Coping and endothelial dysfunction: the SABPA study

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OPSOMMING

Titel: *Coping en endoteeldisfunksie: die SABPA studie.*

Motivering: Daar is getoon dat die coping meganismes wat gebruik word om psigososiale stres te hanteer, verband hou met die ontwikkeling van kardiovaskulêre siekte. Elke coping styl het verskillende fisiologiese uitwerkings op die kardiovaskulêre stelsel. ’n Verdedigende aktiewe coping (AC)-styl toon ’n meer negatiewe impak op kardiovaskulêre gesondheid as 'n vermydingstyl in stedelike swart Suid-Afrikaners (hierna verwys na as Afrikane), weens ’n verhoogde α-adrenergiese respons wat in stedelike swart Afrikane waargeneem is. Teenstrydige literatuur bestaan oor die langtermyn-uitwerking van spesifieke coping-meganismes op die kardiovaskulêre stelsel. Afrikane het 'n groter voorkoms van hipertensie as hul landelike Afrikaan- en Kaukasiese eweknieë, ’n verskynsel wat aan die psigologiese stres en veranderinge in dieet en lewensstyl as gevolg van verstedeliking toegeskryf word. Vorige studies het aangedui dat die endoteel en vaskulêre struktuur nadelig beïnvloed word tydens psigologiese stres en dit het kardiovaskulêre patologie tot gevolg. Dit noodsaak navorsing oor die effek van AC op endoteelfunksie en vaskulêre hermodulering.

Doelwit: Die doel van hierdie studie was om te bepaal of die gebruik van 'n verdedigende AC-styl in reaksie op stres 'n verskil in endoteelfunksie tussen Afrikane en Kaukasiërs tot gevolg het, en of die vaskulêre struktuur verskillend beïnvloed word in hierdie twee groepe.

Metodiek: Hierdie studie vorm deel van die SABPA (Simpatiese aktiwiteit en Ambulatoriese Bloeddruk in Afrikane) studie, wat van 2008 tot 2009 uitgevoer is. Hierdie sub-studie is 'n teikenbevolking-studie op Afrikaan- en Kaukasiese mans. Die studie-steekproef het bestaan uit 80 Afrikaan en 97 Kaukasiese manlike onderwysers, dienooreenkomstig gekies om homogeniteit ten opsigte van sosio-ekonomiese status en werksomgewing te verseker. Die studie is goedgekeur deur die Etiekkomitee van die Noordwes-Universiteit en prosedures is nagekom soos uiteengesit in die bepalings en riglyne van die 2008 Verklaring van Helsinki. Elke deelnemer het psigososiale vraelyste, insluitende die “Coping Strategy Indicator” (CSI),

voltooi onder die toesig van kliniese sielkundiges. Ambulatoriese bloeddruk is gemeet met die Cardiotens CE120 ® apparaat. Antropometriese metings is uitgevoer deur vlak II geregistreerde antropometriste met behulp van gestandaardiseerde metodes, en die fisiese aktiwiteit van elke deelnemer is bepaal deur gebruik te maak van die Actical® omnidireksionele versnellingsmeter. Bloedmonsters is ingesamel deur 'n geregistreerde verpleegkundige en inligting met betrekking tot die deelnemer se medikasiegebruik en mediese geskiedenis is verkry. Rustende bloedmonsters is ontleed vir die biochemiese merkers gamma glutamiel transferase (as maatstaf van alkohol inname), totale cholesterol, hoëdigtheid lipoproteïen cholesterol, C-reaktiewe proteïen, hemoglobien A_{1C}, kotinien (as 'n merker van rookstatus), en die endoteelmerkers stikstofoksied metaboliete (NOx) en von Willebrand-faktor (VWF). Bloedmonsters is ook geneem nadat die deelnemers die Stroop kleur-woord-konfliktoets afgehandel het om NOx en VWF reaktiwiteit teenoor stres na 1 minuut blootstelling, te bepaal. Die reaktiwiteit is uitgewerk as die persentasie verandering vanaf die basislynwaarde. MIV/VIGS-status is bepaal deur gebruik te maak van teenliggaamtoetse. Linker gemeenskaplike karotis intima-mediadikte van die verste wand (L-CIMTf) is gemeet en die linker gemeenskaplike karotis deursnee-wandarea (L-CSWA) is bepaal deur middel van ultrasonografie en deur van die geskikte formules gebruik te maak.

Wat statistiese analises betref, is T-toetse gebruik om die bevolking te beskryf volgens etnisiteit. Van die 80 Afrikaan- en 97 Kaukasiese mans, is 59 Afrikane en 80 Kaukasiërs geïdentifiseer as gebruikers van die AC-styl deur die CSI vraelys. Interaksie met CIMT is slegs gedemonstreer in AC-groepe, en verdere statistiese analises met vermydingsgroepe is gestaak. Analise van kovariansie (ANCOVA) is gebruik om beduidende verskille tussen die etnies gegroepeerde AC-mans te bepaal. Betekenisvolle p-waardes is bepaal deur gebruik te maak van die Benjamini-Hochberg-regstelling vir valse ontdekking. Hiërargiese regressie-analises is uitgevoer in verskillende modelle om onafhanklike assosiasies tussen strukturele en funksionele vaskulêre merkers met 24-uur bloeddrukmerkers te bepaal. Betekenisvolle waardes is noteer as $R \geq 0.15$, aangepaste $R^2 \geq 0.25$ en $p \leq 0.05$.

Resultate: Verdedigende AC-Afrikaanmans het verhoogde endoteeldisfunksie getoon in vergelyking met hul Kaukasiese eweknieë. NOx reaktiwiteit was -52.47 % vir die AC-Afrikane teenoor 895.33 % vir die AC-Kaukasiërs ($p < 0.0001$). VWF reaktiwiteit was 15.78 % vir die AC-Afrikane teenoor 53.58 % vir die AC-Kaukasiërs ($p < 0.001$). Daarbenewens was 24-uur polsdruk sterker geassosieer met L-CIMTf en L-CSWA in AC-Afrikane ($\Delta R^2 = 0.11$ en $\Delta R^2 = 0.08$, respektiewelik, $p < 0.001$) as in die totale Afrikaangroep ($\Delta R^2 = 0.08$ en $\Delta R^2 = 0.06$, respektiewelik, $p < 0.05$). Geen assosiasies met ambulatoriese bloeddrukmerkers het by Kaukasiërs voorgekom nie.

Gevolgtrekking: ’n Verdedigende AC-respons op stressors fasiliteer vaskulêre hermodulering en endoteeldisfunksie in Afrikaanmans. Vaskulêre hermodulering is ook getoon in ’n groter groep Afrikaanmans, wat nie-AC-gebruikers insluit, maar die effek was sterker in die verdedigende AC-Afrikaanmans. Die gevolgtrekking word dus gemaak dat die verdedigende AC-styl ’n kardiovaskulêre risiko vir Afrikaanmans inhou.

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SUMMARY

Title: *Coping and endothelial dysfunction: the SABPA study.*

Motivation: It has been shown that the coping styles used to cope with psychosocial stress are related to the development of cardiovascular disease, with each coping style having a different physiological effect on the cardiovascular system. A defensive Active Coping (AC) style has a more negative impact on cardiovascular health than an avoidance style in urban black South Africans (hereafter referred to as Africans), owing to the enhanced α-adrenergic response of urban Black Africans. However, contradicting literature exists regarding the long-term effect of specific coping styles on the cardiovascular system. Africans have a greater prevalence of hypertension than their rural Black African and Caucasian counterparts, which has been attributed to the psychosocial stress and changes in diet and lifestyle during urbanization. Previous studies have indicated that endothelium and the vascular structure are adversely affected during psychological stress, leading to cardiovascular pathology. This necessitates research into the effect of AC on endothelial function and vascular remodelling.

Objectives: The purpose of this study was to determine whether the use of a defensive AC style in reaction to stress showed a difference in endothelial function between Africans and Caucasians, and whether the vascular structure is differently affected in these two groups.

Methodology: This sub-study forms a part of the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study, which was conducted from 2008 to 2009. This sub-study is a target population study on African and Caucasian men. The study sample included 80 African and 97 Caucasian men, and the sample was selected to ascertain homogeneity regarding socio-economic status and working environment. The study was approved by the Ethics Review Board of the North-West University, and procedures complied with terms and guidelines of the 2008 Declaration of Helsinki. Each participant completed psychosocial questionnaires, including the Coping Strategy Indicator (CSI) under

the supervision of clinical psychologists. Ambulatory blood pressure (24 hours) was recorded with the Cardiotens CE120® apparatus. Anthropometric measurements were performed by level II registered anthropometrists, using standardised methods, and the physical activity of each participant was determined, using the Actical® omnidirectional accelerometer. Blood samples were collected by a registered nurse and information regarding the participant's medication use and medical history was obtained. Resting blood samples were assessed for the biochemical markers gamma glutamyl transferase (as a measure of alcohol consumption), total cholesterol, high-density lipoprotein cholesterol, C-reactive protein, haemoglobin A_{1c}, cotinine (as a marker of smoking status), and the endothelial markers nitric oxide metabolites (NOx) and von Willebrand factor (VWF). Blood samples were also collected after participants had completed the Stroop colour-word conflict test, to determine NOx and VWF reactivity to stress. Reactivity was calculated as the percentage increase from the baseline value. HIV/AIDS status was determined, using antibody tests. Left common carotid intima-media thickness of the far wall (L-CIMTf) was measured and the left common carotid cross-sectional wall area (L-CSWA) was calculated via ultrasonography and by applying the appropriate formulas.

Regarding statistical analyses, T-tests were used to describe the population by ethnic status. Of the 80 African and 97 Caucasian men, 59 Africans and 80 Caucasians were identified as using the AC style by the Coping Strategy Indicator. Interaction with CIMT was only demonstrated in AC groups, and further analyses with avoidance groups were ceased. Analyses of covariance (ANCOVA) were used to show significant differences between the ethnically grouped AC men, with significance levels determined using the Benjamini-Hochberg correction for false discovery. Hierarchical regression analyses were performed in different models to determine independent associations of selected structural and functional vascular markers with 24-hour blood pressure markers. Significant values were noted as $R \geq 0.15$, adjusted $R^2 \geq 0.25$, and $p \leq 0.05$.

Results: Defensive AC African men showed more pronounced endothelial dysfunction when compared to their Caucasian counterparts. Nitric oxide metabolite reactivity was -52.47 % for the AC Africans and 895.33 % for the AC Caucasians ($p < 0.0001$). VWF reactivity was 15.78 % for the AC Africans compared with 53.58 % for the AC Caucasians ($p < 0.001$). Furthermore, 24-hour pulse pressure was strongly associated with L-CIMTf in AC Africans ($\Delta R^2 = 0.11$ and $\Delta R^2 = 0.08$, respectively, $p < 0.001$) in comparison with the total African group ($\Delta R^2 = 0.08$ and $\Delta R^2 = 0.06$, respectively, $p < 0.05$). No associations between ambulatory blood pressure markers were shown for Caucasians.

Conclusion: A defensive AC response to stressors facilitates vascular remodelling and endothelial dysfunction in African men. Vascular remodelling was also present in a larger group which included non-AC users, but the effect was greater in defensive AC African men. Therefore, the defensive AC style holds a cardiovascular risk for African men.

LIST OF TABLES

CHAPTER THREE

ABBREVIATIONS AND NOMENCLATURE

ABPM	Ambulatory blood pressure monitoring
AC	Active Coping
AMS	Artery measurement software
ANCOVA	Analysis of Covariance
BP	Blood pressure
BSA	Body surface area
CCA	Common carotid artery
CRP	C-reactive protein
CSI	Coping Strategy Indicator
CVD	Cardiovascular disease
d	Diameter
DBP	Diastolic blood pressure
ECG	Electrocardiogram
ESH	European Society of Hypertension
HART	Hypertension in Africa Research Team
HbA_{1C}	Glycated haemoglobin A_{1C}
HDL-C	High-density lipoprotein cholesterol
HIV	Human immunodeficiency virus
kcal	Kilocalorie(s)
L	Litre(s)
L-CIMTf	Left carotid intima-media thickness of the far wall
L-CSWA	Left cross-sectional wall area
MHz	Megahertz
mm Hg	Millimetres of mercury
mg	Milligram(s)
ml	Millilitre(s)
mmol	Millimole(s)
N	Unitless quantity
ng	Nanogram(s)
NS	Not significant

NWU	North-West University
PP	Pulse pressure
SABPA	Sympathetic activity and Ambulatory Blood Pressure in Africans
SBP	Systolic blood pressure
TC	Total cholesterol
TEE	Total energy expenditures
TPR	Total peripheral resistance
U/l	Units per litre
URL	Uniform resource locator
U	Unit(s)
VWF	Von Willebrand Factor
WHO	World Health Organisation
y	Years
α	Alpha
β	Beta
γ-GT	Gamma glutamyl transferase
µmol	Micromole(s)
χ^2	Chi square

CHAPTER ONE: PREFACE AND OUTLINE OF THE STUDY

1.1. Preface

This dissertation has been completed for fulfilment of the requirements for the degree *Master of Science in Physiology* at the Potchefstroom Campus of the North-West University and is presented in article format. The article manuscript in Chapter Three has been submitted to the peer-reviewed *Journal of Atherosclerosis and Thrombosis*, and is therefore presented in the prescribed journal format. At the end of Chapters Two, Three, and Four of this dissertation, the relevant references cited therein are indicated. The reference format throughout this dissertation is consistent with the guidelines for publishing in the aforementioned journal, according to the journal's bibliographic style (see *Instructions for Authors* in Chapter Three of this dissertation for further information).

1.2. Outline of the study

This dissertation is divided into four chapters which consist of the following information:

Chapter One contains the preface and an outline of the study. It also specifies the contributions of the student and supervisors to the study.

Chapter Two contains the introduction to and main literature overview of the study, with a detailed discussion of the different coping styles, endothelial and structural vascular markers, and the association of each with ambulatory blood pressure. Chapter Two concludes with questions arising from the literature and hypotheses based on the literature.

Chapter Three contains the article manuscript of the study entitled *Stress appraisal, blunted endothelial response, and vascular remodelling in African men: The SABPA study*, prepared according to the author instructions of the aforementioned journal.

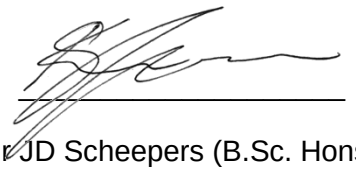
Chapter Four contains a summary of the findings of the study and a brief discussion thereof. The weaknesses of the study are also discussed, along with recommendations for future research. Finally, the conclusion of the study is provided.

1.3. Authors' contributions

The researchers involved in this dissertation contributed as follows:

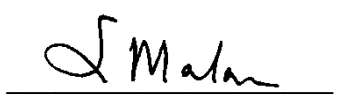
- Mr JD Scheepers (B.Sc. Hons.) was responsible for the writing and presentation of the dissertation, which included all literature searches, statistical analyses, and interpretation of the results.
- Prof L Malan (R.N., Ph.D.), as supervisor for this study and Principle Investigator of the SABPA study, contributed to the study design, data collection, provided funding, led the initial planning of this dissertation, and provided supervision during the analyses, writing, and review processes of this dissertation.
- Mrs A de Kock (M.Sc.) and Prof NT Malan (D.Sc.), as co-supervisors, contributed to data collection and assisted in the planning, writing, and review processes of this dissertation.

I, Jacobus De Wet Scheepers, student number 20765274 at the Potchefstroom Campus of the North-West University, hereby declare that the aforementioned is an accurate reflection of my actual contribution, and that I consent to having the manuscript contained in Chapter Three published as part of the dissertation for the degree *Master of Science in Physiology.*



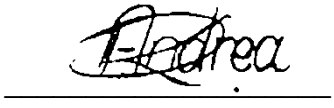
Mr JD Scheepers (B.Sc. Hons.)

As co-authors of the manuscript in Chapter Three, we the undersigned hereby give permission that this manuscript may form part of the dissertation *Coping and endothelial function: the SABPA Study* for the degree *Master of Science in Physiology* by Jacobus De Wet Scheepers.



Prof L Malan (R.N., Ph.D.)

Prof NT Malan (D.Sc.)



Mrs A de Kock (M.Sc.)

CHAPTER TWO: INTRODUCTION AND LITERATURE OVERVIEW

1. INTRODUCTION AND LITERATURE OVERVIEW

Despite progressively more specialized healthcare and growing awareness of the risks, a rapid increase in the prevalence of cardiovascular disease in urban black South Africans (hereafter referred to as Africans) has been observed in the past five decades. Cardiovascular mortalities have become the leading cause of non-communicable disease mortalities by the year 2000.[1, 2] There is consistent evidence that life stressors are related to an increased risk of cardiovascular disease (CVD) and mortality.[3-5] Stress triggers neural, endothelial, and structural vascular changes, which are injurious to the vasculature when consistent or overwhelming.[7-11]

Stress appraisal, or the coping styles used to respond to psychosocial stress, are also related to the development of CVD, with each coping style having a different physiological effect on the cardiovascular system.[12, 13] In urban Africans, a defensive Active Coping (AC) style has a negative impact on general and cardiovascular health,[14, 15] contrary to findings from previous research conducted on European Caucasians.[12, 16] Urban Africans have a greater prevalence of hypertension and stroke than their rural African and urban Caucasian counterparts. This has been attributed to a dissociative neuro-endocrine defensive AC response to psychosocial stress, as well as lifestyle changes which may occur in the transition from rural to urban areas.[15, 17, 18]

Previous studies indicated that endothelial function is adversely affected during psychological stress, leading to restriction of blood flow and tissue perfusion.[10, 19, 20] Pathology of the vascular structure has been observed in conjunction with endothelial dysfunction in previous studies, but it is not yet clear whether endothelial dysfunction is a cause or effect of changes in vascular structure.[21, 22] Hypertension may exacerbate cardiovascular pathology due to vascular remodelling in response to high blood pressure (BP).[23, 24]

Therefore, we will address contributory factors to the above-mentioned as follows:

1.1. Cardiovascular disease prevalence in South Africa

No clear data are available on the current prevalence of CVD in South Africa. The last South African Demographic and Health Survey in 1998 revealed a hypertension prevalence rate of 21% for both men and women,[25] but since more than 15 years have passed, this may not accurately reflect current hypertension prevalence. Burden of Disease estimates for South Africa showed that CVD was the leading cause of mortality from non-communicable diseases in 2000.[1] According to these figures, those of African descent had the lowest CVD mortality rates of all South African nationalities. However, this study did not take into account the difference in CVD prevalence between urban and rural Africans. Most cases of CVD in developing countries, such as South Africa, seem to be mainly in urban areas.[26] In 2010, approximately 10.4% of South African adults self-reported that they were diagnosed as being hypertensive by a health professional.[27] This figure does not account for undiagnosed cases or untruthful self-reporting. Current knowledge on the prevention of CVD is mainly based on European or American cohorts.[28] A concerning lack of information exists for populations of developing countries such as South Africa, especially Africans, as studies performed on other cultural or ethnic groups may not be applicable to the South African population.

1.2. Hypertension in rural and urban areas

There is a higher rate of hypertension among urban Africans than their Caucasian and rural counterparts, with urban Africans having the highest rates of hypertension and rural Africans the lowest.[29] Hypertension is also more frequent in men than in women within the urban African and Caucasian populations, but the reverse is true for rural Africans.[18, 29, 30] The difference in hypertension prevalence between rural and urban Africans was first documented in 1960 by Scotch after interviewing and performing BP measurements on rural and urban Zulus in the Natal province of South Africa.[31] It was observed that the BP of urban Zulus was significantly higher than that of rural Zulus. The BP of most of the urban Zulus fell

above the (then) recommended cut-off point (≥ 90 mm Hg) for diastolic BP (DBP), whereas the BP of the rural Zulu fell below the cut-off.[32] This trend was again shown among the Zulu in 1982,[33] among a group of Venda in 1992,[34] and among a group of randomly selected but mainly Setswana-speaking people in the North West province of South Africa in 2000.[30]

1.3. Psychological stress and cardiovascular disease

The **S**ympathetic activity and **A**mbulatory **B**lood **P**ressure in **A**fricans (SABPA) study was the first psychophysiological study in sub-Saharan Africa which has been designed to analyse the relationship between psychological stress and BP in Africans.[35] Increased psychological stress in the Africans as a result of the adaptation to an urban environment, has been proposed as a possible causative factor for the increase in the prevalence of hypertension.[13, 15, 35] Hypertension and other cardiovascular pathologies are frequently observed in individuals suffering from psychological stress, with evidence provided in animal and human models.[5, 8, 36-39]

Chronic psychological stress augments behavioural risk responses, as it is associated with an increase in habitual smoking and/or alcohol abuse, sedentary lifestyle, and dietary changes.[40-42] However, physiological changes in response to stress also occur. The reports of stress in chronic stress studies are mostly subjective, because psychological stress is mostly considered a subjective experience which cannot be quantitatively measured and compared with reference values. However, studies are increasingly reporting associations between psychological stress and quantitative markers. Blunted baroreceptor reflex (baroreflex) sensitivity was reported in individuals suffering from chronic psychological stress, with participants' heart rate failing to increase when BP increases during exposure to mental stress.[43] Phenylethanolamine-N-methyltransferase, the adrenaline synthesizing enzyme in sympathetic nerves, is also gaining acceptance as an explicit indicator of mental stress.[44] Not only stress, but also appraisal of stress or specifically the coping style preference in response to stress, may affect CVD risk.

1.4. Coping style, sympathetic activity, and cardiovascular pathology

Folkman *et al.* defined coping as a person's "cognitive and behavioural efforts to manage (reduce, minimize, master, or tolerate) the internal and external demands of the person-environment transaction that is appraised as taxing or exceeding the person's resources" [37, 45] Thus, as in psychological stress, when a person feels that he or she is struggling, unable, or unwilling to perform as expected, a coping style will be utilised to manage these demands. Resources for coping may be either psychologically internal, such as personality traits, or external, such as social support.[37, 45]

Coping responses to psychological stressors can be measured by the Coping Strategy Indicator,[6] in which coping is divided into three main styles: AC, a type of problem-focused coping in which the person actively tries to eliminate the problem, and avoidance, a type of emotion-focused coping in which the person withdraws psychologically from the problem.[45] A third coping style, termed "seeking social support" is also sometimes used in conjunction with AC, in which the person seeks help, comfort, and advice to manage the problem.[45]

The Coping Strategy Indicator

The Coping Strategy Indicator was developed by Amirkhan and published in 1990.[6] This self-report measure of coping strategies in specific situations incorporates seeking social support, AC and avoidance strategies. The questionnaire contains 33 items with a combination of inductive and deductive methodologies to unambiguously test for specific coping styles the person uses in response to stressors; a score of ≥ 19 indicates an above mean avoidance response to stressors, ≥ 23 an above mean "seeking social support" response, and ≥ 26 an above mean AC response to stressors.[6]

Activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis is known to occur in response to psychological stress, which results in the release of catecholamines.[9, 12, 46] Furthermore, enhanced cardiovascular reactivity is elicited in

defensive AC African subjects when compared to subjects utilising avoidance,[47], indicating that people of African origin who respond to stress with a defensive AC style may have an exaggerated response to stressors. When using the defensive AC style, a mainly β-1 adrenergic response is provoked in Caucasians,[48] which manifests as an increase in systolic BP (SBP) by way of increased catecholamine release, particularly epinephrine, and increases in heart rate, cardiac output and stroke volume, with smaller increases in total peripheral resistance (TPR) and diastolic BP (DBP).[15, 49-51] When utilising avoidance, a mainly α-adrenergic response is elicited via norepinephrine, leading to increases in DBP, TPR and Windkessel compliance, with weaker effects apparent on central cardiac variables.[15, 49, 51] Hinderliter *et al.* showed that African Americans had increased heart rate, greater peripheral resistance, and increased left-ventricular wall thickness when compared to Caucasians with similar resting BP levels.[52] However, contradicting literature exists regarding the long-term effect of specific coping styles on the cardiovascular system.[16, 47, 53] It was previously thought that AC promoted cardiovascular health, but recent studies showed that urban Africans who used the defensive AC style had a greater risk for hypertension, silent ischaemia, and left ventricular hypertrophy than their Caucasians counterparts.[13, 15, 16] Urban defensive AC Africans display dissociation between behavioural and physiological responses.[13] Contrary to the literature, urban AC Africans exhibit a mainly α-adrenergic vascular response to stressors as evidenced by diminished central but greater peripheral cardiovascular responsiveness, which may provide an explanation for urban defensive AC Africans' increased risk for cardiovascular pathology.[13, 15] The physiological response of AC Africans is therefore dissociative, as a defensive AC style is reportedly utilized to cope with stress, but a physiological avoidance response or "loss of control" is elicited.

1.5. Nitric oxide as a measure of endothelial function, and its relation to coping

Nitric oxide (NO) is a powerful vasodilatory agent and a direct marker of endothelial function which is affected by mental stress.[5] NO plays an important role in the maintenance of vascular tone and its reactivity to physiological, as well as psychological changes.[5, 54]

Moreover, the action of NO is in direct opposition to the actions of contraction factors such as endothelin-1 and angiotensin-II.[54] Therefore, inhibition of NO production in endothelial cells may cause restriction of blood flow owing to insufficient vasodilation (and possibly α-adrenergic vasoconstriction), thereby decreasing tissue perfusion.[19, 20] As the half-life of biologically produced NO is less than one second, quantitative measures of NO can only be obtained *in vivo*.[5, 19] However, the metabolites of NO, nitrite and nitrate, have a longer half-life (approximately four to five hours) and may be measured *in vitro* from blood samples.[5]

Pro-inflammatory cytokines, glucocorticoid hormones and endothelin-1, released in response to mental stress, may downregulate endothelial NO synthase, an enzyme in the NO production pathway, and therefore mental stress may cause a decrease in NO production.[55] No studies regarding the specific effect of coping on NO have been conducted to date. However, as previously mentioned, blunted baroreflex sensitivity and depressed heart rate variability are considered a response to psychological stress. In studies on animals it was shown that a decrease in baroreflex sensitivity may be a result of stress-induced decrease in NO, independent of the direct effect of the stress hormones.[56] Because NO is also an important neurotransmitter for glutamate receptors of the afferent baroreceptor nerves, a reduction in NO bioavailability should cause a reduction in activity of the baroreceptor reflex, which may explain blunted baroreflex responses during psychological stress.[57] Blunted baroreflex sensitivity has previously been reported in urban African men, and could therefore be a result of decreased NO bioavailability during acute stress.[58]

1.6. Von Willebrand Factor as a measure of endothelial function, and its relation to coping

Von Willebrand factor (VWF) is a glycoprotein secreted by endothelial cells and is an essential biomolecule in haemostasis.[59] VWF, unlike NO, is not involved in the regulation and maintenance of vascular tone, but because it is so selectively expressed in endothelial cells, it is considered an important marker of endothelial function.[54, 59] Mental stress induces the release of VWF in a similar extent in healthy and hypertensive individuals.[54] No research

has been performed on the effect of coping styles on VWF response to mental stress. However, the release of VWF from endothelial cells is mediated primarily by β-adrenergic activity during acute stress.[59, 60] As the defensive AC style is associated with a β-adrenergic response in Caucasians but an α-adrenergic response in Africans, VWF activity should therefore be increased in AC Caucasians when compared to AC Africans.

1.7. Mechanical risk factors for vascular remodelling, carotid intima-media thickness, and its relation to endothelial function

For many years SBP and DBP have been considered the only mechanical risk factors for vascular remodelling, i.e. an increase in thickness of the vascular wall.[61] Eutrophic (inward) remodelling is a characteristic of hypertension, causing obstructed blood flow and, if severe, may impede blood flow in the affected arteries.[62] Pulse pressure (PP), the difference between DBP and SBP, later emerged as a risk factor for eutrophic remodelling of the vasculature. SBP and PP are now considered the most important risk markers for eutrophic remodelling.[24, 63] Ambulatory 24-hour SBP and PP predict vascular remodelling better than other types of BP monitoring.[24]

Hypertension and CVD progression has been directly related to the intima-media thickness of the common carotid artery (CCA), as measured by carotid intima-media thickness (CIMT) ultrasonography.[25, 64, 65] CIMT increases with SBP and PP as a result of chronic shear stress and distending pressure on the vascular wall.[25, 61, 66] CIMT of the left CCA of the far wall (L-CIMTf) is most often used because of its superficial location, size, limited movement and ease of access.[64] Structural (and not functional) changes of the vasculature may be confirmed by calculating the left cross-sectional wall area (L-CSWA) with the formula $L\text{-}CSWA = \pi(d/2 + L\text{-}CIMTf)^2 - \pi(d/2)$, with d the luminal diameter of the far wall of the left CCA.[64, 65] Vascular wall remodelling has been associated with endothelial dysfunction in previous studies, however this may be because increased BP affects both the vascular wall and endothelial function.[21, 22, 55] Increased CIMT has been associated with psychological stress in Caucasians, African Americans,[11, 67] and with SBP in AC Africans.[14] Indeed,

defensive AC facilitated sympathetic hyperactivity, i.e. depressed heart rate variability and structural wall changes in urban African males but not their Caucasian counterparts.[68]

1.8. Questions arising from the literature

The preceding literature necessitates the following questions:

- Will the defensive AC style show a greater degree of endothelial dysfunction in African men than in Caucasian men?
- Will endothelial dysfunction show an association with BP in defensive AC African and Caucasian men?
- Will ambulatory SBP and PP be associated with a greater degree of vascular remodelling in defensive AC Africans than in their Caucasian counterparts?

1.9. ***Main aim of the study***

The increase in CVD and hypertension in South Africa and especially among individuals utilising a defensive AC style in stressful everyday situations have a definite negative impact on health in AC African men. This may be evidenced by increased sympathetic nervous system activity and resultant negative effects on the cardiovascular system. This necessitates research into the effect of defensive AC on endothelial function and vascular remodelling.

1.10. Hypotheses

The hypotheses for this study are:

- Defensive Active Coping Africans will have a greater degree of endothelial dysfunction than their Caucasian counterparts.
- Endothelial dysfunction will be associated with 24-hour blood pressure markers in users of the AC style, more so in the African than in the Caucasian men.
- Ambulatory 24-hour systolic blood pressure and/or pulse pressure will show a stronger association with vascular remodelling in defensive Active Coping African men than in their Caucasian counterparts.

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CHAPTER THREE: MANUSCRIPT

INSTRUCTIONS FOR AUTHORS: JOURNAL OF ATHEROSCLEROSIS AND THROMBOSIS

The complete *Guide for Authors* for this journal may be found at the following URL: http://www.j-athero.org/jat/jat/instructions.pdf

The manuscript should be organised according to the following format:

1) A **title page**. Designate one author as the corresponding author, and provide his/her complete address, telephone and fax numbers, and e-mail address.
2) A **conflict of interest statement**,
3) An **abstract,** not more than 250 words, and do not cite references in the abstract, 3 to 5 **supplementary key words**, and a **running title** (not more than 50 characters).
4) The **text** of the manuscript.
5) **Acknowledgments** (include notice of grant supports, if any).
6) **References**.
7) **Footnotes** to text, if any.
8) **Tables**.
9) **Figures**.
10) **Legends for Figures**.
11) **Category of manuscript**.

The title page should indicate the title of paper (not more than two printed lines of about 90 characters), full name(s) of author(s) (without titles) and name(s) and addresses of their institution(s). The text of **Original Articles** should consist of **Introduction**, **Aim**, **Methods**, **Results**, **Discussion** and **Conclusion**. All manuscripts must be typed in double space on A4 paper with standard margins, with each of sections 1) to 11) beginning on a separate page (note that to save printed pages, sections 9-11 of this manuscript do not begin on separate pages, however the electronic submission to the journal is so formatted).

Unit abbreviations should be without periods and in accordance with the “Style Manual for Biological Journals”, 2nd Edition, prepared by the Committee on Form and Style of the Conference of Biological Editors, American Institute of Biological Science, Washington, D. C., 1976. Some examples of commonly used abbreviations are as follows; L, mL, μL, g, mg, μg, ng, mol, μmol, nmol, pmol, mp, bp, h, min, s, cpm, ppm, M, mM, μM, nM, pM, rpm.

Some abbreviations commonly used in clinical biochemistry, such as mg/dL, though not included in the Committee’s manual, is also acceptable. However, other uncommon abbreviations should be avoided or spelled out after the first use.

Tables should be prepared so that they are self-explanatory. No vertical lines should be used to set columns apart, and horizontal lines are limited to three (between the title and the column headings, between the headings and the body and between the body and the legend or footnote). Each table should have a short title, and tables should be numbered in the order in which they are cited in the text. Tables should be typed double-spaced throughout with each table on a separate page. References to footnotes should be made using the symbols *, **, and *** (for statistical significance), and §, † or ‡ for other use.

References cited in the text should be numbered in order of appearance as superscripted numerals and listed in the Reference section of the paper. They should be arranged as follows: all author(s) name and initials (without period), title of paper, name of journal, year of publication, volume, and first and last page numbers connected with a hyphen (-). The titles of journals should be abbreviated as shown below according to the current system for Chemical Abstract and Biological Abstract, which is in accordance with the International List of Periodical Title Word Abbreviations (International Standard ISO 833) published in November 1974 by the International Organization for standardization.

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1) TITLE PAGE

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Stress appraisal, blunted endothelial response, and vascular remodelling in African men: The SABPA study.

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2) CONFLICTS OF INTEREST

The authors declare no conflicts of interest with the content of this article. The findings of this article and the opinions of the authors do not necessarily reflect those of the National Research Foundation.

3) ABSTRACT

Aim: The physiologically dissociative stress-response of defensive active coping (AC) in Africans has been associated with greater cardiovascular risk than in their AC Caucasian counterparts. We examined in AC Africans and Caucasians whether endothelial function and ambulatory blood pressure measurements would differ in their relation to markers of vascular structure and function.

Approach and Results: We examined vascular structure and function in 59 AC African (42.02 ± 8.46 years) and 83 AC Caucasian (46.44 ± 10.28 years) men. Ambulatory blood pressure, left common carotid intima-media thickness of the far wall (L-CIMTf), and left carotid cross-sectional wall area (L-CSWA) were measured. Von Willebrand factor (VWF) and nitric oxide metabolite (NOx) reactivity under stress was calculated to assess endothelial function. Stress appraisal was assessed using the Coping Strategy Indicator questionnaire. Blunted stress reactivity in the AC Africans compared to the AC Caucasians were evident for VWF (15.78 % vs. 53.58 %, $p < 0.001$) and NOx (-52.47 % vs. 895.33 %, $p < 0.001$). Ambulatory pulse pressure significantly contributed to both L-CIMTf ($\Delta R^2 = 0.11$ $p < 0.001$), and L-CSWA ($\Delta R^2 = 0.08$, $p < 0.001$) in AC Africans but not in AC Caucasians.

Conclusions: AC facilitates vascular remodelling via ambulatory pulse pressure. The blunted NOx and VWF responses in AC Africans under stress indicate endothelial dysfunction in this group. These observations may indicate an increased risk of stroke and cardiac incidents via functional and structural changes of the vasculature for AC Africans.

Supplementary key words: Stress appraisal, endothelial dysfunction, vascular remodelling, nitric oxide, von Willebrand factor.

Running Title: Stress, endothelial response, and vascular remodelling

4) TEXT

INTRODUCTION

There is consistent evidence that psychosocial stress is related to an increased risk of cardiovascular disease (CVD) and mortality independent of other lifestyle risk factors.[1] Stress facilitates physiological changes which may damage the vasculature when exaggerated or chronic.[1-4] However, not only the presence of psychosocial stressors, but also their appraisal, influences physiological stress responses.[5]

A problem-focused, in-control response to life stressors is deemed a defensive Active Coping (AC) response.[6] The AC style provokes a mainly β-adrenergic response in Caucasians,[7] which manifests as an increase in systolic blood pressure (SBP) by way of increased catecholamine release, particularly epinephrine, and increases in heart rate (HR), cardiac output (CO), and stroke volume (SV), with smaller increases in total peripheral resistance (TPR) and diastolic blood pressure (DBP).[8] We have previously shown that AC Africans exhibit a physiologically dissociative coping style.[9] Behaviourally, they reported being in control, which is characteristic of AC and thus a β-adrenergic central cardiac response was expected. Physiologically though, an α-adrenergic vascular response was elicited during stressor application, as evidenced by increases in DBP and TPR.[9]

Changes in the vascular structure have previously been associated with psychological stress. Increased carotid intima-media thickness (CIMT) has been associated with psychological stress in Caucasians, African Americans,[4, 10] and with SBP in AC Africans.[11] Indeed, defensive AC facilitated sympathetic hyperactivity, i.e. depressed heart rate variability and structural wall changes in urban African men, but not in their Caucasian counterparts.[12]

Not only vascular structure, but also vascular function may be influenced by psychological stress. The release of haemostatically active von Willebrand factor (VWF) from vascular endothelial cells is mediated primarily by β-adrenergic activity during acute stress, and

therefore, VWF is deemed a marker of endothelial activation and function.[13, 14] The production of nitric oxide (NO), a powerful vasodilatory agent and a marker of endothelial function, may be affected by acute mental stress.[15] Inhibition of NO production in endothelial cells may cause restriction of blood flow owing to insufficient vasodilation and possibly α-adrenergic vasoconstriction, thereby decreasing tissue perfusion.[16, 17] Subsequently, endothelial dysfunction may induce carotid atherosclerosis progression, and increase carotid intima-media thickness (CIMT) and cross-sectional wall area (CSWA).[18] In this study, we used VWF and NO metabolite responses as functional markers of the endothelium during acute psychological stress. Endothelial responses to stressors in ethnic groups related to defensive coping have not previously been studied.

We therefore aimed to assess whether differences in dysfunctional endothelial responses would be present in defensive AC Africans and defensive AC Caucasians. Additionally, we examined whether ambulatory blood pressure measurements were independently related to markers of vascular structure and endothelial function in these ethnicities.

METHODS

Study design and participants

To minimize seasonal effects on physiological processes, the **S**ympathetic activity and **A**mbulatory **B**lood **P**ressure in **A**fricans (SABPA) study was conducted from February to May of 2008 and 2009. This cross-sectional target population study on urban African and Caucasian teachers, from the Dr Kenneth Kaunda education district of the North West province in South Africa, comprised two ethnic groups who were culturally distinctive from each other. Selection of the sample was performed to ascertain comparability with respect to socio-economic status and working environment.

Teachers aged between 20 and 62 years who met the inclusion criteria, were invited to participate and informed about the study protocol before recruitment, after which willing individuals gave their informed consent in writing. Ethical approval for the study was granted

by the Ethics Review Board of the North-West University (Potchefstroom Campus: 0003607S6). All study procedures conformed to the terms and guidelines set in the 2008 Declaration of Helsinki.[19]

Psychotropic substance dependants or abusers, and persons who donated blood or were vaccinated in the preceding three months before data collection, were all excluded from the SABPA study. To guard from influences through gender effects, only male participants were included in this sub-study. We furthermore excluded participants who used α- or β-blockers (N = 2), individuals with a tympanic temperature above 37.5°C (N = 1), participants with a history of myocardial infarction or cardiac events (N = 3), clinically diagnosed diabetics (N = 7), and participants with human immunodeficiency virus infection (N = 13). This sample did not contain participants on anti-coagulant drugs, and comprised 80 African men and 97 Caucasian men.

Ambulatory blood pressure measurements

Participants were fitted with an ambulatory blood pressure- and electrocardiogram device (Cardiotens CE120®, Meditech, Budapest, Hungary) and an accelerometer (Actical®, Montreal, Quebec) on mornings of working days, after which they proceeded with their normal daily activities. The Cardiotens CE120® was validated by The British Hypertension Society in 2003. This device was programmed to measure blood pressure (BP) every 30 minutes between 08:00-22:00 and every 60 minutes between 22:00 and 06:00.[20] Hypertension was classified as an average ambulatory BP of ≥ 130 mm Hg SBP and/or ≥ 80 mm Hg DBP.[21] Obtained data were analysed using CardioVisions 1.15.2 Personal Edition (Meditech, Budapest, Hungary). The mean of successfully recorded ambulatory BP measurements was 80.8%. We additionally calculated ambulatory pulse pressure (PP) as a marker for cardiovascular risk by subtracting the ambulatory DBP from the ambulatory SBP.[22, 23]

Participants completed information forms regarding their medication use and medical history in their own time during the day. At 16:30 participants were transported to the North-West University's Metabolic Unit Research Facility where they received their own individual rooms, and were familiarized with the experimental setup. Hereafter they completed a psychosocial battery under supervision of registered clinical psychologists. A standardized dinner was served to the participants and they were asked to go to bed at 22:00 whilst fasting overnight. Following their awakening at 05:45, the ambulatory BP apparatuses were removed after the last BP was obtained at 06:00. Participants then underwent anthropometric measurements after which they spent 90 minutes in a semi-recumbent position on their beds. A registered nurse collected resting blood samples and determined tympanum temperature, after which laboratory stress testing proceeded.

Assessment of lifestyle risk factors

The Actical® omnidirectional accelerometer measured participants' movement at 15 second intervals to obtain total energy expenditure (TEE) in kcal/24h. Cotinine was measured to determine participants' smoking status, with participants regarded as smokers when cotinine values exceeded 14.99 ng/ml.[24] Gamma-glutamyltransferase (γ-GT) was measured as a marker for alcohol abuse.[25, 26] Anthropometrists, with level II accreditation from the International Society for the Advancement of Kinanthropometry, determined body mass and height in triplicate to the nearest 0.1 kg and 0.1 cm, respectively, while subjects wore minimal clothing and no shoes (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer IP 1465, Invicta, London, UK). Intra- and inter-observer variability was less than 10%. BSA in m^2 was calculated from these measurements using the Mosteller formula.[27]

Laboratory stress testing

To elicit mental stress responses we applied the Stroop Colour-Word conflict test (CWC). Consecutive cycles of five coloured words printed on cardboard, semantically and visually incongruent, were shown to the participant in a random order. We asked participants to

verbally affirm the printed colour of the word under time pressure. To provoke sufficient mental stress during this task, a monetary incentive was awarded to the participants dependent on their performance. The CWC creates a mental conflict between the incongruent colours and meaning of the printed words (e.g. the word 'YELLOW' printed in a red colour), producing central and efferent sympathoexcitatory effects via mixed α- and β-adrenergic receptor activation.[28]

Von Willebrand Factor and nitric oxide metabolite reactivity

To assess endothelial activation and function, VWF and nitric oxide metabolite (NOx) reactivity to the Stroop test were assessed. Before stress testing commenced, fasting resting blood samples had been obtained from the antebrachial vein branches, using a sterile winged infusion set, which had been left *in situ* with a heparin block (0.5 ml Heparin Sodium-Fresenius 5000 IU/ml in 50 ml standard saline solution) to prevent clotting. Ten minutes after completion of the stress test, the infusion set was rinsed with saline and the first two millilitres of blood were discarded before post-stress blood samples were collected. The percentage increase/decrease from rest for VWF and NOx was calculated.

Ultrasonography

Measurement of the left carotid intima-media thickness of the far wall (L-CIMTf in mm) of a 10 mm segment of the common carotid artery (CCA), 1 cm distal to the carotid bulb, was performed to assess the vascular structure.[29] The Sonosite Micromaxx ultrasound system (SonoSite Inc., Bothell, WA, USA) was used to perform ultrasonography using a 6-13 MHz linear array transducer, according to the Rudi Meijer protocol.[30] Ultrasonographs were digitally analysed with Artery Measurement Systems automated software II v1.139 (Gothenburg, Sweden). Left cross-sectional wall area (L-CSWA) was calculated as $L\text{-}CSWA = \pi(d/2 + L\text{-}CIMTf)^2 - \pi(d/2)^2$, with d as the luminal diameter of the far wall of the left CCA.[29, 30]

The Coping Strategy Indicator

To assess coping responses, participants completed the Coping Strategy Indicator (CSI) questionnaire.[6] They were asked to recall a stressful event which had occurred in the previous six months while completing the questionnaire and to consider their method of coping with this event. The questionnaire contains 33 items with a combination of inductive and deductive methodologies to unambiguously test for specific coping styles the person uses in response to stressors. The 33 items form three sub-scales (AC, avoidance/loss of control, seeking social support) with 11 items each. Items are rated with a three-point Likert scale: *A lot* (3), *A little* (2) and *Not at all* (1). Above mean coping responses are ≥ 26 for AC, ≥ 23 for social support, and ≥ 19 for avoidance.[6] The CSI is applicable to African individuals with α-Cronbach coefficients for the SABPA study population being 0.83 for AC, 0.84 for seeking social support and 0.69 for avoidance.

Biochemical sampling and analyses

Whole-blood, plasma, and serum samples were prepared according to standardized procedures and frozen at −80°C until analysis. Serum γ-GT was measured, using the high sensitivity enzyme rate method, serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) was measured, using the timed-end-point method, and C-reactive protein (CRP) as marker for general vascular inflammation was measured turbidimetrically in serum, using a highly sensitive kit (Unicel DXC 800, Beckman and Coulter, Germany). The TC:HDL-C ratio was calculated for use in analyses. Serum cotinine was measured by homogenous immunoassay (Modular ROCHE Automized, Basel, Switzerland). Ethylenediaminetetraacetic acid glycosylated haemoglobin A_{1C} (HbA_{1C}), a reflection of average capillary glucose for the preceding two to three months, was measured in whole-blood using a turbidimetric inhibition immunoassay (Cobas Integra 400plus, Roche, Basel, Switzerland). HIV status was measured, using the First Response kit (Premier Medical Corporation, India), as well as the Pareekshak test (Bhat Biotech, India). VWF antigen percentage was measured in plasma, using a polyclonal rabbit anti-VWF antibody and a

rabbit anti-VWF-horseradish peroxidase antibody (DAKO, Glostrup, Denmark).[31] NOx, as a surrogate marker for bioavailable NO,[32] was calculated as the sum of serum nitrite (NO_3^-) and reduced nitrate (NO_2^-), which were analysed on a Universal ELX800 Plate reader with GEN5TM software (BioTek Instruments Inc, Vermont, USA).

Statistical analyses

Data were analysed, using Statistica version 11.0 (Statsoft Inc., Tulsa, USA, 2011). Gaussian distribution of data was tested using the Shapiro-Wilks W-test for normality, and non-normal distributions were subjected to logarithmic transformation. Baseline comparison between the ethnic groups of the study population was done with independent Student's t-tests. We selected age, body surface area, physical activity, cotinine, and γ-GT as *a priori* confounders, in accordance with the 2013 ESH/ESC guidelines for the management of arterial hypertension.[21] Chi-square (χ^2) tests were used to determine proportions of smoking status, hypertension, and hypertension medication. A single two-way analysis of covariance (ANCOVA), independent of *a priori* defined covariates, was used to determine significant interactions between main effects (coping style × ethnicity) for L-CIMT, L-CSWA, and stressor-induced changes in NOx and VWF. Subsequently, one-way ANCOVAs followed to compare the AC population by ethnic status and to determine differences in VWF-, and NOx reactivity, controlling for *a priori* defined confounders.

Independent associations between endothelial function markers, structural vascular disease indicators, and ambulatory BP variables were assessed by performing hierarchical regression analyses. L-CIMTf, L-CSWA, VWF reactivity, and NOx reactivity were dependent variables, whilst controlling for the *a priori* defined confounders, log CRP, and TC:HDL ratio. Ambulatory SBP, DBP, and PP were added separately to each regression as independent variables in a second step, creating three models for each dependent variable for a total of twelve models. In the second step we calculated ΔR^2 for the addition of each independent variable. We performed the regressions for the total study population by ethnic group, as

well as for the AC population by ethnic group, to determine if AC alone did indeed affect the vasculature differently when compared to the total study group.

To reduce the inclusion of false significant results whilst using multiple models, p-values of ANCOVA results were adjusted using the Benjamini-Hochberg correction for false discovery, with a chosen false discovery rate of 5 %;[33] the significance level was set at $p < 0.05$ for all other statistical tests.

RESULTS

Table 1 describes the characteristics of the total study group (N = 80 African men and N = 97 Caucasian men). The Caucasian men demonstrated higher BSA ($p \leq 0.05$) than African men, but the latter showed less physical activity and greater γ-GT levels. The Africans had a higher mean level of cotinine and comprised more smokers than the Caucasians. Africans reported higher seeking social support scores and less avoidance coping than Caucasians. Baseline NOx and VWF levels were greater in the African men compared to the Caucasian men. Ambulatory SBP and DBP were significantly higher in the African men, and more African than Caucasian men were hypertensive.

Interaction between main effects was only demonstrated in groups with AC scores above the mean (AC score ≥ 26) for L-CIMTf: $F [1.641] = 4.83$, $p < 0.05$. Avoidance and seeking social support did not show a significant interaction for structural/functional vascular or ambulatory BP markers. Therefore, only participants with above mean AC scores were selected within each ethnic group (Table 2).

Table 2 demonstrates differences between the 59 AC African and the 80 AC Caucasian men. All lifestyle risk factors, potentially vasculature-affecting variables, seeking social support score, avoidance score, and hypertensive status showed the same trend as for the total group, namely a more vulnerable profile in the AC African men. NOx and VWF reactivities are additionally included in Table 2; both of these markers showed blunted

responses to stress in the AC Africans. NOx reduced during the Stroop test in the AC African men, whereas there was a large increase in NOx in the AC Caucasian men ($p < 0.0001$). The increase in VWF was significantly more substantial in the AC Caucasian men than in the AC African men ($p < 0.001$).

Hierarchical regression analyses showed that in the African men, ambulatory PP significantly contributed to, and explained greater variations in the models for L-CIMTf and L-CSWA ($p < 0.001$ for all models). Moreover, ambulatory PP explained a greater variation in, and contributed greater to L-CIMTf in the AC African men ($\Delta R^2 = 0.11$ [$p < 0.001$], $\beta = 0.39$, adjusted $R^2 = 0.30$) than in the total ethnic group ($\Delta R^2 = 0.08$ [$p = 0.005$], $\beta = 0.33$, adjusted $R^2 = 0.26$). A similar trend was observed in the models for L-CSWA in the AC African men ($\Delta R^2 = 0.08$ [$p = 0.02$], $\beta = 0.33$, adjusted $R^2 = 0.29$) and the total group African men ($\Delta R^2 = 0.06$ [$p = 0.04$], $\beta = 0.30$, adjusted $R^2 = 0.24$). SBP and DBP did not significantly contribute to the structural or functional measures in any model.

No ambulatory BP measures contributed significantly to the models of the AC and total group Caucasian men, and no variables in the regression models for VWF and NOx contributed significantly to the models in either ethnicity (all $p > 0.05$).

DISCUSSION

Our previous research indicated that the defensive AC style contributed to cardiovascular risk and pathology in Africans.[7, 9, 11, 12] To support these findings, we aimed to examine whether differences in endothelial function between AC African men and AC Caucasian men existed during application of a mental stressor. We also assessed whether ambulatory BP variables were indicative of vascular structure or endothelial function as assessed by L-CIMTf, L-CSWA, and VWF and NOx reactivity, respectively. Our primary finding was that use of the defensive AC style in African men had a more pronounced effect on the vascular structure via ambulatory PP when compared to the total ethnic group. Endothelial responses

as evidenced by NOx and VWF reactivity were blunted in defensive AC African men during mental stress, as opposed to higher responses in AC Caucasian men.

The heightened resting levels of NOx in the AC Africans compared to the AC Caucasians may seem counter-intuitive. Reduced levels of bioavailable NO is usually seen in hypertensives, coupled with endothelial dysfunction and increased oxidative stress.[34] The reduction in NO bioavailability is usually proportional to the severity of hypertension.[35] It has previously been suggested that in African Americans there may be a degree of desensitization of the smooth muscle cells to NO[36] so that the vasculature becomes less responsive to NO in spite of high NO levels. This will result in increased NO bio-availability in response to high BP and increased shear stress in the vasculature. However, the vasodilatory effects of NO would then be reduced.[36] Similarly, while a higher resting level of VWF is present in the AC Africans compared to the AC Caucasians, the relatively attenuated stress reactivity of VWF in Africans indicates a blunted endothelial response to induced mental stress.[13] This supports our previous findings that there is a predominant dissociative peripheral α-adrenergic response in the AC African men, as stress-induced VWF reactivity mainly reflects a β-adrenergic response.[9, 14, 37]

The extreme difference in NOx responses between the AC Caucasian and African men should be interpreted with caution. The metabolites of NO, NO_3,$^-$ and NO_2^- may be a reflection of bio-available NO metabolism in the vasculature, but as NO_3^- is also absorbed from the diet, and dietary patterns may influence the concentration of NO_3^-.[38] However, participants received a standardised dinner and fasted from the previous evening. As plasma half-life of NO_3^- is 5 to 6 hours,[38] it is therefore unlikely that dietary intake of NO_3^- influenced our results. Another likely more important contributing factor to the ethnic difference in NOx reactivity seen in our study is a possible difference in the pathways of NO-production between the AC Africans and Caucasians. Two main pathways for the production of NO exist; the L-arginine/NO pathway is considered the primary means of NO production, whereby the NO_3^-/NO_2^-/NO pathway in which mainly NO_2^- is recycled oxidised in tissue and

blood plasma to produce NO, is secondary.[38] Unlike the L-arginine/NO pathway, the NO_3^- /NO_2^-/NO pathway is not dependent on oxygen and, therefore, is more active in hypoxic and/or acidotic states.[38] We have previously shown the α-adrenergic vascular responses of AC Africans facilitated the incidence of silent ischaemic events during stress.[9] Moreover, AC Africans are at higher risk for the metabolic syndrome than AC Caucasians and non-AC Africans.[39] The metabolic syndrome marker microalbuminuria in this group was associated with end-organ damage, and it has also been shown that an association exists between microalbuminuria and metabolic acidosis-induced insulin resistance.[39, 40] This may provide a partial explanation for the extreme reduction in NOx levels in the AC Africans, although further studies into stress-induced changes in NO production and metabolism pathways in Africans are necessary.

We showed stronger associations between ambulatory PP and the structural vascular disease markers in the defensive AC African men compared to the total group of African men. This indicates that a defensive AC response predicts a greater increase in carotid thickness and arterial wall size than a non-AC response. SBP is most often correlated with increased vascular remodelling,[22] but this was not the case in our study. Along with SBP, it is recommended that PP be considered when measuring cardiovascular risk, especially as hyperpulsatile pressure facilitates vascular remodelling more, compared to normopulsatile pressure.[22, 41] Although the ambulatory PP did not differ significantly between AC Africans and AC Caucasians, the ambulatory SBP and DBP were greater in the AC African men compared to the AC Caucasian men. This causes the PP waveform to start at a higher DBP and end at a higher SBP in the AC African men. Higher mean BP and an increased PP have a more deleterious effect on the vascular wall than a smaller BP curve.[41, 42]

The findings from our study might have been strengthened by a larger sample size and a longitudinal rather than a cross-sectional design, but due to the homogeneity of our ethnic samples, rigorous inclusion criteria, and well-designed and controlled protocols, we believe that our results contribute meaningfully to the literature. We recommend that further studies

be conducted into the seemingly disparate physiological reactions to stress of Africans versus Caucasians, especially on the topic of NO production and its bioavailability.

From a clinical perspective, our data may add to a better understanding of the ever-increasing prevalence of atherothrombotic diseases in the acculturated South African population, particularly an increase in stroke risk.[43] Endothelial dysfunction, as well as vascular remodelling resulting in subclinical atherosclerosis that is evidenced by increased CIMT, are important harbingers of manifest cerebrovascular incidents.[44, 45] Therefore, it would be interesting to see in prospectively designed studies whether the defensive AC style in Africans contributes to their increased risk of stroke via functional and structural changes of the vasculature.

CONCLUSION

We showed that the vascular structure in Africans was greatly facilitated by ambulatory PP; more so by those using a defensive AC style in response to stress. NOx and VWF, as markers of endothelial function, exhibited blunted stress-induced responses in AC African men compared to their Caucasian counterparts, indicating endothelial dysfunction in the former. The extreme reduction of NOx in the defensive AC Africans, compared to the increased NOx in their Caucasian counterparts, may additionally be explained by differences in the activity of NO production pathways, although this needs to be investigated in further studies. We conclude that the AC style poses a health risk to African men by way of endothelial dysfunction and vascular remodelling. Prospective studies need to show whether this health risk translates into clinically manifest endpoints like stroke.

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7) FOOTNOTES TO TEXT

No footnotes are included.

8) TABLES

Table 1: Unadjusted baseline characteristics of the study population by ethnic status

	African men (N=80)	Caucasian men (N=97)
Age, y	42.61 ± 8.48	44.73 ± 11.12
Lifestyle risk factors		
Body Surface Area, m^2	1.95 ± 0.23	2.18 ± 0.21**
Physical activity, kcal/day	2731.18 ± 825.49	3471.55 ± 726.42**
Cotinine, ng/ml	35.11 ± 6.00	29.10 ± 5.40**
‡Smokers, N (%)	23 (28.75)	16 (16.50)**
γ-GT, U/l	59.43 ± 1.93	26.86 ± 1.91**
Coping measures		
Problem solving score	27.99 ± 4.17	29.01 ± 4.21
Seeking social support score	24.64 ± 5.44	18.38 ± 4.96**
Avoidance score	21.19 ± 3.68	23.56 ± 4.75*
Measures of endothelial function		
NOx, µmol/l	10.05 ± 11.30	2.64 ± 5.82**
VWF, %	88.17 ± 24.76	62.91 ± 13.71**
Variables potentially affecting vascular structure		
C-reactive protein, mg/l	4.30 ± 2.16	2.40 ± 1.46**
Total cholesterol, mmol/l	4.83 ± 1.18	5.67 ± 1.19**
HDL cholesterol, mmol/l	1.08 ± 0.39	1.00 ± 0.28
TC:HDL-C	5.07 ± 2.67	5.85 ± 1.45**
HbA_{1C}, %	6.30 ± 1.28	5.64 ± 0.47**
Ambulatory SBP, mm Hg	138 ± 17.0	128 ± 10.3**
Ambulatory DBP, mm Hg	88 ± 11.5	79 ± 7.4**
Ambulatory pulse pressure, mm Hg	50 ± 8.6	48 ± 6.9
‡Hypertensive, N (%)	60 (75.00)	52 (53.61)**
Measures of vascular structure		
L-CIMTf mean, mm	0.71 ± 0.18	0.69 ± 0.16
L-CSWA, mm^2	15.68 ± 5.39	15.00 ± 4.28
Medication		
‡Hypercholesterolemia, N (%)	1 (1.25)	5 (5.15)**
‡Hypertension, N (%)	13 (16.25)	7 (7.22)**

Abbreviations: γ-GT, gamma glutamyl transferase; DBP, diastolic blood pressure; HbA_{1C}, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; L-CIMTf, left carotid intima-media thickness of the far wall; L-CSWA, left cross-sectional wall area; NOx, nitric oxide metabolites; SBP, systolic blood pressure; TC:HDL, total cholesterol to HDL cholesterol ratio; VWF, von Willebrand factor.
Values presented as arithmetic mean ± SD; ‡ values presented as number of observations, N, and percentage of total (%);
* $p \leq 0.05$, ** $p \leq 0.01$ vs. African men

Table 2: Adjusted differences between Active Coping African and Caucasian men

	Defensive Active Coping African men (N=59)	Defensive Active Coping Caucasian men (N=80)
†Age, y	42.02 ± 8.46	46.44 ± 10.28**
Lifestyle risk factors		
†Body Surface Area, m^2	1.95 ± 0.24	2.18 ± 0.21**
†Physical activity, kcal/day	2781.44 ± 864.75	3477.45 ± 731.31**
†Cotinine, ng/ml	27.32 ± 5.15	22.67 ± 5.41**
‡Smokers, N (%)	13 (22.03)	14 (17.5)**
†γ-GT, U/l	42.18 ± 8.49	46.65 ± 10.18**
Coping measures		
Problem solving score	30.00 (29.27, 30.74)	30.51 (29.27, 31.18)
Seeking social support score	25.12 (23.61, 26.65)	17.95 (16.57, 19.32)**
Avoidance score	20.86 (19.60, 22.14)	24.42 (23.27, 25.57)**
Measures of endothelial function		
Baseline NOx, µmol/l	6.25 (4.01, 9.75)	0.77 (0.52, 1.16)**
NOx stress reactivity, %	-52.47 (-71.10, -21.81)	895.33 (539.55, 1449.01)**
Baseline VWF, %	88.82 (82.79, 94.85)	62.96 (57.52, 68.40)**
VWF stress reactivity, %	14.78 (2.29, 28.78)	53.58 (38.53, 70.26)**
Variables potentially affecting vascular structure		
C-reactive protein, mg/l	6.25 (4.01, 9.75)	0.77 (0.52, 1.16)**
Total cholesterol, mmol/l	4.59 (4.32, 4.88)	5.58 (5.28, 5.90)**
HDL cholesterol, mmol/l	1.05 (0.95, 1.14)	1.03 (0.95, 1.13)
TC:HDL-C	4.61 (4.19, 5.07)	5.75 (5.27, 6.27)**
HbA_{1C}, %	6.31 (6.04, 6.58)	5.53 (5.29, 5.77)**
Ambulatory SBP, mm Hg	137 (133.3, 140.5)	128 (124.1, 130.8)**
Ambulatory DBP, mm Hg	87 (84.9, 89.8)	79 (76.8, 81.2)**
Ambulatory pulse pressure, mm Hg	50 (47.2, 51.9)	49 (46.4, 50.6)
‡Hypertensive, N (%)	43 (72.88)	45 (56.25)**
Measures of vascular structure		
L-CIMTf mean, mm	0.69 (0.64, 0.74)	0.70 (0.66, 0.74)
L-CSWA, mm^2	15.38 (14.00, 16.75)	15.28 (14.05, 16.52)
Medication		
‡Hypercholesterolemia, N (%)	1 (1.69)	5 (6.25)*
‡Hypertension, N (%)	10 (16.95)	6 (7.50)*

Abbreviations: γ-GT, gamma glutamyl transferase; DBP, diastolic blood pressure; HbA_{1C}, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; L-CIMTf, left carotid intima-media thickness of the far wall; L-CSWA, left cross-sectional wall area; NOx, nitric oxide metabolites; SBP, systolic blood pressure; TC:HDL, total cholesterol to HDL cholesterol ratio; VWF, von Willebrand factor.
†Values presented as arithmetic mean ± SD; ‡Values presented as number of observations, N, and percentage of total (%); Other values presented as mean, adjusted for *a priori* confounders (± 95% Confidence Interval).
NOx and VWF reactivity values were calculated as the stressor value divided by the baseline value (for each individual).
* $p < 0.05$, ** $p < 0.01$ vs. African men

9) FIGURES

No figures are included

10) LEGENDS FOR FIGURES

No figures are included

11) CATEGORY OF MANUSCRIPT

Basic research

CHAPTER FOUR: SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

4.1. INTRODUCTION

The following is a summary of the findings in the manuscript of the article in Chapter Three, with a succinct discussion of the aim, results and conclusion. Also in this chapter are the recognised weaknesses of the study and recommendations for future research regarding coping and endothelial dysfunction.

4.2. SUMMARY AND CONCLUSION BASED ON MAIN FINDINGS

The article *Stress appraisal, blunted endothelial response, and vascular remodelling in African men: The SABPA study* produced the following main findings:

The main purpose of the study was to determine whether stress appraisal, provoking a defensive Active Coping (AC) response, induced differences in endothelial stress responses in African and Caucasian men. We have previously shown that the AC style is a cardiovascular risk in Africans,[1] contrary to the previous studies which linked only avoidance responses to cardiovascular pathology.[2] This is mainly because of the dissociation that defensive AC Africans exhibit in their responses to stress. Where the typical defensive AC physiological stress response in Caucasians is that of central cardiac β-adrenergic activity, Africans show α-adrenergic AC responses when challenged by stressors.[1]

As the urban environment poses significant challenges for its inhabitants, and they are forced to actively respond to these stressful challenges, we proposed that (a) Defensive AC Africans would have a greater degree of endothelial dysfunction than their Caucasian counterparts, (b) Endothelial dysfunction would be associated with 24-hour blood pressure markers in users of the AC style, more so in the African than in the Caucasian men, and (c) Ambulatory 24-hour pulse pressure (PP) and/or systolic blood pressure (SBP) would show a stronger association with vascular remodelling in defensive AC African men than in their Caucasian counterparts.

Evaluation of the nitric oxide (NO) and von Willebrand Factor (VWF) responses to the Stroop test showed that defensive AC Africans showed a blunted stress-induced endothelial

response compared to their Caucasian counterparts. As the release of VWF from endothelial cells is primarily mediated by β-adrenergic activity,[3] this supports our previous findings of a dissociative physiological response to stress in AC Africans. We therefore accept our first hypothesis.

Although the progression of endothelial dysfunction is associated with the progression of vascular remodelling, it is not clear whether endothelial dysfunction induces structural vascular remodelling, or vice versa.[4] We found no independent correlations between the markers of stress-induced endothelial reactivity and markers of vascular structure. Therefore, our second hypothesis is rejected. We propose that the functional endothelial markers will not correlate with the structural markers, as the former is indicative of an immediate response to stress, whereas the latter is a long-term change due to consistently high blood pressure and possibly chronic stress.[3, 5]

Flowing from the previous point, long-term defensive AC responses to everyday life stressors may chronically increase blood pressure cause eutrophic vascular remodelling of the vascular walls.[6] We found that 24-hour pulse pressure (PP), but not 24-hour SBP or diastolic blood pressure (DBP), was associated with left carotid intima-media thickness (CIMT) of the far wall and left carotid cross-sectional wall area in the African men. This association was more pronounced in the defensive AC African men than in the total African group. This is consistent with previous evidence that PP may be an important marker for vascular remodelling.[7] However, no such associations were found in the Caucasian men. It is possible that the hyperkinetic blood pressure and hyperpulsatile PP of the Africans predispose them to greater structural vascular changes than their Caucasian counterparts.[8-10] We therefore partially accept our third hypothesis.

4.3. CONFOUNDERS AND CHANCE

Confounders in this study were age, body surface area, γ-glutamyltransferase as a marker for alcohol consumption, cotinine as an indicator for smoking status, and physical activity.

These confounders were addressed in the statistical analyses of the data, with adjustments made where necessary. As markers for vascular wall structure were assessed in the regression analyses, we additionally included the biomarkers C-reactive protein and cholesterol, which may strongly affect the vascular structure.[11, 12]

It is possible that an element of chance was present in the study, owing to the relatively small number of participants included. However, we feel that the data collection was well controlled, the data integrity was more than satisfactory, and the analyses were conducted using strenuous protocols. Therefore, if chance is present in this study, it is not to the awareness of the authors.

4.4. LIMITATIONS OF THE STUDY

- The most important limitation of this study is its cross-sectional nature. The coping styles may have different outcomes over a period of time, but more importantly, changes in the vascular structure determined by longitudinal CIMT measurement may reveal more evidence of vascular damage than once-off measurement.
- The sample size in the study was small compared to epidemiological studies, so assumptions should not readily be made until these findings have been replicated in larger studies.
- It is possible that the ambulatory blood pressure measurement device caused participants to experience a small amount of stress every time it recorded, as the device can potentially become somewhat cumbersome, seeing that participants have to stand still when it is recording and they may even receive be startled when the device starts to inflate. The device may also disturb sleep in participants who are easily woken, and may lead to a slightly higher 24-hour blood pressure reading. However, it is improbable that blood pressure values would be significantly influenced owing to the number of measurements taken by the device. Ambulatory blood pressure measurement is the gold standard for blood pressure measurement across the world.

4.5. RECOMMENDATIONS FOR FUTURE RESEARCH

- It is recommended that a follow-up study be conducted on the participants of this study, or that a similar study be conducted longitudinally, to assess the true extent of the endothelial dysfunction and vascular remodelling in AC African men.
- A larger sample size is recommended to be an accurate representation of the population for which the study was intended, i.e. African and Caucasian men. This should eliminate any chance confounders and statistical errors which may have been present because of the small study sample.

4.6. CONCLUSION

To conclude, African men utilising a defensive AC style showed a blunted endothelial response to stress compared to their Caucasian counterparts. The stronger association between PP and structural vascular changes in the AC Africans compared to the total group of Africans indicates that defensive AC Africans are at greater risk for structural pathology than other coping and ethnic groups. Therefore, the defensive AC style poses a significant cardiovascular risk for African men.

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APPENDICES

APPENDIX A – The Coping Strategy Indicator questionnaire

THE COPING STRATEGY INDICATOR (AMIRKHAN, 1990)

INSTRUCTIONS:

Listed below are several possible ways of coping. Indicate to what extent you, yourself, used each of these coping methods. Try to think of one problem you have encountered in the last six months or so. This should be a problem that was important to you, and caused you to worry (anything from the loss of a loved one to a traffic fine, but one that was, important to you). Describe this problem in a few words.

_____

_____

_____

_____

_____

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

Keeping your chosen stressful event in mind, indicate to what extent you…

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

8	Set some goals for yourself to deal with the situation?			
9	Weighed your options very carefully?			
10	Daydreamed about better times?			
11	Tried different ways to solve the problem until you found one that worked?			
12	Confided your fears and worries to a friend or relative?			
13	Spent more time than usual alone?			
14	Told people about the situation because just talking about it helped you to come up with solutions?			
15	Thought about what needed to be done to straighten things out?			
16	Turned your full attention to solving the problem?			
17	Formed a plan of action in your mind?			
18	Watched television more than usual?			
19	Went to someone (friend or professional) in order to help you feel better?			
20	Stood firm and fought for what you wanted in the situation?			
21	Avoided being with people in general?			
22	Buried yourself in a hobby or sports activity to avoid the problem?			
23	Went to a friend to help you feel better about the problem?			
24	Went to a friend for advice on how to change the situation?			
25	Accepted sympathy and understanding from friends who had the same problem?			
26	Slept more than usual?			
27	Fantasized about how things could have been different?			
28	Identified with characters in novels or movies?			
29	Tried to solve the problem?			
30	Wished that people would just leave you alone?			
31	Accepted help from a friend or relative?			
32	Sought reassurance from those who know you best?			
33	Tried to carefully plan a course of action rather than acting on impulse?			

*****END*****

APPENDIX B – Ethical approval for the SABPA study

NORTH-WEST UNIVERSITY
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Ethics Committee
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Dr L Malan

6 February 2008

Dear Dr Malan

ETHICS APPROVAL OF PROJECT

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

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

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

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

Special conditions of the approval (if any): None

General conditions:

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

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

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

Yours sincerely

Prof M M J Lowes
(chair NWU Ethics Committee)

APPENDIX C – Originality report

Turnitin Originality Report

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