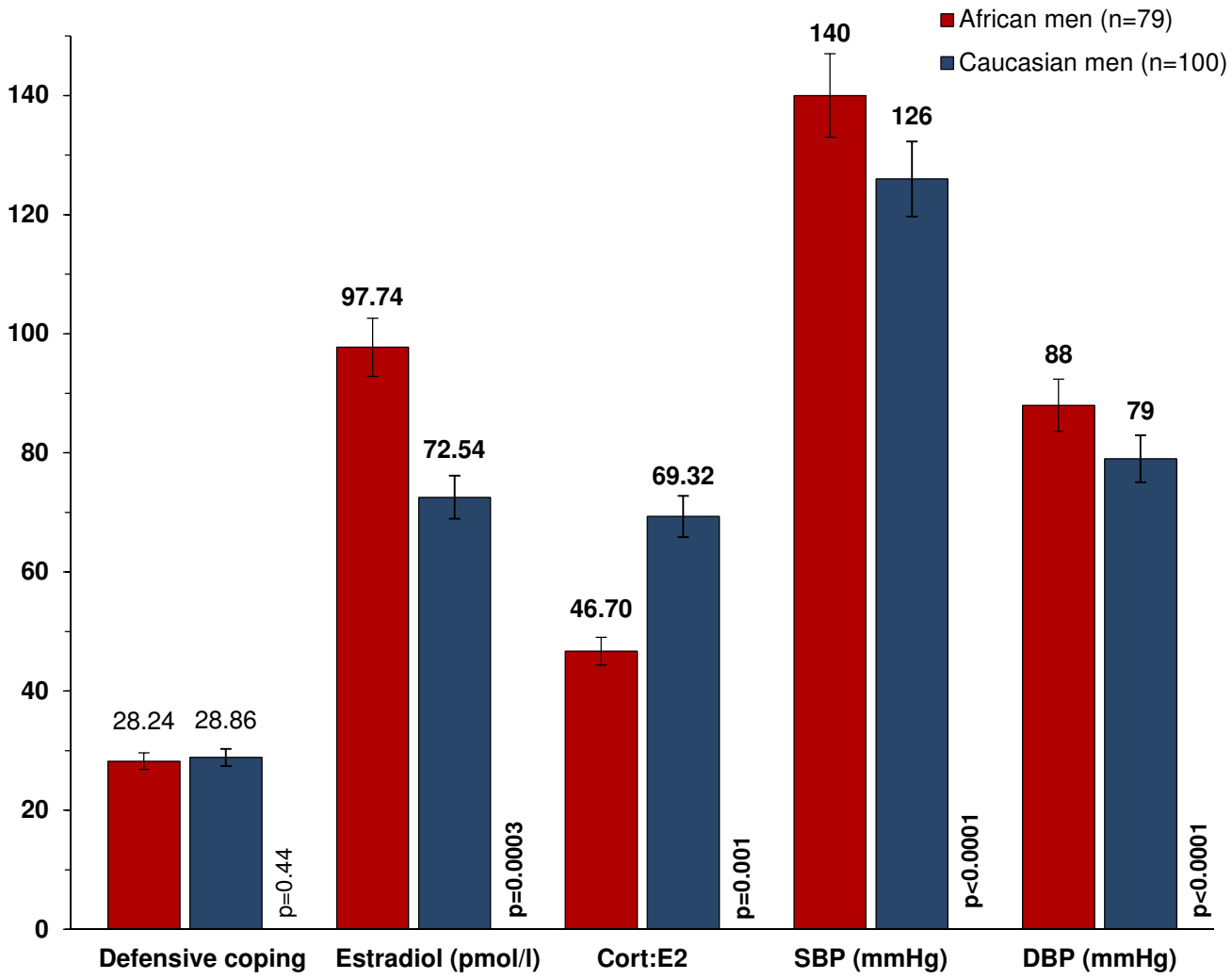

Figure 4.1: Defensive coping, estradiol and hypertension risk in men

Results depicted as least square means (95% confidence intervals) in total groups. Significant differences between ethnic groups presented in bold.

Where: Cort:E2, cortisol-to-estradiol ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

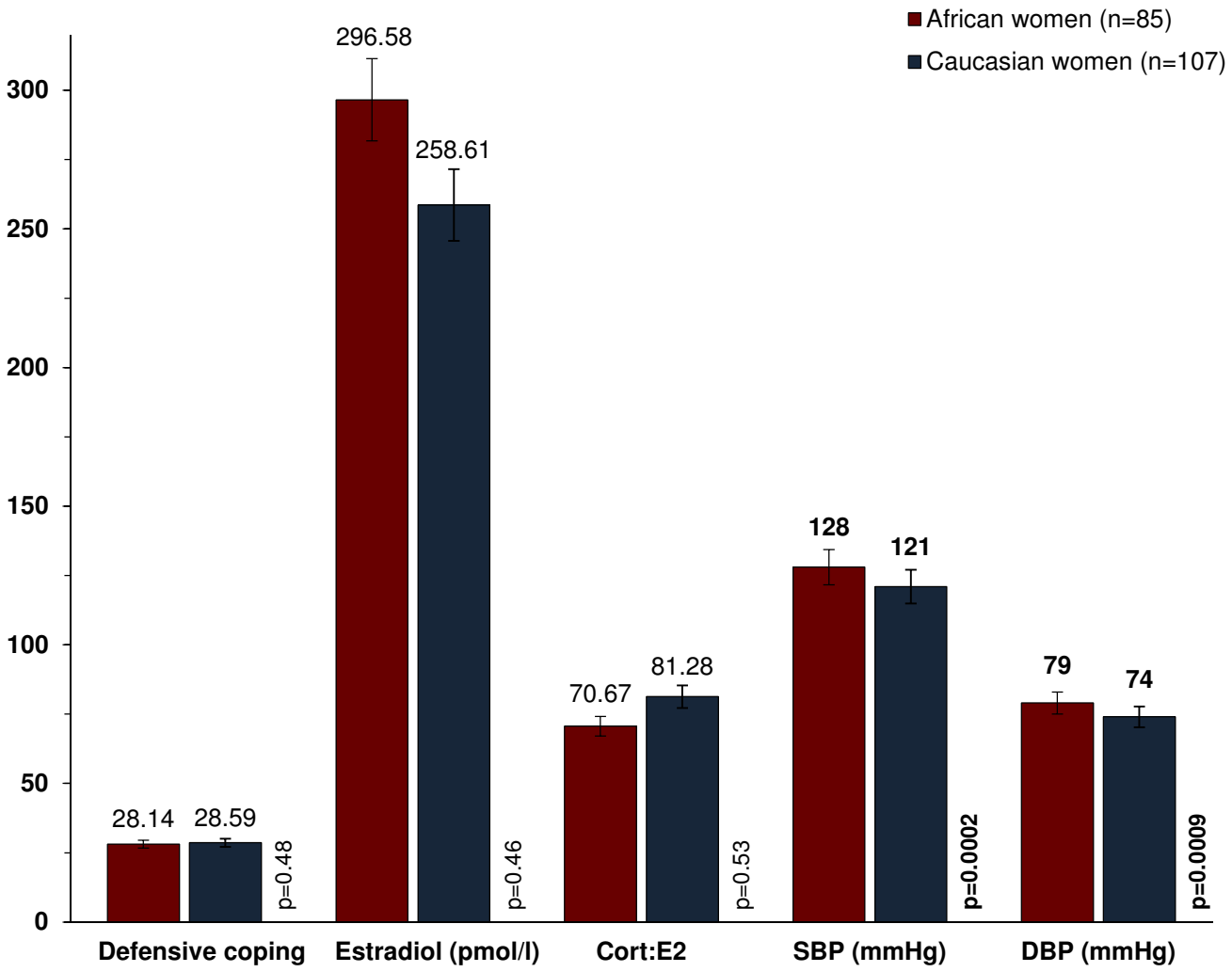

Figure 4.2: Defensive coping, estradiol and hypertension risk in women

Results depicted as least square means (95% confidence intervals) in total groups. Significant differences between ethnic groups presented in bold.

Where: Cort:E2, cortisol-to-estradiol ratio. SBP, systolic blood pressure; DBP, diastolic blood pressure.

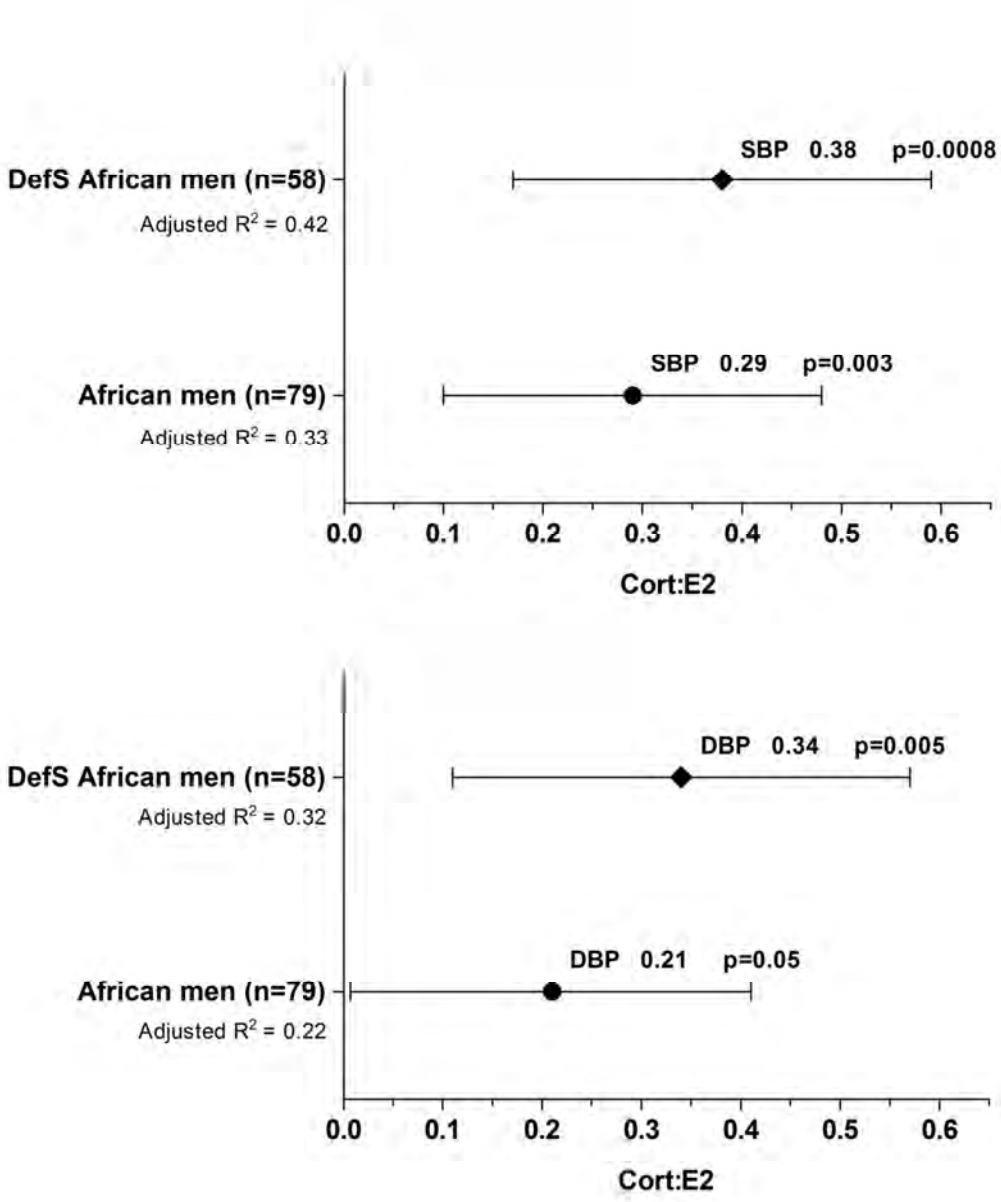

Figure 4.3: Multivariate regressions indicating hypertension risk in African men

Forward stepwise multiple regressions predicted effects of Cort:E2 on hypertension risk. Model covariates included;age, body surface area, physical activity, cotinine, γ-glutamyl transferase, hypertension medication, progesterone, and the cortisol-to-estradiol ratio. Significant values shown in bold, where Adj $R^2 \geq 0.30$ and/or $p \leq 0.05$.

Where: SBP, systolic blood pressure; Cort:E2, cortisol-to-estradiol ratio; DBP, diastolic blood pressure.

An increased E2 concentration was observed in African men together with an attenuated Cort:E2 alongside augmented BP *(Figure 4.1)*. BP was the only variable that was significantly increased in the African women compared to Caucasian women *(Figure 4.2)*. The same trends as in *Figures 4.1* and *4.2* were found in DefS men and women *(data not shown)*.

Associations between cort:E2 and both systolic and diastolic BP were observed in African men ($p \leq 0.05$), which were even more pronounced in the DefS African men ($p \leq 0.005$), even after adjustment for hypertension medication *(Figure 4.3)*. No significant associations were found in African women or Caucasian groups.

4. DISCUSSION

The main findings revealed that African men exhibit augmented E2 levels compared to Caucasian men, especially those utilising a DefS style. African men also demonstrated possible attenuated HPA activity derived from Cort:E2. Additionally, Africans portrayed a higher risk of HT, especially men, which was associated with reduced Cort:E2. In African men, this association of neuro-endocrine dysfunction and hypertension risk was amplified by utilisation of DefS.

4.1 Estradiol and hypertension risk

E2 has been said to have cardio-, reno- and neuroprotective effects, while sustained stress may increase E2 levels to facilitate coping responses [13,34-35], as well as homeostasis. Findings from the National Institutes of Health (Women's Health Initiative), amongst others have however concluded that E2 administration increases hypertension and CVD risk in women [36]. Accordingly, Chan *et al.* (2015) determined that in men, increased E2 levels displayed associations with heightened subclinical vascular disease risk [28]. It was further determined that prominently increased E2 is detrimental in a pathological state [26-27].

Interestingly, our results also showed that 73% of African men, who were preferential users of DefS, evidenced a positive association between E2 and HT risk.

E2 is a strong modulator in coping, probably through effects on neurotransmitters including serotonin, dopamine and NE, such as up-regulation of their receptors and inhibition of NE inactivation [37-38]. Therefore, the increased E2 levels in African men, together with their high self-reported severe stress [6,14], could stimulate SAM and HPA hyperactivity [19-20] and possibly α_1-adrenergic vasoconstrictive responsiveness [21,24]. Increased cortisol and NE will thus be available for receptor binding. However, cortisol's permissive effect of NE's actions may further heighten α_1-adrenergic vasoconstrictory responses and total peripheral resistance [16,39]. Additionally, people of African ancestry have been proven to record an increased sensitivity of glucocorticoid receptors to any given cortisol concentration [7]; even more so in those with augmented cardiovascular risk [17], which may cause more prominent vasoconstrictory responses. Moreover, higher vascular responsiveness to α-adrenergic stimuli and increased hypothalamic drive have been found in Africans, particularly in men [16,20,40]. Therefore, HPA activity and cortisol levels play an important role in BP regulation, especially in Africans. Nevertheless, our findings revealed hypoactivity in the HPA, with reduced cortisol levels (as from Cort:E2) in African, compared to Caucasian, men. This instead signifies possible neuro-endocrine dysfunction and/or adrenal fatigue [41], which was also indicated in these men during previous studies [14]. Nevertheless, their down-regulated cortisol should facilitate normal BP maintenance, but mean systolic and diastolic BP levels indicated HT in African men. Associations between the lower Cort:E2 and augmented BP values were particularly apparent in DefS African men. Thus, we propose that dissociation in coping responses [1] and increased E2 in Africans [42] play an important role here. Therefore, DefS might facilitate an attenuated Cort:E2 and higher BP levels through augmented α_1-adrenerigc vasoconstrictive responsiveness.

4.2 Estradiol, Dissociation in coping and Hypertension risk

While it is still poorly understood, we nonetheless propose that E2 increases during stress in order to enhance coping ability through stimulation of neuro-endocrine coping responses. On the other hand, during sustained stress, E2 probably up-regulates further so as to inhibit the negative effects of prolonged neuro-endocrine coping responses and enhance its own protective effects. However, excessive increases in E2 may also be detrimental [25-27], similar to any other physiological element. Moreover, with concomitant autonomic exhaustion and/or adrenal fatigue in the neuro-endocrine coping responses [8,14,43], further coping may be impaired and E2's protective effects lessened. A vicious cycle of neuro-endocrine dysfunction ensues, which may be accompanied by pathology [9,17,44-45].

One of the pathophysiological alterations associated with neuro-endocrine coping responses is increased BP [46-47]. In established HT, sympathetic activity increases, whilst both cardiac compliance and β-adrenergic responsiveness diminish and resistance vessels undergo hypertrophy [48-51]. Therefore, the systemic resistance rises, which may manifest in HT when chronically increased [52]. This may be related to the dissociation of coping responses of Africans, as the altered BP regulation is indicative of a change from β-cardiac responsiveness to more α-adrenergic resistance responses [5,53-54]. Therefore, these Africans portray emotional avoidance physiological coping responses rather than DefS ones [55].

Ultimately though, a new "normal" cardiac output for essential HT develops [49] as the aforementioned process alters the structure and responsiveness of the heart and vasculature to further stimulation [13]. Sympathetic hyperactivity will decrease, as less sympathetic drive is needed to maintain the new higher BP at a new set point [13], which may be observed as a reduction in NE levels. Therefore, even when NE and cortisol levels are decreased, as described in previous SABPA findings [14,18,43], perhaps through E2-

induced down-regulation as protective homeostatic mechanism, pathology may still persist. While, hypoactivity in the HPA axis has been linked to increased pathology risk [56], augmented disease risk may also stem from prior sustained stress experience and HPA hyperactivity. This can be ascribed to the preceding hypercortisolism which is able to cause irreversible damage [44].

The neuro-endocrine hypoactivity was also evident in previous SABPA findings, as both reduced NE and cortisol were respectively associated with increased subclinical vascular disease and renovascular disease risk in DefS African men [14,43]; indicating coping inability rather than lessened stress. As the neuro-endocrine dysfunction related HT risk was only observed in African men and particularly so in DefS users, they may have insufficient coping resources when actively trying to cope, which may result in alcohol abuse *(Table 4.1*: cGGT levels*)* as a last ditch attempt at dealing with stress. Additionally, social support in an urbanised fast-paced setting may be waning. This creates further concern as regards potential depression and burnout.

4.4 Limitations and Recommendations

Causality cannot be inferred from the cross-sectional design. Furthermore, reliable data regarding phases of the menstrual cycle were not available, and could have impacted on results in women. Recently, evidence showed that immunoassays may under- or overestimate E2 concentrations; this is another limitation of this sub study. We therefore recommend analysis of acute E2 [by means of liquid chromatography-mass spectrometry (LC-MS) assays] and salivary alpha-amylase stress responses in a longitudinal replication of the study, to better establish neuro-endocrine function/dysfunction alongside the effects of DefS.

4.5 Conclusions

Urban DefS Africans revealed distress, augmented E2, HPA hypoactivity, and increased HT risk. We propose that HT risk in urban African men coincides with dissociation in coping responses and possibly increased α_1-adrenergic vasoconstrictory responsiveness, also indicating chronic stress. Excessive E2 may be associated with pathology risk, rather than accommodating cardioprotection in African men. The latter reported high stress; nonetheless their coping resources seem inadequate, ultimately increasing their vulnerability to pathology, both physiologically and psychologically. Additionally, their behaviour portrays more harmful strategies, including high alcohol use and sedentary lifestyles, further augmenting their risk of neuro-endocrine dysfunction and, ultimately, disease manifestation and/or progression.

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6. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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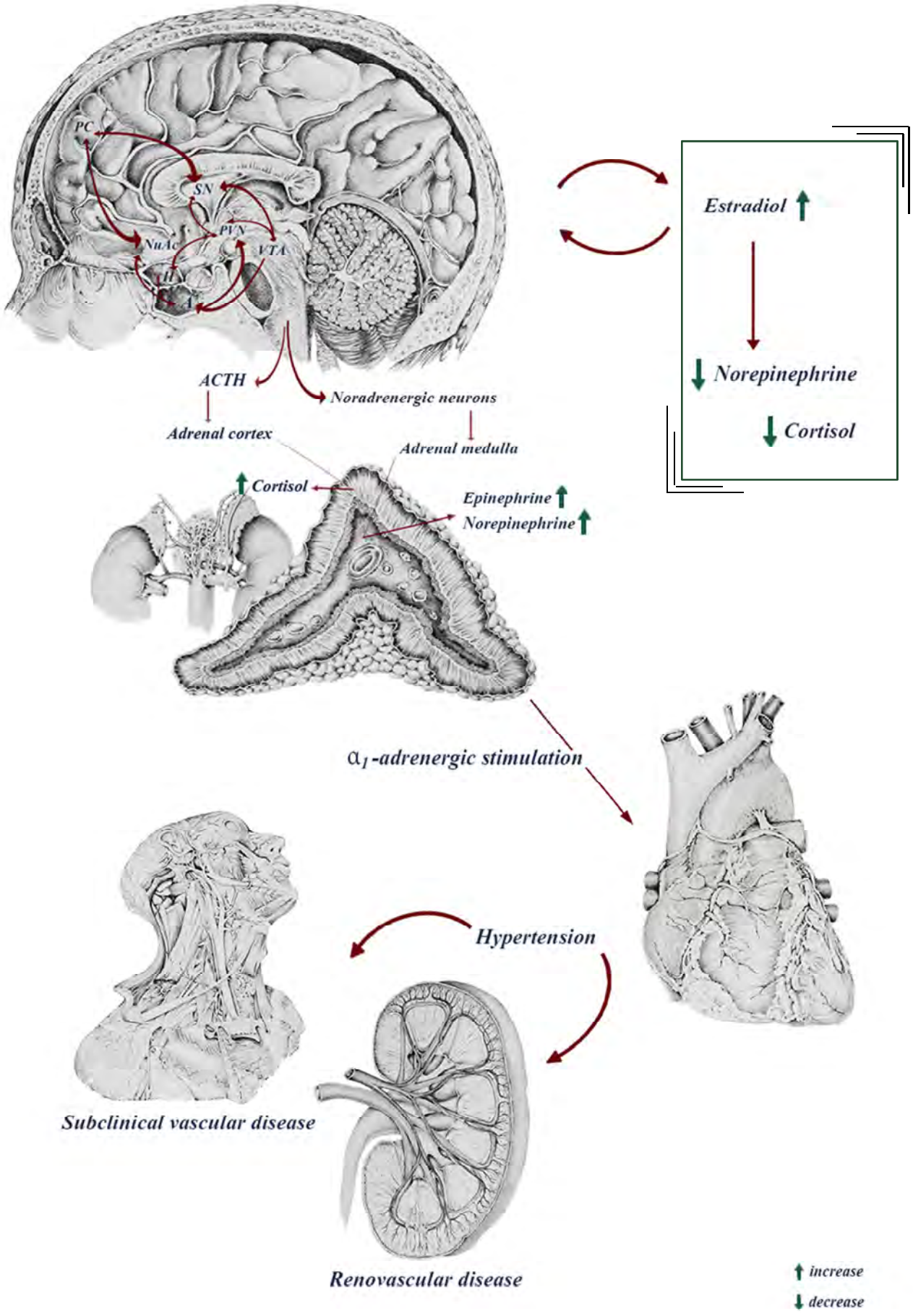
CHAPTER 5


PC
SN
PVN
NuAc
VTA
HT
ACTH
Noradrenergic neurons
Adrenal cortex
Adrenal medulla
Cortisol
Epinephrine
Norepinephrine
Estradiol
Norepinephrine
Cortisol
α1-adrenergic stimulation
Hypertension
Subclinical vascular disease
Renovascular disease
increase
decrease

General Findings and Conclusions

1. INTRODUCTION

This chapter includes the main findings of the three manuscripts presented in this thesis. A thorough discussion and conclusions follow, elucidating the interpretation of all results and comparisons with the relevant literature reviewed in *Chapter 1*. Subsequently, recommendations for future research are made, pertaining to coping and cardiovascular disease (CVD) risk in Africans and Caucasians, with particular regard to confounding factors.

2. SUMMARY OF THE MAIN FINDINGS

The primary aim of this study was to assess the influence of neuro-endocrine coping responses on CVD risk, in an urban South African cohort, particularly regarding the utilisation of defensive coping (DefS). Such responses were scrutinised by means of stress mediators, i.e. norepinephrine (NE) and cortisol, to determine their impact on subclinical vascular and renovascular disease risk, respectively. In addition, estradiol (E2) was studied to determine its modulating effects on neuro-endocrine coping responses and CVD risk. Furthermore, it endeavoured to determine the impact of the utilisation of DefS as regards the aforementioned, in an attempt to unravel the link between neuro-endocrine coping responses and CVD risk, particularly in Africans.

Key findings of the three manuscripts:

2.1. Defensive coping and subclinical vascular disease risk - Associations with autonomic exhaustion in Africans and Caucasians: The SABPA study

The aims of the first sub study were to determine whether urban DefS Africans, rather than Caucasians, would demonstrate signs of autonomic exhaustion [lower 3-methoxy-4-hydroxyphenolglycol (MHPG) levels] and if decreased MHPG would be associated with increased subclinical vascular disease risk in DefS African men. MHPG and carotid intima-

media thickness of the far wall (CIMTf) were studied as markers of sympathetic activity and subclinical vascular disease risk respectively.

The first hypothesis stated that urban DefS Africans, rather than Caucasians, would demonstrate signs of autonomic exhaustion, as indicated by reduced MHPG levels. This hypothesis was only accepted partially, as significantly reduced MHPG levels were only demonstrated in DefS African women when compared to their Caucasian counterparts but not in men.

The second hypothesis proposed that decreased MHPG levels would be associated with increased subclinical vascular disease risk in urban DefS Africans. The findings revealed an inverse association between MHPG levels and CIMTf in DefS African men, after stratification into high subclinical vascular disease risk, but not in women; therefore this hypothesis was also partially accepted.

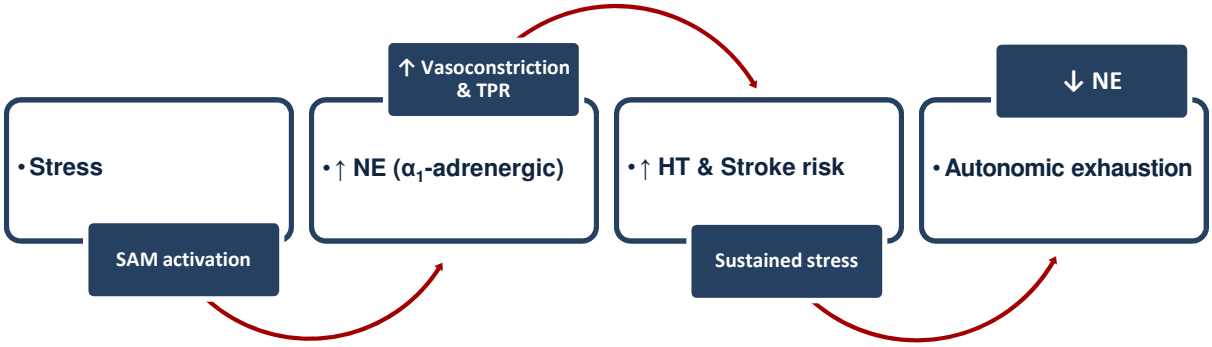


Figure 5.1: Progression of stress, neuro-endocrine coping and vascular risk

Where: SAM, sympathetic-adrenal-medullary; NE, norepinephrine; TPR, total peripheral resistance; HT, hypertension.

Figure 5.1 depicts the progression of stress to sustained stress and the resultant CVD risk. Since DefS Africans' neuro-endocrine coping responses manifests as emotional avoidance responses, possibly augmented α_1-adrenergic activity concomitantly with heightened C-reactive protein and glycated haemoglobin may further exacerbate atherosclerosis risk. During sustained stress, with

possible autonomic exhaustion and/or receptor desensitisation, sympathetic activity will be diminished and NE down-regulated. Nevertheless, pathology may persist due to the neuro-endocrine dysfunction and coping impairment, further increasing risk of stroke.

2.2. Defensive coping and renovascular disease risk - Adrenal fatigue in a cohort of Africans and Caucasians: The SABPA study

The second sub study built on the results of the first published manuscript, and endeavoured, firstly, to determine whether urban Africans, rather than Caucasians, would demonstrate signs of hypothalamic-pituitary-adrenal (HPA) axis dysfunction (lower cortisol with high stress) and, secondly, whether decreased cortisol would be associated with increased renovascular disease risk in Africans. Finally, it undertook to determine if the aforementioned risk would be augmented in those utilising DefS. Here, the second major stress mediator, cortisol, was studied alongside renovascular disease risk markers (estimated glomerular filtration rate: eGFR; albumin-to-creatinine ratio: ACR).

Based on the findings regarding possible autonomic exhaustion in *Manuscript 1*, the first hypothesis stated that urban Africans would reveal decreased cortisol levels (possible adrenal fatigue) when compared to Caucasians. This hypothesis was rejected because neither the total African group nor the African sex groups demonstrated significantly reduced cortisol levels, when compared to their Caucasian counterparts.

The second hypothesis also built on the previous study's results and consequently stated that reduced cortisol levels would be associated with increased renovascular disease risk in urban Africans. This hypothesis was accepted, as Africans with low or possibly down-regulated cortisol exhibited more vulnerability to renovascular disease than their high cortisol counterparts. Conversely, Caucasian men with high cortisol levels, rather than those with reduced cortisol, revealed increased renovascular disease risk.

The third hypothesis proposed that utilisation of DefS would augment renovascular disease risk in urban Africans compared to Caucasians, especially in those with reduced cortisol levels. This was important to determine, because DefS was not employed as a grouping variable throughout this manuscript. The hypothesis was accepted since decreased cortisol in DefS Africans was in fact associated with increased cardiometabolic as well as renovascular disease risk.

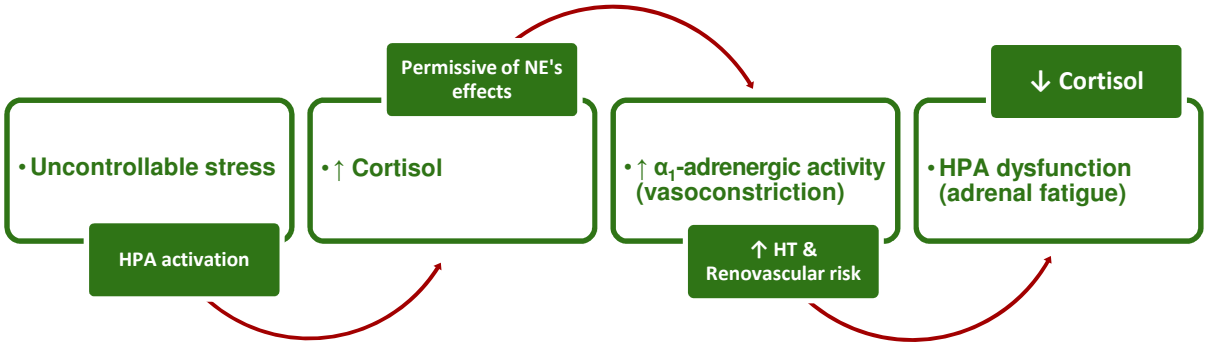


Figure 5.2: Uncontrollable stress, neuro-endocrine coping and vascular risk

Where: HPA, hypothalamic-pituitary-adrenal; NE, norepinephrine; HT, hypertension.

Figure 5.2 illustrates the CVD risk accompanied by uncontrollable stress in DefS Africans. When DefS fails, the HPA is hyperactivated for increased cortisol release, which further amplifies α_1-adrenergic vasoconstrictive responses. Cortisol down-regulation and/or desensitisation may follow adrenal fatigue to inhibit further damage from hypercortisolism. Nonetheless, excessive cortisol might cause irreversible damage. Therefore, even when cortisol lowers or normalises, pathology may be evident.

2.3. Defensive coping and estradiol - Unravelling autonomic dysfunction and augmented hypertension risk in a South African cohort: The SABPA study

As we aimed to unravel the link between neuro-endocrine coping responses and cardiovascular risk in particularly Africans, it was considered important to study a modulator of coping responses (E2) as well as the main precursor to vascular damage, namely HT. The third and final manuscript's aims were firstly to determine whether E2, would be

increased in urban Africans (with higher self-reported stress) compared to Caucasians. Secondly, it investigated whether increased E2 would be associated with HPA axis hypoactivity rather than hyperactivity (in an original cortisol-to-E2 ratio). And lastly, it endeavoured to discover if this neuro-endocrine dysfunction would be associated with hypertension prevalence in urban Africans, with augmented risk through utilisation of DefS.

The first hypothesis was that urban Africans (with higher self-reported stress) would reveal increased E2 levels, when compared to Caucasians. This was partially accepted as indeed DefS Africans showed significantly higher E2 levels than their Caucasian counterparts. However, essential subdivision into sex groups only revealed significance in men, with African men showing higher E2 levels.

Secondly, it was hypothesised that in urban Africans, increased E2 would be associated with HPA axis hypoactivity rather than hyperactivity in an original cortisol-to-E2 ratio. This was again only true in African compared to Caucasian men; therefore the hypothesis was only partially accepted.

And lastly, it was hypothesised that a decreased cortisol-to-E2 ratio would be associated with increased hypertension risk in urban Africans (especially men), augmented by utilisation of DefS. This hypothesis was accepted, since a decreased cortisol-to-E2 ratio in African men was significantly associated with increases in systolic and diastolic blood pressure (BP), which was further augmented by utilisation of DefS.

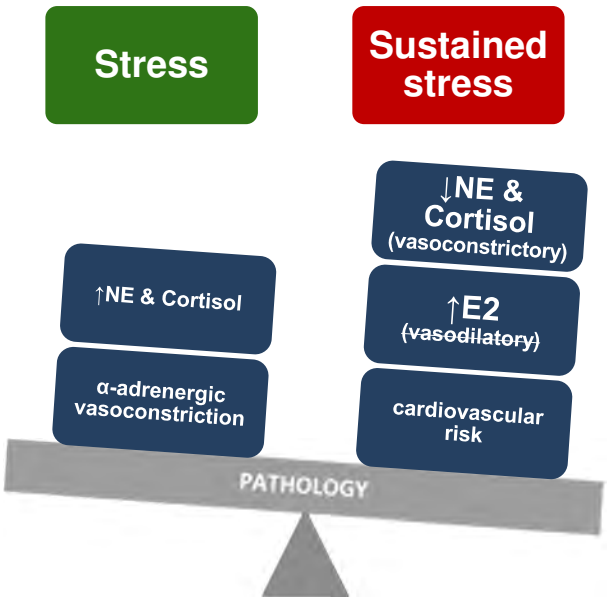


Figure 5.3: Defensive coping in Africans - Cardiovascular effects of neuro-endocrine coping responses in sustained stress

Where: ↑, increased or up-regulated; ↓, decreased or down-regulated; NE, norepinephrine; E2, estradiol.

In Figure 5.3, it is evident that DefS Africans have dysfunctional neuro-endocrine coping responses since these promote pathology rather than homeostasis. DefS Africans with high self-reported stress and dissociation in coping, demonstrated co-occurrence of increased cardiovascular risk factors, particularly in men. E2 is able to enhance neuro-endocrine coping responses to facilitate adaptive coping. However, in uncontrollable sustained stress, E2 may be up-regulated to induce its protective vasodilatory cardioprotective effects, in turn down-regulating stress mediators. Neuro-endocrine dysfunction resulting from autonomic exhaustion and/or adrenal fatigue may ensue; nonetheless α_1-adrenergic vasoconstrictive responses may persist, especially in men. An inability to increase these stress mediators for future coping may add to neuro-endocrine dysfunction and maladaptive coping, further worsening DefS African men's cardiovascular profiles.

3. CHANCE AND CONFOUNDING FACTORS

Before further discussion of the results, it is important to critically reflect on factors that may have influenced or confounded the results. Firstly, and perhaps most importantly, the cross

sectional design of the study could not infer causality; thus conclusions were arrived at with careful consideration.

Three-way and two-way ANCOVA's were used to determine interactions between main effects in all three manuscripts, which defined grouping variables for further analysis. Of note, in these analysis, no significance was revealed either for behavioural social support or for emotional avoidance coping styles. DefS, on the other hand, had a pronounced impact in all thesis sub studies, and showed interactions with neuro-endocrine and CVD risk factors.

Use of the Coping Strategy Indicator (CSI) could have over- or underestimated preferred coping responses. A questionnaire may not always be interpreted in the same way by each respondent and, as each participant had to base their answers on a specific personal event in the past six months, the answers could also be diverse for each individual at any given time. The CSI has nonetheless been used in and validated for Africans, giving peace of mind that this is a well-constructed standardised questionnaire [1,2].

In the statistical analysis of all three manuscripts, *a priori* confounders were adjusted for, including: age, body surface area, physical activity (measured as total energy expenditure), smoking (cotinine) and alcohol consumption (gamma glutamyl transferase). Furthermore, HIV positive, clinically confirmed diabetic, corticosterone users *(Manuscript 2 & 3)*, and renal impairment cases (from microalbuminuria) were excluded from the analysis.

In *Manuscript 1*, cholesterol, C-reactive protein and BP were also considered confounders for subclinical vascular disease; hence the necessary adjustments were made in all analysis pertaining to CIMTf. Additional adjustments were made for oral contraception (OC) use, postmenopausal status, hormone replacement therapy and progesterone as well as HT medication in *Manuscript 3*. OC use could increase corticosteroid binding globulins (CBG) and reduce free cortisol levels [3-4]. However, OC use may also increase E2, which might

have influenced results in women. It is noteworthy that no reliable data were available for women regarding menstrual phases and grouping of women into luteal, ovulation and follicular phases was therefore not possible *(Manuscript 3)*. Sex as a grouping factor was, however, a necessity in *Manuscript 3*, due to the significant differences in sex steroids in men and women. Additionally, E2 levels were determined with immunoassays, which may not have been the gold-standard method. Handelsman *et al.* (2013) provided evidence that commercial immunoassays do not effectively measure lower levels of oestrogen, as may be found in children, men and postmenopausal women, although the higher levels in premenopausal women are accurately measured [5].

The aforementioned exclusions and adjustments were important to diminish erroneous interpretation of results. Consequently, together with the well-controlled setting and design of the SABPA study with standardised protocols and small intra- and inter-variability within these procedures, this added overall value to the final results attained.

4. DISCUSSION OF MAIN FINDINGS AND COMPARISON WITH THE LITERATURE

As the SABPA study is the first in sub-Saharan Africa to explore behavioural and neuro-endocrine coping responses in relation to CVD risk, it is deemed essential in expanding coping, neuro-endocrine and cardiovascular knowledge. The original findings of this study in Africans and Caucasians contribute significantly to the existing literature, both in confirming and in contradicting previous research, as will be discussed next.

Previously, stress and behavioural use of DefS were associated with CVD risk, particularly in Africans [1,6-10], and this study agrees with these findings. The Africans' self-reported severe stress were deemed uncontrollable overwhelming stress [11], while 8 hour collected fasting urinary cortisol levels possibly indicated sustained stress [12]. Urban Africans have definitively demonstrated α-adrenergic rather than β-adrenergic responses to stress [1,9,13-

16], depicting emotional avoidance and helplessness together with behavioural and physiological coping dissociation in those who prefer to utilise DefS [17-18].

The vasoconstrictive effects of stress mediators are deemed to play a major part in the related subclinical vascular and renovascular disease risk, observed in *Manuscripts 1* and *2*, but also in HT development [6,3,19-24]. Increases in NE results in vasoconstrictive effects when binding to α_1-adrenergic receptors [3,21-22] while in sustained stress NE overload may occur with inhibition of α_2-adrenergic negative feedback [20,25-26]. A hypervigilant DefS state may ensue with robust increases in NE and augmented sympathetic activity [9,26], both of which are associated with HT and atherosclerosis risk [6,20,24]. Additionally, uncontrollable stress usually evokes emotional avoidance responses with HPA hyperactivity and augmented cortisol release [27-31]. Hypercortisolism is further related to HT because cortisol is permissive of NE's effects [19,23], and this might induce highly activated α_1-adrenergic vasoconstrictive responses, accompanied by augmented peripheral resistance. HPA hyperactivity and emotional avoidance are also positively related to HT development [9,32]. Results from *Manuscripts 1* and *2,* however, revealed inverse associations between stress mediators and disease risk, which is in contrast to certain studies [6,9,20,24,33-36], whilst supporting others [11,19,25,27,29,37-47].

It is proposed that the decrease in stress mediators, in DefS Africans particularly, is not due to adaptive coping responses, as it was found to be in accordance with disease risk rather than being health promoting [48-49]. Therefore, a possible explanation for the results was hypothesised as: exhaustion of coping resources after a prolonged period of sustained stress and an inability to cope effectively. This hypothesis was supported by the extent of alcohol abuse revealed, particularly by African men, signifying poor coping ability and resources, whilst promoting dissociation in coping responses [50-51]. Moreover, autonomic exhaustion and/or adrenal fatigue could also contribute to reduced stress mediator levels [20,27,35,52]. In sustained stress, desensitisation and/or down-regulation of adrenergic or

glucocorticoid receptors can occur, in part to protect from overexposure to stress mediators [53]. Nonetheless, reduced stress mediators may still incur pathology, especially cortisol, as its diurnal pattern is of major importance. Sonino and Fava (2001) explained that hypercortisolism could have adverse physiological effects and that, despite subsequent decreases in cortisol, damage might be permanent [43].

Furthermore, E2 might enhance neuro-endocrine coping responses for successful adaptation [32,34,53-55] or be up-regulated in sustained stress to reduce stress mediators as a protective mechanism [56-57]. The latter will decrease NE and cortisol release, possibly depicting autonomic exhaustion and/or adrenal fatigue. In accordance, E2's protective effects, mainly elicited by means of vasodilatation [56-57], should theoretically maintain homeostasis and promote health, at least in women. Results from *Manuscript 3* nonetheless evidenced augmented E2 in African men as compared to Caucasian men, but without the cardioprotective effects of E2. Malan and colleagues (2012) further stated that SABPA African men exhibit reduced testosterone concentrations in accord with their heightened E2 [54]. Sustained stress is associated with decreased testosterone levels [32,59-60], further supporting the sustained stress-induced neuro-endocrine dysfunction hypothesis. Androgen conversion, particularly in stressed, abdominally obese DefS African men, may therefore predispose them to CVD risk [61-64]. This may occur through activation of oestrogen receptor β [64]; however, this should be researched in African men.

In *Manuscript 3*, HT prevalence was indeed higher in DefS African men with elevated E2, supporting previous research findings of increased E2-related or -induced pathology risk, morbidity and mortality [61-62,64-73]. E2 has additionally been associated with vasoconstrictive responses through increased affinity of NE to α_1-adrenergic receptors [21,70,74]. This completes a vicious cycle postulated as: elevated E2-induced neuro-endocrine coping responses, hyperactivation of the sympatho-adrenal-medullary (SAM) and HPA systems, increased peripheral resistance and BP, possible down-regulation of

receptors (particularly with decreased sympathetic activity to compensate for a new BP set point), and finally maintained CVD risk (particularly through the impact of possibly preceding hypercortisolism).

African women have been shown to be more resilient [9], even though they did exhibit heightened CVD risk factors in this sub study. The same trend of increased E2-induced CVD risk in African men was, however, not found in African women or Caucasians. In women, various factors can alter sex steroid levels and functionality as described in the following section. Recording of menstrual phases could, however, clarify whether African women are in fact more resilient to stress, or whether re-stratification and redefining of this group would render them more vulnerable to CVD. Caucasians on the other hand did not show dissociation in their coping responses [17-18] or heightened CVD risk as Africans did, and thus the former seem to be more adept in coping. Dissociation in coping responses is therefore deemed a major influence in the vulnerability to pathology in Africans, although the reasons and mechanisms are still poorly understood, paving the way for future research.

5. RECOMMENDATIONS FOR FUTURE RESEARCH

Validation of this SABPA sub study's findings is still necessary in other urban African groups for extrapolation to the entire South African population; the following recommendations are made for future research:

Repetition of this study as a longitudinal study to establish cause and effect, plus inclusion of different stress protocols and more ethnicities in larger groups would be beneficial to clarify the findings from the three manuscripts. Because different stress protocols elicit stressor-specific HPA responses, the use of, for example, the Stroop Colour-Word Conflict protocol together with the Trier Social Stress Test (TSST), would be constructive. Glucose or availability of glucose could, however, impact on cortisol stress responses and this should be kept in mind with fasting measurements [34]. Further improvement would be through use of

solely salivary or 24 hour urinary measures (catecholamines and cortisol), which are non-invasive and do not in themselves incur stress. Measurement of hair cortisol may further aid in determining sustained (2-3 months) stress [75-76].

It could be beneficial to include adrenocorticotropic hormone (ACTH), gamma aminobutyric acid, and corticotrophin releasing factor, as well as metabolic enzymes, such as monoamine oxidase (NE metabolism), aromatase, and alpha-amylase in supporting the findings. Reduced testosterone has been associated with sustained stress and all-cause mortality, especially in men. Moreover, increased E2 levels in African men may be due to irreversible androgen conversion, which can be measured in aromatase activity [77-78]. Additionally, salivary alpha-amylase (sAA) may be considered a more practical marker of SAM activity than NE, because non-invasive samples can be taken anywhere without complex apparatuses and handling thereof is easier. Stress increases sAA, revealing a stable circadian pattern similar to that of salivary cortisol, making it more efficient [79].

Another important marker that could add value to stress research is the growth hormone. This has been stated to increase with onset of acute stress; however, release is inhibited during sustained stress and therefore, it may play a pivotal role in autonomic exhaustion, adrenal fatigue, burnout, and depression. The synacthen test (ACTH administration) and cortisol-to-dehydroepiandrosterone ratio can further prove important here.

Immunoassays for analysis of E2 concentrations, as were used in the SABPA study, may be inefficient, since steroid cross-reactions may occur and lower E2 levels may be under- or overestimated. Another recommendation is therefore to repeat analysis with the use of liquid chromatography-mass spectrometry LC-MS assays [5], to validate the results.

Hypertension medication use could also impact on neuro-endocrine function. It has been noted that calcium channel blockers worsen outcomes in some patients, with reflexive

increases in sympathetic activity, which in turn heightens CVD risk. In point of fact, almost 9% of SABPA DefS African men used calcium antagonists. The different hypertension medications used by participants should thus be analysed and adjusted for separately, to determine its impact on neuro-endocrine coping responses and CVD risk.

Finally, the cortisol-to-E2 ratio used in *Manuscript 3* is a novel approach to coping and cardiovascular research, integrating neuro-endocrine function with CVD risk. The cortisol-to-E2 ratio should be studied further as a risk indicator for neuro-endocrine dysfunction; calculation of a cut point where this ratio best predicts CVD risk could be advantageous as a diagnostic and/or prognostic tool in future research.

6. CONCLUSIONS

Neuro-endocrine dysfunction was evident in urban Africans, and especially in men, who reported severe stress, but their coping resources (stress mediators) were inadequate. Sustained stress may up-regulate E2 with vasodilatory and cardioprotective characteristics. Nevertheless, excessive E2 is also detrimental and impacts on CVD risk, particularly in men. Furthermore, neuro-endocrine stress mediators (vasoconstrictory) may be homeostatically down-regulated. However, coping abilities may be impaired, with SAM and HPA suppression (autonomic exhaustion and adrenal fatigue) negatively influencing future coping responses of already overwhelmed Africans. Increased E2 may therefore augment CVD risk in DefS African men, possibly through highly stimulated α_1-adrenergic vasoconstrictive activity. Indeed, the observed neuro-endocrine coping dysfunction and increased E2 were respectively associated with increased risk of HT, subclinical vascular, and renovascular disease in DefS Africans, particularly in men. Reasons for concern are the Africans' poor lifestyle factors, including low physical activity, abdominal obesity and alcohol abuse, as these may further exacerbate neuro-endocrine dysfunction and CVD risk. Further research is crucial to improve coping abilities and/or resources in this rather defenceless group.

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Appendix A

THE COPING STRATEGY INDICATOR

Instructions:

Listed below are several possible ways of coping. Indicate to what extent you, yourself, used each of these coping methods.

Try to think of one problem you have encountered in the last six months or so. This should be a problem that was important to you, and caused you to worry (anything from the loss of a loved one to a traffic fine, but one that was, important to you). Describe this problem in a few words.

_____
_____
_____
_____
_____

With this problem in mind, indicate how you coped by ticking the appropriate box for each coping behaviour listed on the following pages. Answer each and every question even though some may sound similar.

Keeping <u>that</u> stressful event in mind, indicate to what extent you…

		A lot	**A little**	**Not at all**
1	Let your feelings out to a friend?			
2	Rearranged things around you so that your problem had the best chance of being resolved?			
3	Brainstormed all possible solutions before deciding what to do?			
4	Tried to distract yourself from the problem?			
5	Accepted sympathy and understanding from someone?			
6	Did all you could to keep others from seeing how bad things really were?			

7 Talked to people about the situation because talking about it helped you to feel better?

8 Set some goals for yourself to deal with the situation?

9 Weighed your options very carefully?

10 Daydreamed about better times?

11 Tried different ways to solve the problem until you found one that worked?

12 Confided your fears and worries to a friend or relative?

13 Spent more time than usual alone?

14 Told people about the situation because just talking about it helped you to come up with solutions?

15 Thought about what needed to be done to straighten things out?

16 Turned your full attention to solving the problem?

17 Formed a plan of action in your mind?

18 Watched television more than usual?

19 Went to someone (friend or professional) in order to help you feel better?

20 Stood firm and fought for what you wanted in the situation?

21 Avoided being with people in general?

22 Buried yourself in a hobby or sports activity to avoid the problem?

23 Went to a friend to help you feel better about the problem?

24 Went to a friend for advice on how to change the situation?

25 Accepted sympathy and understanding from friends who had the same problem?

26 Slept more than usual?

27 Fantasized about how things could have been different?

28 Identified with characters in novels or movies?

29 Tried to solve the problem?

30 Wished that people would just leave you alone?

31 Accepted help from a friend or relative?

32 Sought reassurance from those who know you best?

33 Tried to carefully plan a course of action rather than acting on impulse?

Appendix B

SCORING INSTRUCTIONS FOR THE COPING STRATEGY INDICATOR:

1. For each response assign a numerical score accordingly:

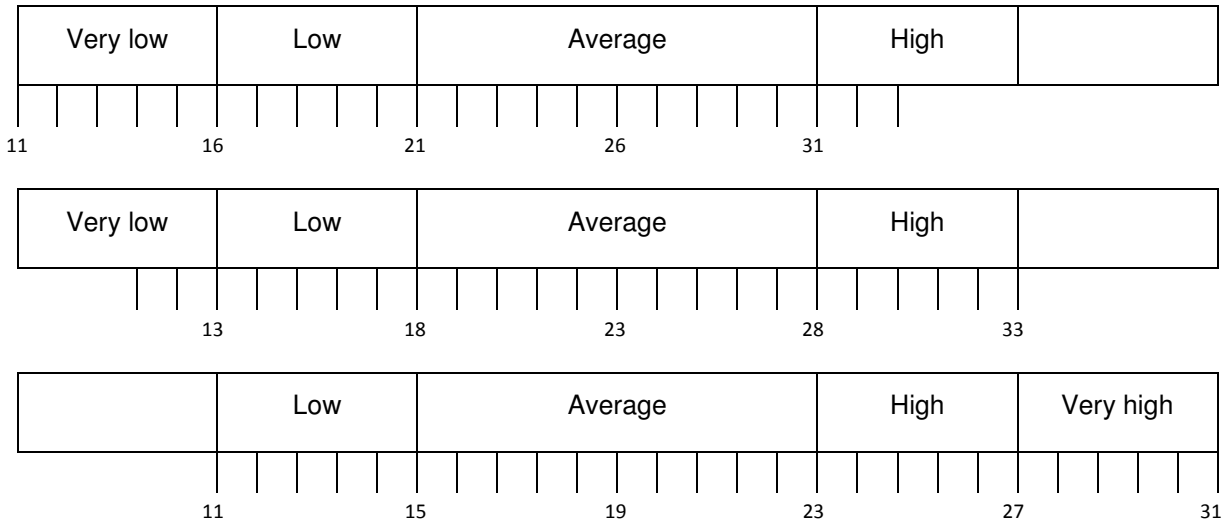
Response	**Score**
A lot	3
A little	2
Not at all	1

2. Enter the scores in the appropriate column below:

SCALE I		**SCALE II**		**SCALE III**	
Item	Score	Item	Score	Item	Score
2		1		4	
3		5		6	
8		7		10	
9		12		13	
11		14		18	
15		19		21	
16		23		22	
17		24		26	
20		25		27	
29		31		28	
33		32		30	
Total I		Total II		Total III	

3. Sum each column and enter the totals in the allocated field.

4. Mark each bar of the graph below at the point indicated by each column total. Fill in the bar to the point indicated, to graphically depict the preferred coping strategy.



Appendix C

NORTH-WEST UNIVERSITY
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NOORDWES-UNIVERSITEIT

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South Africa 2520

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

Dr L Malan

6 February 2008

Dear Dr Malan

ETHICS APPROVAL OF PROJECT

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

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

***Ethics* number**: N W U - 0 0 0 3 6 - 0 7 - S 6
(Institution – Project Number – Year – Status)
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

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

Special conditions of the approval (if any): None

General conditions:

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

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

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

Yours sincerely

Prof M M J Lowes
(chair NWU Ethics Committee)

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
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Ethics Committee
Tel: 018 2994237

E-mail: 10055355@nwu.ac.za

To whom it may concern

31 August 2012

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

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

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

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

Kind regards

Prof. H.H. Vorster
Chair person

Appendix D

NORTH-WEST UNIVERSITY
POTCHEFSTROOM CAMPUS
SCHOOL FOR PHYSIOLOGY, NUTRITION AND CONSUMER SCIENCES

PARTICIPANT INFORMATION AND CONSENT FORM

PART 1
PRINCIPAL RESEARCHER: Dr Leoné Malan, Subject Group Physiology
PROJECT LEADER**:** Dr. Leoné Malan, Subject Group Physiology

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

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

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

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

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

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

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

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

Our research has shown that lifestyle diseases in urbanised Africans present higher obesity levels, high blood pressure or hypertension prevalence rates and the experiencing of more stress. This pattern is enhanced during psychosocial stress/urbanisation in participants with a specific coping style.

Hence the planned SABPA project, which is the first study in South Africa where coping and direct markers of nervous system activity in Africans will be measured.

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

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

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

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

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

How was I chosen?
Inclusion criteria:
Phase I: 200 black Africans aged 25-60 years (male=100, female = 100)
Phase II: 200 white Africans (male=100, female=100) aged 25-60 years.

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

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

- Recruitment, screening and informed sessions with all participants will be done two months prior to the study (October - November 2007, Phase I, and November, 2008, Phase II) and informed consent forms will be signed.

- After selection of all participants, the details of the project will be discussed with you in English or your home language, i.e. what the exact objectives of the study are, what procedures will be taken and what will be expected from each of you (e.g. overnight stay, resting blood pressure procedures and fasting urine and blood samples are required, importance of complying with the correct sampling methods, incentives). You will be given the opportunity to ask questions.

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

DAY I

- On day I at 07:00, the blood pressure apparatus, which will measure your blood pressure and heart function as well as a physical activity meter, will be applied to your arm and waist at your school and you can then resume your normal daily activities. In the afternoon you must complete the Neethling Brain Instrument questionnaire which measures thought processes of the brain.
- At the end of Day I (± 16:30) you will be transported from your schools to overnight in the Metabolic Unit Research Facility of the North-West University. This unit is a research unit for human studies and equipped with 10 well-furnished bedrooms, a kitchen, two bathrooms and a television room. Each of you will be subjected to the following procedures:
  - At the end of Day I between ± 17:15 and 18:00 you will be welcomed and each of you will receive your own private bedroom.
  - The procedures, which will be done, will be explained again and each of you will then complete a general socio-demographic health questionnaire. Afterwards you will receive dinner.
  - After dinner, psychological questionnaires will be completed under supervision of registered education specialists and psychologists. Completion of questionnaires will take approximately 40 min, including a break of 20 minutes with coffee/tea and biscuits. This willl be you last meal for Day I as you must be fasting on Day II for obtaining good results.
  - Thereafter, you can relax and watch television or socialise with your c-participants. It will be wise to go to bed not later than 22:00 as the blood pressure apparatus will take measurements every hour during the night and it can be tiring.

DAY II

- At 06:45 on Day II the AMBP will be removed and an urine sample collected. Once this has been done you will be directed to the anthropometric station where your weight, height and body circumferences will be measured.
- The next station involves the blood pressure measurement station. Whilst in a sitting position your blood pressure will be taken in duplicate with the sphygmomanometer (the same as used at clinics) with a resting period of 5 minutes in between. Our registered research doctor/nurse will take a fasting saliva sample as well as a blood sample of 45ml from a vein in your dominant arm. The infusion set will be left in your arm to lessen the effect of inserting a needle again for blood sampling after exposure to the two stressors. A small amount of diluted heparin will be left in the infusion set in your arm to prevent clotting.

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

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

- The 2nd measurements are non-invasive and will be done by means of the Finometer device which also involves the assessment of heart functioning such as pulse (beats per minute), stroke volume (blood volume ejected by the heart per beat), cardiac output (blood volume ejected by the heart per minute), total peripheral resistance (resistance against the blood flow created by small arteries), central resistance (resistance against which the heart has to work while ejecting the blood into the aorta) as well as the elasticity of your large arteries (compliance). For this procedure a blood pressure cuff will be placed around your left arm and middle finger which will be inflated and stepwise deflated. You will not have more discomfort than during a common blood pressure measurement. This will take about 5 minutes.
- The stressor application procedure follows: You will now be exposed to a stressor for 1 minute whilst your blood pressure and ECG will still be taken. After exposure a saliva and blood sample (45ml) will be taken. After 10 minutes another saliva sample will be taken. Then the stressor application procedure will be repeated with the second stressor.
- At another station your 3rd measurement includes the assessment of pulse wave velocity, i.e. how fast your blood travels through your arteries. This measurement gives us an indication about how stiff your vessel walls are. The stiffer your vessel wall is the faster the blood travels from one point of your body to another. These painless measurements will require two technicians using blunt probes (tonometer) putting light pressure on the neck and on the foot to measure the velocity of the pulse waves. This takes only a few minutes. An ultrasound device will be taken of your arteries in the neck with a blunt probe to indicate the intrinsic thickness of your arteries which contributres to high blood pressure.

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

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

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

- You have reached the end of the sampling phase.
- **Thank you for your participation! You now will have the opportunity to shower and a take away breakfast will be given**.
- Immediate feedback on your HIV/AIDS status, obesity, blood pressure and blood glucose/sugar values will be given. *HIV/AIDS post-test counselling will be arranged if you are tested positive.*
- You are now transported back to your school and after one week you will receive your Neethling Brain Instrument and 24-hour blood pressure reports.

Possible Risks

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

Precautions to protect the participant

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

Other Treatments Whilst on Study

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

Incentives

1. All teachers will receive feedback on their health profile and if necessary references will be given to physicians/clinics/hospitals.

2. Printout feedback on 24 hour blood pressure monitoring report (normally costing R637.60), sonar of the artery (R1200.00), resting ECG (R600.00) and other variables (R500.00). Your benefit of participation is a comprehensive assessment of the cardiovascular and metabolic condition including investigation of blood pressure, inflammatory status and psychological well-being. These examinations will help us to assess the degree of vascular impairment of the arteries and to predict your risk of possible cardiovascular events such as heart attacks and stroke. The results may assist your doctor in decision making for further treatment or for instituting preventive measures. Our study will also contribute to the identification of possible factors leading to high blood pressure. As 24 hour ambulatory blood pressure monitoring is required for the diagnosis of hypertension, medical aids insist on this method of diagnosis to qualify for chronic medication. Additional testing could also reveal illnesses of a chronic nature and would serve as a motivation to qualify for chronic medication, such as metabolic syndrome, anti-inflammatory and cholesterol-lowering drugs.

3. Monetary incentive on completion of the colour word conflict chart (± R55.00).

4. Dinner and breakfast (± R24.00).

5. Neethling Brain Instrument profiles done by registered user of the Whole Brain (normally costing ± R350.00).

6. Coping skills workshop will be arranged on request.

Privacy, Confidentiality and Disclosure of Information

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

Participation is Voluntary

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

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

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

If you decide to withdraw from this project, please notify a member of the research team before you withdraw.

Ethical Guidelines
This project will be carried out according to Ethical Guidelines of the Helsinki declaration from 2000, with additional notes in 2002. This statement has been developed to protect the interests of people who agree to participate in human research studies.

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

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

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

PART 2
To the subject signing the consent as in part 3 of this document

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

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

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

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

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

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

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

PART 3
Consent

Title of the project:
"THE SABPA STUDY (**S**YMPATHETIC ACTIVITY AND **A**MBULATORY **B**LOOD **P**RESSURE IN **A**FRICANS)"

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

(Signature of the subject)

Signed at ..... on ……2008/9

Witnesses

1. .....

2. .....

Signed at ..... on ……2008/9

Appendix E

Originality Report

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Appendix F

CERTIFICATE

D N R LEVEY (PROF.)
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TO WHOM IT MAY CONCERN

This is to certify that I have edited the following document for English style, language usage, logic and consistency; it is the responsibility of the author to accept or reject the suggested changes manually, and interact with the comments in order to finalise the text.

Author: Mrs A de Kock
Student Number: 20273371

Thesis: NEURO-ENDOCRINE COPING RESPONSES IN AFRICAN AND CAUCASIAN TEACHERS FROM THE NORTH-WEST PROVINCE: THE SABPA STUDY

Sincerely

Levey

DAVID LEVEY
2015-10-30