Depressive symptoms and cardiometabolic health in urban black Africans: The SABPA study

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It all starts here ™

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT ®

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Correction does much, however, encouragement does more (Johann Wolfgang von Goethe).

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS i

TABLE OF CONTENTS iii

SUMMARY vii

OPSOMMING xi

PREFACE xv

LIST OF TABLES xviii

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW 1

1. INTRODUCTION 2

2. CARDIOMETABOLIC RISK FACTORS 4

3. STRESS AND CARDIOMETABOLIC RISK 8

3.1. The concept of stress 8

3.2. The definition of stress 12

3.3. The social environment and chronic stress 13

3.4. Chronic psychosocial stress and acute mental stress testing 15

3.4.1. The Stroop Colour Word Conflict Test 15

4. ROLE OF STRESS IN THE ONSET OF DEPRESSION 16

4.1. Neurobiology of Depression 17

4.2. Definition of Depression 22

4.3. Assessment of Depression 22

4.3.1. Patient Health Questionnaire 23

5. PATHOLOGICAL MECHANISMS LINKING DEPRESSION AND CARDIOMETABOLIC RISK 25

5.1. Depression, hypothalamic-pituitary-adrenal axis (HPA-axis) and sympatho-adrenal-medullary (SAM) hyperactivity 25

5.2. Hypothalamic-pituitary-adrenal axis (HPA-axis) and sympatho-adrenal-medullary (SAM) hyperactivity and cardiometabolic risk 29

5.2.1. Cortisol and cardiometabolic risk 29

5.2.2. Catecholamines and cardiometabolic risk 31

5.3. Depression, inflammation and hypercoagulation 32

5.3.1. Depression, inflammation and cardiometabolic risk 32

5.3.2. Depression, haemostasis and cardiometabolic risk 38

5.4. Cardiometabolic dysfunction and left ventricular hypertrophy (LVH) 42

6. MOTIVATION, AIMS AND HYPOTHESES FOR EACH PAPER IN THIS THESIS 44

6.1. Manuscript 1 (Chapter 2): Depression, cardiometabolic function and left ventricular hypertrophy in African men and women: The SABPA Study. 44

6.2. Manuscript 2 (Chapter 3): Blunted neuroendocrine responses linking depressive symptoms and ECG left ventricular hypertrophy in black Africans: the SABPA study. 45

6.3. Manuscript 3 (Chapter 4): Hypercoagulation vulnerability exacerbated by hypertension state in black Africans with depressive symptoms: the SABPA study. 46

7. REFERENCES 47

CHAPTER 2: Depression, cardiometabolic function and left ventricular hypertrophy in African men and women: The SABPA Study 73

CHAPTER 3: Blunted neuroendocrine responses linking depressive symptoms and ECG left ventricular hypertrophy in black Africans: the SABPA study. 101

CHAPTER 4: Hypercoagulation vulnerability exacerbated by hypertension state in black Africans with depressive symptoms: the SABPA study 136

CHAPTER 5: GENEREAL FINDINGS AND CONCLUSIONS 167

1. INTRODUCTION 168

2. SUMMARY OF THE MAIN FINDINGS 168

2.1. Manuscript 1 (Chapter 2): Depression, cardiometabolic function and left ventricular hypertrophy in African men and women: The SABPA Study. 168

2.2. Manuscript 2 (Chapter 3): Blunted neuroendocrine responses linking depressive symptoms and ECG left ventricular hypertrophy in black Africans: the SABPA study. 169

2.3. Manuscript 3 (Chapter 4): Hypercoagulation vulnerability exacerbated by hypertension state in black Africans with depressive symptoms: the SABPA study. 170

3. COMPARISON WITH THE RELEVENT LITERATURE 170

4. CHANCE AND CONFOUNDING 174

5. DISCUSSION OF THE MAIN FINDINGS 176

6. GENERAL CONCLUSIONS 180

7. CONTRIBUTION OF THE STUDY AND FURTURE RECOMMENDATIONS... 181

8. REFERENCES..... 183

SUMMARY

Depressive symptoms and cardiometabolic health in urban black Africans: the SABPA study.

Motivation

Depression is a mental disorder that has been associated with cardiovascular morbidity and mortality in the Western world. Cardiometablic mechanisms have been implicated as possible intermediating factors in the relationship between depressive symptoms and cardiovascular disease; however this has not yet been determined in black Africans (hereafter referred to as Africans).

Aim

The overarching aim of this study was to investigate the relationship between depressive symptoms and cardiometabolic risk. We therefore aimed to assess cardiometabolic function, neuroendocrine responses, inflammatory and haemostatic markers in Africans with depressive symptoms compared to those without symptoms of depression.

Methodology

Manuscripts presented in Chapter 2, 3 and 4 utilised data from the cross-sectional, target population multi-disciplinary "Sympathetic activity and Ambulatory Blood Pressure in Africans" (SABPA) study. The participants comprised of 200 African teachers from the Dr Kenneth Kaunda District in North-West province, South Africa. As cardiovascular disease is compromised by a positive HIV status, 19 participants were excluded from further statistical analysis. Stratification was based on the Patient Health Questionnaire 9-item (PHQ-9), which has been validated in a sub-Saharan African setting. PHQ-9 scores > 10 were used to classify participants as having signs of depressive symptoms. Subjects were further stratified by gender (Manuscript 1 and 3) and cortisol responses (Manuscript 2). Cardiometabolic health measures included 24-hour blood pressure, metabolic syndrome markers, neuroendocrine markers [cortisol and 3-methoxy-4-hydroxy-phenylglycol (MHPG)], left ventricular

hypertrophy (LVH),inflammatory and haemostatic markers (fibrinogen, C-reactive protein, plasminogen activator inhibitor-1 and D-dimer). Resting 12-lead ECG Cornell Product-Left ventricular hypertrophy (CP-LVH) was measured as a marker of target end-organ damage and cardiovascular dysfunction (Manuscript 1 and 2).

Means and prevalence were computed through t-test and Chi-square analysis respectively. Significant differences of mean cardiometabolic measures between depressive symptom status groups were also determined by analysis of covariance (adjusted for traditional cardiovascular risk factors and additional factors as specific per manuscript). Multivariate analysis was used to demonstrate associations between left ventricular hypertrophy (LVH) and cardiometabolic markers in Africans with depressive symptoms (Manuscript 1 and 2) and a logistic regression analysis were performed to examine the association between depressive symptoms and inflammatory/haemostatic factors (Manuscript 3).

All subjects who participated gave informed consent, the study was approved by the Ethics Committee of North-West University (NWU-0003607S6), in accordance with the principles outlined by the World Medical Association Declaration of Helsinki of 1975 (revised 2008).

Results and conclusions of the individual manuscripts

The aim of the study was to investigate the associations between depressive symptoms and cardiometabolic function including cardiovascular dysfunction. Markers of cardiometabolic function assessed were 24 hour blood pressure measurements, metabolic syndrome markers, neuroendocrine markers [cortisol and 3-methoxy-4-hydroxy-phenylglycol (MHPG)], inflammatory and haemostatic variables (fibrinogen, C-reactive protein, plasminogen activator inhibitor-1 and D-dimer).

Manuscript 1, focused on LVH as a marker of cardiovascular dysfunction and metabolic syndrome components as markers of cardiometabolic function. The aim of the study was to

assess the associations between LVH and metabolic syndrome (MetS) risk markers in participants with and without depressive symptoms. Results revealed that in African men with depressive symptoms the most significant determinants of LVH were systolic blood pressure (SBP) and the percentage glycosylated haemoglobin (HbA1c). While in African women (with depressive symptoms), this association was determined by low high-density lipoprotein (HDL-cholesterol). The study concluded that in black African men, independent of depressive symptoms, cardiometabolic factors (namely SBP and HbA1c) may be the driving significant factors in the development of cardiovascular diseases. Furthermore, the data showed that depressive symptoms in African women were associated with a measure of target end organ damage, and that this association was driven by a metabolic factor.

Manuscript 2, the aim of this manuscript was to examine the relationship between depressive symptoms, neuroendocrine responses [with cortisol and 3-methoxy-phenylglycol (MHPG) as markers] and cardiovascular risk, i.e. LVH. The results revealed that Africans with depressive symptoms demonstrated blunted cortisol and MHPG levels in response to acute mental stress, in comparison to those without symptoms of depression. Additionally, these low cortisol and blunted MHPG responses were associated with LVH in this ethnic group. The conclusion for this manuscript was that, blunted neuroendocrine responses linked depressive symptoms and ECG left ventricular hypertrophy in Africans. When coupled to their hypertensive status, these vasoconstrictive responses (cortisol and MHPG) may underpin the increased long-term depression and vascular disease risk in urban Africans.

Manuscript 3, the aim of this manuscript was to investigate the relationship between depressive symptoms and inflammatory/haemostatic markers in a cohort of urban-dwelling black African men and women. Our data demonstrated hypercoagulation vulnerability in African men with depressive symptoms. The African men with signs of depression displayed higher plasminogen activator inhibitor (PAI-1) levels and marginally elevated D-dimer

levels. It was concluded that hypercoagulation may partially be the mediating factor between depressive symptoms and cardiovascular risk in African men; a situation that may be exacerbated by hyperkinetic blood pressure.

In conclusion, through the assessement of cardiometabolic function and neuroendocrine responses, it seems that Africans withdepressive symptoms are at great risk for cardiovascular related morbidity and mortality, this was particulary evident in the African men (Manuscript 1 and 3). Additionally, it appears that blunted neuroendocrine responses and hypercoagulation could be seen as possible cardiovascular risk markers in Africans with depressive symptoms.

Keywords: Cardiometabolic function, neuroendocrine responses, inflammatory and haemostatic markers, depressive symptoms, left ventricular hypertrophy.

OPSOMMING

Depressie simptome en kardiometaboliese gesondheid in verstedelikte swart Afrikane: die SABPA studie.

Motivering

Depressie is ‘n geestesversteuring wat geassosieer word met kardiovaskulêre morbiditeit en mortaliteit in die Westerse wêreld. Kardiometaboliese meganismes is geïmpliseer as moontlike intermediêre faktore in die verhouding tussen depressiewe simptome en kardiovaskulêre siekte; dit is egter nog nie in swart Afrikane (voortaan verwys na as Afrikane) vasgestel nie.

Doel

Die oorkoepelende doel van hierdie studie is om die verhouding tussen depressie simptome en kardiovaskulêre siekte te ondersoek. Ons het daarom gestreef om die kardiometaboliese funksie, neuroendokriene reaksies en inflammatoriese/ hemostatiese merkers in Afrikane met depressiewee simptome te vergelyk met die daarsonder.

Metodologie

Manuskripte wat deur Hoofstukke 2, 3 en 4 voorgestel word, het data van die multidissiplinêre, dwarssnit, teikenpopulasie studie genaamd die “Simpatiese Aktiwiteits- en Ambulatoriese Bloeddruk in Afrikane” (die SABPA studie) gebruik. Die deelnemers het 200 Afrikane vauit die Dr Kenneth Kaunda District in die Noord-Wes provinsie in Suid-Afrika ingesluit. Die rede vir hierdie seleksie is gebasseer op die behoefte om ‘n homogene deelnemergroep te verseker.

Aangesien kardiovaskulêre siekte gekompromiteer word deur ‘n positiewe HIV-status, is 19 deelnemers uit enige verdere statistiese analises uitgesluit. Klassifikasie is gedoen volgens die 9-item Pasiënt Gesondheids Vraelys (PHQ-9) wat reeds geldig bewys is vir sub-Sahara Afrikane. Deelnemers met PHQ-9 tellings > 10 is geklassifiseer as die groep met depressie

simptome. Deelnemers is verder onderverdeel volgens geslag (Manuskrip 1 en 3) en kortisolreaksies (Manuskrip 2).

Kardiometaboliese gesondheidsmaatstawwe het 24-uur bloeddruk-, metaboliese sindroom merkers, neuroendokriene merkers [kortisol en 3-metoksie-4-hydroksi-fenielglikol (MHPG)] en inflammatoriese/ hemostatiese merkers [fibrinogeen, C-reaktiewe proteïen (CRP), plasminogen aktiveerder inhibeerder-1 (PAI) en D-dimeer] ingesluit. Rustende 12-afleiding EKG Cornell Produk-linker ventrikulêre hipertrofie (CP-LVH) is gemeet as ‘n merker van teikenorgaanskade en kardiovaskulêre disfunksie (Manuskrip 1 en 2).

Gemiddeldes en algemene karakteristieke is gemeet met die t-toets en die Chi-square analises, onderskeidelik. Beduidende verskille in die gemiddelde kardiometaboliese metings tussen die twee depressie-simptoomgroepe is bepaal deur die analise van kovariansie (aangepas vir tradisionele kardiovaskulêre risko faktore en addisionele faktore, soos per manuskrip). Multivariansie analises is gebruik om die assosiasies tussen linker ventrikulêre hipertrofie (LVH) en kardiometaboliese merkers in Afrikane met depressie simptome (Manuskrip 1 en 2) te demonstreer. ‘n Logistiese regressie analise is gedoen om die assosiasie tussen depressiewe simptome en inflammatoriese/ heemostatise fakore te ondersoek (Manuskrip 3).

Alle deelnemers het ‘n toestemmingsvorm onderteken. Die studie is, in ooreenstemming met die beleid van die Wêreld Mediese Vereniging se Verklaring van Helsinki 1975 (hersien in 2008), deur die Etiese Kommissie van die Noord-Wes Universiteit goedgekeur (NWU-0003607S6).

Resultate en slotsomme van die onderskeie manuskripte

Die doel van die studie was om die assossiasie tussen LVH, depressiewe simptome en kardiovaskulêre funksie te ondersoek. Die kardiometaboliese funksie merkers wat

geassesseer is het 24-uur bloeddruk, metaboliese sindroom merkers, neuroendokriene merkers [kortisol en MHPG] en inflammatoriese/ hemostatiese veranderlikes (fibrinogeen, C-reaktiewe proteïen, plasminogeen aktivateerder inhibeerder-1 en D-dimeer) ingesluit.

Manuskrip 1 het gefokus op LVH, as ‘n merker van kardiovaskulêre disfunksie, en na metabolisee sindroom komponente as merkers van kardiometaboliese funksie. Die doel van die studie was om die assossiasie tussen LVH en metaboliese sindroom (MetS) risiko merkers in deelnemers, met en sonder depressie simptome, vas te stel. Resultate het getoon dat sistolies bloeddruk (SBP) en persentasie ge-glikosileerde hemoglobien (HbA1c) die beduidendste bepalers was van LVH in Afrikaan mans met depressie simptome. In Afrikaan vroue (met depressie simptome) is die assosiasie egter deur lae hoë-digtheid lipoproteïen aangedui. Die slotsom was dat kardiometaboliese faktore (naamlik SBP en HbA1c) die drywende faktore is in die ontwikkeling van kardiovaskulêre siekte in Afrikaan mans, ongeag van hulle depressie simptome, Dit was ook duidelik vanuit die data dat depressie simptome in Afrikaan vroue geassosieer is met ‘n mate van teikenorgaanskade en dat hierdie assosiasie gedryf is deur ‘n metaboliese faktor.

Manuskrip 2 se doel was om die verhouding tussen depressie simptome, neuroendokriene reaksies [met kortisol en 3-methoxy-fenielglikol (MHPG) as merkers] en kardiovaskulêre risiko, LVH, te ondersoek. Die resultate het bewys dat Afrikane met depressie simptome onderdrukte kortisol en MHPG vlakke toon tydens blootstelling aan akute mentale stres in vergelyking met diegene sonder depressie simptome. Die onderdrukte kortisol en MHPG reaksies is geassosieer met LVH in hierdie etniese groep. Die slotsom van die manuskrip is dat onderdrukte neuro-endokriene reaksies geassosieer is met depressie simptome en EKG LVH in Afrikane. Gekombineerd met hipertensiewe status, mag hierdie vasokonstriktiewe agent reaksies die basis vorm vir die toename in lang-termyn depressie en vaskulêre risiko in verstedelikte Afrikane.

Manuskrip 3 se doel was om die verhouding tussen depressie simptome en inflammatoriese/ hemostatiese merkers in ‘n groep stedelike Afrikane te ondersoek. Die data het hiperkoagulasie weerloosheid in Afrikaan mans met depressie simptome gedemonstreer. Die Afrikaan mans met tekens van depressie het hoër plasminogeen aktiveerder inhibeerder (PAI-1) vlakke en ‘n effense verhoging in D-dimeervlakke getoon. Die slotsom was dat hiperkoagulasie dalk deels die bemiddelende faktor tussen depressie simptome en kardiovaskulêre risiko in Afrikaan mans is; ‘n situasie wat vererger kan word deur hiperkinetiese bloeddruk.

Ten slotte, deur die assessering van kardiometaboliese funksie en neuro-endokriene reaksies blyk dit dat Afrikane met depressie simptome, veral mans, ‘n groot risiko inhou vir kardiovaskulêre morbiditeit en mortaliteit (Manuskrip 1 en 3). Verder blyk dit dat onderdrukte neuro-endokriene reaksies en hiperkoagulasie as moontlike kardiovaskulêre risiko merkers in Afrikane met depressie simptome gesien kan word.

Sleutelwoorde: Kardiometaboliese funksie, neuro-endokriene reaksies, inflammatoriese/ hemostatiese merkers, depressie simptome, linker ventrikulêre hipertrofie.

PREFACE

This dissertation is presented in the article-format, consisting of peer-reviewed published or submitted articles. The chosen format for submission is approved, supported and outlined by the North-West University guidelines for postgraduate doctorate level studies. The first chapter in this dissertation comprises a detailed literature overview (in addition to the literature overviews discussed in each manuscript). The Chapters 2 to 4 consists of manuscripts in the form of original research articles. The promoter, co-promoters and assistant promoter were included as co-authors in each article. The first author was responsible for initiating and writing this dissertation. The first author was also responsible for literature searches, data mining and statistical analyses of the research papers. All co-authors gave their consent to the inclusion of the research articles in this dissertation. References included in chapter 1 and 5 are according to the Vancouver format, except where otherwise stated.

The first article was submitted to *Clinical and Experimental Hypertension* (published: (2013, 35(3):213-219), the second to *Cardiovascular Endocrinology* (accepted for publication) and the third will be submitted to *Biological Psychiatry*.

Relevant references are given at the end of each manuscript according to the author's instructions of each specific journal where the manuscripts were submitted or intended for submission.

STATEMENTS BY THE AUTHORS

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

Ms N Mashele	**Author**. Responsible for proposal of study, literature searches, design and planning of research articles and thesis, statistical analyses, interpretation of results and writing of entire thesis.
Prof L Malan	**Promoter**. Guidance, intellectual input. Supervised the initial planning of the thesis and design, collection of data and guidance in the writing of the thesis. Project leader of the SABPA study.
Prof JM van Rooyen	**Co-promoter**. Intellectual input, data collection and assessment of content of the thesis.
Prof JC Potgieter	**Co-promoter**. Intellectual input regarding psychological data
Prof BH Harvey	**Co-promoter**. Intellectual input regarding biochemical and pharmacological data. Input regarding the writing of this thesis.

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

I hereby declare that I approved the aforementioned manuscripts and that my role in this study as stated above, is representative of my actual contribution and that I hereby give my consent that these manuscripts may be published as part of the doctorate dissertation of Nyiko Mashele.

Prof L Malan	Prof JM Van Rooyen	Prof BH Harvey	Prof JC Potgieter

LIST OF TABLES

CHAPTER 1:

Table 1: The IDF definition of Metabolic Syndrome

Table 2: The Joint Statement Consensus (JSC) definition of Metabolic Syndrome.

Table 3: Global cardiometabolic risk factors.

Table 4: Diagnostic criteria for Major Depressive Disorder from the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR).

CHAPTER 2:

Table A.1: Characteristics of participants at baseline in depressed (D) and non-depressed (ND) Africans (mean ± SD)

Table B.1: Adjusted differences in cardiometabolic variables in depressed (D) and non-depressed (ND) African men and women (mean ± 95% CI).

Table C.1: Forward stepwise regression analysis demonstrating associations between left ventricular hypertrophy and various cardiovascular factors

CHAPTER 3:

Table 1: Descriptive statistics of the study sample (mean ± standard deviation).

Table 2: Adjusted means (± 95%CI): Comparing salivary stress cortisol (low vs. high) in Africans with/ without depressive symptoms, independent of covariates (age, smoking prevalence).

Table 3: Forward stepwise regression analyses to demonstrate associations between ECG-LVH and potential independent predictors in Africans with/without depressive symptoms and low/high cortisol responses.

CHAPTER 4:

Table 1: Unadjusted characteristics of the study sample according to Depressive Symptoms

Table 2: Adjusted differences in inflammatory and haemostatic biomarkers in African men and woman with/without depressive symptoms (mean ± 95% CI).

Table 3: Odds Ratios evaluating the associations between depressive symptoms and inflammatory/haemostatic markers in African men and women.

LIST OF FIGURES

CHAPTER 1:

Figure 1: Dissociation between physiological and behavioral neuroendocrine defensive AC responses in African urban men.

Figure 2: Interaction between chronic stress and affective disorders such as depression.

Figure 3: Neurochemical pathways implicated with the stress response and their role in the etiology of depression

Figure 4: The stress induced glutamate-NMDA receptor mediated activation of the nitric oxide (NO) synthase pathway in the neural cells.

Figure 5: An overview of the neuroendocrine pathways, metabolic syndrome and inflammation.

Figure 6: Tryptophan metabolic pathway.

Figure 7: A schematic overview of the haemostatic and fibrinolytic pathways involved in coagulation.

CHAPTER 3:

Figure 1: Comparing adjusted resting cortisol and median split responses (cut point > 1.5 ng/ml) during acute mental stress STROOP responses in Africans with depressive and those without depressive symptoms. Covariates included age and smoking prevalence. STROOP responses were adjusted for baseline levels.

CHAPTER 4:

Figure 1: Association between Depression score (PHQ-9 total score) and PAI-1 in African men and women; adjusted for age, BMI, smoking prevalence, γ-GT, physical activity and statin use.

Figure 2: Associations between Depression score (PHQ-9 total score) and D-dimer in African men and women; adjusted for age, BMI, Smoking prevalence, γ-GT, physical activity and statin use.

LIST OF ABBREVIATIONS

α	– Alpha
α2AP	– α_2-antiplasmin
β	– Beta
5-HT	– 5- hydroxytryptamine
5-HTP	–5-hydroxytryptophan
γ-GT	– Gamma glutamyl transferase
ABPM	– Ambulatory blood pressure monitoring
AMPA	– α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic
ANCOVA	– Analysis of covariance
BDNF	– Brain-derived neurotrophic factor
BMI	– Body mass index
BP	– Blood pressure
°C	– Degrees celsius
Ca^{2+}	– Calcium
CI	– Confidence interval
CP	– Cornell Product
CRP	– C-reactive protein
CVD	– Cardiovascular disease
CV	– Cardiovascular
D	– Depressive symptoms
DA	– Dopamine
DAG	– Diacylglycerol
DBP	– Diastolic blood pressure
ECG	– Electrocardiogram

ESH	– European Society of Hypertension
FPA + B	– Fibrinopeptides A and B
FDPs	– Fibrinogen degradation products
GABA	– Gamma amino butyric acid
GAS	– General adaptation syndrome
GLU	–Glutamate
GR	– Glucorticoid receptor
HDL-chol	– High density lipoprotein
HT	– Hypertension
IDO	– Indoleamine 2, 3-dioxygenase
IL-6	– Interleukin-6
IL-1	– Interleukin-1
IL-8	–Interleukin-8
IP_3	–Inositol triphosphate
IR	– Insulin receptor
KAT	– Kynurenine aminotransferase
kcal	– Kilocalories
kg/m^2	– Kilograms per meter squared
LDL-chol	– Low density lipoprotein
LPL	–Lipoprotein lipase
LVH	– Left ventricular hypertrophy
MetS	– Metabolic syndrome
mGluR	–Metabotropic glutamate receptor
MHPG	– 3-methoxy-4-hydroxy-phenylglycol
MAO	– Monoamine oxidase

mmHg	– Millimetre mercury
mmol/L	– Millimole per litre
mV	– Millivolt
n	– Number of
ng/ml	– Nanogram per milliliter
NE	– Norepinephrine
NAD	– Nicotinamide adenine dinucleotide
NADPH	–Nicotinamide adenine dinucleotide phospholipase
NMDA	– N-methyl-D-aspartate
NO	– Nitric Oxide
p	– Probability
PAP	– Plasmin-antiplasmin complex
PAI-1	– Plasminogen activator inhibitor-1
PDE	–Phosphodiesterase
PIP_3	–Phosphatidylinositol bisphosphate
PI3K	–Phosphoinositide 3-kinase
PLC	–Phospholipase
PTF	– Prothrombin fragments
PPARγ	– Proliferator-activator receptor
QPRT	– Quinolinate phosphoribosyltransferase
r	– Correlation coefficient
R^2	– Relative predictive power of a model
SABPA	– Sympathetic activity and Ambulatory Blood Pressure in Africans
SBP	– Systolic blood pressure
SD	– Standard deviation

SR	–Sarcoplasmic reticulum
TAT	– thrombin-antithrombin complex
TDO	–Tryptophan 2, 3-dioxygenase
TF	– Tissue factor
TGF-β	–Transforming growth factor
TNF-α	– Tumour Necrosis factors
tPA,	– Tissue plasminogen activator
TRIG	– Triglycerides
WC	– Waist circumference

Chapter 1: Introduction and Literature review

1. INTRODUCTION

The worldwide escalation in the incidence of metabolic syndrome and its association with cardiovascular morbidity and mortality has elicited an interest in the underlying mechanisms that link metabolic disorders and cardiovascular diseases (CVD).[1-3] 'Cardiometabolic risk' is a term generally used to indicate a clinical entity of substantial heterogeneity represented by the co-occurrence of both cardiovascular and metabolic risk factors.[2,3] These include traditional CVD risk factors (age, gender, genetics, hypertension, smoking, dyslipidemia and diabetes), abdominal obesity, inflammation and pro-thrombotic profile.[2,4]

In addition to the above mentioned risks, evidence from the literature suggests that other factors of a more psychosocial nature may be associated with the initiation and progression of CVD. These psychosocial risk factors for CVD can generally be classified into three main categories or domains that refer to the social environment, personality traits and negative affect.[5] Psychosocial risk factors have also been linked with a constellation of cardiovascular and metabolic (cardiometabolic) risk indicators such as hypertension (HT), diabetes, dyslipidemia, visceral fat accumulation and inflammation.[6-13]

One psychosocial stressor that has received increased attention as a source of stress in low- and middle-income countries in the past few years is urbanisation.[14] Urban living in black Africans (hereafter referred to as Africans) has been linked with cardiometabolic risk factors such as hypertension, type 2 diabetes, and obesity in women.[7,8,15-17] Urbanisation has also been associated with low cultural and social support that may lead to psychosocial disruption, higher stress levels and the subsequent onset of depression.[18,19] Peen et al. (2010) showed that urbanisation is associated with an increased risk for negative affective disorders such as

anxiety and depression.[20] This overlap between the social environment (urbanisation, job strain and social support) and negative affect (depression and anxiety) may suggest that any life situation that evokes negative emotions may promote ill health.[21]

Depression is one of the negative emotional responses that have received attention in the past few years as an independent risk factor for CVD.[22] Depression has been associated with adverse cardiac outcomes in healthy populations and in cardiac patients.[23-25] However, the pathophysiological mechanisms linking depression and cardiovascular outcome remain unclear. Advances in biological psychiatry have identified alterations in hypothalamic-pituitary-adrenal axis (HPA-axis), sympathetic-adrenomedullary (SAM) and inflammatory/ haemostatic activity in depression. These alterations may reflect important pathophysiological dysregulations that contribute to the increased vulnerability of depressed individuals to CVD.[26]

This chapter will constitute a literature review that focuses on the association between depressive symptoms and cardiometabolic health in Africans by looking at various aspects of cardiometabolic risk including neuroendocrine responses to acute mental stress and inflammatory/ haemostatic variables in this sample population.

2. CARDIOMETABOLIC RISK FACTORS

The clinical importance of the metabolic syndrome (MetS) was first highlighted by Reaven in 1988, who coined the term 'Syndrome X' as describing a clustering of disturbances in metabolic functioning (glucose and insulin metabolism), dislipidemia and hypertension (HT).[27] Since then scientists have recognised that this cluster of metabolic risk factors increase the risk for type 2 diabetes and CVD.[27,28] The fundamental element in Reaven's definition of MetS was insulin resistance, which he emphasised was at the centre of a cluster of metabolic abnormalities that included elevated triglycerides (TRIG), low high-density lipoprotein cholesterol (HDL-chol), hyperglycemia and HT.[27] Later on the pivotal role of central obesity in metabolic disorders and CVD was recognised.[2,4,29] The emphasis on central obesity put the location of body fat (specifically abdominal obesity) as an important predictor of MetS, a factor that was highlighted by expert consensus of the International Diabetes Federation (IDF). The IDF established a unified definition of MetS that would be suitable for use in epidemiological studies and clinical practice.[30] The IDF definition for MetS focused on obesity (especially central obesity) as a prerequisite for diagnosis of the syndrome.[30] The group also included different cut-off points for different ethnic groups (Table 1).

Table 1: The IDF definition of metabolic syndrome 2005

The IDF definition of Metabolic Syndrome [30]
'central obesity', defined as waist circumference ≥ 94 cm in sub-Saharan African men or ≥ 80 cm in sub-Saharan African women plus any two of the following four factors:
elevated blood pressure (systolic of ≥ 130 mmHg or diastolic ≥ 85 mmHg and or treatment of previously diagnosed HT)
elevated serum TRIG (≥ 1.7 mmol/l or specific treatment for lipid abnormality)
reduced HDL-chol (< 1.1 mmol/l in men or < 1.3 mmol/l in women and/ or specific treatment for this lipid abnormality)
elevated fasting glucose (≥ 5.6 mmol/l/ or previously diagnosed type 2 diabetes)

The IDF definition for MetS was later revised and a new criterion for the syndrome was then established.[31] Contrary to the first definition, abdominal obesity was no longer a prerequisite. The new revised definition, established by the Joint Statement Consensus (JSC), stated that any three of the five components previously listed in the first IDF definition would establish MetS.[31]

Table 2: The Joint Statement Consensus (JSC) definition of Metabolic Syndrome 2009

The Joint Statement Consensus definition of Metabolic Syndrome. [31]
Any three of the following five criteria listed below:
Elevated waist circumference according to population- and country-specific definition: ('waist circumference' defined as ≥ 94 cm in sub-Saharan African men or ≥ 80 cm in sub-Saharan African women)
elevated blood pressure (systolic of ≥ 130 mmHg or diastolic ≥ 85 mmHg and/ or treatment of previously diagnosed HT)
elevated serum TRIG (≥ 1.7 mmol/l or specific treatment for lipid abnormality)
reduced HDL-chol (< 1.1 mmol/l in men or < 1.3 mmol/l in women and/ or specific treatment for this lipid abnormality)
elevated fasting glucose (≥ 5.6 mmol/l or previously diagnosed type 2 diabetes)

Although abdominal obesity was no longer a prerequisite for the diagnosis of MetS, its role in metabolic and cardiovascular risk is still recognised. The International Chair on Cardiometabolic Risk subsequently coined the term 'global cardiometabolic risk' to describe cardiovascular diseases resulting from the presence of traditional CVD risk factors. Features of MetS and emerging risk factors associated with the syndrome are described in *Table 3*.[4]

Table 3: Global cardiometabolic risk factors.[4]

Global cardiometabolic risk factors:
Traditional cardiovascular risk factors
• Age • Gender • Family history • Smoking • Genetics
Metabolic risk factors
• Hypertension • Glucose intolerance • Dyslipidemia; low-density lipoprotein (LDL-chol), high-density lipoprotein (HDL-chol) and triglycerides (TRIG)
Emerging risk factors
• Central obesity • Insulin resistance • Inflammatory state and pro-thrombotic profiles

Other risk factors

Although not included in the International Chair on Cardiometabolic risk profile for CVD, evidence from the literature suggests that psychological mechanisms also play an important role in the development of CVD.[32-34] For instance, psychosocial stress has been linked with coronary heart disease (CHD).[35-39] Additionally, negative emotional factors and chronic stress may increase the risk for cardiometabolic-related illness such as HT and type 2 diabetes mellitus. [6-13] Furthermore, these psychosocial factors might interact with others to encourage the development of CVD. For instance, chronic stressors such as urbanisation and job strain are associated with the onset of negative emotions and affective disorders such as depression and anxiety.[21,39]

3. STRESS AND CARDIOMETABOLIC RISK

3.1. The concept of 'stress'

'Stress' is an ambiguous term with a wide variety of definitions ranging from a physiological description to a more cognitive one. Ever since Walter B. Cannon first used the term 'fight or flight' responses–physiological reactions in response to a perceived threat, harm or attack– the concept of 'stress' has evolved to include a variety of factors that may influence the individual's response pattern.[40] They include the person's perception of the situation and the coping resources available, genetic make-up, personality and social support.[5,41,42]

Fight or flight response

As mentioned above, the 'fight or flight' response (also known as the acute stress response) was first described by Cannon in the 1930's. He defined it as the organism's physiological reaction to threat resulting in the instantaneous arousal of the sympathetic nervous system.[40] He postulated that emotions such as fear and anger evoked the same type of response as physical threats, resulting in the automatic arousal of the sympathetic nervous system (SNS).[40] This initial physiological response was later incorporated in the initial stage of Hans Selye's general adaptation syndrome theory.[8,43]

General Adaptation Syndrome (GAS) [43]

Selye expanded the concept of 'stress' to include responses mediated by the HPA-axis.[43] Selye [43]postulated that stress can be categorised into two main groups depending on how it is experienced: eustress and distress.[43] 'Eustress' refers to a positive experience of stress in which adaptive responses are initiated to promote the activation of internal resources (coping) to meet any emotional or environmental demand. 'Distress' is a negative experience of stress and describes the inability to adapt or cope with persistent emotional or environmental demands.[44] Selye [43] also described a 'critical stress level' as one that promotes the onset of distress. This is a threshold level at which any additional stress, stimuli or event produces a breaking strain that is characterised by physiological and behavioural responses, and may lead to disorder or disease.[43,45]

GAS categorises the stress response into three stages: the alarm stage, defined as the exposure to a stressor such as the stress of urban living in Africans and the realisation of a threat (Figure 1).[43,44] This leads to the activation of both the SNS and HPA-axis that produces

epinephrine and cortisol respectively. Second is the resistance stage that is described as a state in which chronic and persistent exposure to a stressor elicits the activation of various coping mechanisms (physiological and emotional). In Africans this stage is linked with an increase in HT prevalence, prolactin, cortisol testosterone ratio and a decrease in testosterone levels[7,18] The final stage of exhaustion is described as the point when the body's resources are depleted (of coping and adaptive mechanisms) and is unable to maintain homeostasis (normal bodily function). The prolonging of this phase may lead, for example, to damage in the long-term, disassociation between behavioural and physiological patterns, diminished coping ability and the resultant development of depression and an increase in cardiometabolic risk in Africans.[8,18,43]

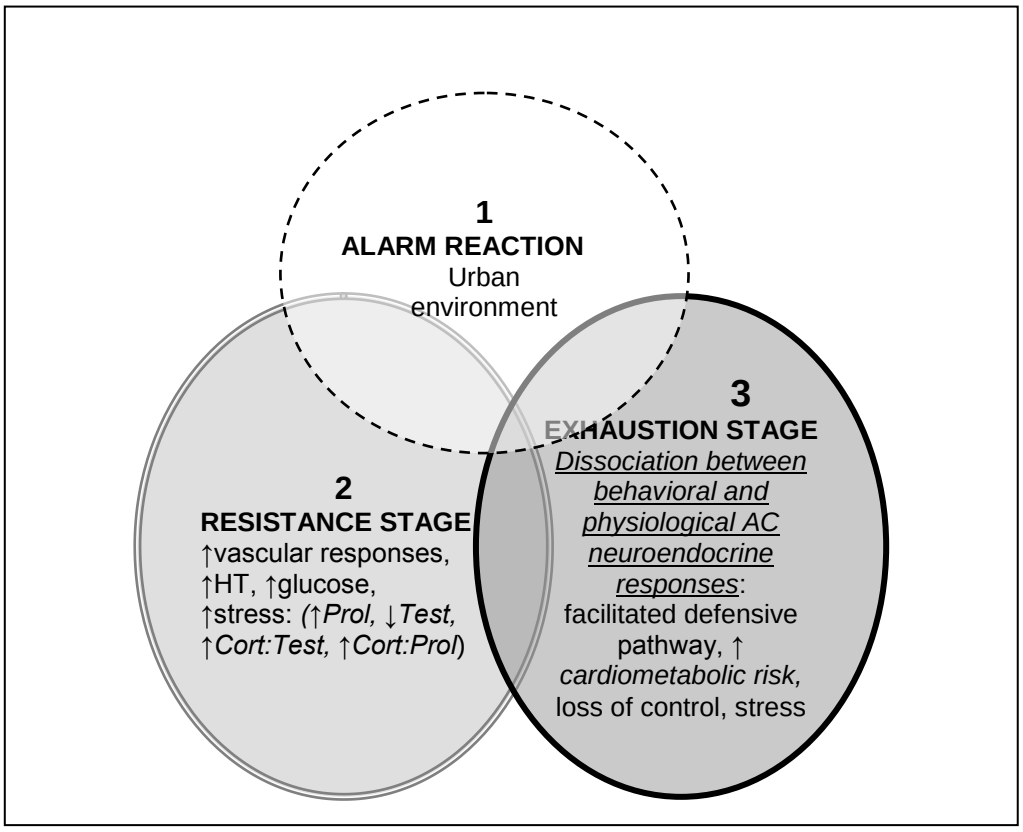

Figure 1: Dissociation between physiological and behavioral neuroendocrine defensive AC responses in African urban men. Abbreviations: HT,

hypertension; AC, active coping; Prol, prolactin; Test, testosterone; Cort, cortisol. Symbols: ↑increase, ↓decrease.[8,18,43]

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Selye's general adaptation theory pioneered the future conceptualisation of the stress response by researchers such as Chrousos and Gold[46] who defined 'stress' as a state of threatened homeostasis and a maladaptive state that can affect many aspects of physiology, emotional well-being and coping abilities. The researchers postulated that stress evokes a maladaptive response when the threat exceeds a threshold; this response is governed by two major components of the stress system: the corticotropin-releasing hormone (CRH) neurons and the locus coeruleus-norepinephrine/autonomic system.[46] Deregulation of these two systems, which is characterised by a persistent hyper- and or hypoactivity, contributes to a range of pathophysiologic states that increase the susceptibility to various conditions including various psychiatric disorders (depressive and anxiety disorders), endocrine abnormalities (hypercortisolemia) and inflammatory diseases.[47]

Allostasis and Allostatic load

Sterling and Eyer [48] first introduced the term 'allostasis' to describe the ability of the body to adjust its vital functions to a new state, in which the body is exposed to a stressor, in an effort to maintain homeostasis.[48] McEwen[49] recognised that the long-term effects of repeated allostasis may be detrimental to the human body. He coined the term 'allostatic load' to define the 'wear and tear' experienced by the body as a result of prolonged and inappropriate secretion of stress hormones.[49]

It is clear from the above-mentioned models that the experience of stress evokes a wide range of initially adaptive changes (physiological, behavioural and emotional) in order to maintain homeostasis. Persistent activation of these responses, as a consequence of chronic exposure to stress, leads to a maladaptive stress response. This pattern of response may promote a pathophysiologic state that leads to the dysfunction of vital bodily systems (cardiovascular system) and distress.[7,8,18,49]

However, not all individuals are susceptible to experience distress and maladaptive stress responses during periods of high demands.[42] Individual susceptibility is determined by factors such as genetics, social support and personality.[42] Additionally, the individual's perception of stress determines the pattern of their stress response.[41] 'Cognitive stress appraisal', as termed by Lazarus[41] is how the individual perceives the stress, threat and challenge and the evaluation of coping resources available to meet the situation.[41] Cognitive appraisal is generally divided into two types: primary and secondary appraisal. 'Primary appraisal' can be defined as the individual's interpretation of the stressful stimulus. 'Secondary appraisal' can be defined as the assessment of the adequacy of coping resources available to meet the perceived stressful situation; this may be influenced by factors such as demands, constraints and opportunities eliciting emotions attributed to a particular stressor.[7,8,18,41,50,51]

3.2. Definition of a 'stressor'

A stressor is any demanding environmental situation that elicits some form of an effect on the individual.[49] The initial activation of these responses (behavioural, physiological and emotional) is to try and maintain homeostasis in the individual. However, these stress responses become maladaptive if sustained or exaggerated.[5]

A number of factors may induce the stress response in humans, for example, job strain, low socioeconomic status, loss of social support, and marital strain.[21] Any life event, circumstance or stimulus that potentially relates psychological phenomena to the social environment and physical health is known as a psychosocial factor.[52] These psychosocial factors have been associated with cardiovascular risk, in healthy populations and with future cardiac events in patients with CVD.[36-38,54]

3.3. Social Environment and chronic stress

As previously mentioned, any demanding environmental situation that induces a stress response in an individual is a stressor.[49, 50] When these demands become constant or chronic they begin to challenge the individual's coping abilities ultimately leading to distress.[41,54] This was noted by Malan et al.[7,18] who found that urbanisation, a potent psychosocial stressor, was linked with higher stress levels and psychological distress in Africans .[7,8,18] The same authors suggested that the loss in social and cultural support may underpin the higher stress levels reported in this ethnic group leading to distress and ultimately the onset of negative affects such as anxiety and depression.[8,18,,20] Rozanski et al.[21]stated that this overlap between the social environment and negative affect may promote the development of CVD.[21] Indeed, in Africans, the higher stress levels associated with urbanisation have been linked with HT prevalence, exaggerated cardiovascular reactivity and increased cardiometabolic risk.[7,8,15-18]

Chronic psychosocial stress is a potent inducer of negative affective emotions such as depression. These conditions often cluster together increasing the risk for developing CVD (Figure 2).[21] For instance, a lack of social support often coexists with depression; both are

implicated as predictors in the onset and progression of CHD.[55] Likewise, work stress is associated with an increased risk for developing depression and the onset of a first CHD event.[56,57] Chronic stress and negative affective emotions may promote CVD by activating the stress response (Figure 2), HPA-axis, SNS, and promoting unhealthy behaviours. Activation of these responses results in multiple adverse peripheral effects including neuroendocrine, increased sympathetic tone, somatic effect and subsequent neuroplastic changes. Neuroplastic changes that ensue may induce a state of heightened physiologic responsiveness to acute stressors.[21]

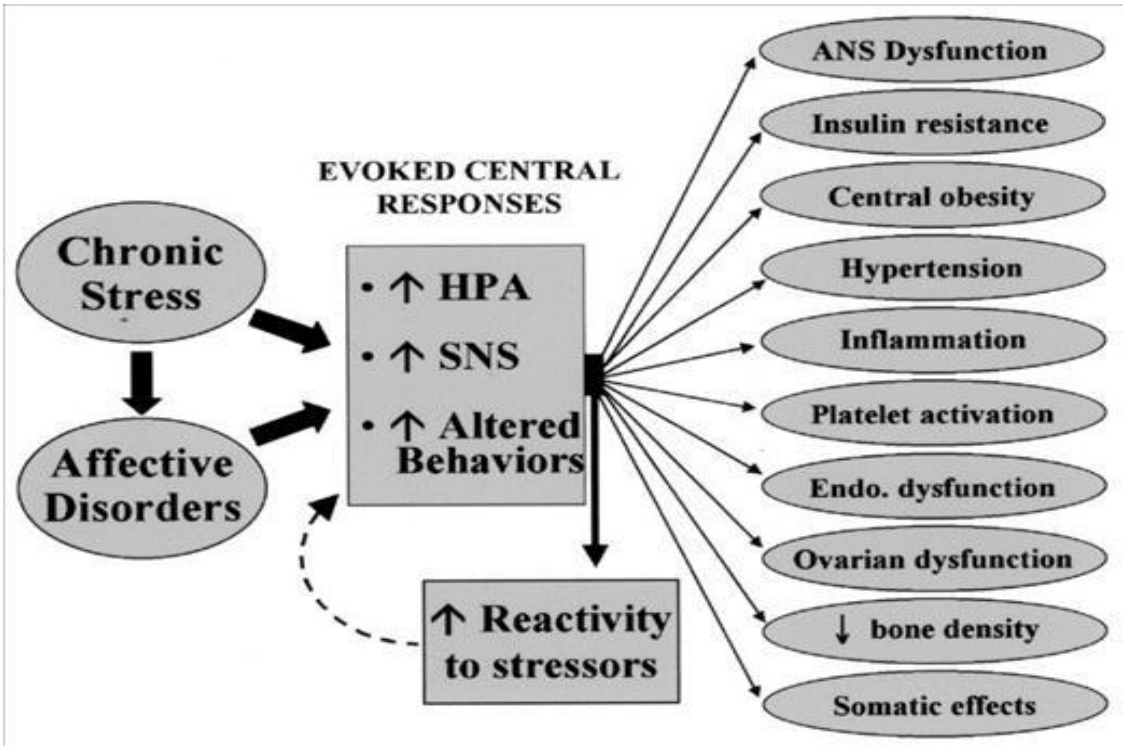

Figure 2: Interaction between chronic stress and affective disorders such as depression. Abbreviations: ANS, autonomic nervous system; Endo; endothelial; HPA, hypothalamic-pituitary-adrenal; SNS, sympathetic nervous system. Symbols: ↓decrease; ↑increase.[21]

3.4. Chronic psychosocial stress and acute mental stress testing

One method of evaluating the effects of chronic psychosocial stress on the development of CVD is through psychophysiological stress testing. This method of assessment involves the measurement of cardiovascular and biological responses to acute laboratory-induced stress that evokes a particular pattern of response.[58] This allows for individual responses to be assessed and related to psychosocial factors and cardiometabolic risk factors like hypertension that increase the risk for developing CVD.[59-61] A meta-analysis by Chida and Steptoe[62] found that augmented responses to acute mental stress have an adverse effect on future CV risk status.[62]

The magnitude of the acute response is governed by the activation of the sympathetic/ autonomic nervous system and of the HPA-axis. This response may be augmented in vulnerable individuals such as those exposed to chronic psychosocial stressors like urbanisation, and those with affective illnesses like depression.[15,59,62,63] For instance, greater norepinephrine response to acute mental stress challenges was observed in Africans with depressive symptoms.[63]

3.4.1. The Stroop Colour Word Conflict Test

The Stroop Colour Word Conflict Test is used in psychophysiological research as a psychological or cognitive stressor that elicits an emotional and physiological response.[64,65] The particular task demands that the subject recognise and name the colour of the word contrary to the word written. The Stroop test consists of three basic components: the presence of colour-word conflict, the pacing of task execution and the rate of task pacing. These three

components together with the added pressure of time constraint increase the demand of the task contributing significantly to the test's stressfulness.[65]

The physiological response patterns often associated with the Stroop test include heightened autonomic responses, particularly blood pressure (BP); increases in heart rate; cardiac index (cardiac output and stroke volume); and catecholamines, increased norepinephrine (NE).[58,61] Increases in catecholamines levels elicit dilatory effects, via epinephrine stimulation of β_2-adrenergic pathways, and vasoconstrictive effects through norepinephrine stimulation of α-adrenergic pathways in the vasculature.[66] However, in participants with depressive symptoms norepinehprine release is enhanced, suggesting that depression is primarily related to central adrenergic dysfunction. This central dysfunction, which is characterised by a dominant cholinergic tone compared to adrenergic mechanisms, may increase their risk to stress-related pathology.[67,68]

4. ROLE OF STRESS IN THE ONSET OF DEPRESSION

As previously mentioned, chronic stress is an inducer of negative affective emotions including depression, anxiety, and hostility.[57] However, the exact role of stress in the pathogenesis of affective disorders remains unclear. A number of theories have been proposed as possible mechanisms responsible for neuroplastic alterations that may occur during chronic stress; these include the neurotrophic hypothesis, monoamine theory and the glutamatergic models. For the purpose of this study, we will discuss the molecular processes implicated in the onset of depression.

4.1. Neurobiology of Depression

Although genetic make-up may undoubtedly underpin the pathology of most affective disorders, the environment may play an important role in the etiology and progression of psychiatric illnesses such as depression.[69] Chronic exposure to stress may induce maladaptive stress-induced changes within the brain, especially in areas important in the regulation of the stress responses such as the hippocampus.[70] More importantly, these changes may result in structural and functional alterations that may lead to, depending on the duration and severity of the stressor, neuropsychiatric dysfunction.[69]

Chronic stress results in the persistent activation of HPA-axis and SAM with a subsequent over- or underproduction of stress hormones cortisol and catecholamine, respectively. One particular area in the brain that is particularly vulnerable to unmitigated HPA-axis activity is the hippocampus.[71] The hippocampus is at the intersection of the limbic, cognitive/affective and neuroendocrine regulatory pathways, and as such it is particularly vulnerable to neuroendocrine changes associated with stress.[72] The hippocampus has a high density of glucocorticoid receptors (GR) and elevations in cortisol may cause impairment in neuroplasticity and cellular resistance that over time may lead to hippocampal atrophy.[73] These structural alterations may further augment neuroendocrine dysfunction.[74] In addition, alterations in hippocampal functioning may ensue as a result of a down-regulation in GR sensitivity during stress.[75] A decrease in GR sensitivity during chronic stress may result in poorly regulated negative feedback mechanisms in which GR signaling process are unable to 'turn off' the initial stress response.[75] This uncontrolled constant activation of the HPA-axis may lead to a subsequent increase in sympathetic tone.[46] Increased sympathetic drive will promote the release of pro-inflammatory cytokines from macrophages and lymphocytes.[76]

This rise in pro-inflammatory cytokines may diminish neurotrophic support and monoamine neurotransmission leading to neuronal damage and cell death.[77]

Neurotrophic support is mediated by the activities of brain-derived neurotrophic factor (BDNF); a primary neurotrophin in the hippocampus involved in cell maintenance, growth, plasticity and apoptosis.[78] BDNF is densely distributed throughout the brain. Once it is secreted in its mature form it acts as a facilitator of receptor dimerisation and consequent receptor phosphorylation by binding to the tropomyosin-related kinase B and promoting cellular resilience and long-term potentiation.[79,80] However, in its precursor form (pro-BDNF) the protein elicits apoptosis and reduction in dendritic spines by binding to the p75 neurotrophin receptor.[81] This became the basis for the yin-yang hypothesis which states that neurotrophins promote dendritic growth while their precursors (proneurotrophins) promote cell death.[81]

The observation that chronic stress and depression are associated with a low BDNF expression gave rise to the neurotrophic hypothesis for the pathogenesis of depression.[82-85] The hypothesis states that in addition to genetic vulnerability, stress may lead to hippocampal cell degradation via elevations in cortisol that alter cellular plasticity and down-regulate BDNF levels and receptor sensitivity.[84,86] This reduction in BDNF levels may negatively impact structural and functional processes within the hippocampus and the rest of the limbic system, leading to further disruption in neurocircuitry and atrophy.[72]

Diminished monoamine neurotransmitters have also been postulated as a major contributor to the onset of affective illnesses. The monoamine theory states that depression is associated

with synaptic deficiency in neurotransmitters norepinephrine (NE), serotonin transporter (5-HT) and dopamine (DA). The theory was later updated to incorporate the role monoamine oxidase (MAO)-A, a principle degenerative enzyme for monoamines, plays in the regulation of monoamine activity.[87] High density of MAO-A receptors have been noted in patients that are not treated for depression. Expression of MAO-A in these individuals may contribute to altered brain and behavioural function associated with the illness.[88]

Figure 3 shows the neurochemical pathways implicated in the etiology of depression. As previously mentioned, activation of the stress response leads to the secretion of glucocorticoids (cortisol), corticotrophin releasing hormone (CRH) and pro-inflammatory cytokines; tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6. Disruption in 5-HT, NE and DA transmission, as a consequence of rising levels of cortisol and pro-inflammatory cytokines, results in an impaired negative feedback process and failure to 'turn-off' the initial stress response. Increase in sympathetic tone contributes to the release of pro-inflammatory cytokines from macrophages and lymphocytes, further perpetuating monoaminergic and neurotrophic disruptions, and decreasing glucocorticoid sensitivity mediated negative feedback mechanisms.[72,89]

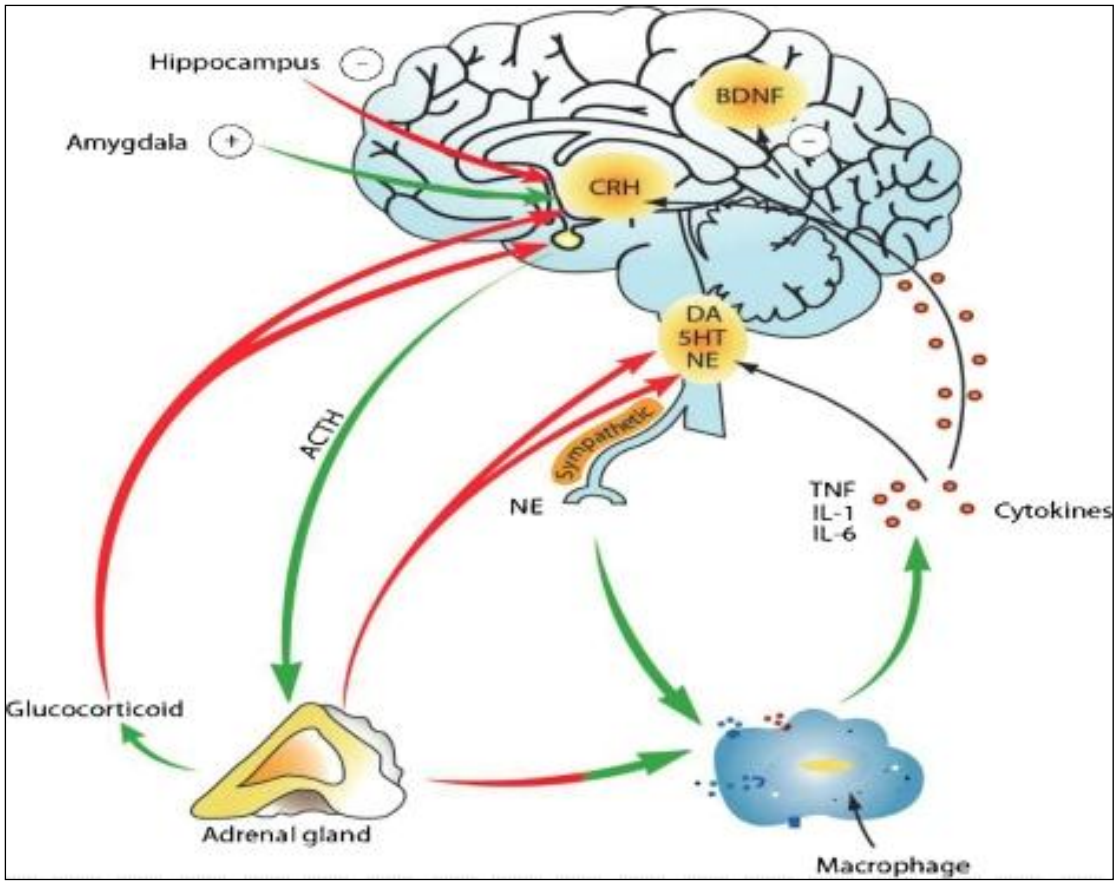

Figure 3: Neurochemical pathways implicated with the stress response and their role in the etiology of depression. Abbreviations: CRH, corticotrophin releasing hormone; TNF, tumor necrosis factor; IL, interleukin; 5-HT; serotonin; NE, norepinephrine; DA dopamine.[72,85-89]

Stress-induced activation of glutamatergic pathways, N-methyl-D-aspartate (NMDA)-glutamate and inhibitory gamma amino butyric acid (GABA) have also been implicated in the pathology of depression.[69] These pathways play an important role in synaptic plasticity and modification of pre-existing neural networks.[90-92] Chronic elevations in glucocorticoids may evoke the release of glutamate.[93-95] Glutamate activation of NMDA receptors in the central nervous system (CNS) mediates the release of nitric oxide (NO) by activating Ca^{2+}-dependent neuronal NO synthase (Figure 4).[96] NO then binds to and activates guanylate

cyclase leading to the synthesis of cyclic guanosine monophosphate (cGMP) that is responsible for cell-specific responses. NO is broken down to nitrogen oxides with actions of cGMP. Another source of NO synthase activation is via Ca^{2+} release from the endoplasmic reticulum via metabotropic receptor induced activation of phospholipase (PLC) and the synthesis of inositol triphosphate (IP_3) from membrane lipid, phosphatidylinositol bisphosphate (PIP_3). Normally, NO regulates synaptic plasticity, hormone secretion and contributes to learning and memory mechanism and affective regulation.[91,97] However, excessive release of NO, flowing overt NMPA receptor activation, leads to non-specific binding of NO to iron-containing cellular proteins with neurotoxic effects.[69,97,98]

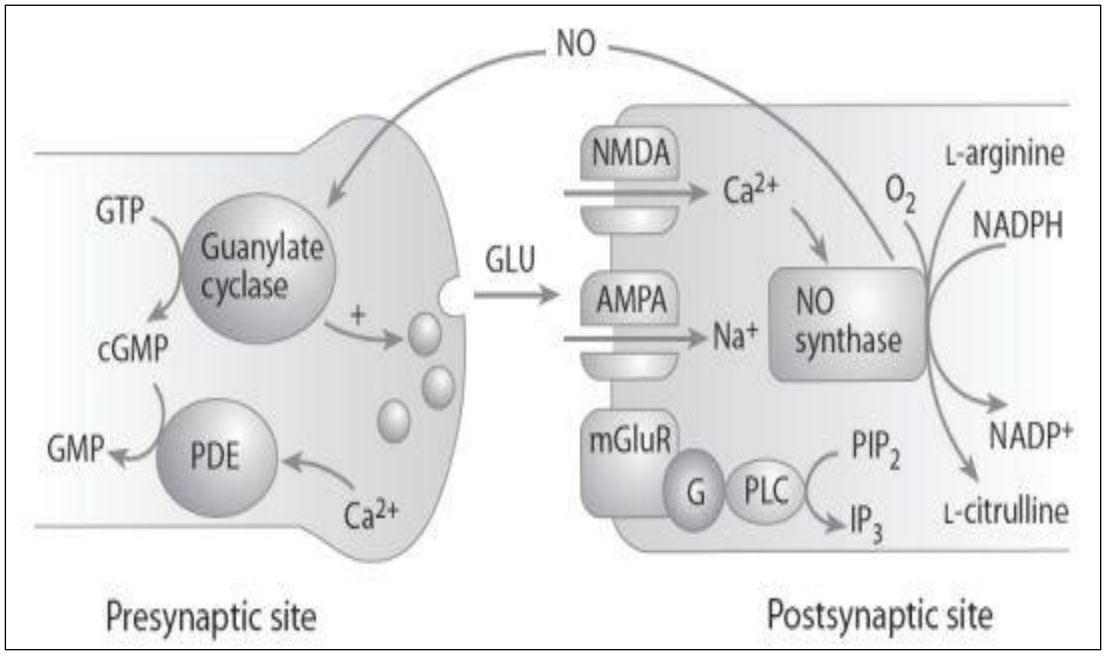

Figure 4: The stress induced glutamate-NMDA receptor mediated activation of the nitric oxide (NO) synthase pathway in the neural cells. Abbreviations: NMDA, N-methyl-D-aspartate; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate; PDE, phosphodiesterase; GLU, glutamate; AMPA, α-amino-3-

hydroxy-5-methyl-4-isoxazolepropionic acid; NADPH, nicotinamide adenine dinucleotide phosphate; mGluR, metabotropic glutamate receptor; PLC, phospholipase; IP_3, inositol triphosphate; PIP_3, phosphatidylinositol bisphosphate.[97,98]

4.2. Definition of Depression

The *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR) defines 'depression' as an affective disorder characterised by a severely depressed mood and or anhedonia (loss of interest or pleasure) that last for two weeks or more and involves functional impairment and somatic manifestations (Table 4) (DSM-IV-TR, 2000). According to Kerr and Kerr[99], a distinction can be made between subclinical depression (depressive symptoms measured by self-report screening tools) and major depressive disorder (measured by the DSM-IV-TR).[99]

4.3. Assessment of Depression

A number of instruments have been developed to evaluate the presence and severity of depressive symptoms in population studies. These screening tools include the Beck Depression Inventory (BDI), Center for Epidemiological Studies Depression Scale (CES-D), Duke Anxiety-Depression Scale, Geriatric Depression Scale, Hopkins Symptoms Checklist, Primary Care Evaluation of Mental Disorders (PRIME-MD), Patient Health Questionnaire (PHQ) and Zung Self-Rating Depression Scale. For the purpose of this study PHQ, which assesses the 9 diagnostic criteria (Table 3) outlined by the DSM-IV-TR, will be discussed.[100]

4.3.1. Patient Health Questionnaire [100]

The PHQ is a streamlined and self-report version of the Primary Care Evaluation of Mental Disorders (PRIME-MD). The PHQ has become a useful, reliable and valid diagnostic tool for measuring depression severity in any setting including sub-Saharan Africa.[100-102] Multiple versions of the questionnaire with varying numbers of items can be found including the 2-item and the 9-item (PHQ-2 and PHQ-9) versions. The PHQ-2 (the first two items of the PHQ-9) assesses the presence and frequency of depressive mood and anhedonia.[102] The 9-item version includes the 9 diagnostic criteria outlined by the DSM-IV-TR for depressive disorders [major depressive disorder (MDD) and other depressive disorders (ODD)] outlined in Table 3.

Each item in the questionnaire is coupled with the following responses: 0 (not at all), 1 (several days), 2 (more than half of the days) and 3 (nearly every day). For diagnostic purposes the PHQ-9 total scores are grouped into the following categories of increasing depression severity: 0-4 (minimal), 5-9 (mild), 10-14 (moderate to severe). Scores higher than 15 (severe) usually suggest the presence of major depression.[100,101] A recent meta-analysis by Manea et al.[103] found that the PHQ-9 had acceptable diagnostic properties for indentifying major depression with cut-off scores between 8 and 11.[103] In this study we will use the recommended cut-off score of 10 that has been found to have a sensitivity and specificity of 88% in identifying major depression and is comparable to other larger depression measures.[100]

Table 4: Diagnostic criteria for Major Depressive Disorder from the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR).[104]

Diagnostic criteria for MDD from the DSM-IV-TR
Symptoms: At least five of the following symptoms must be identified: • Depressed mood nearly every day for most of the day. • Markedly diminished interest or pleasure in almost all activities nearly every day for most of the day • Insomnia or hypersomnia nearly every day • Psychomotor agitation or psychomotor retardation nearly every day • Unintentional weight loss or weight gain and substantial change in appetite • Diminished ability to concentrate or indecisiveness • Feelings of guilt and worthlessness • Fatigue or loss of energy, nearly every day • Recurrent thoughts of death or suicide **Duration:** • symptoms must be present for at least two weeks and causes significant impairment in normal functioning

5. PATHOPHYSIOLOGICAL MECHANISMS LINKING DEPRESSION AND CARDIOMETABOLIC RISK

There is substantial evidence to suggest that negative emotional factors such as depression, anxiety, hostility and anger are associated with poor cardiac outcomes across the spectrum of cardiac disease.[105-107] These negative emotional factors have been shown to be significantly associated with CHD outcomes in both healthy populations and in individuals with CHD.[33,108-110] Among the abovementioned emotional factors, depression has been the most studied in the past few years. Its high prevalence in cardiac patients and its relationship with cardiovascular morbidity and mortality has elicited an interest in the behavioural and pathophysiological mechanisms underlying the relationship.[111]

Evidence from literature suggests that depression may contribute to cardiometabolic dysfunction and subsequent increased risk for CVD through the persistent activation of the following stress response mechanisms: HPA-axis and SAM hyperactivity, inflammation and facilitation of hypercoagulation.[46,49]

5.1. Depression, HPA-axis and SAM hyperactivity

Two key components of the stress response described by Cannon[40] and Selye[43] are the HPA-axis and the SAM system. Activation of the HPA-axis also induces the activation of SAM, resulting in the release of two major stress hormones: cortisol and catecholamines into circulation. These stress hormones exert their effects at different sites initiating sequelae of alterations that enable the body to respond or adapt to a stressor accordingly.[46,47,49]

The activation of the HPA-axis is initiated when the neurons within the medial paraventricular nucleus of the hypothalamus synthesise CRH. CRH is released into the anterior pituitary where it stimulates the production and secretion of corticotropin or adrenocorticotropic hormone (ACTH), β-endophins and other pro-opiomelanocortin products.[112,113] ACTH stimulates the production and secretion of corticosteroids (including cortisol) from the adrenal cortex into the vascular system where it exerts its effects at various sites throughout the body.[112,113] These effects include the conversion of stored fats and proteins into carbohydrates (gluconeogenesis), anti-inflammatory effects and the suppression of growth and reproductive processes as well as the modulation of limbic and prefrontal regions associated with the regulation of negative affect and stress.[47,114-116] Regulation of cortisol secretion by the adrenal cortex is governed by circadian rhythms–regulated in the suprachiasmatic nucleus of the hypothalamus– that in turn influence ACTH secretion, the stress response and negative feedback mechanisms.[117,118]

In addition, the CRH-containing neurons within the hypothalamus also provide stimulatory input to the central control nuclei of the SNS, which in turn is regulated by catecholamines.[46] Nerve impulses from these central control nuclei regulate catecholamine secretion from the SAM, which comprises the SNS and the adrenal medulla. During stress sympathetic stimulation to the adrenal medulla causes the secretion of the catecholamines, that is, epinephrine and norepinephrine (NE), into the plasma. Plasma concentrations of epinephrine are derived from the adrenal medulla whereas most of the NE concentrations are derived from sympathetic nerve terminals with the remainder secreted by the adrenal medulla and extra-adrenal chromaffin cells.[119,120] Plasma NE levels are not only determined by synaptic discharge from sympathetic nerves, but also by metabolism (degradation of NE by tissue enzymes), re-uptake by synaptic terminals and diffusion into circulation.[121] These various

routes of catecholamine elimination lead to the formation of the metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG) by the liver through the process of oxidation. MHPG levels can be measured in the plasma and saliva as an indication of sympathetic arousal.[67,121]

According to McEwen,[49] the activation of these stress-related pathways is fundamentally tailored to each individual based on behavioural (which include lifestyle factors and coping mechanisms), historical (previous exposure to stressors, major life events, circumstance and trauma) and constitutional (genetics, developmental and experience) factors that establish an individual's vulnerability and resilience to stress.[49]

Adaptive, prolonged and persistent activation of both these systems (HPA-axis and SAM) may lead to heightened susceptibility towards stress-related diseases and disorders such as hypertension and depression respectively.[49,122,123] Elevated levels of cortisol interact with NE in the amygdala; a region in the human brain that also plays a role in emotional reactions and social stress processing.[116,124,125] It increases the risk for negative affective disorders such as depression through either the effect of amygdala activity on CRH levels or the effect of chronic cortisol elevations on the amygdala.[126] Other changes associated with chronic activation of neuroendocrine pathways include the suppression of hippocampal neurogenesis, shortening and debranching of dendrites within the hippocampus, and structural atrophy/ hypertrophy, which further diminishes the body's ability to cognitively process and physiologically respond to stress.[93,126-129] These brain changes have been reported in depression and it is suggested that, among other mechanisms, the exposure to repeated episodes of hypercortisolemia and stress-induced reduction in neurogenesis may be one of the key components linking the experience of stress with the onset of depression.[94,129-131]

HPA-axis hyperactivity and hypercortisolemia has been reported in depression. For instance, patients with depression have been found to exhibit elevated CRH levels in their cerebrospinal fluid.[132-134] This leads to hyperactivity of the HPA-axis, blunted ACTH response to CRH administration and non-suppression of cortisol secretion following dexamethasone infusion.[135] However, HPA hyperactivity and hypercortisolemia is not a universal trait in depression. Hypocortisolemia has also been observed in depression, specifically in major depression with atypical features.[122] Blunted cortisol response to naturalistic stressors in women with high depressive symptoms has also been demonstrated.[136] Findings on depression in Africans are sparse; however, it appears that Africans with cognitive distress display disturbed cortisol and cortisol-testosterone ratio. [7,18,63] This may be a consequence of stress-induced SNS activation and subsequent elevation in circulating plasma NE levels, previously reported in this ethnic group.[137,138] Persistent SNS activity leads to increases in catecholamine levels that are provoked by rising cortisol levels. Further exposure to stress may result in the habituation of the neuroendocrine pathways as a consequence of the disruption in the SAM-HPA axis interaction and the dysregulation of the HPA-axis.[139-141] The authors Mashele et al. [63] demonstrated that blunted neuroendocrine responses (cortisol and salivary norepinephrine) to acute mental stress linked depressive symptoms and left ventricular hypertrophy (LVH) in Africans. This finding supports the notion that depressive symptoms may result in the habituation of neuroendocrine responses and that further exposure to stress may result in dysfunctional physiologic responses. Such responses may increase the risk for developing vascular disease and/ or other lifestyle illness in this ethnic group.[63] According to Gerra et al.[139] this demonstrates possible individual differences in neuroendocrine stress response patterns affecting the brain (mood regulation) and the body (strain on interdependent systems).[139]

5.2. HPA-axis, SAM hyperactivity and cardiometabolic risk

Maladaptive neuroendocrine mechanisms involving the HPA-axis and SAM hyperactivity may play an important role in the connection between depression and CVD. Dysregulation in HPA-axis and SAM functioning, over time, may lead to cardiometabolic adjustments that increase risk for hypertension, type 2 diabetes and atherosclerosis.[142-146]

5.2.1. Cortisol and cardiometabolic risk

Chronic elevations in cortisol levels result in a number of adverse cardiometabolic health effects that play an important role in the pathology of insulin resistant conditions such as type 2 diabetes, hypertension and dyslipidemia by interfering with several levels of insulin functioning.[21,118,143,147-150] These include impairment of insulin-dependent glucose uptake in the periphery, increased gluconeogenesis in the liver and inhibition of insulin secretion by the pancreatic β-cells.[114,151,152] The ensuing resistance to the effects of insulin on glucose metabolism results in an increase in glucose and insulin levels in the peripheral tissues, and subsequently promotes atherogenesis and hypertension.[153,154]

Cortisol also plays a pivotal role in the etiology of abdominal obesity, a key feature of metabolic syndrome, that has been linked with type 2 diabetes, hypertension and atherosclerosis.[4,30,31,155-157] On cellular level glucocorticoids (primarily cortisol) exert their effects by binding with specific cytoplasmic GR. Intra-abdominal adipose tissue has been found to have a higher density of GR than any other region the body.[158,159] By binding with these receptors cortisol stimulates lipid accumulation and lipolysis of fat in adipose tissue.[160,161] These functions are mediated by the increase in lipoprotein lipase (LPL) activity; the primary enzyme responsible for converting lipoprotein triglycerides into fatty acids and

accumulating triglycerides in adipose tissue.[162] In the presence of insulin the stimulatory effect of cortisol on LPL activity is attenuated resulting in a state where fat accumulation is augmented.[149,162]

Cortisol is also associated with the development of arterial hypertension by activating, enhancing and suppressing various mechanisms linked with the regulation of blood pressure. Cortisol binds with mineralocorticoid receptors (MR), which display similar binding affinities for aldosterone and cortisol, and initiate a series of intracellular mechanisms that lead to an increased tubular reabsorption of sodium causing sodium retention as well as the expansion of extracellular and blood volumes.[163,164] Other hypertension-promoting effects of cortisol include stimulation of angiotensinogen production by the liver.[143,164] Such stimulation influences vascular volume homeostasis and vascular tone. It also increases the sensitivity of the vasculature to catecholeamines and angiotensin II vasoconstrictive actions.[165,166] Cortisol also down-regulates expression of Na^{+}-Ca^{2+} exchanger in vascular smooth muscles. This causes elevations in intracellular calcium and vascular resistance and reduces activities of vasodilatory systems such as the nitric oxide synthase system.[167-169] These vasoconstrictive effects of cortisol lead to an increase in vascular resistance and HT risk.[170]

Meyburg et al.[171] demonstrated dysregulation of cortisol and blood pressure in Africans exposed to chronic psychosocial stress. These effects were exaggerated when defensive coping style strategies were utilised.[171]

5.2.2. Catecholamines and cardiometabolic risk

Secretion of catecholamines, in particular NE, may increase cardiometabolic risk by eliciting its effects on the vasculature and on adipose-tissue.[172,173] Salivary MHPG levels are associated with the risk of MetS, mainly the obesity component of the syndrome, in Africans with moderate-severe depressive symptoms.[138]

Stimulation of the locus coeruleus, the principle noradrenergic nuclei located in the brain stem, leads to secretion of NE and activation of α-adrenergic pathways. This may contribute to an increase in peripheral vasoconstriction that may in turn increase peripheral vascular resistance and contribute to the development of essential HT.[174] Visceral adipose tissue has a high density of lipolytic β_3-adrenergic receptors that respond to the stimulation of NE by initiating lipolytic activity within these cells.[171,175] Expression of these lipolytic β-adrenergic receptors in adipose tissue is mediated by excess cortisol and results in the release of free fatty acids into the liver via the portal circulation.[176] Accumulation of free fatty acids in the liver may influence hepatic metabolism and contribute to metabolic disturbances in several ways. Fatty acid oxidation in the liver reduces insulin's binding affinity to its receptors leading to a reduction in insulin function that includes the inhibition of gluconeogenesis. This process is regulated by peroxisome proliferator-activator receptor (PPARγ) activation and an elevation in peripheral insulin levels.[11,177,178] Together, this increase in peripheral glucose (gluconeogenesis), insulin and free fatty acids may lead to insulin resistance in the peripheral tissues.[151] The inadequate glucose utilisation that results from insulin resistance underlies the neuronal changes in limbic system observed among patients with depression.[179] Drugs that increase insulin sensitivity by targeting the nuclear hormone receptor PPARγ, such as thiazolidinediones, have been shown to act as antidepressants in animal models, and as enhancers of antidepressant action in patients with depression.[180,181]

5.3. Depression, inflammation and hypercoagulation

5.3.1. Depression, inflammation and cardiometabolic risk

Evidence from studies suggests that vascular inflammation may play a pivotal role in the pathogenesis of atherosclerosis.[182-184] Elevated inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) have been established as predictors of CVD, for example, CHD.[185,186]

Neuroendocrine hormones relevant to depression may also regulate inflammatory processes; cortisol is implicated in the regulation of the immune system. Release of cortisol from the adrenal cortex is associated with immune-suppression.[115] However, chronic secretion of cortisol may increase inflammatory risk through direct and indirect mechanisms. For instance, Walker et al.[187] demonstrated that circulating cortisol levels may directly promote perivascular inflammation.[187] Indirectly, cortisol may be associated with inflammation through its link with cardiometabolic risk factors (Figure 5), particularly abdominal obesity.

Abdominal adipose tissue also functions as an endocrine organ, producing and secreting a number of peptide hormones, including resistin and leptin. Adipose tissue also secretes a variety of cytokines, such as angiotensinogen, plasminogen activator inhibitor-1 (PAI-1), TNF-α, IL-6 and IL-18.[188] Circulating pro-inflammatory cytokines inhibit LPL activity and increase levels of fatty acids, contributing to insulin resistance and dyslipidemia.[189] Additionally, these cytokines (TNF-α, IL-6 and IL-1β) stimulate the synthesis of acute-phase proteins such as CRP in the liver, adipose tissue and vascular smooth muscle cells.[190,191,192] CRP interferes with insulin signaling and expression of corticosteroid-binding globin that

leads to an increase in free cortisol levels, subsequent insulin resistance and other manifestations of the MetS.[193]

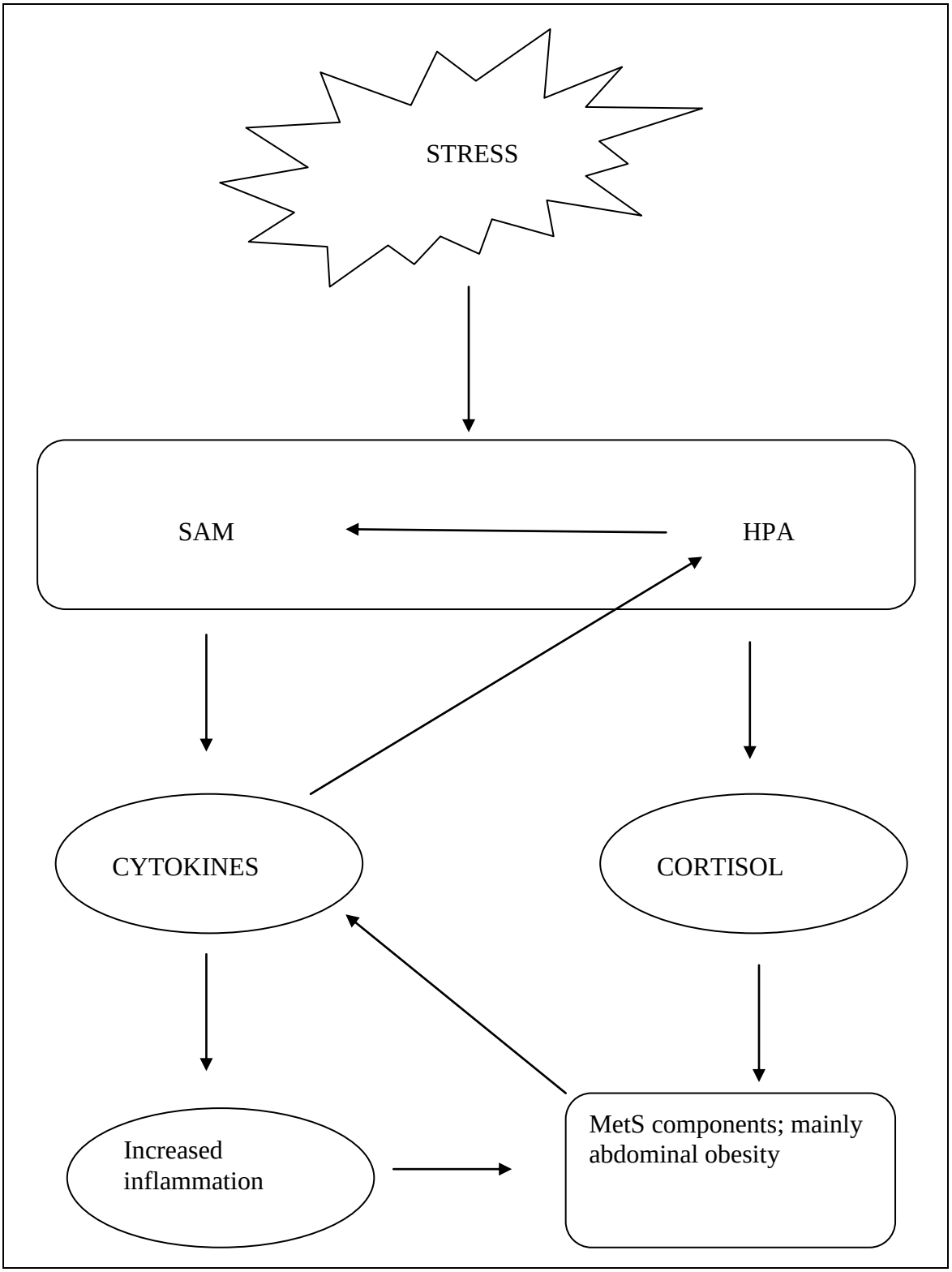

Figure 5: An overview of the neuroendocrine pathways, metabolic syndrome and inflammation.[193]

Catecholamines (particularly NE) may also regulate immune activity and, subsequently, inflammation.[194] Secretion of catecholamines from the sympathetic nerve terminals in lymphoid tissues act on lymphoid cells that express β-adrenoreceptors, stimulating the secretion of both pro-inflammatory and anti-inflammatory cytokines.[76, 195] Lymphoid tissue is innervated with noradrenergic nerve fibres that secrete NE when stimulated. Together with the NE secreted from the blood vessels and epinephrine from the adrenal medulla, the increases in NE modulate immune responsiveness by stimulating the humoral and suppressing cellular immunity.[194,196] Endogenous catecholamine secretions inhibit the production of type 1/pro-inflammatory cytokines. They stimulate immune cells that target intracellular pathogens such as natural killer cells and cytotoxic T cells. They also potentiate the production of type 2/anti-inflammatory cytokines that mediate humoral immunity. This is done by stimulating B cell antibody production against antigens.[194,197] In certain local responses and under certain conditions, such as acute and chronic stress, catecholamines may actually augment regional immune responses through the stimulation of primary pro-inflammatory cytokines TNF-α, IL-1, and IL-8; and through inhibiting transforming growth factor (TGF-β) production.[194] Thus, situations that are associated with significant changes in neuroendocrine activity, such as psychological stress and depression, may stimulate autoimmune activity and/ or progression of inflammation through modulation of the systemic or local pro-/ anti-inflammatory cytokine balance.[12,13]

Depression has been described as an inflammatory illness.[13] Increased pro-inflammatory cytokines such as TNFα, IL-6 and IL-1 have been noted in patients with depression.[72,75] These inflammatory cytokines have been shown to access the brain and interact with virtually every pathophysiologic domain known to be involved in depression including monoamine metabolism, neural plasticity and neuroendocrine regulation.[72,75] As previously mentioned,

activation of inflammatory pathways may contribute to neural changes by decreasing neurotrophic support and altering glutamate release/re-uptake leading to oxidative stress, excitotoxicity and glial damage.[69,72,77] Both oxidative stress and inflammatory cytokines may activate tryptophan breakdown (Figure 6). Indoleamine 2, 3-dioxygenase (IDO) and its hepatic equivalent tryptophan 2, 3-dioxygenase (TDO) metabolize tryptophan to form kynurenine.[198] Under normal circumstances, TDO is the dominant enzyme is the more dominant enzyme. However, during conditions when pro-inflammatory cytokines are released, IDO subject to induction (depicted by the dotted line in Figure 6) which leads to an increase in the kynurenine pathway activity eliciting an increase in tryptophan metabolism and reduction in tryptophan concentration available for conversion into serotonin and kynurenine toxicity.[199-201]

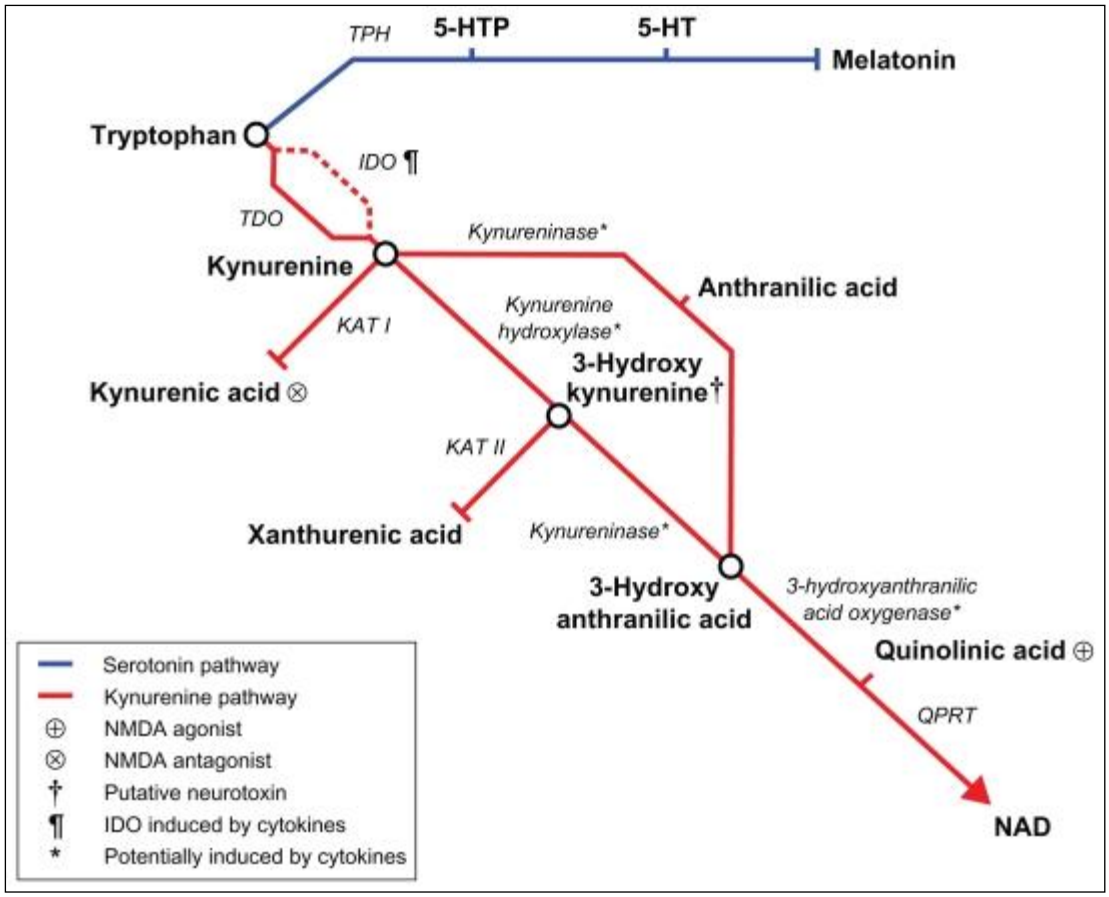

Figure 6: Tryptophan metabolic pathway. Abbreviations: IDO, Indoleamine 2, 3-dioxygenase; TDO, tryptophan 2, 3-dioxygenase; 5-HTP, 5-hydroxytryptophan; 5-HT, serotonin; NAD, nicotinamide adenine dinucleotide; KAT, kynurenine aminotransferase;QPRT, quinolinate phosphoribosyltransferase; NMDA, N-methyl-D-aspartate.[199-201]

Distress, disturbed cortisol and NE have been found in an African male cohort.[63, 201] In the same cohort, low-grade inflammation was strongly linked with target organ damage, increasing this groups' vulnerability to CVD .[202] Additionally, altered redox activity which is commonly associated with inflammation has been described in this population.[203] Reimann et al. [203] demonstrated that the L-Arginine/NO system is affected in Africans with cognitive distress.[203] These findings may suggest a pro-inflammatory state in Africans with cognitive

distress which may decrease insulin and GR sensitivity further perpetuating metabolic and neuroendocrine disturbances previously described in this ethnic group.[7,8,15-17] These findings still need to be substantiated in Africans with depressive symptoms.

5.3.2. Depression, haemostasis and cardiometabolic risk

Within the last couple of years, haemostatic factors have emerged as 'new' risk factors for coronary artery disease (CAD). Increased levels of haemostatic markers such as fibrinogen, PAI-1 and D-dimer have been implicated as predictors of coronary artery syndromes in individuals with CAD and in healthy populations.[204,205] Evidence from the literature indicates that depression is associated with alterations in haemostasis, in particular hypercoagulation.[206] Indeed, blood coagulation, fibrinolysis, D-dimers, PAI-I, platelet activation, vascular endothelial growth factor (VEGF), plasma nitric oxide and its synthase are altered in psychological distress and depression.[203,207]

Africans showed greater hypercoagulability at rest but diminished pro-coagulant reactivity to acute mental stress when compared with Caucasians.[208] Ethnic differences in vascular adrenergic stress response might partially explain the finding. Depressive symptoms were associated with exaggerated endothelial dysfunction in Africans compared to Causasians.[208] We need to know what the association is between coagulation and inflammation in Africans presenting depressive symptoms. This may contribute to the current speculations on low-grade inflammation-inducing depression and vice versa.

Blood coagulation and fibrinolysis

The haemostatic process and the formation of a thrombus is mediated by two blood clotting pathways: the intrinsic (contact activation) pathway, which is slower and depends on circulating clotting factors (factor VIIIa), and the more rapid extrinsic (tissue factor) pathway, triggered by the interaction between extravascular factor (tissue factor) and activated Factor VIIa.[209-211] Tissue factor, the major physiological initiator of the coagulation cascade, is a membrane-integrated protein that is expressed on vascular cells only upon injury or on monocytes and endothelial cells in response to a variety of stimuli.[211] Activation of the coagulation system results in the formation of thrombin from prothrombin, which converts fibrinogen into insoluble fibrin and induces platelet activation. By binding to platelet glycoprotein IIb/IIIa, fibrinogen induces platelet aggregation (Figure 7).[210,211]

Activation of the fibrinolytic system leads to the conversion of plasminogen into plasmin by tissue plasminogen activator (tPA), the main fibrinolytic stimulator. Plasmin promotes the degradation of fibrin clots and thrombi into soluble fragments, fibrin degradation products, of which D-dimer is the widest known. PAI-1, the primary inhibitor of the fibrinolytic process, inhibits tPA activation by binding tPA and forming PAI-1/tPA complexes. Therefore, any impairment in fibrinolytic function may be reflected by an elevation in PAI-1 or tPA antigen (inactive PAI-1/tPA complex) and or low plasma levels of tPA activity and activation products such as D-dimer. Elevated levels of these markers indicate a reduced fibrinolytic function, favouring fibrin persistence and thrombosis.[211,212]

Most of these coagulation and fibrinolytic markers can be measured by immunological [enzyme-linked immunosorbent assay (ELISA)] or functional (molecule activity) techniques.[213] These haemostatic variables and the prothrombotic tendency they may create

may be involved in the initiation of early atherosclerotic lesions and contribute to the pathogenesis of atherosclerotic cardiovascular disease.[214] For instance, D-dimer is associated with an increased risk for CHD and ischemic heart disease.[215,216] Fibrinogen and von Willebrand factor (vWF) have been linked with atherosclerosis and myocardial infarction, respectively.[217,218] Plasma fibrinogen, resulting from a decrease in fibrinolytic activity, also promotes platelet aggregation; vascular smooth muscle migration, proliferation and contribute to blood viscosity and thrombi.[219] Higher fibrinogen levels as well as self-reported cognitive distress were evident in urban Africans.[7]

Elevations in catecholamines and cortisol may underlie the hypercoagulabilty, which may be mediated by increases in clotting factors like VIII and vWF, as observed in depression.[210] As part of normal haemostatic functioning, sympathetic activation provokes a simultaneous increase in molecules of both the coagulation and fibrinolysis pathways within minutes, resulting in net hypercoagulability.[220] However, sympathetic hyperactivity, mediated by catecholamines and adrenergic receptor activation, may cause an exaggeration in clotting activities that might confer an increased arterial thrombotic risk in individuals with a pre-existent atherosclerotic disease. In those experiencing ongoing stressful life circumstances sympathetic hyperactivity may increase the risk for cardiovascular morbidity and mortality.[220] Malan et al. [221] revealed an endothelial dysfunction associated with attenuated stress hormones, testosterone and peripheral vascular responses in African men. This may underpin vascular changes and increased morbidity risk in this ethnic group .[221] However, it is not clear if a notion of inflammatory-hypercoagulation status may increase their vulnerability should depressive symptoms be evident.

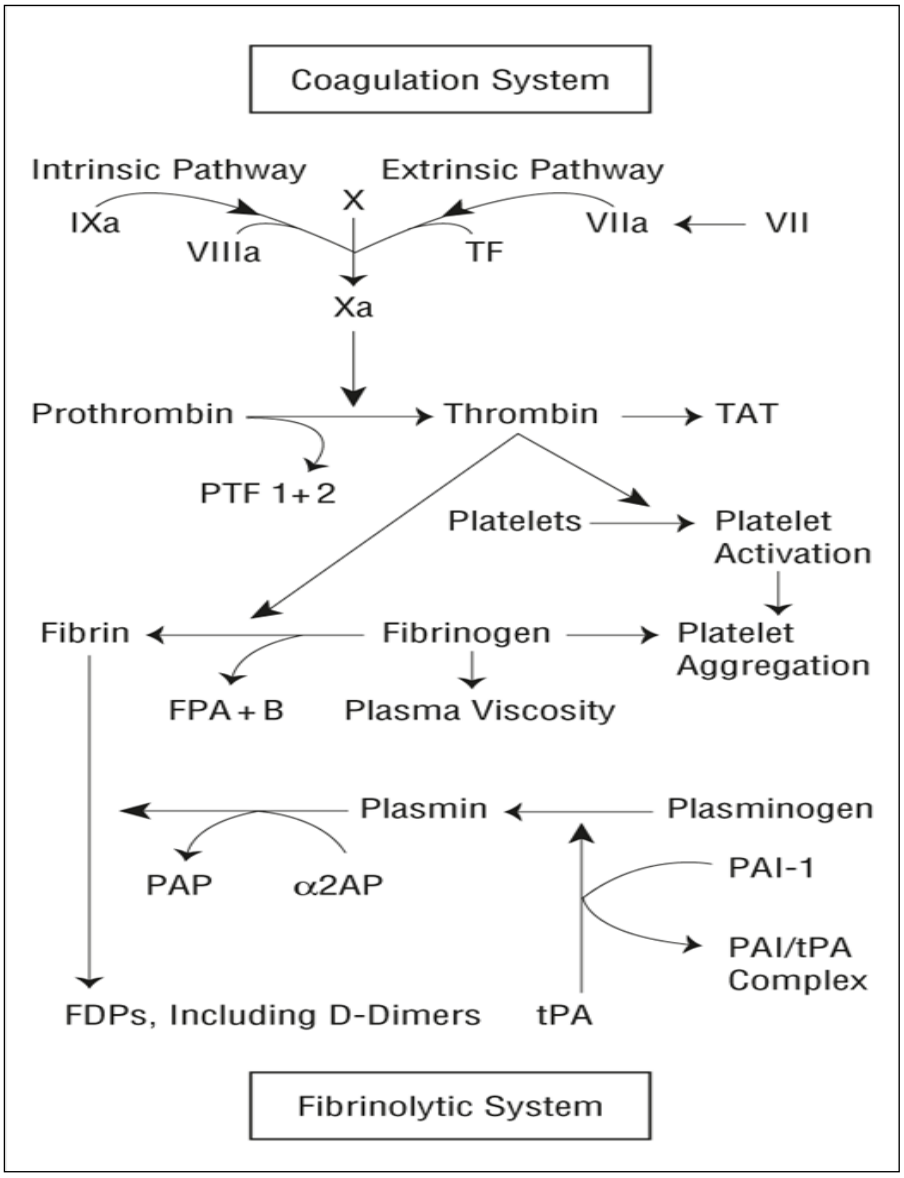

Figure 7: A schematic overview of the haemostatic and fibrinolytic pathways involved in coagulation. Outlined in solid arrowheads is the sequence of activation. Abbreviations: TF, tissue factor; TAT, thrombin-antithrombin complex; PTF, prothrombin fragments; FPA + B, fibrinopeptides A and B; PAP, plasmin-antiplasmin complex; α2AP, α_2-antiplasmin; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activatorand FDPs, fibrinogen degradation products .[211]

5.4. Cardiometabolic dysfunction and left ventricular hypertrophy

LVH is a marker of structural abnormality and an increase in left ventricular mass. Hypertrophy of the left ventricle is thought to occur as a result of volume and pressure overload during which terminally differentiated cardiomyocytes undergo hypertrophic changes.[222,223] LVH is a strong predictor of cardiovascular morbidity and mortality.[224]

Neurohormones, associated with depression, may increase the development of LVH via direct and indirect actions on the heart or though their association with cardiometabolic risk. Directly, neuroendocrine pathways that involve angiotensin II, endothelin-1 and catecholamines produce hypertrophy through G-protein mediated phosphoinoistide 3-kinase (PI3K) activation.[225] PI3K regulates various physiological process including membrane trafficking, adhesion, actin rearrangement, cell growth and cell survival.[226] Its activation under pathological conditions leads to the activation of phospholipase Cβ and the dephosphorylation of phosphatidylinositol trisphosphate (PIP_3) into diacylglycerol (DAG) and inositol triphosphate (IP_3).[227] IP_3 binds to receptors in the sarcoplasmic reticulum (SR) resulting in the release of calcium (Ca^{2+}), which activates phosphatase calcineurin together with calmodulin. Calcineurin dephosphorylates the nuclear transcription factor of T cells (NFAT), allows the nuclear translocation and modulation of gene expression of hypertrophic response genes.[225] DAG, together with the IP_3-mediated release of calcium from the SR, activates members of the PKC family, which can also alter hypertrophic gene expression.[228]

Indirectly, persistent activation of the hypothalamic and sympatho-hormonal regions results in cardiovascular adjustments that may increase the risk for HT and maintain volume and pressure overload in hypertensive states.[229] Volume and pressure overload are important

stimuli for ventricular remodeling and ventricular dysfunction.[222] Over time persistent activation of these stress systems, coupled with HT status, results in structural and functional changes in the heart and blood vessels leading to LVH.[222] Conversely, Malan et al. [230] demonstrated disturbed vascular responses associated LVH in chronic defensive-coping African men, but not in their Caucasian counterparts.[230] These men also showed augmented sympathetic nervous system activity and cognitive distress.[63,138,173] Vulnerability in the urban African men may be detrimental to cardiovascular morbidity and mortality.

Studies show that other cardiometabolic risk factors such as dislipidemia, glucose intolerance (insulin resistance) and obesity are associated with LVH.[231-233] For instance, Sciacqua et al. [232] found that glucose tolerance status was associated with higher left ventricular mass (an index of LVH) and greater prevalence for LVH.[232] Ledgedz et al.[233] demonstrated that other metabolic disturbances such as elevated levels of triglycerides and insulin resistance may contribute to cardiac hypertrophy and arterial stiffening independent of hemodynamic and hormonal markers.[233] These cardiometabolic risks often cluster together in certain individuals which may account for LVH linked with the metabolic syndrome.[234]

6. MOTIVATION, AIMS AND HYPOTHESES FOR EACH PAPER IN THIS STUDY

This thesis consists of three manuscripts submitted for publication. Since the relevant background is discussed in the papers, only a brief motivation, aims and proposed hypotheses for each manuscript will be discussed in this section.

6.4. Manuscript 1 (Chapter 2)

Depression, cardiometabolic function and left ventricular hypertrophy in African men and women: the SABPA study.

Literature indicates that urban living in Africans is associated with cardiometabolic risk factors such as hypertension, type 2 diabetes and obesity.[7,8,15-17]The accumulative effects of these cardiometabolic components, including abdominal obesity, may increase the risk for cardiovascular morbidity and mortality in this population group.[31] More importantly, these cardiometabolic risk factors have been associated with depression.[235-237] Whether this association is evident in Africans with depressive symptoms remains to be investigated. Therefore, this study aimed to investigate the possible link between depressive symptoms and cardiometabolic risk in urbanised African men and women. The secondary aim of this study was to determine the mediating factors for LVH in the determined sample.

Hypothesis

Africans with depressive symptoms (men and women) will have a significantly higher prevalence of cardiometabolic risk factors. These factors, in particular blood pressure, will be significantly associated with LVH in both genders.

6.5. Manuscript 2 (Chapter 3)

Blunted neuroendocrine responses linking depressive symptoms and ECG left ventricular hypertrophy in black Africans: the SABPA study.

Repeated exposure to stress leads to the dysregulation of normal endocrine functioning and increased risk for cardiometabolic alterations such as hypertension and type 2 diabetes.[143-145] Black Africans (referred to in this manuscript as Africans) have been shown to respond with increased sympathetic responsiveness to stressful situations.[15] The influence of depression in neuroendocrine functioning and the interaction thereof with target organ damage in this ethnic group were investigated. The primary aim of this study was to examine the differences between urban Africans with depressive symptoms and those without depressive symptoms in terms of neuroendocrine responses and cardiometabolic risk factors. The secondary aim was to determine which of these factors were significantly associated with the development of LVH.

Hypothesis

Africans with depressive symptoms will display greater neuroendocrine (cortisol and MHPG) responses to stress as opposed to Africans without depressive symptoms. Those with a greater cortisol response will demonstrate significantly higher metabolic risk profiles. These higher risk profiles will, in turn, prove to be a predictor for LVH.

6.6. Manuscript 3 (Chapter 4)

Hypercoagulation vulnerability exacerbated by hypertension state in black Africans with depressive symptoms: the SABPA study.

Depression has been described as an inflammatory illness.[13] However, literature on this relationship has produced conflicting findings. It appears that cognitive distress is associated with inflammation and target end-organ damage in black Africans (referred to in this manuscript as Africans).[202] This relationship is yet to be determined in Africans with depression.

Depression has been associated with a dysregulation in blood coagulation, fibrinolysis, D-dimers, PAI-1, platelet activation, vascular endothelial growth factor, plasma nitric oxide (NO) and its synthase.[203,207] Reimann et al.[203] demonstrated an alteration in L-Arginine/NO activity in Africans with psychological distress.[203] Whether this dysregulation in haemostatic functioning is evident in Africans with depressive symptoms is unknown. Therefore, the aim of this study was to investigate the relationship between depressive symptoms, inflammation and haemostatic markers in African men and women.

Hypothesis

Africans with depressive symptoms will display a thrombophilic and inflammatory state in comparison to those without depressive symptoms.

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Chapter 2: Research Article 1

Depression, Cardiometabolic function and Left Ventricular Hypertrophy in African Men and Women: the SABPA study

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Chapter in Book: Kaplan NM, Ed. Primary hypertension: natural history and evaluation. In:

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Conference Proceeding: Kario K, Rapoport D. Sleep-disordered breathing as a determinant of non-dipping status of nocturnal blood pressure independent of age and body mass index. The 73rd Scientific Sessions, American Heart Association, Atlanta, GA, November 12-15, 2000.

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Abstract

Depressive symptoms are associated with an increased risk for developing cardiovascular diseases, driven by its link to the metabolic syndrome. This phenomenon, however, still needs to be investigated in the black African (hereafter referred to as Africans) population. The aim of this study is to investigate the association between left ventricular hypertrophy (LVH) and metabolic syndrome (MetS) risk markers. The researchers stratified African men and women into a group with depressive symptoms (D) and a group without (ND) depressive symptoms, based on the DSM-IV criteria score. Fasting MetS, chronic hyperglycemia (HbA1c), ambulatory blood pressure and ECG Cornell product-LVH (CP-LVH) measures were obtained.

Depressive symptoms were reported in 45.3% of the sample. Irrespectiveof depression status, African men and women revealed a pre-diabetic state (glycated hemoglobin > 5.7%). CP-LVH was associated with decreased high-density lipoprotein (HDL-chol) in D African women. In D African men systolic blood pressure (P = 0.001) and HbA1c (P = 0.08) explained 64% and 31% of the variation in LVH respectively.

In conclusion, depressive symptoms in African women were associated with a measure of target end-organ damage, CP-LVH; an association driven by a metabolic factor. In African men, independent of depressive symptoms, LVH was driven by cardiometabolic factors, namely SBP and HbA1c.

Key words: Depressive symptoms, cardiometabolic risk, left ventricular hypertrophy, Africans.

Introduction

According to the World Health Organisation (WHO), approximately 5 to10 % of the population at any given time suffers from depression that is severe enough to require psychiatric treatment or psychosocial intervention (1). A considerable body of evidence suggests that depression may be associated with cardiovascular diseases (CVD), (2-4). Although the association between depression and CVD has been established, the underlying relationship is not well defined or understood (5). The metabolic syndrome (MetS) is increasingly placed at the forefront in the relationship between depression and CVD, thus linking all three conditions with adverse health outcomes (6).

Cardiometabolic risk is a term used to describe a combination of factors common to both metabolic and cardiovascular risk, and includes elevated fasting plasma glucose and triglycerides, low levels of high-density lipoprotein cholesterol (HDL-chol), hypertension (HT), a sedentary lifestyle, higher dietary fat intake and psychosocial factors such as urbanisation and depression (7,8).

Studies indicate that depression is associated with various risk factors common to both the MetS and CVD (9-12). An association between depression, large waist circumference (WC) and low HDL-chol has been found (9), but most research only consider data on females due to a small sample population (11). Several explanations have been proposed for the link between depression and cardiometabolic risk. Viltaliano et al. (12) propose a behavioural factor linking depression and CVD risk, with poor health habits leading to the MetS and subsequently, CVD (12). As far as could reasonably be ascertained by the authors of the current study, no studies have addressed the relationship between depressive symptoms, metabolic syndrome indicators and cardiac endpoints like left ventricular hypertrophy (LVH)

in black Africans (hereafter referred to as Africans). Depressive symptoms are the psychological constructs of interest in this investigation and the aim of this study is to investigate the possible association between cardiometabolic function and the Cornell product LVH (CP-LVH) in Africans with depressive symptoms.

Methods

Study population and procedures

Participants for this study were recruited as part of the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study. The study was a cross-sectional, multi-disciplinary, target population study conducted between February 2008 and May 2009. The study sample comprised 179 urban African teachers aged between 25 and 60 years and working within the Dr Kenneth Kaunda Education district in the North-West Province in South Africa. The purpose of this choice was based on the need to obtain a homogeneous sample from the same socio-economic grouping. The exclusion criteria for this study sample included an ear temperature above 37 °C, users of psychotropic agents, pregnancy, lactation, the use of α- and β-blockers and individuals who had been vaccinated or had donated blood in the three months prior to participating in this study. For the purpose of this study, participants were HIV positive (N= 19) and statin uses (N= 2) were also excluded from statistical analysis. Participants were fully informed regarding the objectives and procedures of the study two months before data collection commenced. Assistance was offered to participants who requested information in their native language, whereafter each was requested to sign an informed consent form. In accordance with ethical guidelines of the World Medical Association Declaration of Helsinki 1975 (revised in 2008), the necessary clearance was granted by the Ethics Committee of the North-West University (NWU) (13).

The participants were transported at approximately 16h40 on the day concerned to the Metabolic Unit Research Facility of North-West University, a research unit for human studies. Upon arrival participants were familiarised with the available facilities and the applicable protocol. This was followed by the completion of a collection of psychosocial questionnaires under the supervision of a registered psychologist and fieldworker, and a dinner. The participants were encouraged to go to bed at approximately 22h00, fasting overnight, and were awoken the following morning at 05h45 to undergo a battery of clinical assessments.

Clinical assessment procedures

Registered biokineticists and nurses collected anthropometric data and venous blood samples from each participant. The anthropometric measurements (including height, weight and waist circumference) were standardised and taken in triplicate to the nearest 0.1 cm. Circumferences were measured with a metal tape at the midpoint between the lower costal border and the iliac crest perpendicular to the long axis of the trunk (14).

Participants then underwent assessments for left ventricular hypertrophy (LVH), which was calculated from a 12-lead ECG (NORAV PC 1200) device connected to each participant while in a semi-Fowler's position. Using strip leads RaVL + SV3 in the calculation of a gender-specific formula, the Cornell product was calculated as the sum of all leads (RaVL + SV3)* QRS. Values > 244 mV.ms (15, 16), implies higher LVH.

On the morning of the working day prior to the clinical assessment, the participants were each fitted with a 24-hour ambulatory blood pressure monitoring device (Meditech CE0120 CardioTens® Budapest, Hungary) on the non-dominant hand. The device was programmed to take blood pressure at 30-minute intervals during the day (08h00 to 22h00) and at 60-

minute intervals during the night (20h00 to 06h00). The mean successful inflation rate was 75.4% (± 9.6%) in African men and 69.7% (± 13.2%) in African women. Data from the device was analysed using the CardioVisions 1.15.2 Personal Edition Software (Meditech). The participants were also fitted with an Actical ® accelerometer (Montréal, Québec) around their hips during the working day. Data from the device was used to assess physical activity.

Depressive symptoms

Depressive symptoms were assessed using a self-reported Patient Health Questionnaire (PHQ-9); a questionnaire that has been validated in primary health care settings and for use on sub-Saharan Africans (17-19). In the current sample study PHQ-9 displayed good reliability, and the Cronbach alpha-reliability index for this sample was 0.81. The questionnaire is based on the nine criteria specified in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV). Each item of the PHQ-9 evaluates the presence of one of the nine DSM-IV criteria of major depression. The PHQ-9 provides a continuous score of depression severity, and scores of 15 or greater are considered to be indicative of major depression (17). The recommended and established PHQ-9 cut-off point of greater than ten was used to indicate the presence of depressive symptoms, stratifying the gender group into sections with depressive symptoms (D) and without depressive symptoms (ND) (18).

Metabolic syndrome

Consistent with the operational definition outlined by the International Diabetes Federation (IDF), 'metabolic syndrome' was defined as central obesity (or WC > 94 cm in men or > 80 cm in women in sub-Saharan Africans), plus any two of the following factors: elevated blood pressure (systolic of ≥ 125–130 mmHg or diastolic > 80 mm Hg); elevated triglycerides (1.7

mmol/l); reduced high density lipoprotein (1.1 mmol/l in men or 1.3 mmol/l in women); or elevated fasting glucose (≥ 5.6 mmol/l) (8).

Biochemical analysis

Fasting blood samples were handled and prepared according to standardised methods and were stored at –80°C. EDTA whole blood turbidometric inhibition immunoassay analyses determined glycated haemoglobin (HbA1c) values. The HbA1c values reflect an average capillary glucose equivalent for the preceding 2-3 months, respectively (Integra 400 Roche, Switzerland). Fasting sodium fluoride (glucose) and serum samples for triglycerides, high density lipoprotein (HDL-chol) and gamma glutamyl transferase (γ-GT) were analysed using two sequential multiple analysers (Konelab™ 20i; Thermo Scientific, Vantaa, Finland; Unicel DXC 800 – Beckman and Coulter®, Germany). Participants' smoking status was defined by measuring serum cotinine levels using the homogeneous immunoassay (Modular ROCHE Automised, Switzerland); and values greater than 14.99 μg/l were considered as indicative of smoking status (20). The intra- and inter coefficients of variation for all assays were below 10%.

Statistical Analysis

All data was analysed using the software package STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA, 2010). γ-GT were logarithmically transformed to normalise distribution. A single one-way ANCOVA tested interaction on the main effects (gender × depressive symptoms) for all metabolic syndrome markers and CP-LVH. Differences in baseline characteristics of participants in relation to depressive symptoms were analysed using t-tests for continuous variables and chi-square for categorical data. Significance between genders (high depressive

symptoms versus low depressive symptoms) was computed with one-way ANCOVA, independent of bio-behavioural risk markers [including age, body mass index (BMI), physical activity (kcal/h), log-transformed γ-GT and smoking status (%)].

By using single- and multiple regression analysis, the associations between bio-behavioural risk markers, MetS indicators and CP-LVH were measured. Stepwise-forward regression analyses were computed in four models (gender × depression) with CP-LVH as the dependent variable and considered age, BMI, physical activity (kcal/h), log γ-GT, smoking status (%), 24-hour systolic BP and HDL-chol as covariates. All data was considered statistically significant if $p \leq 0.05$ and trend as $p \leq 0.08$.

Sensitivity analysis

We repeated the forward stepwise regression analyses by adding HbA1c as covariate in the depression and non-depression models (models 1-4). Other covariates were age, BMI, physical activity (kcal/h), log γ-GT, smoking status (%), 24-hour systolic BP and HDL-chol.

Results

General characteristics

Depressive symptoms were reported in 45.3% of the sample with women (56.8%) reporting more depressive symptoms than the men (43.2%). Women with depressive symptoms tended to smoke more ($P = 0.07$) than those without (see Table A.1). Factors like age, BMI, physical activity (kcal/h), γ-GT , MetS and hypertension (HT) had no significant influence on the presence (or lack thereof) of depressive symptoms.

Table A.1: Characteristics of participants at baseline in depressed (D) and non-depressed (ND) Africans (mean ± SD)

	African men			African women		
	D (*n* = 35)	ND (*n* = 52)	*p*-value	D (*n* = 46)	ND (*n* = 46)	P-value
Age (years)	41.97 ± 8.2	43.81 ± 8.3	0.30	45.43 ± 8.2	45.51 ± 8.1	0.94
Body mass index (kg/m^2)	28.13 ± 5.8	27.23 ± 5.7	0.54	32.73 ± 7.0	33.17 ± 7.6	0.78
Gamma-glutamyl transferase (μ/L)	70.8 (57.5–87.1)	56.2 (47.9–67.6)	0.24	38.0 (32.4–45.7)	33.1 (27.5–39.8)	0.19
Smoking *n* (%)[a]	13 (50.0)	13 (50.0)	0.26	13 (68.4)	9 (31.6)	0.07
Physical activity (kcal/h)	2722.4 ± 833.8	2670.4 ± 788.5	0.83	2724.4 ± 854.1	2622.4 ± 726.4	0.54
Hypertension status *n* (%)[b]	28 (41.8)	39 (58.2)	0.59	27 (51.9)	25 (48.1)	0.67
Metabolic syndrome *n* (%)[c]	19 (50.0)	19 (50.0)	0.10	19 (44.2)	24 (55.8)	0.30

[a] 'Smoking' was defined as serum cotinine (ng/ml) values > 14.99 μg/l. [b] Hypertension is based on the 24-hour classification; systolic andor diastolic > 125 and or > 80 mmHg (European Hypertension Society Guidelines, 2011).
[c] Metabolic syndrome is based on IDF criteria (8).
Data is considered as statistically significant $p \leq 0.05$.

Depression and cardiometabolic variables

A single two-way ANCOVA interaction on the main effects (gender x depressive symptoms) was evident for CP-LVH [*F* (1.14) = 5.97, P = 0.02]. As is clear from Table B.1, African men with depressive symptoms demonstrated a trend (P= 0.06) of increased abdominal obesity at a 94% level. Men without depressive symptoms, however, revealed increased CV risk with higher CP-LVH (Mean = 91.74; P = 0.05). Irrespective of their respective depression status, the men displayed mean high risk characteristics such as chronic hyperglycemia (> 5.7%), 24-hour systolic and diastolic BP ≥ 125 mmHg and > 80 mmHg respectively, triglyceride levels above 1.7 mmol/l and elevated fasting plasma glucose levels ≥5.6 mmol/l (8).

Table B.1: Adjusted[a] differences in cardiometabolic variables in depressed (D) and non-depressed (ND) African men and women (mean ± 95% CI).

	African men			African women		
	D men	ND men	*p*-value	D women	ND women	*p*-value
Waist circumference (cm)	95.07 (93.0; 97.0)	92.65 (90.8; 94.1)	0.06	93.99 (91.6; 96.4)	93.79 (91.4; 95.8)	0.67
HDL cholesterol (mmol/l)	1.14 (1.0; 1.3)	1.04 (0.9; 1.1)	0.23	1.22 (1.1; 1.3)	1.21 (1.1; 1.3)	0.80
Triglycerides (mmol/l)	1.91 (1.3; 2.5)	1.71 (1.2; 2.2)	0.61	1.05 (0.9;1.1)	0.92 (0.8; 1.1)	0.15
Glucose (mmol/l)	6.23 (5.5; 7.0)	5.96 (5.4; 6.6)	0.57	5.69 (5.0; 6.3)	4.90 (4.3; 5.5)	0.08
HbA1c (%)	6.47 (6.0; 6.92)	6.16 (5.8; 6.5)	0.29	5.98 (5.7; 6.3)	5.73 (5.4; 6.0)	0.22
Ambulatory SBP (mmHg)	139 (134; 144)	137 (133; 142)	0.61	127 (122; 132)	131 (126; 135)	0.50
Ambulatory DBP (mmHg)	89 (85; 93)	88 (84; 91)	0.87	78 (75; 80)	80 (78; 83)	0.28
CP-LVH (mV)	**68.95 (52.3; 86.4)**	**91.74 (77.0; 105.9)**	**0.04***	57.75 (49.2; 66.3)	56.53 (48.6; 64.4)	0.70

Values are geometric mean (5^{th} to 95^{th} percentile interval); HbA1c, glycated hemoglobin; CP-LVH, Cornell product- left ventricular hypertrophy.

[a] Values adjusted for age, body mass index, physical activity, log γ-GT and smoking (%).

*Data in bold is regarded as statistically significant.

Women with depressive symptoms revealed a pre-diabetic state with a mean HbA1c > 5.7%, a tendency towards higher plasma glucose levels (P = 0.08) in comparison to those without depressive symptoms.

In Table C.1 the results from the forward stepwise regression analyses, investigating potential independent predictors for CP-LVH, are reported. An independent association was observed between CP-LVH and SBP in men with and without depressive symptoms, whereas in women with depressive symptoms CP-LVH was independently associated with low HDL-chol levels. The sensitivity analyses revealed a trend in depressed men where a pre-diabetic state explained 31% of the variation in the CP-LVH.

Table C.1: Forward stepwise regression analysis demonstrating associations between left ventricular hypertrophy and various cardiovascular factors.

	CP-LVH (mV)							
	Model 1		Model 2		Model 3		Model 4	
	D men		ND men		D women		ND women	
Adjusted R^2	**0.29**		**0.11**		**0.45**		**NS**	
Independent variables	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	**–0.37 (-0.7,-0.0)**	**0.04***			**0.52 (0.3,0.8)**	**< 0.001***	-	-
Body mass index (kg/m^2)	0.22 (0.2,0.3)	0.30			**0.24 (0.0,0.5)**	**0.06***	**0.41 (0.2,0.7)**	**0.01***
HDL-chol (mmol/l)			0.20 (-0.1,0.5)	0.15	**–0.39 (-0.6,-0.1)**	**0.004***	-0.18 (-0.5,0.1)	0.25
SBP (mmHg)	**0.64 (-0.3,1.0)**	**0.001***	**0.44 (0.2,0.7)**	**0.003***			0.21 (-0.1,0.5)	0.13
***Sensitivity analyses*	**0.15**		No entry		No entry		No entry	
HbA1c	**0.31 (-0.02,-0.64)**	**0.08**						

β: beta coefficient (5^{th} to 95^{th} percentile interval). Models 1-4 adjusted for physical activity, log γ-GT and smoking.

**Sensitivity analyses model adjusted for age, body mass index, physical activity, log γ-GT, smoking, 24-hour systolic blood pressure (SBP) and high density lipoprotein cholesterol (HDL-chol).

*Data in bold is regarded as statistically significant, $p \leq 0.05$ and a trend level significance when $0.05 < p \leq 0.08$.

Discussion

This is the first study to examine the relationship between cardiometabolic and cardiac structural wall abnormalities and related depressive symptoms in urban Africans. Although the relationship found was modest, it remains an important finding because of the increasing prevalence of cardiometabolic risk factors including HT and type 2 diabetes mellitus, in the African population (21, 22). The researchers have shown that CP-LVH in African women with symptoms of depression is driven primarily by a metabolic factor, specifically low HDL-chol. In D and ND, African men 24-hour systolic BP explained 30% and 11% of the variation in CP-LVH respectively. The association between depressive symptoms and cardiometabolic risk was independent of MetS status. This distinction is important because of the bi-directional relationship between depressive symptoms, diabetes and CVD risk (23).

The relationship between depression and MetS has been examined in the past and most studies have documented an association between depression and MetS indicators in women (24, 26). Vogelzangs et al. (26), for instance, found that the prevalence of MetS and some of its components (abdominal obesity, low HDL-chol and hypertension) were higher in the depressed women than their non-depressed counterparts (26). Furthermore, Muhtz (24) found that depressed women have a significantly larger WC, higher plasma glucose levels and low HDL-chol than non-depressed women (24). This study in turn found no significant differences regarding the components of the MetS in women, but did find that women with depressive symptoms had a trend towards elevated plasma glucose and revealed a pre-diabetic state.

The results revealed an independent association between low HDL-chol and CP-LVH in women with depressive symptoms. The study's findings correspond to those of Muhtz et al.

(24) who reported an association between depression and low HDL-chol in women, independent of known confounders (24). Although the HDL-chol levels of the women, irrespective of depression status, did not differ significantly, the mean levels indicated a metabolic syndrome risk (< 1.29 mmol/l) (8). The mechanism for the underlying association is not well understood, but it has been speculated that high glucose levels may lower HDL-chol levels (27-29). Low HDL-chol levels and elevated plasma glucose levels have both been associated with target end-organ damage, specifically CP-LVH (28, 29).

The clustering of metabolic components such as large WC (> 80 cm), low HDL-chol (< 1.29 mmol/l) levels and elevated acute (≥ 5.6 mmol/l) and chronic (> 5.7%) plasma glucose levels in women with depressive symptoms may further augment the negative effects that metabolic factors may have in this gender group (8). Abdominal obesity has been implicated as a key component for MetS-related health risk in sub-Saharan Africa and is associated with psychosocial factors such as urbanisation (30, 31). In women in the United States, obesity increases the risk of being diagnosed with major depression by 37% whereas obese men have a 37% lower risk of depression than men of normal weight (32). Conversely, depression is associated with an increased incidence of diabetes, which in turn seems to be mediated largely through central adiposity. In this study, only the depressed African males demonstrated a trend of central adiposity with a mean waist circumference exceeding the cut point (94 cm) of the International Diabetes Federation Guidelines as well as the ethnic specific cut point of 90 cm (8, 33).

The possible pathophysiological basis for the association between abdominal obesity and depressive symptoms is not fully elucidated. However, it has been suggested that the dysregulation of the HPA-axis may play a role (34). Hypercortisolemia mediates the accumulation of visceral adipose tissue that secretes inflammatory cytokines that have been

implicated in insulin resistance (34). The latter is considered a key factor in metabolic abnormalities, disturbed vascular responses and endothelial dysfunction thus playing an important role in cardiometabolic risk. Interestingly, Abildgaard et al. (35) found that Flinders's sensitive line rats, a genetic rodent model of depression, become hyperinsulinaemic following exposure to metabolic stress induced by a high-fat diet and developed larger infarct sizes following ischaemic-reperfusion injury (35). This animal model of depression may help us to understand the underlying mechanisms behind the relationship between depression and cardiometabolic risk in humans. HPA-axis hyperactivity augments sympatho-adrenal hyperactivity via central regulatory pathways, subsequently leading to an increase in BP (36). In this study, the African men with depressive symptoms who tend to be substantially obese, SBP and HbA1c were associated with CP-LVH. It seems possible that depressive symptoms could alter HPA-axis activity and subsequently cardiometabolic risk. Considering their pre-diabetic status (> 5.7%), an increased risk for a cardiovascular event exists in the black male cohort according to the American Heart Association. It is supported by HbA1c explaining 31% of the variance in structural wall abnormalities.

The results also revealed an unexpected scenario in the cardiovascular profiles of men without symptoms of depression. These men revealed higher values for CP-LVH than their counterparts with symptoms of depression, and systolic BP was associated with CP-LVH in this group. The reasons for this particular finding is unclear. Age may have been a factor as the men without depressive symptoms were slightly older (mean = 43.81 vs 41.97 years). Age is a well-known traditional risk marker for increased vascular resistance and vascular dysfunction (37). Results, however, revealed that even after adjusting for age, systolic BP remained associated significantly with CP-LVH ($P = 0.004$) in men without depressive symptoms. These results suggest that there may be more underlying factors involved such as low renin hypertension and impaired salt handling (22). It is also possible that self-reported

questionnaires may not be sensitive enough to identify all dimensions associated with depressive symptoms. One such omitted dimension may be culture-specific manifestations of depressive symptoms (including cognitive and affective factors, cultural idioms of distress and somatic presentations), coupled with cultural-specific notions of masculinity. This omission, for example, may obscure the detection of depressive symptoms in the gender group (38, 39). Psychological questionnaires may have to consider such and other aspects in order to minimise over- or under-reporting of depressive symptoms.

The limitations of this study should be noted. Although a number of mechanisms have been suggested to explain the association between depressive symptoms and cardiometabolic risk, the results presented here is cross-sectional and causality cannot, as a result, be inferred. The data may however be used to determine whether depressive symptoms contribute to CP-LVH through its link with metabolic indicators.

In conclusion, the results suggest that CP-LVH is associated with increased systolic BP in African men irrespective of depression, while low HDL-chol was associated with CP-LVH values in women with depression. The African men are more at risk as group mean valuesrevealed more cardiometabolic symptoms exceeding cut points. The high mean values for metabolic risk indicators should become a source of public health concern in CVD risk management in the future. Future research should investigate HPA activation and underlying mechanisms in the relationship between depressive symptoms, cardiometabolic dysfunction and cardiac morbidity and mortality.

Acknowledgements

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Chapter 3: Research Article 2

Blunted neuroendocrine responses linking depressive symptoms and ECG left ventricular hypertrophy in black Africans: the SABPA study.

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Acknowledgements

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

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Articles in journals

Ali YS, Linton MF, Fazio S. Targeting cardiovascular risk in patients with diabetes: management of dyslipidemia. Curr Opin Endocrinol Diabetes Obes 2008; 15:142-146.

More than seven authors:

Shenouda SM, Widlansky ME, Chen K, Xu G, Holbrook M, Tabit CE, et al. Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus. Circulation 2011; 124:444-453.

Supplements:

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Letter/Abstract:

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Chapter in a book:

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Abstract

Objective: Chronic psychosocial stress as experienced in an urban environment plays an important role in the etiology of depression-related cardiovascular risk. If acute mental stress responses may aggravate this risk, the risk is uncertain. Therefore, this study aimed to explore the associations between depressive symptoms, neuroendocrine acute mental stress responses and cardiovascular risk, i.e., echocardiogram (ECG)-left ventricular hypertrophy (LVH) in a black African cohort (hereafter referred to as Africans).

Methods: The sub-study sample consisted of 179 African men and women from the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study. Depressive symptoms were evaluated using the 9-item Patient Health Questionnaire (PHQ-9) and the participants were stratified into Africans with depressive symptoms and without. Cortisol and 3-methoxy-phenylglycol (MHPG) responses were analysed during rest and exposure to the Stroop mental stressor. Cortisol median split responses were determined and gender groups were stratified accordingly into above (> 1.5 ng/ml) and below (≤ 1.5 ng/ml) responders. Blood pressure (BP) and ECG-LVH data were obtained from 24-hour ambulatory monitoring.

Results: The Africans with depressive symptoms demonstrated mean hypertension levels, blunted cortisol and MHPG acute mental stress responses ($P \leq 0.05$). In Africans with depressive symptoms and low cortisol stress responses, blunted MHPG acute mental stress responses were associated with ECG-LVH in Africans (adj. $R^2 = 0.20$; $\beta = 0.92$ (95% CI 0.74, 1.10); $P = 0.02$).

Conclusion: Blunted neuroendocrine responses were associated with ECG-LVH in Africans with depressive symptoms. When coupled to their hypertensive status, these vasoconstrictive agent responses may underpin the increased long-term depression and vascular disease risk in urban Africans.

Key words: Africans; cortisol, MHPG; depressive symptoms; ECG-LVH

INTRODUCTION

There is growing awareness of the impact of psychosocial stressors, such as urbanisation, on the mental and physical health of black Africans (hereafter referred to as Africans). Chronic psychosocial stressors constitute a burden on the ability of the brain to adapt to stress and to initiate a recovery process, especially in susceptible individuals. These neuroendocrine responses are central to how the body copes with stress and also recovers from it [1, 2]. Inability to properly regulate aspects of these responses has been proposed as an essential factor in the pathophysiology of various stress-related disorders, including depression [3]. Indeed, evidence from the literature indicates that depression is linked with alterations in neuroendocrine responses to mental stress. More specificall, these alterations have been linked to an increased risk for cardiovascular disease (CVD) [2, 4-7]. Depression has been associated with increases in norepinephrine responses to stress [8]. There still remains no clear-cut or generally accepted model for cortisol responses in depression as both blunted and increased cortisol activity have previously been noted [5, 9-11]. While a recent study by Hamer et al. [6] supported the link between depressive symptoms, norepinephrine responses and metabolic syndrome in Africans, [6], no study has (to our knowledge) attempted to investigate the association between depressive symptoms, neuroendocrine responses and increased cardiovascular risk (left ventricular hypertrophy) in Africans.

Left ventricular hypertrophy (LVH) is a marker of cardiac structural abnormality and has previously been linked to depressive symptoms in urban Africans [12]. Examining the role of the neuroendocrine responses in the abovementioned association is of importance because of the high prevalence of depressive symptoms and the emerging burden of CVD in this population group [6, 13]. Therefore, the aim of this study was to examine the association

between depressive symptoms, neuroendocrine responses [represented by salivary cortisol and 3-methoxy-phenylglycol (MHPG)] and ECG-LVH in urban Africans.

MATERIAL AND METHODS

Study design and participants

Subjects for this study were recruited as part of the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study. The SABPA study was a cross-sectional, multi-disciplinary, target population study conducted between February 2008 and May 2009. For the purpose of the current study 200 urban African teachers working in the Dr Kenneth Kaunda Education district, North-West Province, South Africa, were invited to participate. The purpose for this selection was based on the need to obtain a homogeneous sample from the same socio-economic grouping. All participants between the ages of 25 and 60 years were invited to participate. The study procedures are described elsewhere [6]. Exclusion criteria for this study sample were pregnancy, lactation, α- and ß-blockers, psychotropic substance users; ear temperature > 37 °C, individuals who had been vaccinated or had donated blood in the three months prior to participating. For the purpose of our study we also excluded participants who were HIV positive (N = 19) and statin users (N = 2). The total sample comprised 179 participants.

The ethical clearance for this study was granted by the Ethics Committee of North-West University (0003607S6) in accordance with the principles outlined by the World Medical Association Declaration of Helsinki 1975 (revised in 2008). All participants signed a consent form before data collection commenced. Assistance was offered to participants who requested information in their native language.

Clinical assessment procedure

Each morning of the working week, four participants were fitted with a British Hypertension Society validated ambulatory blood pressure (ABPM) monitoring device (Meditech CE120® Cardiotens, Budapest, Hungary) as well as a 2-lead electrocardiogram (ECG). The ABPM device was fitted to the participants' non-dominant arm and programmed to take blood pressure measurements at 30-minute intervals during the day (08h00-22h00) and 60-minute intervals during the night (22h00-06h00) accompanied by a sequential recording of the ECG strips every 5 minutes for 20 seconds [14]. The successful mean inflation rate in this sample was 82.7% (± 3.8%). A 12-lead electrocardiogram (NORAV PC 1200) device was used to determine LVH, using strip leads RaVL + SV3 in the calculation of a gender-specific formula, the Cornell product: sum of the leads LVH= (RaVL + SV3)* QRS > 244 mV.ms [15,16]. Higher Cornell product (>244mV.ms) values implies worse LVH [15, 16].

The participants were also fitted with an Actical® omnidirectional accelerometer monitor (Mini Mitter, Bend OR, Montréal, Québec) to measure physical activity during the ABPM period. Participants were requested to continue with normal daily activities, recording any abnormalities such as visual disturbances, headache, nausea, fainting, palpitations and stress on their ambulatory diary charts.

At approximately 16h40 they were transported to the Metabolic Unit Research Facility of North-West University (research unit for human studies) and were familiarised with the available facilities and the experimental setup, to minimise the 'white-coat effect' [17]. This was followed by the completion of a collection of psychosocial questionnaires under the supervision of a registered clinical psychologist. Hereafter the participants received a

standardised dinner and were advised to retire for the night at approximately 22h00, fasting overnight.

At 06h00 the following morning, the ABPM and the Actical® devices were removed. Thereafter the participants' anthropometric measurements were obtained followed by the resting 12-lead electrocardiogram (ECG) and psychophysiological stress testing while the participants were in a semi-recumbent position. Fasting venous blood and saliva samples were obtained. Saliva samples were collected using the Sarstedt® salivette device, which involves placing a small cotton roll in the mouth of the participant for several minutes. Participants stayed overnight at the Metabolic unit and therefore refrained from consuming alcohol and caffeine, smoking and exercising eight hours prior to data collection. Additionally, they were advised to take caution with their dental hygiene the previous night preventing bleeding gums for sampling the following morning. Participants were requested not to brush their teeth before data sampling [18].

Mental stress testing

The Stroop mental stress task has been used extensively in psychophysiological stress testing. The task was administered over a 1-minute period followed by a 30-minute recovery, using cards. The participants were required to identify the colours of the colour word cards in contrasting colours of ink under time pressure. As a motivation to complete the task, each participant received a monetary incentive of 1 euro with the completion of every column. Saliva samples were collected at baseline and 30 minutes post task for detection of cortisol and 3-methoxy-phenylglycol (MHPG). Salivary MHPG has been shown to be a reliable indicator of sympathetic activity during acute mental stress with optimum responses occurring 30 minutes post stress task [4].

Depressive symptoms were assessed with the use of the 9-item self-administered Patient Health Questionnaire (PHQ-9). The PHQ-9 is a measure of depressive symptom severity and has been validated in various ethnic groups including sub-Saharan Africans [19-21]. The questionnaire is designed for use in primary health care settings adapting diagnostic criteria from the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition). Each item of the PHQ-9 evaluates the presence of one of the nine DSM-IV criteria of major depression [19]. In the current study, the Cronbach alpha-reliability index for the total PHQ-9 score was 0.81. Items on the questionnaire were scored to reflect the frequency of symptom occurrence during the prior two weeks on a scale of zero to three where zero meant "not at all" and three "nearly every day". A continuous score between 0-27 was thus provided [19]. We used the recommended and established PHQ-9 cut-off point of >10 to indicate the presence of depressive symptoms, stratifying the study sample into participants with and without depressive symptoms [20].

Anthropometric measurements

Anthropometric data was standardised and taken in triplicate to the nearest 0.1 cm. Body weight and height measurements were taken and used to calculate body mass index (BMI) with the formula kg/m^2. Waist circumference was measured with a metal tape at the midpoint between the lower costal border and the iliac crest perpendicular to the long axis of the trunk [22]. Intra- and inter-observer variability was less than 10%.

Biochemical sampling and analyses

Fasting blood samples were handled and prepared according to standardised methods, and frozen at –80 °C until analysis. Fasting sodium fluoride (glucose) and serum samples for triglycerides, high-density lipoprotein (HDL-chol), gamma glutamyl transferase (γ-GT) and

cotinine were analysed using the sequential multiple analyser computer (Konelab 20i; Thermo Scientific, Vantaa, Finland) and by immunoassay (Integra 400, Roche, Switzerland). As marker of alcohol abuse, γ-GT was measured [23] whereas serum cotinine levels were measured as a marker of smoking status with values above 14.99 μg/l considered an indication of exposure to first- or secondary smoking [24]. The intra- and inter-coefficients of variation for all assays were below 10%.

Cortisol levels were determined from salivary samples which were obtained before 09h00. To avoid the cortisol awakening response, resting saliva samples were collected 45 minutes after awakening [25]. Cortisol levels were determined using a high sensitivity enzyme-linked immunosorbant assay (ELISA). Cortisol showed intra- and inter-coefficients of variation of 7.7% and 9.8% respectively. The major metabolite of norepinephrine, MHPG, closely reflects plasma metabolite levels and was analysed using high-performance liquid chromatography coupled to an electrochemical detector [26, 27]. The inter- and intra-day coefficient of variation for all assays was less than 10%.

Statistical analysis

Stress responses were calculated with the following formula as changes from baseline (delta, Δ): X stressor – X resting. All data was analysed using the software package STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA, 2010). γ-GT was logarithmically transformed to normalise distribution. Differences in characteristics of participants in relation to depressive symptoms were analysed using t-tests (mean ± standard deviation) to examine continuous variables and chi-square to compare proportions (%). A single two-way analysis of covariance (ANCOVA) was applied to assess interaction between the main effects

(depressive symptoms × cortisol median split response), independent of *a priori* covariates (age, smoking prevalence, resting cortisol and MHPG levels). Subsequently, one-way ANCOVA analyses were conducted where cardiovascular and biochemical data of low and high cortisol responses were compared using least squared means.

Multiple unadjusted and adjusted regression analyses were computed to identify independent predictors of ECG-LVH in the participants with and without depressive symptoms in the low and high cortisol response groups. In forward stepwise regression analyses, ECG LVH was the dependent variable and the following independent covariants were included: age, gender, smoking prevalence, body surface area, resting cortisol and MHPG levels, MHPG responses and 24-hour systolic BP. Significance was noted as $P \leq 0.05$ and tendency for $P < 0.1$.

Sensitivity analyses

We computed sensitivity analyses on the relationship between dipping status, depressive symptoms and cortisol levels in separate gender groups.

RESULTS

The participant sample is described in Table 1. Africans with depressive symptoms were more likely to smoke ($P = 0.05$) although no other differences in baseline characteristics were observed. Both groups displayed similar resting MHPG and cortisol levels.

Mental stress responses

Two-way ANCOVAs revealed significant interaction on main effects (depressive symptoms × cortisol median split responses) for ECG-LVH [$F(1.179) = 5.86$; $P = 0.02$] with a trend for systolic BP [$F(1.179) = 2.96$; $P = 0.09$] and diastolic BP [$F(1.179) = 3.18$; $P = 0.08$].

Therefore, our group was stratified into those with depressive symptoms and those without depressive symptoms. Median split cortisol responses, using a cut-point of >1.5 ng/l, were also used to stratify the group. In Table 2 the Africans with depressive symptoms and low cortisol responses demonstrated a significantly blunted MHPG response (P = 0.004) in comparison to the high cortisol response group as well as lower triglycerides levels (P = 0.04). Interestingly, Africans without depressive symptoms and high cortisol responses displayed significantly higher ECG-LVH (P = 0.01) and a tendency for elevated systolic (P = 0.08) and diastolic BP (P = 0.06).

Table 1: Descriptive statistics of the study sample (mean ± standard deviation).

Variable (*n* = 179)	With depressive symptoms (*n* = 81)	Without depressive symptoms (*n* = 98)	P-value
Age (years)	43.91 ± 8.36	44.51 ± 8.12	0.63
Body mass index (kg/m^2)	30.68 ± 6.92	30.00 ± 7.29	0.53
Men (%)	35 (43.2)	52 (53.1)	0.19
Physical activity (kcal/h)	2717 ± 843.44	2647.68 ± 760.11	0.56
Smoking n (%)[a]	**26 (32.1)**	**19 (19.4)**	**0.05**
γ-glutamyl transferase (μ/L)*	1.70 ± 0.29	1.64 ± 0.33	0.24
Hypertension status n (%)[b]	51 (62.96)	62 (63.27)	0.97
Non-dip status n (%)	32 (40.51)	41 (41.84)	0.86
Biochemical variables			
Resting cortisol (ng/ml)	2.10 ± 2.08	2.07 ± 1.64	0.93
Resting MHPG (ng/ml)	7.64 ± 4.32	6.79 ± 3.46	0.14
Metabolic syndrome			
Waist circumference (cm)	94.65 ± 15.73	92.79 ± 15.81	0.43
HDLchol (mmol/l)	1.18 ± 0.37	1.11 ± 0.32	0.19
Triglycerides (mmol/l)	1.48 ± 1.60	1.36 ± 1.03	0.53
Resting glucose (mmol/l)	5.87 ± 2.51	5.48 ± 1.78	0.23
24h Systolic BP (mmHg)	132 ± 16.90	133 ± 16.08	0.73
24h Diastolic BP (mmHg)	82 ± 10.44	84 ± 11.43	0.39
Metabolic syndrome n (%)[c]	38 (46.9)	43 (43.8)	0.81
Cardiovascular variables			
ECG LVH (mV)	64.02 ± 30.03	73.90 ± 48.16	0.14

Psychological variables

Depression score[d]	**14.25 ± 4.16**	**5.51 ± 2.70**	**< 0.01**

[a] 'Smoking' defined as serum cotinine (ng/ml): values >14.99 μg/l [b] Hypertension based on the 24-hour ABPM; systolic and/or diastolic ≥ 130 and or ≥ 80 mmHg [14].

[c] Metabolic syndrome based on IDF criteria [43].

[d] Depression score is based on the total points scored on the Patient Health Questionnaire.

[*] Variable is geometric mean

In Figure 1 cortisol responses at rest and during acute mental stress STROOP responses were compared in Africans with depressive symptoms and those without depressive symptoms, independent of covariates. A blunted cortisol response was revealed in Africans with depressive symptoms (P = 0.02).

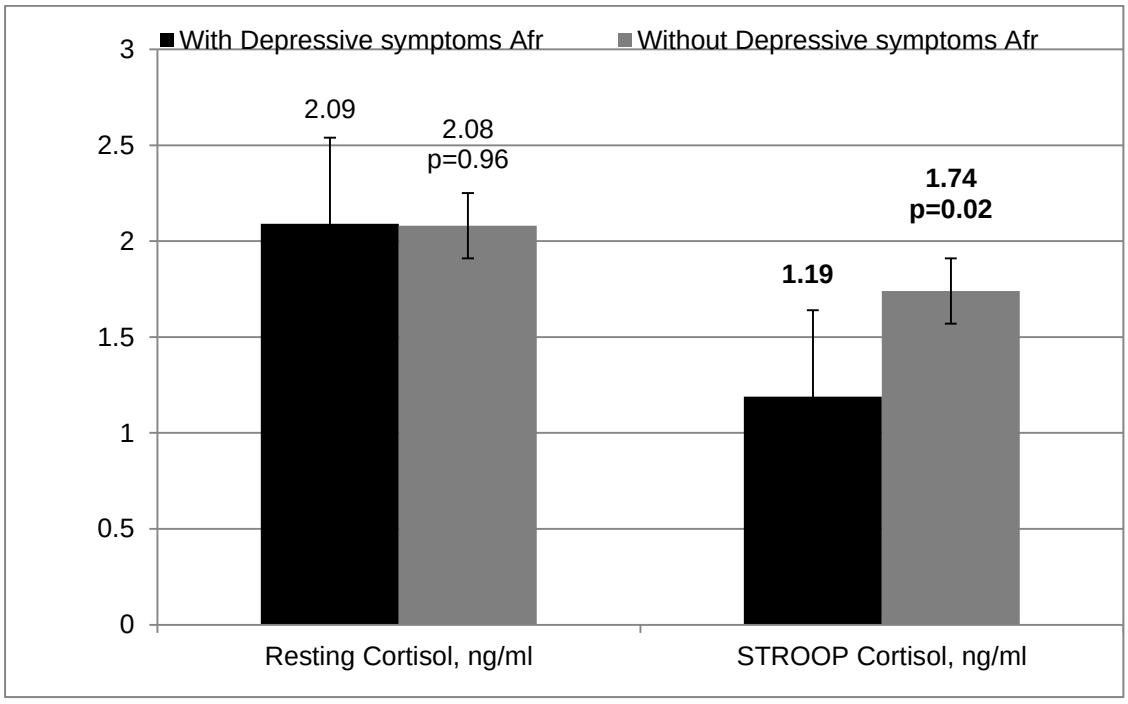

Figure 1: Comparing adjusted resting cortisol and median split responses (cut point > 1.5 ng/ml) during acute mental stress STROOP responses in Africans with depressive and those without depressive symptoms. Covariates included age and smoking prevalence. STROOP responses were adjusted for baseline levels.

Predictors of ECG Left Ventricular Hypertrophy

In forward stepwise regression analysis models (Table 3) blunted MHPG acute mental stress responses were positively associated with ECG-LVH in Africans with depressive symptoms and with low cortisol stress responses (adj. R^2 = 0.20; β = 0.92 (95% CI=0.74, 1.10); P = 0.02).

No associations existed between MHPG responses and ECG-LVH in participants without depressive symptoms.

Sensitivity analyses

No relationship existed between dipping status, depressive symptoms and cortisol levels in separate gender groups.

Table 2: Adjusted means (± 95% CI): Comparing salivary stress cortisol (low vs. high) in Africans with vs without depressive symptoms, independent

of covariates (age, smoking prevalence).

	With depressive symptoms			Without depressive symptoms		
	CORTISOL STROOP RESPONSES					
	Low ≤ 1.49 ng/ml	**High > 1.50 ng/ml**		**Low ≤ 1.49 ng/ml**	**High > 1.50 ng/ml**	
Variable	*n* = 60	*n* = 28	*P*-value	*n = 32*	*n = 49*	P-value
Waist circumference (cm)	95.64 (91.7; 99.6)	91.84 (85.1; 98.6)	0.34	94.04 (90.0; 98.1)	90.83 (85.7; 95.9)	0.33
HDL-chol (mmol/l)	1.19 (1.1; 1.3)	1.16 (1.0; 1.3)	0.73	1.11 (1.0; 1.2)	1.12 (1.0; 1.2)	0.79
Triglycerides(mmol/l)	**1.28 (0.9; 1.7)**	**2.09 (1.4; 2.8)**	**0.04**	1.32 (1.1; 1.6)	1.42 (1.1; 1.7)	0.63
Glucose (mmol/l)	5.88 (5.2; 6.5)	5.85 (4.8; 6.9)	0.97	5.59 (5.1;6.1)	5.30 (4.7; 5.9)	0.43
Systolic BP (mmHg)	133 (129; 138)	130 (124; 137)	0.44	131 (127; 135)	137 (132; 142)	0.08
Diastolic BP (mmHg)	83 (80; 86)	81 (76; 86)	0.50	82 (79; 85)	87 (83; 90)	0.06
Hypertension *n* (%)	43 (71.67)	12 (57.14)	0.22	38 (63.33)	26 (68.42)	0.61

ECG LVH (mV)	66.20 (58.1; 74.4)	57.22 (42.7; 71.8)	0.29	**63.32 (50.4; 76.3)**	**90.25 (74.2; 106.4)**	**0.01**
Stress MHPG (ng/ml)[a]	**8.72 (8.0; 9.4)**	**10.80 (9.6; 12.0)**	**0.004**	7.60 (7.0; 8.3)	7.70 (6.9; 8.5)	0.85

CI, confidence interval; HDL-chol, high-density lipoproteins; BP, blood pressure; ECG LVH, echocardiogram left ventricular hypertrophy; MHPG, 3-Methoxy-4-hydroxy-phenylglycol.

[a] Additionally adjusted for resting levels of MHPG.

*Values in bold are considered statistically significant, $P \leq 0.05$.

Table 3: Forward stepwise regression analyses to demonstrate associations between ECG-LVH and potential independent predictors in Africans with vs without depressive symptoms and low/ high cortisol responses.

	ECG-LVH (mV)			
	With depressive symptoms		**Without depressive symptoms**	
	CORTISOL STROOP RESPONSES			
	Low ≤ 1.49 ng/ml	High > 1.50 ng/ml	Low ≤ 1.49 ng/ml	High > 1.50 ng/ml
	(n = 39)	(n = 28)	(n = 32)	(n = 49)
Adjusted R^2	0.20	NS	0.26	NS
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Stroop MHPG	**0.92 (0.74; 1.10), P = 0.02**	-	-0.44 (-0.44; 0.05), P = 0.09	-

F to enter: 2.5.

β: beta coefficient; ECG LVH, echocardiogram left ventricular hypertrophy.

Independent variables included in models, are: age, gender, smoking prevalence, body mass index, resting cortisol, resting and acute stress MHPG responses.

DISCUSSION

Possible associations were assessed between neuroendocrine acute mental stress responses (represented by salivary MHPG and cortisol), depressive symptoms and ECG-LVH (a marker of structural left ventricular wall abnormalities) in urban Africans. To our knowledge, this is the first well-controlled study focusing on the contribution of selected stress responses and depression to cardiovascular risk in Africans. Main findings demonstrated no direct association between depressive symptoms and resting cortisol levels. However, blunted neuroendocrine responses linked depressive symptoms and ECG-LVH in Africans. When coupled with their hypertensive status these vasoconstrictive agent responses may underpin the increased long-term depression and vascular disease risk in urban Africans.

Earlier work demonstrated elevated salivary MHPG levels in individuals with greater depressive symptoms [4, 28]. This study could not establish similar significant findings. Conversely, salivary MHPG is a major metabolite of norepinephrine that closely resembles plasma levels and was investigated in this African target population [27]. The positive association revealed between salivary MHPG stress responses and depressive symptoms may be related to psychosocial stressors [29] such as urbanisation, a challenged nervous system and heightened sympathetic activity. Findings are supported by other studies where urbanisation has been associated with increased hypertension prevalence, vascular responsiveness and myocardial ischemic risk in this ethnic group [30-32].

A secondary aim of the study was to link depressive symptoms to neuroendocrine responses and potential target end-organ damage. Depressive symptoms may act via attenuation of norepinephrine responses to stress, potentially contributing to allostatic load that can predispose Africans to structural LVH changes [33, 34]. Indeed, neither participants with or

without depressive symptoms showed a difference with respect to basal cortisol and MHPG levels, yet blunted salivary stress MHPG and cortisol responses were apparent in individuals with depressive symptoms *after* exposure to the Stroop test. This could imply that the presence of depressive symptoms sensitises the individual to stress and the subsequent development of vascular disease and or other lifestyle illness.

We were able to establish MHPG responses were associated with ECG-LVH in these participants. As cortisol has a permissive effect on norepinephrine functioning, the vasoconstrictive effect of norepinephrine secretion will add to a higher α-adrenergic vascular responsiveness and a hyperkinetic state. Indeed, a hypertension prevalence of 71.67% may support the notion of a shift from central cardiac (ß-adrenergic) to vascular (α-adrenergic) BP responses [30], increasing cardiovascular disease risk.

In a previous study we demonstrated an association between systolic BP and ECG LVH in African men with depression [12]. This study supported these findings as it demonstrated that blunted cortisol and MHPG responses, acting as vasoconstrictive agents, may induce structural wall abnormalities by increasing pre- and afterload to the heart [30]. Depression may enforce these changes and augment cardiovascular morbidity. Blunted cortisol responses to laboratory and naturalistic psychosocial stressors have been demonstrated in both clinical and sub-clinical depression [35, 36]. Burke et al. [5], for instance, demonstrated that women with high depressive symptoms exhibited blunted cortisol stress responses to a naturalistic stressor [5]. This response is not unexpected since depression is typically associated with elevated cortisol while at the same time individuals with depression demonstrate a blunted response to dexamethasone challenge [1, 37, 38]. However, it could be speculated that since depression is a constant state of perceived stress, further exposure to a challenging urban environment or psychosocial stress may result in habituation of the neuroendocrine pathways

[39]. Alternatively, although the HPA-axis in depression is moderately activated, possibly due to the initial (primary) hippocampal degeneration in this condition, the ensuing structural changes are likely to illicit a maladaptive cortisol response as described here [37]. Chronic mental or psychosocial stress triggers sympathetic hyperactivity resulting in increased release of catecholamine and ultimately depletion, a process that is abrogated by cortisol [9]. Unmitigated increases in cortisol, as a result of maladaptive responses to stress, lead to the dysregulation of HPA axis activity resulting in the above-described patterns of cortisol release eventually leading to the development of an anxiety and/ or mood disorder [9, 39]. The failure to appropriately regulate aspects of this stress axis represents a crucial factor in the pathophysiology of various stress related disorders [3].

Therefore, our findings suggest that Africans with depressive symptoms and blunted MHPG-cortisol responses are at a greater risk for vascular disease and stress related disorders. Subsequently, various maladaptive neurobiological and behavioral changes ensue that lay the foundation for altered metabolic and redox function, as has recently been described in this population. This in turn drives the increased risk for cardiovascular illness [40].

Certain strengths and limitations of this study should be acknowledged. The nature of the study design and the uniqueness of the study sample is a noteworthy strength, and is the first well-controlled study in sub-Saharan Africa examining the role of neuroendocrine responses and depressive symptoms and their association with cardiovascular health. The limitations of our study include the inability to make inferences about causality, because of the cross-sectional nature of the study. Prospective data is needed to address this issue. Furthermore, we used a self-report questionnaire to assess depressive symptoms which may introduce reporting biases. The study sample consist only of Africans and it is suggested that the collectivistic nature of this population group, where most actions are reflected by group/ social interaction coupled with culture-specific manifestations of depressive symptoms, may

influence the interpretation of the items in the PHQ-9 [41, 42]. However, the questionnaire is a well-established diagnostic tool, which has been validated in various ethnic groups, including Africans [21].

In conclusion, blunted neuroendocrine responses linked depressive symptoms and ECG LVH in Africans. When coupled to their hypertensive status, these vasoconstrictive agent responses may underpin the increased long-term depression and vascular disease risk in urban Africans. These findings may have important health implications given the emerging burden of cardiovascular disease among urban Africans and that a 3-fold risk of depressive symptoms has been observed in this population group in comparison to Caucasians [6, 13]. Identifying and treating depressive symptoms in Africans may play a role in reducing stress-related cardiovascular risk in this ethnic group.

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Any opinion, findings and conclusions or recommendations expressed in this material are those of the author(s) and therefore the NRF do not accept any liability in regard there to.

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Chapter 4: Research Article 3

Hypercoagulation vulnerability exacerbated by hypertension state in black Africans with depressive symptoms: the SABPA study.

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On the title page, include the full names of all authors and their academic or professional affiliations, along with the corresponding author's complete contact information. Multiple corresponding authors are not allowed. Six key words should also be included. Separately list the number of words in both the abstract and article body (excluding abstract, acknowledgments, financial disclosures, legends and references), and the number of figures, tables, and supplemental information (if zero, state zero for each item). Article titles should not contain acronyms, and be less than 100 characters.

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Abstracts for Archival Reports should be structured with sections entitled as follows: Background, Methods, Results, Conclusions. Abstracts for all other article types are unstructured, but should not exceed the word limits as defined above. The Methods section should explicitly state the sample size of the trial. For those manuscripts that require clinical

trials registration (see Clinical Trials Registration section, below), the registry name, URL, and registration number should be included at the end of the abstract.

Acknowledgments

This section should follow the Discussion and precede the References, and should include acknowledgments for personal and technical assistance, in addition to detailed information regarding all sources of grant and other material or financial support. If a research group is listed as an author, then the individual members of the research team must be named here. Written permission should be obtained from all individuals named here. Data that was published previously, such as in an abstract or poster, should be identified here.

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This section **must** include the required conflict of interest statements for each author (see section on disclosure, below).

References

References should be numbered and listed by their order of appearance in the text. Refer to references in the text with the appropriate number in parentheses. References in tables and figures should also be numbered. List all authors; if there are more than seven authors, list the first six then *et al*. Periodical abbreviations should follow those used by Index Medicus. The following are sample references for a journal article (1), a book (2), and an edited book (3).

1. Krystal JH, Carter CS, Geschwind D, Manji HK, March JS, Nestler EJ, *et al*. (2008): It is time to take a stand for medical research and against terrorism targeting medical scientists. *Biol Psychiatry* 63: 725-727.

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FIGURES AND TABLES Figures and tables should be cited in the text, numbered consecutively (i.e., 1, 2, 3) in the order of their mention, and have brief descriptions. If not included in the manuscript file, tables should be uploaded individually in a text format, such as DOC.

ABSTRACT

Background: Altered inflammatory and haemostatic processes are associated both with depression and cardiovascular risk, and may constitute possible mechanisms through which depression contributes to cardiovascular diseases. The aim of this study is to investigate the relationship between depressive symptoms, inflammatory and haemostatic markers in black African (hereafter referred to as Africans) men and women.

Methods: An indication of 'moderate depression', as defined by the Patient Health Questionnaire (PHQ-9) score of 10 or greater, was obtained from 181 Africans (88 men, 93 women) aged 25-60 years, drawn from the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study. Plasma concentrations of C-reactive protein (CRP), fibrinogen, D-dimer, plasminogen activator inhibitor-1 (PAI-1) and 24-hour BP were measured.

Results: Irrespective of depressive symptoms, hypertensive status and low grade inflammation (> 3 mg/l) were demonstrated in Africans. Of the 181 participants, 45.3% were moderately depressed. PAI-1 [OR 1.08 (1.02,1.14), P = 0.02] in men and D-Dimer [OR 0.998 (0.996, 1.02,1.14), P = 0.02] in women were assciated with depressive symptoms, independent of confounders.

Conclusion: Depressive symptoms may increase hypercoagulation vulnerability in Africans exacerbated by hyperkinetic blood pressure. Their hypertensive status may induce endothelial dysfunction by up-regulating inflammatory processes. Even though CRP as marker of low grade inflammation was not directly linked to depressive symptoms it may act as modulator of inflammation and thrombosis increasing coronary heart disease risk in Africans.

Keywords: Depressive symptoms, Africans, inflammation, haemostasis, coagulation

INTRODUCTION

Depression is a common affective disorder that has been associated with cardiovascular morbidity and mortality in the western world (1, 2). Both clinical and sub-clinical depressive symptoms have been linked to a higher incidence of cardiac events in individuals with cardiovascular disease (CVD) and in healthy populations (3, 4). In Africans, symptoms of depression have been associated with an increase in cardiovascular risk and target end-organ damage (5). Several mechanisms have been proposed as possible mediators in the depression-CVD association in Africans, including sympathetic hyperactivity and metabolic factors (5, 6). Although depression is increasingly being viewed as an inflammatory illness (7), data regarding the association between depressive symptoms, inflammation and haemostatic processes has not yet been established in an African population. Exploring the role of these processes may shed some light into the depression-CVD risk association in this ethnic group.

Evidence from prospective studies suggests that inflammation may play a pivotal role in the pathogenesis of CVD (8, 9). Elevated inflammatory markers, particularly C-reactive protein (CRP) and fibrinogen, have been established as predictors of CVD such as coronary heart disease (CHD) (10-12). Studies reporting on the relationship between depression and inflammation have produced mixed findings. A number of studies have found a positive relationship (13, 14), while others have reported a no relationship (15, 16). However, the abovementioned studies have been reported in western and Chinese populations; no data is available on Africans. Investigating the inflammation-depression relationship in this ethnic group may be of great importance due to the greater CRP levels previously reported in this group (17). Moreover, altered redox, which is commonly associated with inflammation, has been described in this population (18). Reimann *et al.* (19) demonstrated that the L-Arginine/NO system is affected by psychosocial distress in Africans (19).

CRP facilitated the release of PAI-I and inhibited endothelium dependent NO-mediated dilatation in coronary arterioles (20). Increased levels of haemostatic markers such as fibrinogen, plasminogen activating inhibitor-1 (PAI-1) and D-dimer have been implicated as predictors of coronary artery syndromes in individuals with coronary artery disease and in healthy populations (21, 22). Despite the lack of available data it appears that depression is associated with hypercoagulation or a thrombophilic state (23). Indeed, blood coagulation, fibrinolysis, D-dimers, plasminogen activator inhibitor-1 protein, platelet activation, vascular endothelial growth factor, plasma nitric oxide and its synthase are altered in psychological distress and depression (19, 24). The possible link between depressive symptoms and hypercoagulation is yet to be investigated in an African population within the SABPA study where seasonal changes, affecting haemostasis, were avoided.

Therefore, the purpose of this study is to investigate the association between depressive symptoms, inflammatory and haemostatic markers (CRP, fibrinogen, PAI-1 and D-dimer) in an African cohort from South Africa.

MATERIALS AND METHODS

Study participants

The Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study is a target population study and was conducted from February to May 2008. We intentionally recruited 200 Africans aged 25-60 years working in the Dr Kenneth Kaunda Education district, North West Province, South Africa. The objective was to obtain a homogeneous sample from a similar socio-economic grouping. The exclusion criteria included pregnancy, lactation, psychotropic substance users; tympanum temperature > 37.5°C, individuals vaccinated or donated blood in the three months prior to participating. For the present study,

we excluded subjects that were HIV positive (*n* = 19). The final study sample consisted of 181 subjects who signed a consent form.

Ethical clearance for this study was approved by the Ethics Committee of North West University (0003607S6), in accordance with the principles outlined by the World Medical Association Declaration of Helsinki 1975 (revised in 2008).

Procedure

Participants were fitted with an ambulatory blood pressure (ABPM) monitoring device (Meditech CE120® Cardiotens, Budapest, Hungary) and a 2-lead electrocardiogram (ECG), as well as an Actical® omnidirectional accelerometer monitor (Mini Mitter, Bend OR, Montréal, Québec) in the morning of a working day prior to their overnight stay at the Metabolic Unit Research Facility. The ABPM device was programmed to measure blood pressure at 30-minute intervals during the day (08h00-22h00) and 1-hour intervals during the night (22h00-06h00). The successful mean inflation rate was 72.6% (± 11.75) for the total sample. Participants were requested to resume with their normal daily activities, and to record any abnormalities such as visual disturbances, headache, nausea, fainting, palpitations and stress on their ambulatory diary charts.

At approximately 16h40 (*n* = 4, at a session) they were transported to the Metabolic Unit Research Facility of the North-West University and were familiarised with the available facilities and the protocol, to minimise the 'white-coat effect' (25). This was followed by the completion of the psychosocial battery under the supervision of a registered clinical psychologist and postgraduate students followed by a standardised dinner between sessions. Participants were encouraged to retire for the night at approximately 22h00, fasting overnight. Participants were woken the following morning at 05h45. Following the last

ABPM measurement at 06h00, the ABPM and the Actical® devices were disconnected. Thereafter the participants underwent a battery of clinical assessments. Registered nurses and well-trained personnel obtained anthropometric measurements (including height, weight, waist and hip circumferences), information about medical history and prescribed medication.

Biochemical analysis

Sodium fluoride, plasma and serum samples from fasting blood were obtained before 09h00, handled and prepared according to standardised methods, and frozen at –80 °C for the analysis of inflammatory and biochemical risk markers. High sensitivity C-reactive protein was analysed using two sequential multiple analysers (Konelab 20i; Thermo Scientific, Vantaa, Finland). The intra- and inter-coefficient of variation was below 10%. Fibrinogen was measured by viscosity-based clotting method using a STA compact with the reagent FIB (STAGO diagnostic; Roche, France). PAI-1 concentrations were measured by TriniLIZE PAI-1 Antigen assay (Tcoag Ireland Limited, Ireland) on a microtest plate spectrophometer. The intra-assay coefficient was 2.4%-3.3% and the inter-assay CV of 1.9-2.9%. D-dimer (marker of coagulation) was analysed using Viscosity-based clotting (Immuno-turbimetric method 540 nm) with the STA Compact (Manufacturer: TAGO Diagnostic Supplier: Roche; Country: France).

Depressive symptoms

Symptoms of depression were measured with the 9-item self-report Patient Health Questionnaire (PHQ-9), a well-validated screening questionnaire suitable for sub-Saharan population studies (26,27). The questionnaire is designed for use in primary health care settings adapting diagnostic criteria from the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) (26). To identify respondents with depressive symptoms

we used the generally accepted cut-off point of > 10, stratifying the study sample into participants with and those without depressive symptoms (28). The internal consistency (Cronbach alpha-reliability index) of the PHQ-9 in the present study was 0.81.

Covariates

Body mass index (BMI) was calculated using body weight and height measurements that were taken in triplicate to the nearest 0.1 cm and inter- and intra observer variability was less than 10%. Serum samples for high-density lipoprotein (HDL) cholesterol and gamma glutamyl transferase (γ-GT) were analysed using the sequential multiple analyser computer (Konelab 20i; Thermo Scientific, Vantaa, Finland). Smoking status was assessed using serum cotinine which was analysed by immunoassay (Integra 400, Roche, Switzerland). Participants with serum cotinine values > 14.99 μg/l were considered as an indication of exposure to primary- or secondary smoking (29).

Statistical analysis

All data was analysed using the software package STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA, 2010). All γ-GT and hs-CRP values were logarithmically transformed to the normalised distribution. Log normalised computation of D-dimer values (30.4%) was done for levels below detection (LOD), which is recommended rather than LOD/2 or LOD/square root 2 (30). Differences in characteristics of participants in relation to depressive symptoms were analysed using independent t-tests (mean ± standard deviation) to examine continuous variables and identify potential confounders. Chi-square analyses compared proportions prevalence (%). A single two-way analysis of covariance (ANCOVA) was applied to assess interaction between the main effects (depressive symptoms × gender), independent of potential confounders. Subsequent one-way ANCOVA analyses followed where

inflammatory and haemostatic variables were compared in the different gender groups, using least squared means, independent of confounders.

Logistic regression analyses were done with depressive symptoms (PHQ score > 10) as the dependent outcome variable. Independent variables included haemostatic and inflammatory markers as well as confounders (age, smoking (%), body mass index (BMI), physical activity, γ-GT, HDL-chol, blood pressure, the use of statin, and ante-inflammatory and anti-coagulant medication).

RESULTS

A significant interaction for gender × depressive symptoms was indicated for PAI-1 [F (1.173) = 7.28, *P* = 0.012] and D-dimer [F (1.170) = 6.05. *P* = 0.015]. In Table 1, it can be seen that 82 (45.3%) participants reported depressive symptoms as indicated by a PHQ-9 score of > 10. Presence of depressive symptoms was also marginally associated with smoking status (*P* = 0.051). Independent of depressive scores, mean γ-glutamyl transferase were just surpassing the cut point for men ≤ 65 μ/L indicative of alcohol abuse (31).

Table 1: Unadjusted characteristics of the study sample according to depressive symptoms

Variables (*n* = 181)	With depressive Symptoms (*n* = 82)	Without depressive symptoms (*n* = 99)	*P* value
Age (years)	44.13 ± 8.57	44.56 ± 8.14	0.729
Gender M:F	36:46	52:47	0.248
Body mass index (kg/m^2)	30.71 ± 6.88	30.04 ± 7.27	0.526
Physical activity (kcal/24h)	2723.51 ± 840.16	2650.29 ± 759.63	0.540
Smoking status *n* (%) [a]	26 (57.78)	19 (42.22)	0.051
Hypertension status *n* (%) [b]	56 (46.28)	65 (53.72)	0.708
Biochemical measurements			
γ-glutamyl transferase (μ/L) [c]	65.93 ± 59.13	65.14 ± 95.33	0.175
HDL-chol (mmol/l)	1.18 ± 0.37	1.12 ± 0.33	0.299
Triglycerides (mmol/l)	1.48 ± 1.59	1.36 ± 1.03	0.536
CVD measurements			
ABPM SBP (mmHg)	132.73 ± 17.11	133.33 ± 16.04	0.807
ABPM DBP (mmHg)	82.48 ± 10.48	83.79 ± 11.38	0.425
Inflammatory and Haemostatic Biomarkers			
CRP (mg/l) [c]	7.57 ± 9.11	9.91 ± 11.88	0.320
Fibrinogen (g/l)	3.59 ± 0.84	3.59 ± 0.94	0.924
PAI-1 (ng/ml)	36.11 ± 10.54	34.42 ± 8.69	0.241
D-Dimer (μg/L)	463.68 ± 552.14	466.47 ± 546.53	0.973

Medication			
Antihypertensive *n* (%)	17 (42.50)	23 (57.50)	0.686
Antidiabetic treatment *n* (%)	5 (55.56)	4 (44.44)	0.526
Statins *n* (%)	1 (50.00)	1 (50.00)	0.893
Antidepressants *n* (%)	1 (50.00)	0 (0)	0.271
Anti-inflammatory *n* (%)	4 (30.77)	9 (69.23)	0.275

HDL-chol; high-density lipoprotein cholesterol, hs-CRP; high sensitivity C-reactive protein, PAI-1; plasminogen activator inhibitor-1 ,ABPM SBP; 24-hour ambulatory systolic blood pressure monitoring; ABPM SBP 24-hour ambulatory diastolic blood pressure monitoring.

[a] 'Smoking' defined as serum cotinine (ng/ml): values > 14.99 μg/l.

[b] Hypertension based on the 24-hour ABPM; systolic and/or diastolic > 125/ 80 mmHg.

[c] Data not normally distributed, *P* values were calculated based on log-transformed values.

P-values < 0.05 are considered statistically significant.

The differences between depressive symptoms and inflammatory and haemostatic biomarkers (CRP, fibrinogen, PAI-1 and D-dimer) and depressive symptoms are given in Table 2. African men displayed higher PAI-1 levels (P = 0.001) and marginally elevated D-dimer levels (P = 0.055). Figures 1 and 2 reveal that in the men the depression score (PHQ-9 total score) was positively associated with PAI-1 (r = 0.24, P = 0.038) and D-dimer (r = 0.28, P = 0.013), irrespective of traditional covariates. African women with depressive symptoms displayed significantly lower D-dimer levels (P = 0.008) and marginally lower CRP levels (P = 0.075) in comparison to women without depressive symptoms. In Figure 2 the depression score shows a negative relationship with D-dimer levels (r = -0.25 , P = 0.022) in this gender group. No relationship was found between fibrinogen and depressive symptoms in both gender groups *(results not shown)* .

In logistical regression analyses it was confirmed that depressive symptoms were associated with elevated levels of PAI-1 (Odds Ratio of 1.08 (CI 95%: 1.02 - 1.14; P = 0.009) in the African men (Table 3). In African women D-dimer levels showed a relationship with depressive symptoms with an OR of 0.998 (CI 95%: 0.996 – 1.001; P = 0.031).

Sensitivity analysis was computed to additionally adjust for diabetic medication usage but it did not change the outcome of the results.

Table 2: Adjusted differences in inflammatory and haemostatic biomarkers in African men and woman with vs without depressive symptoms (mean ± 95% CI).

	African men			African women		
	With depressive symptoms (*n* =36)	Without depressive symptoms (*n* = 52)	*P*-value	With depressive symptoms (*n* = 46)	Without depressive symptoms (*n* = 47)	*P*-value
CRP (mg/l) [a]	3.44 (0.53; 6.35)	6.74 (4.33; 9.14)	0.254*	10.63 (7.17; 14.09)	13.72 (10.35; 17.10)	0.075*
Fibrinogen (g/l) [a]	3.21 (2.97; 3.45)	3.13 (2.93; 3.34)	0.555	3.86 (3.61; 4.11)	4.13 (3.89; 4.37)	0.143
PAI-1 (ng/ml)[b]	**40.71 (37.54; 43.89)**	**33.05 (31.05; 36.29)**	**0.001**	32.75 (30.00; 35.50)	35.17 (32.51; 37.82)	0.228
D-dimer (μg/L) [b]	523.82 (336.90; 710.73)	282.93 (127.34; 438.51)	0.055	**404.66 (245.24; 564.07)**	**710.44 (554.70; 866.17)**	**0.008**

CRP; C- reactive protein, PAI-1; plasminogen activator inhibitor.

[a] Variables adjusted for age, smoking (%), BMI, γ-GT, HDL-chol, anti-inflammatory, systolic and diastolic blood pressure and statin medications.

[b] Variable adjusted for age, smoking (%), BMI, γ-GT, physical activity and statin use.

* Data not normally distributed, *P* values were calculated based on log-transformed values. *P*-values < 0.05 are considered statistically significant.

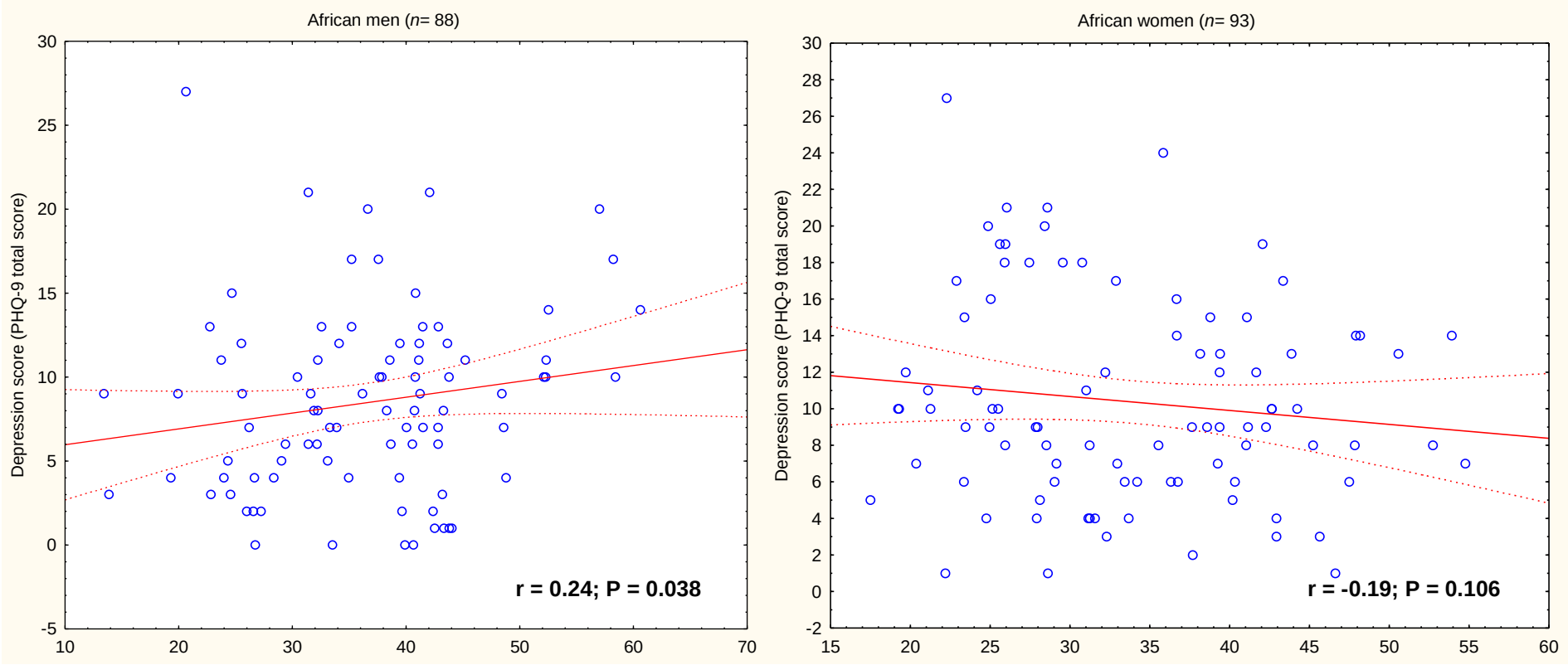

Figure 2: Association between depression score (PHQ-9 total score) and PAI-1 in African men and women; adjusted for age, BMI, smoking prevalence, γ-GT, physical activity and statin use

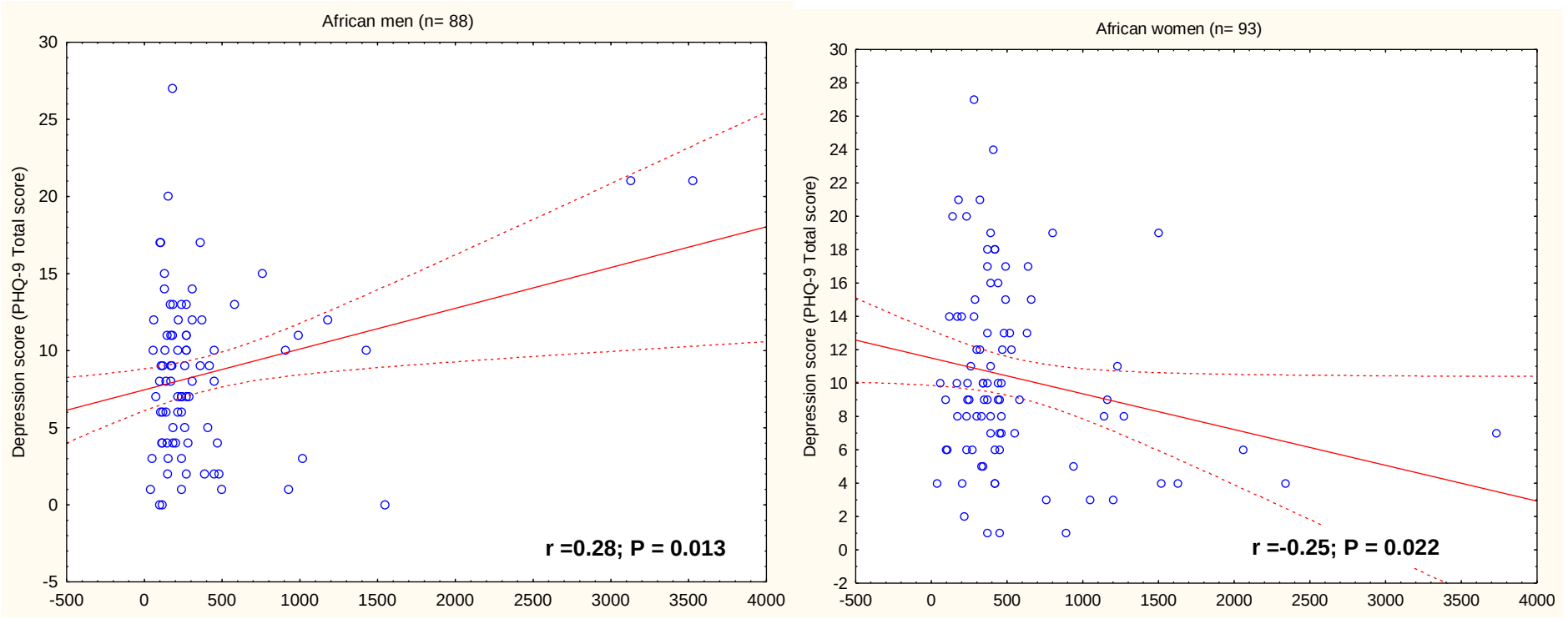

Figure 2: Associations between Depression score (PHQ-9 total score) and D-dimer in African men and women; adjusted for age, BMI, smoking prevalence, γ-GT, physical activity and statin use

Table 3: Odds Ratios evaluating the associations between depressive symptoms, inflammatory andhaemostatic markers in African men and women.

	Depressive symptoms			
	African men (n = 88)		**African women (n = 93)**	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
CRP (mg/l)	0.38(-1.28; 2.04)	0.253	0.37 (-0.71; 1.46)	0.073
Fibrinogen (g/l)	1.46 (0.48; 2.43)	0.448	1.09 (0.28; 1.90)	0.833
PAI-1 (ng/ml)	**1.08 (1.02; 1.14)**	**0.009**	2.68 (0.93; 1.04)	0.575
D-dimer (μg/L)	1.0001(0.999; 1.002)	0.068	**0.998 (0.996;1.001)**	**0.031**

CRP, high sensitivity C- reactive protein; PAI-1, plasminogen activator inhibitor-1.

P values < 0.05 are considered statistically significant.

DISCUSSION

To the best of our knowledge, the present study is the first study to evaluate the association of depressive symptoms with inflammatory markers and haemostatic factors in an African population. In a sample of 181 Africans, the study demonstrated that hypercoagulation vulnerability in Africans this may be exacerbated by hyperkinetic blood pressure, as demonstrated by the elevated mean systolic and diastolic blood pressure and hypertension prevalence. These findings suggest that the pathways linking depression to CVD risk in African men may include hypo-fibrinolytic processes. In this study, even though CRP as marker of low grade inflammation was not directly linked to depressive symptoms it may act as modulator of inflammation and thrombosis.

Depressive symptoms and inflammatory markers

Although abundantly researched, studies examining the association between depression and inflammation have produced inconsistent findings. For instance, findings from the ATTICA study showed that depression correlated positively with CRP and fibrinogen levels in both gender groups free of cardiovascular disease (32). Other studies revealed that lifetime recurrent depression was strongly related to higher levels of CRP in men, however, this was not evident in their female counterparts (33, 34). In contrast, some studies have reported no associations between inflammation and depressive symptoms, and one study found an inverse relationship between the two (35).

This study's findings differ from those studies that found a positive association between inflammation and symptoms of depression, but are consistent with the absence of associations reported in the Whitehall study II and the Cardiovascular Health Study of the elderly (16, 36). Reasons for this lack of findings are unknown; however, one can speculate that the association between depression and inflammation may vary according to the

measures of inflammation investigated and gender (15, 33). Results from a study by Pan *et al*. revealed that in their unadjusted analyses, depression was only associated with interleukin (IL)-6 (15). Two other studies reported a link with certain inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-1 and acute phase proteins such as haptoglobin (37, 38). It should be noted that the parameters investigated here have been associated with depressive symptoms in earlier research and are particularly pertinent to vascular inflammation that underlies atherosclerosis (8). Further research exploring the role of cytokines such as IL-6 and TNF-α may be warranted. Interleukin-6 plays a role in the induction of CRP secretion and also has effects on fibrinogen production (39, 40).

Other studies have noted gender differences in the association between depressive symptoms and inflammation (33, 34). However, in this study no relationship was found between depression and inflammation in either gender groups. In the women it is thought that the fluctuations in CRP levels during the menstrual cycle may hinder the detection of any link that may exist between the two (41). Considering the fact that this study also included pre-menopausal participants it is plausible that the menstrual effect may partially explain the lack of findings reported in the African women. Unfortunately, in this study information on last menses was not available, rendering it difficult to test this hypothesis.

Depressive symptoms and haemostatic markers

The relationship between negative emotional states and haemostasis is an area that has sparsely been studied and the direction of the relationship remains vague. Elevated PAI-1 and D-dimer levels have been positively linked with emotional exhaustion, recent negative life events and depressive symptoms (23, 42, 43). In contrast, other emotional states such as depressed mood have been reported to have no association with PAI-1 and D-dimer levels (44). Our results reveal a positive relationship between PAI-1 and depressive symptoms in

African men. As PAI-1 is a primary inhibitor of the fibrinolytic process, elevated levels may reflect an impaired fibrinolytic function (45). Elevated PAI-1 levels have been identified as an independent predictor of subsequent cardiac events in prospective studies of both healthy populations and in patients with CVD (22, 46). Therefore, these findings suggest that depressive symptoms might be associated with a hypo-fibrinolytic function in African men, which may partially explain the increased cardiovascular risk previously observed in this gender group (5).

D-dimer is the degradation product of fibrin at the end of the fibrinolytic process and consequently elevated levels of D-dimer reflect an increase in fibrinolytic activity (45). Therefore, it would be expected that in this study the D-dimer levels would decrease as a result of the increase in PAI-1 levels. Reasons for the rise in D-dimer levels in the presence of elevated PAI-1 are unknown; however, it has been found that elevated levels of both biomarkers are predictive of coronary artery syndrome in patients with coronary artery disease and in healthy populations and have also been observed in depression (22, 46). Interestingly, African women displayed a negative association between depression score and D-dimer levels. This may support the notion of an increased fibrinolytic function in depressed women and furthermore that depression severity is associated with hypo-fibrinolysis in African men.

Strengths and limitations

This study has several aspects that should be considered in the interpretation of the results. Firstly, the cross-sectional nature of this data makes it difficult to determine causality. Longitudinal data would be useful in understanding the temporal relationship between depressive symptoms and the biomarkers investigated in this study. It is possible that instead of depression leading to elevated levels of inflammatory markers, inflammatory state may

lead to depression, or both. It has been suggested that inflammatory cytokines are also responsible for the activation of the hypothalamic-pituitary-adrenal axis which in turn might contribute to depression (47). Secondly, it should also be noted that although the PHQ-9 is a well validated instrument used for assessing depressive symptoms in a variety of ethnic groups including sub-Saharan Africans, it is a self-reported questionnaire that is subject to reporting biases. A structured depression interview might have been the ideal method, however this would have not been feasible in a large community based research such as the present study. Thirdly, focusing on only two inflammatory biomarkers maybe an oversimplification of an extremely complex pathophysiological process involved in the atherosclerotic process. Especially when findings from this study suggest that fibrinogen in this ethnic group may be more of a marker of the coagulation cascade than inflammation, as seen by the unaltered fibrinogen levels in the presence of decreasing CRP levels. Although fibrinogen has been associated with depression and is particularly relevant to the vascular inflammatory process linked with atherosclerosis, it seems that in Africans it may play more of a haemostatic role (8, 48). Further studies may be required to evaluate the potential haemostatic role of fibrinogen in Africans.

Finally, the size of the study sample might have been insufficient to detect any significant associations in inflammatory markers. Previous studies that reported a relationship between depressive symptoms and inflammation have identified it in larger samples than reported in this study (32, 43, 49). The strengths of the study include the uniqueness of the study population, the well-controlled study design, the ability to control for potential confounding factors associated with depressive symptoms and the investigated biomarkers. Another strength is that the use of a continuous measure of depression (PHQ-9 total score) in the analyses has allowed for the investigation of the relationship between inflammatory and haemostatic markers across the continuum of depression severity.

In conclusion, depressive symptoms may increase hypercoagulation vulnerability in Africans, especially men, exacerbated by hyperkinetic blood pressure reflected by the elevated BP and high HT prevalence. Their hypertensive status may induce endothelial dysfunction by up-regulating inflammatory processes. Even though CRP as marker of low grade inflammation was not directly linked to depressive symptoms it may act as modulator of inflammation and thrombosis increasing coronary heart disease risk in African men.

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Chapter 5: General Findings and Conclusions

1. INTRODUCTION

This chapter comprises the main findings from each of the three research articles reported in this thesis. The results from each manuscript will be discussed, interpreted, elucidated and compared with the relevant literature reviewed in Chapter 1 and the individual chapters. Conclusions are drawn and recommendations are made to health professionals and researchers investigating psychosocial risk factors of cardiovascular diseases.

2. SUMMARY OF THE MAIN FINDINGS

In order to assess the relationship between depressive symptoms and cardiometabolic risk, focus was placed on different aspects of the cardiometabolic matrix and their role in target end-organ damage. These included metabolic risk factors, neuroendocrine responses, inflammatory and haemostatic markers and their possible associations with left ventricular hypertrophy (LVH) (Chapter 2 and 3). The findings of the three research articles reported in this thesis will be discussed in the following sections.

2.1. Depression, cardiometabolic function and left ventricular hypertrophy in African men and women: The SABPA Study (Chapter 2).

The aim of this sub-study was to determine the association between depressive symptoms and cardiometabolic risk in urban-dwelling African men and women, and furthermore, whether cardiometabolic risk factors were significantly associated with LVH. An association between depressive symptoms and cardiometabolic risk factors in this ethnic group was observed. Additionally, it was found that this relationship would contribute to target end-organ damage, LVH in African men and women.

Results from this section of the study revealed a modest relationship between depressive symptoms and cardiometabolic risk. Preliminary data showed that African men with depressive symptoms demonstrated a trend towards increased abdominal obesity while African women with depressive symptoms revealed a pre-diabetic state and a trend towards higher plasma glucose levels in comparison to those without depressive symptoms.

Interestingly, men without depressive symptoms, revealed increased cardiovascular (CV) risk with higher LVH. Irrespective of their respective depression status, the men also displayed high cardiometabolic risk based on mean levels of HbA1c, elevated 24-hour systolic and diastolic blood pressure (BP); elevated triglycerides and plasma glucose levels.

Further investigation revealed that LVH in participants with depressive symptoms is driven primarily by metabolic factors namely high-density lipoprotein cholesterol (HDL-chol) in African women and 24-hour systolic BP in African men.

2.2. Blunted neuroendocrine responses linking depressive symptoms and ECG left ventricular hypertrophy in black Africans: the SABPA study (Chapter 3).

The aim of this manuscript was to examine the relationship between depressive symptoms, neuroendocrine responses [with cortisol and 3-methoxy-phenylglycol (MHPG) as markers] and cardiovascular risk, i.e., LVH. It was hypothesised that Africans with depressive symptoms would display greater neuroendocrine (cortisol and MHPG) responses to acute mental stress and this increase will relate to target end-organ damage, i.e., LVH.

Our findings opposed our hypothesis as Africans with depressive symptoms demonstrated blunted cortisol and MHPG acute mental stress responses in comparison to those without

symptoms of depression. Additionally, blunted MHPG response within the low cortisol response group was associated with LVH in this group.

2.3. Hypercoagulation vulnerability exacerbated by hypertension status in black Africans with depressive symptoms: the SABPA study (Chapter 4).

The aim of this manuscript was to investigate the relationship between depressive symptoms, inflammatory and haemostatic markers in urban-dwelling black African (referred to in this manuscript as Africans) men and women. Our data demonstrated hypercoagulation vulnerability in Africans with depressive symptoms. African men with signs of depression displayed higher plasminogen activator inhibitor (PAI-1) levels and marginally elevated D-dimer levels.

In the Africans, contrary to what was hypothesised, no direct relationship was found between depressive symptoms and acute phase inflammatory markers [fibrinogen and C-reactive protein (CRP)]. Although not significantly different, Africans with depressive symptoms demonstrated lower levels of CRP. This finding contradicts the notion of depression being an inflammatory illness. Further investigation, using other more sensitive inflammatory markers including interleukin (IL)-6, in this ethnic group may be warrented.

3. COMPARISON WITH RELEVANT LITERATURE

When the results from this study are compared to findings from previous studies (as presented in Chapter 1), it is evident that certain findings are in accordance with those reported in the literature while others contradict certain physiological states often described in

individuals with depression. The findings in this study add to the sparse literature available on the relationship between depressive symptoms and cardiometabolic disease in Africans.

There is an increasing prevalence of cardiometabolic risk factors including hypertension (HT), type 2 diabetes mellitus and abdominal obesity in Africans.[1-4] Psychosocial factors such as urbanisation have been linked with cardiometabolic risk and the onset of depressive disorders.[5] The relationship between depressive disorders, in particular clinical (major depression) and subclinical depression (depressive symptoms), and cardiometabolic risk factors have been reported in the literature.[6-8] In support of these previous findings, this study has illustrated a modest association between depressive symptoms and cardiometabolic risk in African men and women. In accordance with the findings by Muhtz et al.[6] who demonstrated higher plasma glucose levels and low HDL-chol in women with depressive symptoms[6], results from this study (Manuscript 1) revealed that African women with depressive symptoms displayed a trend towards elevated plasma glucose levels and revealed a pre-diabetic state [mean glycated haemoglobin (HbA1c)>5.7% . Additionally, although not significantly different from those without depressive symptoms, in this study, low mean HDL-chol (below the recommended cut-off value of < 1.29 mmol/l for metabolic risk) was associated with LVH in women with depressive symptoms.

From literature it is evident that abdominal obesity plays a key role in metabolic-related health risk in sub-Saharan Africans and is linked with psychosocial risk factors such as urbanisation and psychological distress.[3,9,10] In African American women, central adiposity has been linked to depression and increased incidence of diabetes.[11] In this study only the African men with depressive symptoms displayed a trend towards central adiposity with a mean waist circumference exceeding the both the International Diabetes Federation (IDF) guidelines and ethnic specific cut point of 90 cm.[9, 12]

In preceding research it has been reported that African men, when exposed to psychosocial stress, display a more chronic sympathetic activation and an exaggerated peripheral resistance response pattern contributing to a sustained elevation in BP and increasing risk for LVH.[1,2,10,-14] In support of this notion, this study found that in urban African men (irrespective of depressive symptoms) ambulatory systolic BP was associated with LVH. Furthermore, this elevation in BP coupled with cardiometabolic factors that exceed IDF cut-off points, African men seemat risk for cardiometabolic-related health complications than African women.

Earlier work demonstrated elevated resting cortisol levels and heightened cortisol and salivary MHPG responses in individuals with greater depressive symptoms.[15-17]Our study did not reveal heightened resting cortisol levels or greater responses in Africans with depressive symptoms (Manuscript 2). Interestingly, preliminary analysis showed no significant differences in mean resting cortisol levels between the Africans with depressive symptoms and those without. Further analyses showed that in participants with depressive symptoms and low cortisol responsiveness, to acute mental stress, MHPG was associated with LVH. These participants demonstrated blunted neuroendocrine, i.e., cortisol and MHPG acute mental stress responses. Furthermore, these blunted responses were associated with the risk of developing LVH in these individuals. Previously, a hypocortisolemic state in Africans exposed to psychosocial stress was described.[18] In the present study, however, hypocortisolemia is evident during chronic or prolonged stress and has been linked to depression and type 2 diabetes.[19,20] These findings, along with the results demonstrated in Manuscript 2, may further support the notion that prolonged activation of physiological systems (as described in depression and chronic stress) may result in the disassociation or habituation of these processes, which may increase CV risk.[19-21] This is additionally supported by the finding that in Africans with depressive symptoms and low cortisol

responsiveness, MHPG was a predictor LVH. This result adds further support to the previously noted sympathetic activation demonstrated in participants with depressive symptoms in this ethnic group.[22] Coupled with higher hypertension prevalence compared to Africans without depressive symptoms and vascular responsiveness previously reported in this ethnic group, these vasoconstrictive responses may highlight the increased long-term depression and vascular disease risk in urbanised Africans.[1,18]

Depression is increasingly being viewed as an inflammatory illness[23] with a number of studies reporting an association between depression and acute phase inflammatory markers, such as CRP and fibrinogen.[24-26] CRP is a low grade inflammatory marker of which elevated levels have previously been reported in urban-dwelling Africans.[27] However, this finding could not be confirmed in Africans with depressive symptoms. Our study found no direct association between depressive symptoms and inflammation, specifically CRP levels. The lack of association observed confirms the findings of two other major studies that reported no link between depression and inflammation.[28,29] The occurrence of this lack of association in Africans with depressive symptoms emphasises the necessity for future research to incorporate other more sensitive measures of inflammation in addition to CRP. Pro-inflammatory cytokines such as interleukin (IL)-6, for example, has been found to be a stronger predictor of cardiovascular mortality over a 5-year follow-up than CRP, and should be included as a measure in future research.[30]

In addition to its link with inflammation, depression also appears to be associated with hypercoagulation and alteration in fibrinolytic processes.[31] Indeed, depression has been linked with an alteration in blood coagulation, fibrinolysis, D-dimers, PAI-1, platelet activation, vascular endothelial growth factor, plasma nitric oxide (NO) and its synthase.[32,33]

Furthermore, an altered L-Arginine/NO system has been demonstrated in Africans who are psychologically distressed.[33] Findings from this study (Manuscript 3) further support the findings of Reimann et al.[33]; in which an association between depressive symptoms and prothrombotic changes in African men and women was demonstrated. These changes may be exacerbated by hyperkinetic blood pressure, previously described in this study (Manuscript 1) and this population group.[33,34] These findings suggest that prothrombotic changes, namely hypercoagulation, may play a role in the association between depression and CVD risk in Africans, particularly the men.

Differences regarding the results of these manuscripts and findings from the literature may be explained by differences in study populations as some of the findings are based on Caucasian populations, differences in gender groups and types of psychological instruments utilised.

4. CHANCE AND CONFOUNDING

It is imperative to address certain confounding factors that may have influenced the results of each manuscript of this study (represented by the individual chapters) before discussing the main findings of this study.

Firstly, the cross-sectional nature of this study renders it difficult to infer causality. A longitudinal approach would have been ideal for understanding the temporal relationship between depressive symptoms and cardiometabolic risk investigated in this study.

Secondly, although a well-validated instrument, the PHQ-9, was used for assessing depressive symptoms in this study, it is a self-reported questionnaire that is subject to reporting biases. A structured depression interview might have been the ideal method for assessing depression in this population group as all dimensions associated with depressive

symptoms may be easily identified. One example of such a dimension is the culture-specific manifestations of depressive symptoms that include cognitive/ affective factors, cultural idioms of distress and somatic presentations. Coupled with cultural-specific notions of masculinity, this may obscure the detection of depressive symptoms or result in over-/ under-reporting of depressive symptoms in specific African gender groups.[35-37]

However, the use of a structured interview would not have been feasible in a large community-based study such as this. Additionally, the PHQ-9 has been validated in various ethnic groups including sub-Saharan Africans.[37] This renders the PHQ-9 a useful diagnostic tool for assessing depression severity in large population studies including the present one.

Finally, the size of the study sample may be insufficient to establish significant trends within this study. However, power calculations based on the largest standard deviation of 24-hour systolic blood pressure have shown that 50 participants per group in this type of study are more than sufficient in determining significant trends in biological profiles.[38] Furthermore, the probability of chance should also be considered in the interpretation of the results in this study. Through multiple regression analysis, statistics have indicated the possibility that one out of twenty correlations may be due to chance.

Confounding factors such as age, HIV status, smoking status, alcohol intake, physical activity, diabetic status and the use of statin medication may have influenced the results by causing over- or underestimation of the association between depressive symptoms and the cardiometabolic variables investigated in this thesis. Additionally, turning data into binary variables is not ideal as data may be lost which may have influenced the results observed.

By adjusting for age, smoking status, alcohol intake, physical activity, diabetic status and the use of statin medication, these potential confounders were addressed. Additionally, participants who were HIV positive were excluded from the analysis.

All interpretation of the statistical results from this thesis is based on physiological point of view; therefore, it is important to keep in mind that a statistical significance is not necessarily equivalent to physiological significance.

5. DISCUSSION OF THE MAIN FINDINGS

Depression, cardiometabolic function and left ventricular hypertrophy in African men and women: The SABPA Study.

Research has indicated that there is an increase in the prevalence of cardiometabolic risk factors among the African population, including HT and type 2 diabetes mellitus.[1,2] The accumulative effects of these cardiometabolic components, including abdominal obesity, may increase the risk for cardiovascular morbidity and mortality in this population group.[12] Research on the link between depression and cardiovascular risk in Africans is limited. Therefore, the aim of this study was to investigate the possible link between depressive symptoms and cardiometabolic risk in urbanised African men and women.

In the Western world the association between depression and cardiometabolic risk, more specifically the metabolic syndrome, and some of its components, has been intensively described in women.[6-8] This study demonstrated a link between depressive symptoms and cardiometabolic risk in African women and men. In particular, African women with

depressive symptoms displayed a trend towards elevated plasma glucose and revealed a pre-diabetic state. Coupled with the clustering of other metabolic components such as large WC (> 80 cm) and low HDL-chol (< 1.29 mmol/l), depressive symptoms may further augment the negative effects that metabolic factors may have in this gender group.[38] Indeed, when determining the major predictors of LVH, a marker of structural wall abnormalities, it became evident that low HDL-chol was the major metabolic predictor in this gender group. This finding is in accordance with the results demonstrated by Horio et al.[39] who demonstrated that low HDL-chol was associated with two major markers of LVH, LV structure and diastolic dysfunction.[39]

African men with depressive symptoms demonstrated a trend towards abdominal obesity with a mean waist circumference exceeding recommended cut-off points highlighted by the International Diabetes Federation Guidelines (94 cm) and the ethnic specific cut-off point (90 cm).[9,38] Abdominal obesity has been implicated as a key component for metabolic-related health risk in sub-Saharan Africa and is associated with hypertension and psychosocial factors such as urbanisation.[9,10] The correlation between obesity, in particular abdominal obesity, and hypertension is well documented. It is suggested that abdominal obesity may play an important role in the etiology and pathophysiology of hypertension through mechanisms such as the sympathetic and renin-angiotensin-aldosterone pathways.[40,41] Both of these mechanisms have been implicated with HT development and cardiovascular risk in Africans.[2]

A number of mechanisms have been proposed as mediating pathways between obesity and sympathetic functioning including hypothalamic-pituitary-adrenal axis dysfunction, insulin resistance and renin hypoactivity, subsequently leading to an increase in BP and the development of LVH.[40,41] This notion was further supported by findings demonstrating that

in African men (irrespective of depression status), LVH was driven primarily by BP and pre-diabetic status (only in depression). These findings support the notion that African men with depressive symptoms are at greater risk for a cardiovascular as seen by the risk towards poor glucose handling and the development of structural wall abnormalities.

Blunted neuroendocrine responses linking depressive symptoms and ECG left ventricular hypertrophy in black Africans: the SABPA study.

Activation of neuroendocrine pathways plays an important role in the maintenance of cardiovascular homeostasis during stress. However, prolonged activation of these systems, as noted in conditions of chronic stress, leads to dysregulation of normal endocrine functioning and increased risk for hypertension, type 2 diabetes and atherosclerosis.[42-45] This functioning (response) can be measured within laboratory settings by exposing an individual to laboratory-induced physical or mental stress. Ultimately, the acute physiological response can be related to psychosocial factors and future CVD risk.[46] Depression has been related to an elevation in resting cortisol levels and exaggerated sympathetic (represented by represented by salivary MHPG) and cortisol responses to mental stress.1[6,17,47-49] In this study, no direct association was found between depressive symptoms and resting cortisol levels in urban black Africans. However, a blunted neuroendocrine response (presented by salivary MHPG and cortisol) was demonstrated in Africans with depressive symptoms. This was further associated with risk of developing structural wall abnormalities (LVH). It is proposed that since depression is a constant state of perceived stress, further exposure to a challenging urban environment, psychosocial stress or induced mental stress may result in habituation of the neuroendocrine pathways.[19,20] One other hypothesis suggests that, hippocampal degeneration often associated with depression may result in a maladaptive cortisol responses

leading to the dysregulation of HPA axis activity and the above-described patterns of cortisol release.[50] Stress triggers sympathetic activity. Under chronic conditions this leads to hyperactivity resulting in an elevation in catecholamine release and ultimately depletion; a process that is abrogated by cortisol.[19,20] Depressive symptoms may sensitise the individual to stress via the attenuation of norepinephrine and the maladaptive cortisol responses, potentially contributing to allostatic load resulting in an increase in pre- and afterload to the heart which can predispose Africans to structural LVH changes.[51,52] Together with an increased HT prevalence, these potentially vasoconstrictive responses may be the underlying mechanisms responsible for the association between depression and vascular disease in this population group

Hypercoagulation vulnerability exacerbated by hypertension state in black Africans with depressive symptoms: the SABPA study.

As previously mentioned, depression has been described as an inflammatory illness.[23] However, literature pertaining to this relationship has produced inconsistent findings with some studies reporting a positive relationship,[25,26] no association[28,28] and even an inverse relationship was described.[53] The lack of association found reported in this study may be as a result of differences in gender and inflammatory measures investigated (CRP and fibrinogen). Even though CRP is not directly linked with depre ssive symptoms in Africans it may act as modulator of inflammation and thrombosis, increasing coronary heart disease risk previously described in this population group. The lack of correlation between CRP and fibrinogen levels (results not reported) in depressed individuals further suggests that fibrinogen, may be more of a marker of the haemostatic/ coagulation cascade than inflammation.

Vulnerability towards hypercoagulation was demonstrated in Africans, especially men, which may be related to the onset of coronary artery syndrome in patients with coronary artery disease and in healthy populations and has also been observed in depression.[54] The hypertension prevalence in African men may exacerbate this vulnerability and induce endothelial dysfunction by up-regulating inflammatory processes thus increasing the risk for atherosclerotic cardiovascular diseases in this gender group.

6. GENERAL CONCLUSIONS

A modest relationship was found between cardiometabolic risk factors and depressive symptoms in the African population. The importance of such an association cannot be ignored due to the increasing prevalence of cardiometabolic risk factors such as HT and type 2 diabetes mellitus in this ethnic group. This is particularly pertinent to the African males who displayed more cardiometabolic symptoms exceeding recommended cut-off points. Results suggest that in African women with depressive symptoms target end-organ damage, specifically left ventricular hypertrophy, was driven by a metabolic factor, specifically low HDL-chol. In African men (irrespective of depression), increased ambulatory systolic BP was the driving factor for LVH.

In Africans with depressive symptoms, a blunted neuroendocrine response was demonstrated as the link between depression and target end-organ damage, LVH. Together with increased HT prevalence, these vasoconstrictive agent responses may be the underlying mechanism responsible for the relationship between long-term depression and vascular disease risk in this ethnic group.

Finally, results suggest that depressive symptoms may increase hypercoagulation vulnerability in Africans, particularly the men. This may be exacerbated by hyperkinetic

blood pressure previously reported in this gender group. Their hypertensive status may induce endothelial dysfunction by up-regulating inflammatory processes. Even though CRP as marker of low grade inflammation was not directly linked to depressive symptoms it may act as modulator of inflammation and thrombosis increasing coronary heart disease risk in African men.

7. CONTRIBUTION OF THE STUDY AND FURTURE RECOMMENDATIONS

The contributions of this study lie in the results of the urban-dwelling Africans, especially those with depressive symptoms. There is an emerging burden of cardiovascular disease among urban Africans and a 3-fold risk of depressive symptoms has been observed in this population group in comparison to Caucasians.[17,55] Identifying and treating depressive symptoms in Africans may play a role in reducing stress-related cardiovascular risk in this ethnic group.

Secondly, the role of depressive symptoms in target end-organ damage (LVH) and 'global' cardiometabolic risk in Africans provides novel findings about the input that emotional factors have on the development of cardiovascular diseases in this subject cohort.

Thirdly, hyperkinetic BP and HT status exacerbated the results found in this study. A relationship between ambulatory BP and depressive symptoms has been previously reported in the African cohort.[34] The findings in this study add further information on this subject and emphasise the importance of blood pressure management in this ethnic group.

Fourthly, the high mean values for metabolic risk indicators found in this African cohort adds to the already established emerging prevalence of cardiometabolic risk factors previously reported on Africans. This should become a source of public health concern in CVD risk management in the future.

Lastly, increased hypercoagulation vulnerability in Africans, especially the men, with depressive symptoms was established. These findings add a new perspective on the role of depressive symptoms on haemostasis and cardiovascular risk in Africans.

Recommendations

The following recommendations are proposed for future studies:

A longitudinal design is needed to determine causality in this ethnic group. It is imperative to understand the nature of the relationship between depressive symptoms and cardiometabolic risk in Africans in order to implement effective intervention strategies that focus on stress and cardiovascular health in particular HT prevention in this ethnic group.

A larger population sample may be ideal in allowing sufficient statistical power for sub-stratification.

Future studies should include other markers of inflammation including IL-6. The inflammatory cascade is an extremely complex pathophysiological process involved in the atherosclerotic process. The addition of more inflammatory markers may give a better understanding of the role of depression in the inflammatory process.

To further support the notion of sympathetic activation in depressed participants, other supportive measures should also be assessed including baroreceptor sensitivity and cardiovascular reactivity responses to stress.

Carotid intima-media thickness as a marker of target end-organ damage should be included in future studies to substantiate the role that inflammation and haemostasis play as risk factors for coronary heart disease risk in African men.

More direct measures of LVH, through echocardiography must be implemented in future studies.

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