Exploring the link between oxidative stress and the vasculature in a bi-ethnic population

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"I can do all things through Christ who strengthens me."

- Philippians 4:13 -

This study would not have been possible without my **Almighty Father's** blessing and support. His presence with me through every step of the process is what gave me the strength that I needed to succeed.

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- To my parents, **Teresa** and **Louis**: Thank you for your motivation and for raising me to believe in going the extra mile and not giving up on my academic career.
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- Thank you very much to **Clarina Vorster** for her help with the language editing of this thesis.

I would like to dedicate this thesis to my late Grandfather, **Malcolm Noel Victor**. It was through his guidance that I decided to pursue my Doctoral degree and although he did not live to see the completion of it, I hope he is smiling upon me proudly from Heaven.

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PREFACE

This study forms part of the African-PREDICT and SABPA studies conducted by the Hypertension in Africa Research Team (HART) of the North-West University. This thesis has been submitted in the article format as approved and recommended by the North-West University for the fulfilment of the degree Doctor of Philosophy in Physiology. It has been written in English and contains 3 manuscripts, published or submitted in international peer-reviewed journals, namely *Journal of Human Hypertension, Blood Pressure and Hypertension Research.* Also contained within this thesis is an extensive literature review, comprehensive methodology as well as a concluding chapter interpreting the results and providing recommendations for future research in this field.

The structured format of this thesis is as follows:

Chapter 1 consists of a comprehensive literature review focusing on oxidative stress and various components of the vasculature and cardiovascular disease in black and white cohorts. Chapter 1 also introduces the problem statement and motivation for this study.

Chapter 2 contains a detailed layout of the study protocol and all methodologies involved in both the African-PREDICT and SABPA studies.

Chapter 3 is the first manuscript published in the *Journal of Human* Hypertension in March 2018. It involves the relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population of the African-PREDICT study.

Chapter 4 is the second manuscript published in the journal *Blood Pressure* in April 2019. This article explores cardiovascular reactivity and oxidative stress in young and older cohorts, making use of both the African-PREDICT and SABPA studies.

Chapter 5 is the last manuscript submitted to the journal Hypertension Research and is under review. Based on the SABPA study, this article explains how changes in oxidative stress markers associate with target organ damage in black and white participants over 3 years.

Chapter 6 comprises of the final chapter of this thesis, where a general discussion of results from all three manuscripts is given along with recommendations for future research in the field and a final conclusion based on the findings of this study.

Annexures include a declaration from the language editor, a turn-it-in report as well as the final printed versions of the first and second manuscripts as found in the respective journals.

The PhD candidate is listed as first author in each of the manuscripts, with Prof CMC Mels, (acting as corresponding author) and Prof HW Huisman included as co-authors in each manuscript. Each chapter contains a separate reference list according to the Vancouver reference style, while each manuscript adhered to the relative instructions for authors from their respective journals when submitted (found at the beginning of Chapter 3, 4 and 5).

AFFIRMATION BY AUTHORS

Each researcher contributed to this thesis in the following manner:

Mrs. C Myburgh

Responsible for the study proposal, compiling an ethics application for approval by the Health Research Ethics Committee (HREC) of the North-West University, conducting the in-depth literature reviews, performance of all statistical analyses and processing of data involved, interpretation of results and the overall design, planning, writing and execution of the thesis and manuscripts. The student was also actively involved in data collection for the African-PREDICT study by participation in the screening phase of the study, performing Sphygmocor readings when on duty in the HART clinic and partaking in the African-PREDICT study's biochemical analyses in the on-site laboratory.

Prof. CMC Mels (Promotor) and **Prof. HW Huisman (Co-promotor)**

Both the promotor and co-promotor were responsible for supervising the study design, collection of data, reviewing statistical analyses of the data and reviewing all literature involved. Both researchers made valuable recommendations for all aspects of the manuscripts and thesis.

Hereby, I declare that the statements above are accurate representations of my actual contribution to the study. Therefore, I give my consent for this thesis to be published as part of the degree Doctor of Philosophy in Physiology of Caitlynd Myburgh.

Caitlynd Myburgh	Prof CMC Mels	Prof HW Huisman

CONFERENCE PRESENTATIONS RELATING TO THIS STUDY

Conference attendance and oral presentations pertaining to this study are as follows:

Myburgh C, Huisman HW, Mels CMC. Cardiovascular reactivity and oxidative stress in young and older cohorts: The African-PREDICT and SABPA studies. The Stroke and Hypertension Congress 2018 (Southern African Hypertension Society), Stellenbosch, Cape Town, South Africa, 3-5 August 2018. *Accepted for top 6 oral presentation.*

Myburgh C, Huisman HW, Mels CMC. Cardiovascular reactivity and oxidative stress in young and older cohorts: The African-PREDICT and SABPA studies. The Provincial Health Research Conference 2019, Mafikeng, North-West province, South Africa, 12-13 March 2019. O*ral presentation.*

SUMMARY

Motivation

Unlike developed countries, the burden of hypertension and cardiovascular disease continues to grow within the South African population. The high prevalence of both communicable and non-communicable diseases, especially among the black population, places a large strain on the healthcare system. Black populations are more prone to early vascular aging, hypertension and cardiovascular disease development as compared to white populations. The mechanism related to this phenomenon is unclear but may involve oxidative stress, amongst others.

Oxidative stress can be defined as an imbalance between the production of reactive oxygen species (ROS) and the scavenging of these molecules by the antioxidant system, leading to cellular and molecular damage and altered signaling processes. Oxidative stress related markers may serve as early predictors of cardiovascular disease development and play a role in pathologies including hypertension, atherosclerosis and kidney disease.

There is a significant overlap between the risk factors for cardiovascular disease development and those of oxidative stress. Obesity, inflammation, advancing age, chronic stress and unhealthy lifestyle behaviors such as smoking, excessive alcohol use and physical inactivity have all been associated with oxidative stress as well as cardiovascular disease. However, it remains to be established whether oxidative stress plays a pivotal role in cardiovascular disease development in the South African context, especially in the black population who seems to be predisposed to cardiovascular disease.

Aim

The central aim of this study was to investigate the association between markers of oxidative stress and cardiovascular function and structure in the understudied black and white South Africans of varying age. Both a younger and older cohort of South Africans were included in this study, as the relationship of cardiovascular function and structure with markers of oxidative stress were explored cross-sectionally in both cohorts, as well as longitudinally in the older population.

Methodology

The study made use of data from the baseline phase of the African PRospective study on the Early Detection and Identification of Cardiovascular Disease and HyperTension (African-PREDICT) study and the baseline and follow-up phases of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study.

In manuscript one, cardiovascular measurements included ambulatory blood pressure and carotid intima-media thickness while oxidative stress markers and antioxidant enzyme activity included ROS, total glutathione (tGSH), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), total antioxidant status and uric acid. This study included 89 black men, 78 white men, 105 black women and 124 white women of the African-PREDICT study.

In manuscript two, the study population comprised of 191 black and 196 white participants of the African-PREDICT study together with 200 black and 209 white participants of the baseline SABPA study. Participants were exposed to acute stress in the form of the color-word conflict test and cardiovascular responses were calculated as percentage change in cardiovascular reactivity from rest. Biochemical measurements included ROS, tGSH, GPx, GR, SOD and γ-glutamyl transferase (γ-GT).

Lastly, the study population for manuscript three comprised of 89 black men, 91 white men, 84 black women and 95 white women who participated in both phases of the SABPA study. Target organ damage was assessed by measurements of carotid intima-media thickness, cross-sectional wall area (CSWA) and kidney function, while percentage change in oxidative stress markers (ROS, tGSH, GPx, GR, SOD and γ-GT) were measured over a 3-year study period.

Results and conclusions based on each manuscript

The central aim of this study was achieved as shown in the results of the three manuscripts. In the first manuscript, black participants had worse oxidative stress profiles as shown by higher ROS and lower GPx antioxidant enzyme activity than white participants. Ambulatory pulse pressure associated with lower GPx activity in black men, while the other 3 groups displayed associations of blood pressures with GR activity. The negative association of ambulatory pulse pressure in young black men suggested a role for oxidative stress in the acceleration of early vascular changes in this group.

Results from manuscript two showed age-related disparities between oxidative stress markers and cardiovascular reactivity to acute stress. Black participants had a greater response to stress than their white counterparts, with total peripheral resistance, stroke volume and Windkessel arterial compliance showing unfavorable responses when compared to white groups.

Heightened cardiovascular reactivity in older black groups associated with the glutathione system while cardiovascular reactivity in both white groups associated with ROS and their determinants (such as SOD and tGSH). These results highlight a possible up-regulation of the glutathione system to correct unfavorable responses to stress in blacks, while associations in white groups emphasize the age-independent role of ROS in regulating vascular tone during stress.

Black men displayed a decrease in ROS, SOD and GR activity while white men showed a decreased SOD and GPx but increased GR activities over 3 years. Associations of increased CSWA with diminished SOD activity in the white men suggest a role for oxidative stress in vascular remodeling over 3 years. Additionally, in white men, associations of estimated glomerular filtration rate with the glutathione system uncover a role for stability within this system to maintain normal renal function. No associations were found in the women studied for Manuscript 3. Positive associations of CSWA with ROS and SOD activity in black men suggest a delay in target organ damage due to adequate antioxidant system functioning.

Final conclusion

This study shows that oxidative stress plays a role in cardiovascular function and structure in black and white South Africans, regardless of age. While research within the South African population shows that black groups are already prone to vascular compromise at a younger age, this may be accelerated by oxidative stress. This study confirms that oxidative stress may serve as a possible risk factor for the development of hypertension, atherosclerosis, heightened cardiovascular responses to stress and target organ damage in younger and older black and white South Africans.

Keywords

Oxidative stress, antioxidant enzyme activity, carotid intima-media thickness, atherosclerosis, cardiovascular reactivity, target organ damage, race, age, South Africans.

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LIST OF ABBREVIATIONS

24hr	-	24 hours
8-oxodG	-	8-oxo-7,8-dihydro-2′-deoxyguanosine
AAMI	-	Advancement of Medical Instruments
ABPM	-	Ambulatory blood pressure monitoring
African-PREDICT	-	African PRospective study on the Early Detection and Identification of Cardiovascular disease and hyperTension
AMS	-	Artery measurement systems
ANCOVA	-	Analysis of covariance
AngII	-	Angiotensin II
AP-1	-	Activator protein-1
AT1	-	Angiotensin II receptor type 1
BH4	-	Tetrahydrobiopterin
BMI	-	Body mass index
CAT	-	Catalase
CI	-	Confidence interval
cIMT	-	Carotid intima-media thickness
CRP	-	C-reactive protein
CSWA	-	Cross-sectional wall area
Cwk	-	Windkessel arterial compliance
DBP	-	Diastolic blood pressure
DNA	-	Deoxyribonucleic acids
ECG	-	Electrocardiogram
eGFR	-	Estimated glomerular filtration rate
ET-1	-	Endothelin-1
Et al.	-	Et alia (and others)
G-6-PDH	-	Glucose-6-phosphate dehydrogenase
GFR	-	Glomerular filtration rate
GPx	-	Glutathione peroxidase
GR	-	Glutathione reductase
GSH	-	Reduced glutathione

GSH:GSSG	-	Reduced glutathione: oxidized glutathione ratio
GSSG	-	Oxidized glutathione
H_2O_2	-	Hydrogen peroxide
HREC	-	Health Research Ethics Committee
HART	-	Hypertension in Africa Research Team
HbA1c	-	Glycated haemoglobin
HDL-C	-	High-density lipoprotein cholesterol
HOCl	-	Hypochlorous acid
IL-6	-	Interleukin-6
kg	-	Kilogram
LDL-C	-	Low-density lipoprotein cholesterol
MAP	-	Mean arterial pressure
ml	-	Millilitres
m	-	Meters
m^2	-	Meters squared
mmHg	-	Millimetres of mercury
mmol/l	-	Millimole per litre
N	-	Number of
NADPH	-	Nicotinamide adenine dinucleotide phosphate
NF-κB	-	Nuclear factor-kappa B
NO·	-	Nitric oxide
eNOS	-	Endothelial nitric oxide synthase
NOX	-	NAD(P)H oxidase
O_2·-	-	Superoxide
OH·	-	Hydroxyl radical
$ONOO^-$	-	Peroxynitrite
p	-	Probability
PDGF	-	Platelet derived growth factor
PP	-	Pulse pressure
r	-	Regression coefficient
R^2	-	Relative predictive power of a model
RNS	-	Reactive nitrogen species

ROS	-	Reactive oxygen species
SABPA	-	Sympathetic activity and Ambulatory Blood Pressure in Africans
SAMRC	-	South African Medical Research Council
SARChI	-	South African Research Chairs Initiative
SBP	-	Systolic blood pressure
SD	-	Standard deviation
SES	-	Socio-economic status
SHIP	-	Strategic Health Innovation Partnerships
SOD	-	Superoxide dismutase
SV	-	Stroke volume
TEE	-	Total energy expenditure
tGSH	-	Total glutathione
TNF-α	-	Tumor necrosis factor- α
TPR	-	Total peripheral resistance
µmol/l	-	Micromole per litre
XO	-	Xanthine oxidase
γ-GT	-	γ-Glutamyl transferase

CHAPTER 1:

Introduction and literature study

1. General introduction

Within recent years, cardiovascular disease has emerged as one of the leading causes of global morbidity and mortality.[1-5] This epidemic is not only limited to developed countries, but is becoming a growing concern in developing countries such as South Africa,[2,6-8] especially among urban black groups.[9-12] Although the vulnerability of black South Africans to develop cardiovascular disease is poorly understood, it may be partly explained by urbanization, which is accompanied by lifestyle changes such as smoking, obesity, increased alcohol usage, high dietary salt intake and stress.[12-19] The combination of these risk factors may contribute to early vascular compromise, as seen in Black South Africans.[20-22] Interestingly, many of the same cardiovascular risk factors also result in oxidative stress, which in itself is an integral role player in the development of cardiovascular disease.

Oxidative stress is defined as an imbalance in the production of reactive oxygen species (ROS) and the scavenging of these molecules by the antioxidant system.[23-26] While ROS are known as important signaling molecules in the physiological functioning of the vasculature, oxidative stress has been implicated in endothelial dysfunction, inflammation and arterial remodeling.[24-26] Hence, oxidative stress plays a role in elevated blood pressure, arterial stiffness and carotid wall thickening.[24-26] However, the impact of oxidative stress on cardiovascular disease in the context of the South African population is limited. Therefore, we aimed to investigate the association of cardiovascular function and structure with markers of oxidative stress in black and white South Africans, both in young and older stages of life.

This study was based on two studies performed by the Hypertension in Africa Research Team, namely the prospective Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study and the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) study. The SABPA study aimed to investigate the brain-heart link and neural responses as a means to identify mechanisms related to cardiovascular disease development in South Africans. The African-PREDICT study expands on these results in order to build an understanding of early pathophysiology and the identification of novel markers for the prediction of cardiovascular disease development in young South Africans between 20 and 30 years of age.

This chapter provides a broad overview of the literature, focusing on ROS production and function, the antioxidant system, oxidative stress in the vasculature, oxidative stress and cardiovascular risk factors and the literature surrounding oxidative stress and cardiovascular disease in the South African context.

2. Literature overview

2.1 Reactive oxygen species

During oxygen metabolism, ROS are produced in all human cells including endothelial, adventitial and smooth muscle cells of the vasculature.[27-32] These highly reactive free radical molecules contain unpaired electrons and have the ability to react with and cause damage to various molecules in the body, including lipids, proteins and deoxyribonucleic acids (DNA).[23,28] However, under normal physiological conditions, ROS have also been identified as important intracellular signaling molecules in various metabolic pathways.[29,32] These include gene expression, transcription factor activation, cellular differentiation, proliferation and migration, apoptosis and ion channel activation to name a few.[32-36]

Different types of ROS include superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$), while other important reactive compounds, known as reactive nitrogen species (RNS), include nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^{-}$).[28-32,37]

2.2 Production of reactive oxygen species

There are various enzymatic, humoral, inflammatory and hemodynamic sources of ROS within the cells. Enzymatic sources of ROS production include xanthine oxidase, uncoupled endothelial nitric oxide synthase (eNOS), the mitochondrial electron transport chain and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX). Hormones, cytokines, enzymes and hemodynamic factors amplify the activity of NADPH and/or other enzymatic sources of ROS, thus further exaggerate the production of ROS and oxidative stress in the vasculature (Figure 1).[29,37,38]

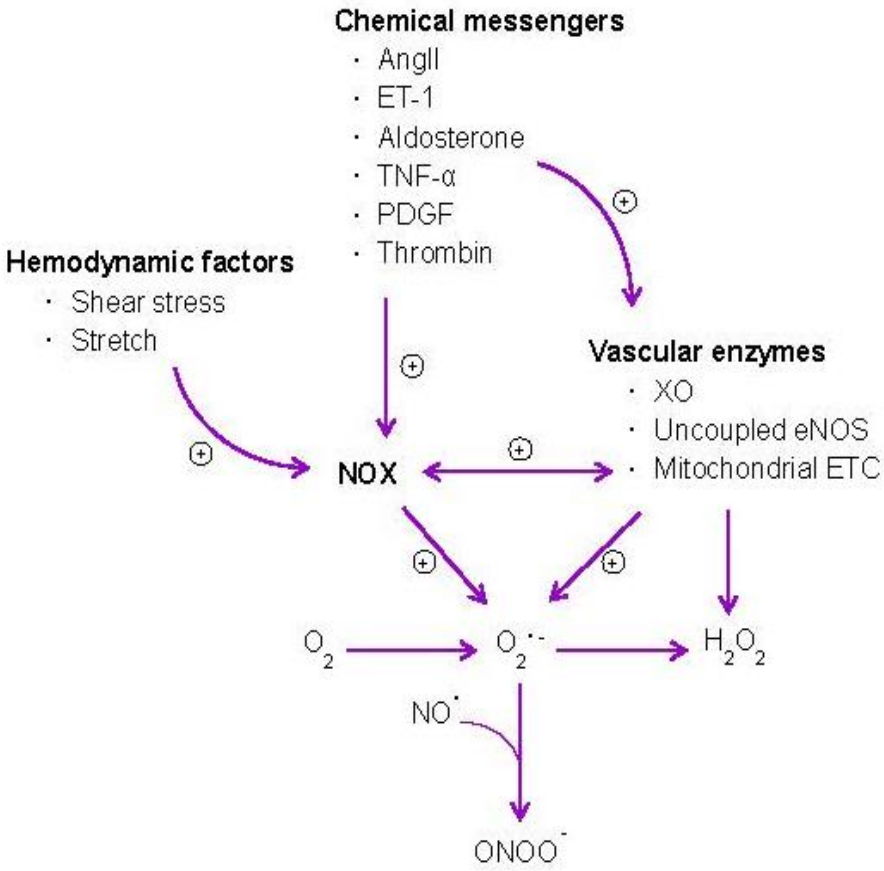

Figure 1: Production of superoxide^{-}, hydrogen peroxide and peroxynitrite within the vascular wall. Adapted from Touyz et al.[28] AngII, angiotensin II; ET-1, endothelin-1; TNF-α, tumor necrosis factor-α; PDGF, platelet derived growth factor; XO, xanthine oxidase; eNOS, endothelial nitric oxide synthase; ETC, mitochondrial electron transport chain; NOX, NAD(P)H oxidase.

Enzymatic ROS production

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)

Vascular ROS production is mainly as a result of enzymatic mechanisms, with the majority deriving from the NADPH oxidase enzyme within the cell membrane.[37,38] This enzyme consists of five cytosolic and membrane-bound protein subunits that are assembled to form the NOX enzyme complex that produces superoxide by reducing molecular oxygen through an electron donor, NADPH.[25,38-41] The ability of NOX enzymes to produce ROS within the phagocytes also serves as a weapon in the fight against pathogens, highlighting a function of ROS within the immune system.[41]

Different iso-forms of this NOX complex exist throughout the vasculature depending on the catalytic subunit they possess, however the primary function of these complexes are to produce ROS, unlike most other enzymatic sources which produce ROS as by-products of their primary function.[40] Not only is this enzyme the major producer of ROS, but it also performs a dual function of enhancing oxidative stress through its ability to stimulate xanthine oxidase activity, increase mitochondrial production of ROS and cause eNOS uncoupling.[42]

In the vasculature, the NOX-derived ROS plays a crucial role in endothelial cell survival, proliferation and angiogenesis.[41] On the other hand, elevated NOX activity and consequently oxidative stress have been implicated in vascular injury accompanying cardiovascular pathologies such as hypertension and atherosclerosis.[32,38,40] This is thought to be partly due to angiotensin II (AngII), as this is one of the main peptides responsible for regulation and activation of NOX.[38,43,44] The renin-angiotensin system is one of the main blood pressure control mechanisms utilized by the kidneys and is responsible for the production of the potent vasoconstrictor AngII.[45-47]

The ability of AngII to enhance ROS production is thought to be mediated by its interaction with AngII receptor type 1, although the gene expression of this receptor is regulated by ROS, thus forming an endless loop of ROS production in the vasculature.[38,43,44,48] Other factors increasing NOX activity include hemodynamic factors (mechanical stretch and shear stress), hormones (ET-1, aldosterone), cytokines (TNF-α), enzymes (thrombin) and growth factors.[25]

Xanthine oxidase

The xanthine oxidase enzyme within the vascular endothelium acts as an electron donor for molecular oxygen and is therefore another major source of both superoxide and hydrogen peroxide production.[39,49] This enzyme catalyzes the conversion of hypoxanthine to xanthine and subsequently the conversion of xanthine to uric acid, producing ROS as by-products in this purine metabolic pathway.[39,49,50]

Not only is xanthine oxidase activity upregulated with an increased AngII and NOX activity, but under inflammatory conditions, xanthine oxidase-derived ROS production also increases. This has been implicated in endothelial dysfunction, atherosclerosis and hypertension, thus elucidating its role in oxidative stress and cardiovascular disease development.[26,39,51,52]

Uncoupled endothelial nitric oxide synthase (eNOS)

Under normal conditions, nitric oxide (NO) acts as a potent vasodilator that stimulates guanylyl cyclase and cyclic guanosine monophosphate in vascular smooth muscle cells.[53] It is produced in the vasculature by the endothelial nitric oxide synthase (eNOS) system where it plays a vital role in maintaining normal vascular tone and suppressing atherogenesis, vascular smooth muscle cell proliferation and platelet activation.[39,53]

The eNOS enzyme uses a cofactor, tetrahydrobiopterin (BH_4), to transfer electrons to a substrate, L-arginine, thus producing NO in the process.[50] However, during oxidative stress or in the absence of L-arginine or BH_4, this system can also generate ROS in a process known as eNOS uncoupling.[38,54] This may be achieved due to the oxidation of BH_4 by peroxynitrite, thereby diminishing the cofactor needed to produce NO.[50,54]

Not only is oxidative stress a cause of eNOS uncoupling, but it is also a consequence thereof. This is due to the ability of uncoupled eNOS to produce superoxide and hydrogen peroxide which has been implicated in pathological conditions such as hypertension, atherosclerosis and diabetes.[38,50,54] Uncoupled eNOS may also become partially uncoupled, in which case both NO and superoxide are simultaneously produced and consequently react with each other to generate more peroxynitrite. Thus, the disturbance in the eNOS system participates in oxidative stress directly by producing ROS and indirectly by uncoupling more eNOS enzyme molecules.[50]

The mitochondrial electron transport chain

The mitochondrial electron transport chain is a series of five electron donor and acceptor membrane-bound enzyme complexes that function to produce adenosine triphosphate (ATP) as a source of energy for cellular processes.[41] Mitochondrial complexes I and III produce vast amounts of superoxide mainly through electron flow leakage in which molecular oxygen is converted to superoxide instead of water.[39,41]

Non-enzymatic ROS production mechanisms

Shear stress

To a lesser extent, ROS may also be produced by various non-enzymatic mechanisms within the vasculature.[29] Endothelial cells in the vasculature are exposed to various physical forces, one of which being shear stress accompanying blood flow.[55]

Under normal physiological conditions, shear stress acts within the vasculature to maintain proper vascular function and homeostasis, by way of activating cellular signaling pathways, transcription factors, as well as gene and protein expression.[55,56] However, when blood flow is disrupted (or non-laminar), ROS production is enhanced while various cytokines and mediators are also activated, which themselves have the ability to produce ROS.[54,55,57] Shear stress may also enhance oxidative stress indirectly by activating NOX and thus subsequently activating xanthine oxidase, the primary enzymatic sources for the production of ROS in the vasculature.[56,58,59]

Low or oscillatory shear stress can occur due to anatomical hindrances to blood flow (as in the case of vascular bifurcations), abnormal blood viscosity, surgical vascular interventions, endothelial dysfunction or sites of plaque formation in the vessel wall.[60-62] Abnormal shear stress within the vasculature has shown to increase inflammation, leukocyte adhesion, vascular smooth muscle cell proliferation and collagen buildup while also decreasing vasodilation through the reduction of eNOS production.[58] Taken together, it is no surprise that abnormal shear stress is thought to play an intricate role in the vascular pathogenesis of atherosclerosis.[56]

Phagocyte activation

Phagocytes such as neutrophils and monocytes undergo a process of activation when exposed to phagocytised particles of microbes, in which physiological and biochemical changes occur wherein they consume oxygen and produce ROS.[63-65] The production of hydrogen peroxide and superoxide by activated phagocytes is mainly catalyzed by NOX,[63,64,66] but through the action of myeloperoxidase, they can also produce cytotoxic hypochlorous acid (HOCl).[23,64] The production of ROS by these leukocytes is commonly known as the respiratory burst and forms part of the antimicrobial defense system.[66]

2.3 Physiological and pathological roles of reactive oxygen species

Although involved in pathological conditions associated with oxidative stress, ROS also have various physiological functions when present at normal concentrations. In this regard, ROS play an important role as second messenger, maintaining vascular tone and controlling vital biochemical pathways such as endothelial function, transcription factor activation and gene expression, ion transport and kinase activation (Figure 2).[29,37,38]

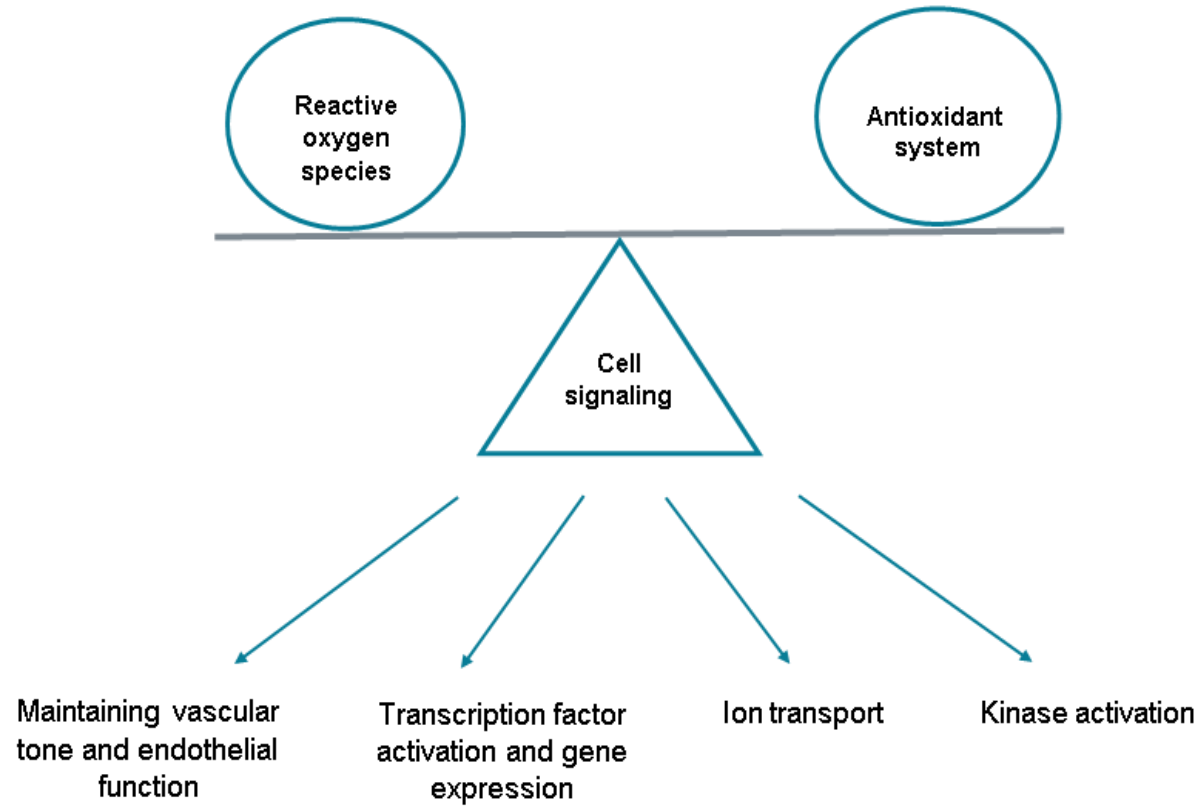

Figure 2: The physiological role of controlled ROS in normal cell signaling.

When the production of ROS becomes excessive or when the scavenging action of the antioxidant system is diminished, oxidative stress ensues.[67-69] During oxidative stress, the balance between oxidants and antioxidants within the body is disturbed, disrupting normal redox signaling and causing damage.[67-69]

Oxidative stress has been well documented as an important risk factor for cardiovascular disease development since it has numerous adverse effects within the vascular system, including disrupting normal endothelial function, enhancing inflammation and stimulating various processes involved with vascular remodeling (Figure 3).[70-72]

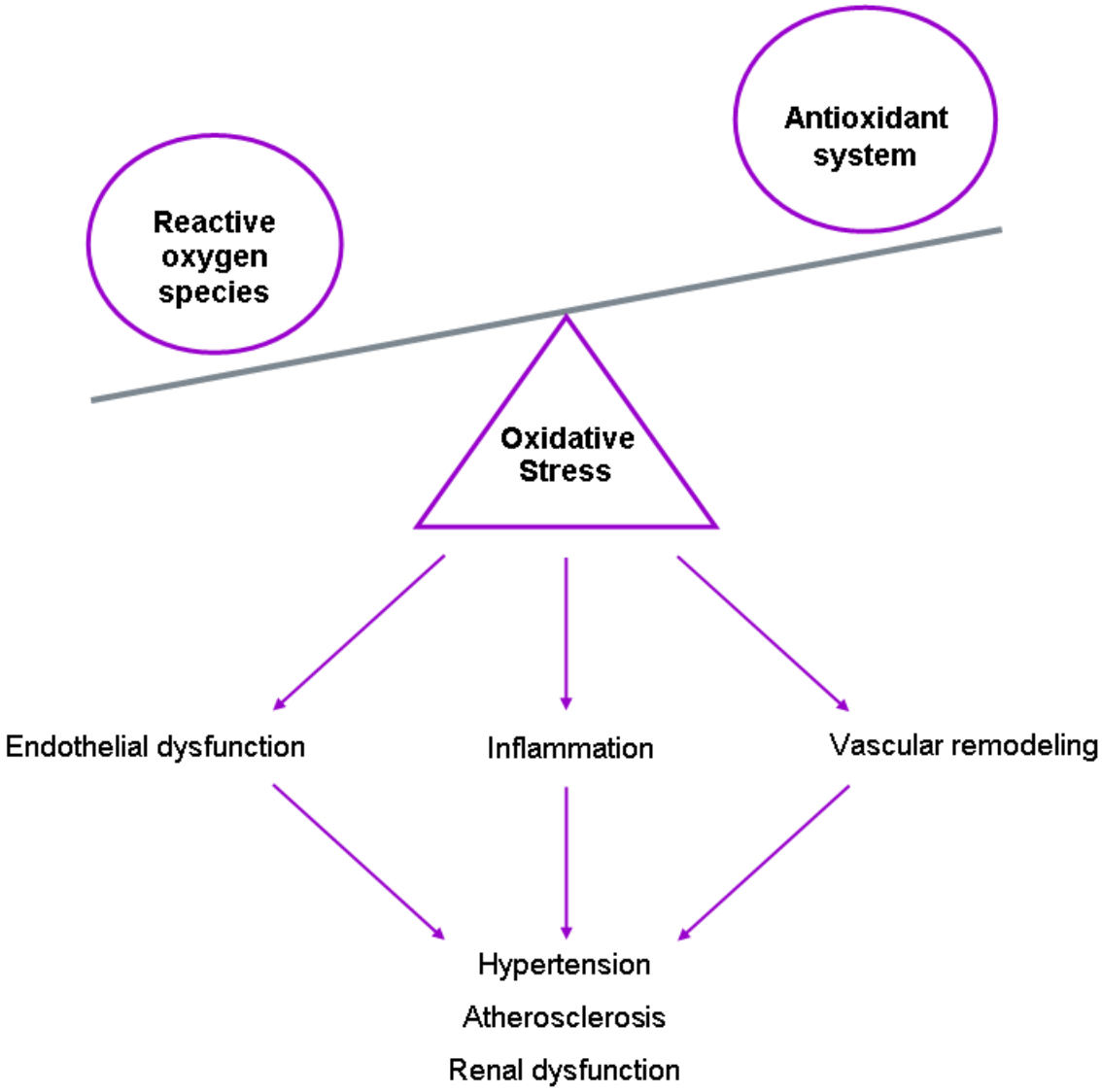

Figure 3: The pathological role of excessive ROS in cardiovascular disease development as a result of increased production of ROS or diminished scavenging thereof by the antioxidant system. Adapted from Nedeljkovic et al.[37]

Maintaining vascular tone and endothelial function

One of the most important reactive molecules within the vasculature is nitric oxide, which is produced within the endothelium via the eNOS enzyme.[73,74] While nitric oxide is already well documented as a potent vasodilator, it also plays a role in maintaining normal vascular smooth muscle cell growth and inhibiting platelet aggregation.[29,37]

Vascular tone is not solely influenced by nitric oxide, since superoxide and hydrogen peroxide also participate in the normal functioning of the vascular endothelium.[25,71] Through its ability to react with and inactivate nitric oxide, superoxide supports vasoconstriction.[75] Meanwhile, the promotion of calcium release, activation of potassium channels, hyperpolarization of the vascular smooth muscle cells and stimulation of eNOS makes hydrogen peroxide an ideal vasodilator substance.[75-77]

Furthermore, ROS are important for maintaining normal endothelial function by acting as second messengers during cell growth and proliferation, migration, cellular survival and apoptosis and increasing the expression of matrix metalloproteinase.[75,78,79] The mechanism by which ROS regulates these important angiogenic processes can be explained by its ability to activate growth regulating proteins, growth factors and protein expression.[75,80] Involvement of ROS in angiogenesis following damage to blood vessels or ischemia has placed ROS in a new light as important components in tissue repair.[80,81]

Pathological role of ROS in endothelial dysfunction

The endothelium plays a vital role in vascular function and under normal conditions secrete bioactive molecules including vasodilators and antithrombotic compounds, vasoconstrictors, prothrombotic compounds and anticoagulants.[41]

When injured by oxidative stress and inflammation, these cells are unable to regulate vascular tone, prevent coagulation or maintain an anti-inflammatory profile, a process known as endothelial dysfunction.[37,50,72] This disruption in endothelial function promotes conditions such as vasospasm, atherogenesis, thrombosis, inflammation and vascular growth.[37]

Impaired endothelium-dependent vasodilation may be directly or indirectly modulated by ROS. Directly, hydrogen peroxide is able to induce vasodilation while superoxide is able to induce vasoconstriction in the vasculature.[27,82] Indirect alterations in vascular tone occur as a result of nitric oxide quenching by superoxide (forming peroxynitrite), by eNOS inactivation or by eNOS uncoupling, all of which diminishes the bioavailability of nitric oxide and the vasodilatory effect on the vasculature.[50,83]

Transcription factor activation and gene expression

Transcription factors, as the name suggests, are nuclear molecules that interact with DNA sequences in order to control gene transcription.[84] Two of the most well-known transcription factors activated by ROS includes nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), which play important roles in inflammation and cell proliferation.[84]

While ROS is thought to alter messenger RNA stability, gene expression can be directly influenced due to ROS-induced upregulation of transcription factors that contain redox sensitive cysteine regions on their DNA binding sites.[78] This functions in order to increase the expression of various cytokines within the cell, such as TNF-α and AngII, while also inducing the expression of antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione reductase (GR).[78,85]

Antioxidants also have the ability to regulate gene transcription by interacting with antioxidant response elements on the promotor region of many genes (such as glutathione-S-transferases), or through suppressing gene expression (such as during inflammation).[86,87] The mechanisms are through the ability of antioxidants to regulate the binding and activation of transcription factors such as such as AP-1 and NF-κB during their binding with DNA.[86,88]

Pathological role of ROS in inflammation

One of the major pathological functions of oxidative stress is the development of a vicious cycle of inflammation whereby oxidative stress drives inflammation and inflammatory responses aggravate oxidative stress through the formation of more ROS.[89] This environment of oxidative stress and inflammation promotes vascular injury and worsens endothelial dysfunction.[72]

Oxidative stress enhances inflammation through activating the transcription factors AP-1 and NF-κB, which are responsible for the activation of proinflammatory cytokines (TNF-α and IL-6), chemokines and adhesion molecules (vascular cell adhesion molecule-1 and intercellular adhesion molecule-1).[83,90] In return, the expression of the inflammatory cytokines enhance oxidative stress by activating NOX or by inactivating eNOS.[43,90-92] The expression of adhesion molecules in the vascular endothelium attracts monocytes to the site of release, which together with ROS-induced low-density lipoprotein (LDL-C) oxidation, forms an integral step in the formation of atherosclerotic plaque in the vasculature, as illustrated in Figure 4.[43,90-92] Carotid intima-media thickness (cIMT) is an accurate measurement of the structural changes associated with atherosclerosis development and an increase in cIMT may predict cardiovascular events.[93]

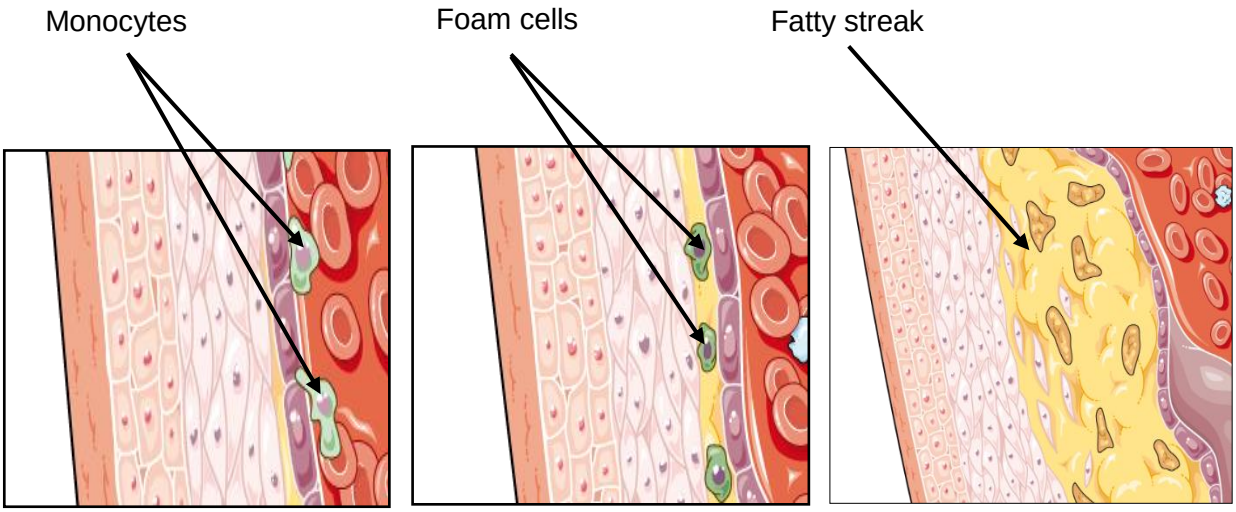

Figure 4: The combined effects of vascular inflammation and endothelial dysfunction in atherosclerosis development, showing the recruitment of monocytes to the endothelium, ingestion of oxidized LDL-C and subsequently forming foam cells and fatty streaks within the vascular wall. Taken and adapted from Servier Medical Art, which is licensed under a Creative Commons Attribution 3.0 Unported License.

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Oxidative stress and inflammation also play an important role in renal dysfunction.[94] Studies have shown that not only does oxidized LDL-C and markers of oxidative stress (such as ROS, plasma 8-isoprostane, F_2-isoprostanes and malonyldialdehyde) increase, but antioxidant capacity decreases in patients with kidney disease.[95-98]

Evidence of an inverse relationship between oxidative stress and glomerular filtration rate (GFR) suggests a progressive increase in oxidative stress as renal function deteriorates.[96-100] However, not only does oxidative stress hinder GFR, but glomerular hyperfiltration (GFR ≥ 150 ml/min/1.73m^2) is also shown to occur during oxidative stress.[103,104]

While it is commonly known that a diminished GFR serves as a predictor of renal disease and cardiovascular morbidity and mortality, research has shown that glomerular hyperfiltration is also a strong predictor of renal failure, cardiovascular disease development and adverse cardiovascular events.[103,104]

The mechanism behind oxidative stress-driven renal dysfunction is thought to occur as a result of direct and indirect processes. Directly, oxidative stress inactivates nitric oxide which acts as a potent vasodilator of the afferent arterioles to increase renal blood flow and promote pressure natriuresis.[101] Oxidative stress can also directly cause salt retention, kidney damage and ischemia, which further diminishes renal function.[101] Indirectly, oxidative stress promotes hypertension and atherosclerosis, both of which are shown to promote the development of kidney disease.[101,102]

Ion transport

Plasma membrane and intracellular ion channels can be modulated by ROS.[78] One of the chemical messengers influenced by this ROS-induced ion transport is calcium, which plays an important role in regulating muscle contraction, cellular metabolism, gene expression and apoptosis.[105]

Calcium influx into the cell can be increased via either ROS-induced opening of voltage-gated ion channels, increased calcium release from intracellular stores or an increased activity of calcium ATPase pumps.[78] This process forms a positive feedback loop, since not only does ROS regulate calcium signaling, but calcium also has the ability to activate NOX and subsequently regulate the formation of ROS.[78,105]

The interaction between ROS and calcium is thought to play a role in the augmentation of hydrogen peroxide production in the neutrophils, as well as the apoptosis of neutrophils after phagocytosis in order to prevent inflammation.[106] Additionally, since the calcium channels involved with cardiac excitation-contraction coupling are sensitive to redox damage, this highlights a function for ROS in adequate cardiac functioning and ischemic pathologies.[107]

Kinase activation

Protein kinases such as mitogen-activated protein (MAP) kinase and tyrosine kinase receptors (TKR) can be phosphorylated and activated by ROS produced by the NOX enzymes.[78,108] These activated kinases then function to trigger inflammation, cell differentiation, apoptosis and gene expression.[78,109,110] These kinase pathways have been implicated in cardiovascular pathologies including hypertension, cardiac ischemia and hypertrophy, increased vascular contractility and vascular remodeling.[79,111,112]

Pathological role of ROS in vascular remodeling

Additional to enhancing inflammation and endothelial dysfunction, ROS has been shown to play a pathological role in vascular remodeling, which is defined as a structural change in vascular smooth muscle cells and the extracellular matrix leading to vascular stiffness and hypertension (Figure 5).[72]

Vascular remodeling is associated with oxidative stress through processes of hypertrophy, apoptosis, proliferation, migration and altering the extracellular matrix.[90]

Chemical messengers such as platelet derived growth factor, thrombin, AngII and TNF-α are believed to play an important role in vascular remodeling through their ability to stimulate ROS production and promote vascular smooth muscle cell hypertrophy.[43,71,90,91,113] Along with the growth of vascular smooth muscle cells, ROS are also able to increase both apoptosis, as well as the build-up of collagen and connective tissue within the vasculature, further aggravating vascular remodeling and aggravating lumen narrowing as shown in Figure 5.[82,90]

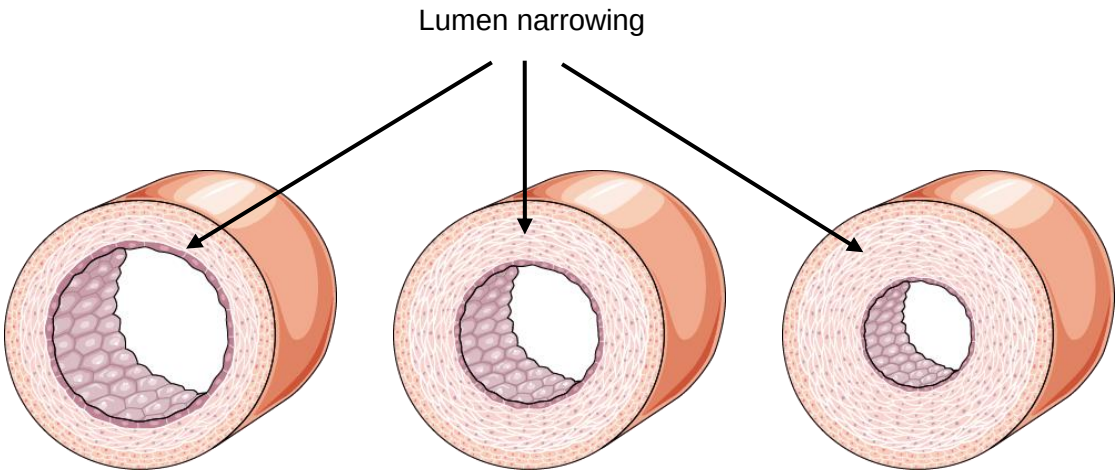

Figure 5: Vascular remodeling within the vasculature, showing a narrowing lumen as the condition worsens. Taken and adapted from Servier Medical Art, which is licensed under a Creative Commons Attribution 3.0 Unported License.
(https://creativecommons.org/licenses/by/3.0/legalcode).

Phosphatase inhibition

Phosphatases are redox-sensitive molecules that regulate the phosphorylation of other signaling proteins involved with cell metabolism, proliferation and differentiation.[78] These molecules remove phosphates from their respective substrates, unlike protein kinases that attach phosphates to their substrates.[114]

Phosphatases can be inactivated by ROS as part of their normal signal transduction function, thus indirectly activating protein kinases allowing protein phosphorylation and downstream signaling to occur in vascular cells.[78]

2.4 Antioxidant system

Fortunately, the body is not defenseless against the harmful effects of ROS. It possesses various enzymatic and non-enzymatic systems that ultimately function to neutralize these reactive molecules in order to protect against oxidative damage of lipids, proteins and DNA.[23,72]

Enzymatic antioxidants

In this study, six enzymes forming the antioxidant system, namely superoxide dismutase (SOD), catalase (CAT), GPx, GR, glucose-6-phosphate dehydrogenase (G-6-PDH) (a secondary enzyme within the antioxidant system) and γ-glutamyl transferase (γ-GT) are discussed. The combined function of the enzymatic antioxidant system is summarized in Figure 6 below.

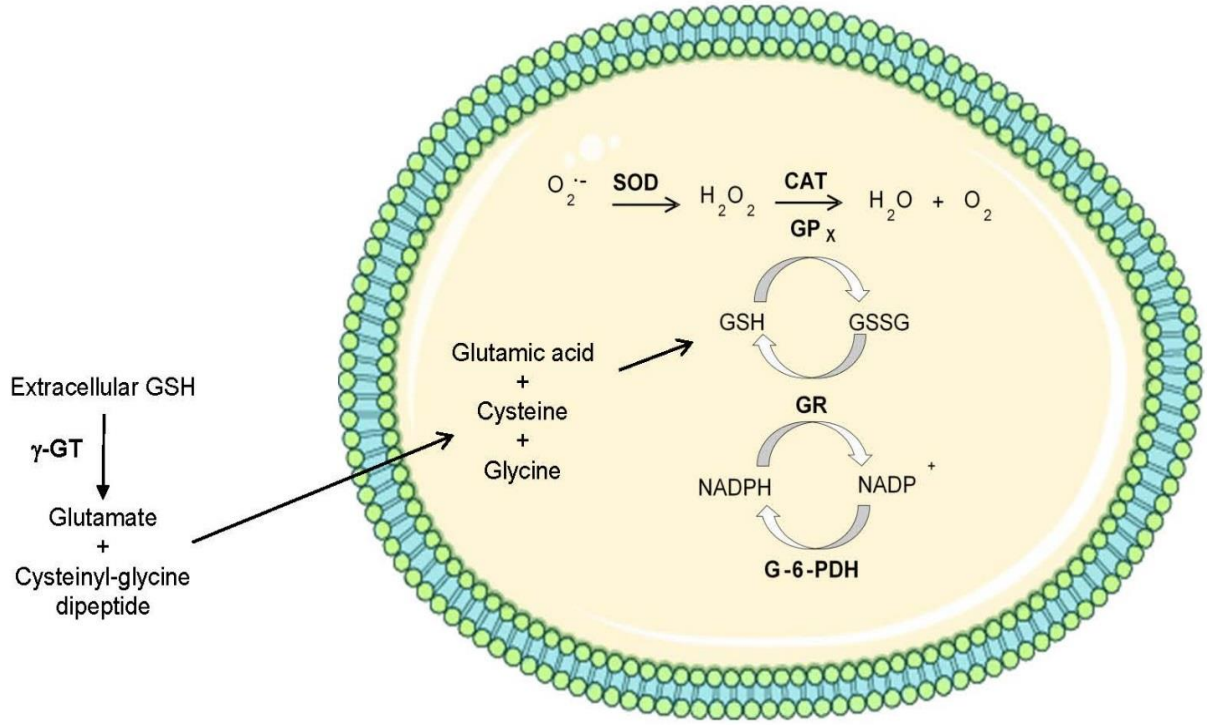

Figure 6: Major and secondary antioxidant enzymes as part of the antioxidant system. Adapted from Li et al and Cappellini et al.[115,116] Superoxide is converted into hydrogen peroxide by the activity of the superoxide dismutase enzyme. This end product is catabolized to form water and oxygen by two mechanisms, one being the catalase enzyme and the other being the glutathione system. The enzyme glutathione peroxidase converts reduced glutathione to its oxidized form during its activity, which is replenished by the actions of glutathione reductase and the γ-glutamyl transferase enzymes. γ-GT, γ-glutamyl transferase; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GR, glutathione reductase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NADP +, oxidized nicotinamide adenine dinucleotide phosphate; G-6-PDH, glucose-6-phosphate dehydrogenase.

Superoxide dismutase (SOD)

The first line of defense in the enzymatic antioxidant system is SOD. As demonstrated in Figure 6, the enzyme SOD is responsible for the conversion of superoxide to hydrogen peroxide.[23,83,117]

There are three known isoforms identified namely cytoplasmic SOD, mitochondrial SOD and extracellular SOD, of which the major vascular SOD is the extracellular isoform.[83] The expression of SOD within the vasculature can be altered in situations of shear stress, chemical stimuli (such as growth factors and cytokines) and nitric oxide bioavailability, shedding light on the role of diminished activity in endothelial dysfunction accompanying hypertension and atherosclerosis.[117-120]

The antioxidant function of SOD is an important step in redox balance and vascular homeostasis considering that the action of this enzyme is a major determinant of nitric oxide availability in the vasculature.[120] Since the unfavorable reaction between nitric oxide and superoxide occurs more rapidly than what superoxide can be quenched, SOD acts to protect nitric oxide from becoming the cytotoxic oxidant peroxynitrite.[120] Peroxynitrite is also able to further uncouple eNOS, solidifying a role for adequate SOD functioning in the prevention of endothelial dysfunction, inflammation and vascular remodeling in cardiovascular pathologies.[121]

Catalase (CAT)

The enzyme CAT is the second of the two enzymes responsible for the conversion of hydrogen peroxide to water and is located in the peroxisomes.[23,83] It consist of four identical subunits and possesses the unique ability to withstand saturation of hydrogen peroxide at any concentration, thereby protecting cells from oxidative damage.[122,123] Diminished CAT activity has been associated with diabetes, metabolic syndrome, atherosclerosis, hypertension and coronary artery disease.[123-126]

Glutathione peroxidase (GPx)

Once hydrogen peroxide has been formed by the action of SOD, it is converted to water by one of two enzymes, the first of which being GPx.[83] This enzyme makes use of reduced glutathione (GSH) as a substrate and subsequently forms oxidized glutathione (GSSG), which is later converted back to GSH by the secondary enzyme glutathione reductase (GR) in an effort to replenish the substrate.[83,127]

GPx is a selenoprotein, which means that the enzyme contains selenium (in the form of a selenocysteine residue) at the catalytic site that plays a principle role within the enzyme to reduce hydrogen peroxide.[128]

Four different isoforms of GPx have been identified, the first being cytosolic GPx, or GPx-1. GPx-1 is the most important isoform in combating the effects of oxidative stress, and is found in the cytosol, mitochondria and intermembrane space of most tissues of the body.[128] Gastrointestinal GPx, or GPx-2, is found mainly in the gastrointestinal tract and protects against ingested hydroperoxide.[128] Plasma GPx, or GPx-3, is found in the plasma and is in high concentrations in the kidneys, suggesting an antioxidant role in the renal system.[128] The last isoform is phospholipid hydroperoxide GPx, or GPx-4 and is responsible for the protection of cellular and mitochondrial membranes against oxidative stress in the presence of adequate vitamin E.[128] Another protein worth mentioning due to the similarities to GPx is epididymal secretory GPx, or GPx-5, which is found in the epididymis and is responsible for protecting the spermatozoa from oxidative stress.[128]

Glutathione reductase (GR)

The secondary enzyme GR is responsible for the reduction of GSSG in an NADPH-dependent manner and in so doing provides GSH as substrate for the functioning of GPx.[85,127,129]

The first step is reducing GSSG by NADPH, in which the electrons are carried over to GSSG resulting in an oxidized form of GSH.[129] This flavoprotein disulfide oxidoreductase enzyme thus replenishes GSH stores needed for maintaining a normal redox state and a decrease in GR activity could tip the balance towards oxidative stress and cardiovascular disease development.[85,129,130] This notion is further strengthened by research in hypertensive and diabetic patients with signs of oxidative stress that have shown depleted GSH reserves, along with an up-regulation of GR in an effort to compensate for this diminished antioxidant level.[130,131]

Glucose-6-phosphate dehydrogenase (G-6-PDH)

In order for GPx to function efficiently, G-6-PDH (a secondary enzyme within the antioxidant system) is utilized as a cofactor in order to regenerate NADPH.[132,133]

It has been noted that the majority of NADPH is produced by G-6-PDH, as improper functioning of this enzyme impairs proper functioning of NADPH-dependent cellular processes including eNOS functioning and BH_4 production.[134] The enzyme G-6-DPH forms part of the pentose phosphate pathway and is an important form of defense against oxidative stress, especially in the red blood cells where this enzyme is its only source of NADPH.[132,135]

γ-Glutamyl transferase (γ-GT)

While γ-GT was originally considered as a marker for liver function and alcohol abuse, it can also be seen as a marker of oxidative stress.[136-139] This is due to γ-GT being able to both counteract oxidative stress by catalyzing the breakdown of extracellular GSH in an effort to avail the amino acid constituents needed for intracellular GSH synthesis and also enhance oxidative stress due to its ability to directly produce ROS.[136,137,139-142]

During conditions of oxidative stress, GSH may be consumed at a higher rate, leading to a compensatory up-regulation in γ-GT synthesis in an effort to increase GSH levels, with this increase in γ-GT having been shown to predict cardiovascular risk and mortality.[139,141-143]

Non-enzymatic antioxidants

As seen in Figure 6, the most integral non-enzymatic antioxidant working in close association with the enzymatic antioxidant system is GSH which is discussed in detail in this study. While the rest of this segment goes beyond the scope of the study, some of the remaining non-enzymatic components are discussed shortly in order to cover the broader perspective of the antioxidant system.

Glutathione (GSH)

Probably the most well-known non-enzymatic antioxidant is GSH. This antioxidant is predominantly found in the cytosol but also in the mitochondria, peroxisomes and the nuclear matrix.[85,127,144] The tripeptide GSH is synthesised from the amino acids glutamic acid, cysteine and glycine by way of two enzymatic systems, γ-glutamylcysteine synthetase and GSH synthase.[85,127]

Direct ROS scavenging by GSH is made possible by the presence of a powerful reducing thiol group that acts as an electron donor to free radicals.[85,129,144] Aside from being able to directly scavenge ROS molecules, GSH also indirectly scavenges hydrogen peroxide by acting as a cofactor for the GPx enzyme as previously mentioned.[85] Other vital functions of GSH in the antioxidant system include participation in the repair of oxidized protein, lipid and DNA damage while also regenerating other non-enzymatic antioxidants.[144]

This antioxidant can exist in one of two chemical states, either reduced glutathione (GSH) or oxidized glutathione (GSSG).[85,127] Under conditions of increased hydrogen peroxide levels, GSH is utilized which results in the enhanced uptake of cysteine (or oxidized cystine) into the cell as well as heightened activity of γ-glutamylcysteine synthetase.[85,127,145] In this way, the depletion of GSH during oxidative stress is able to promote production of new GSH in a negative feedback manner.[85]

Bilirubin, uric acid, ascorbate (Vitamin C), α-Tocopherol (Vitamin E), β-Carotene and coenzyme Q10

Bilirubin is a by-product of hemoglobin catabolism and is generally regarded as a toxic waste product, however in low concentrations it has the ability to act as a powerful antioxidant in the plasma.[146-148] For this reason it is no surprise that bilirubin is shown to decrease the risk for development of cardiovascular disease.[149]

Uric acid has been noted as a powerful endogenous antioxidant, especially in the plasma and is a product of purine metabolism.[150] Here it is able to protect cells in the plasma from oxidative damage while also protecting extracellular SOD from inactivation by hydrogen peroxide.[151,152] The soluble form of uric acid, known as urate, is also capable of scavenging multiple ROS including hydrogen peroxide and hydroxyl radicals.[151]

Ascorbate, also known as vitamin C, is an important non-enzymatic antioxidant obtained from the diet and is abundant in fruits and vegetables.[153,154] Ascorbate is able to directly scavenge ROS in the body fluids, inhibit LDL-C oxidation, prevent vascular cell adhesion during endothelial dysfunction, enhance vascular nitric oxide production through stabilizing BH_4 and regenerate vitamin E.[153-156]

Another important dietary vitamin that acts as an antioxidant is vitamin E.[153] Of the eight different forms of vitamin E that exist, the most active form of this lipid-soluble antioxidant found in humans is α-tocopherol.[153,155,156] Unlike ascorbate, α-tocopherol is hydrophobic in nature and is closely associated with cellular membranes, where it functions to protect the lipid structures from oxidative stress.[153]

β-carotene, the precursor of vitamin A, is also a dietary acquired lipid soluble antioxidant bound in cellular membranes where it can scavenge ROS.[157,158] However, it is no coincidence that this enzyme resembles similar characteristics to that of α-tocopherol, because they often work synergistically to combat lipid peroxidation within the cellular membranes.[158]

Coenzyme Q_{10}, also known as ubiquinone, is an important component of the mitochondrial electron transport chain.[159,160] Coenzyme Q_{10} is the only endogenously produced lipid-soluble antioxidant and is found in all cell membranes where it has the ability to protect LDL-C and membrane phospholipids from oxidative stress.[159-161]

2.5 Oxidative stress and cardiovascular risk factors and pathophysiology

There are various factors which may contribute to the development of cardiovascular disease development, one of which is oxidative stress and studies have shown that hypertensive patients not only exhibit increased ROS production, but also a decrease in antioxidant enzyme activity.[27,31,162,163]

The following sections elaborate on well-known cardiovascular risk factors and how they integrate with oxidative stress and cardiovascular pathophysiology.

Obesity

Obesity is known to be independently associated with oxidative stress,[164-168] and it also increases the risk for cardiovascular disease development, hypertension and metabolic syndrome.[164-170] Adipose tissue plays an important role in the endocrine system, through the production of various adipokines such as TNF-α, IL-6, resistin, leptin, plasminogen activator inhibitor-1 and adiponectin.[164,165,171]

Adipokines themselves have the capability to produce ROS and thus induce oxidative stress, suggesting that adipose tissue is an independent contributor to the development of oxidative stress.[171] The oxidative stress accompanying obesity has been suggested to cause dysregulation in the production of adipokines,[164] and the inflammatory and pro-oxidant effects of the increased adipose tissue in obesity have been suggested as a link between cardiovascular disease and obesity.[165]

Obesity has been shown to induce oxidative stress through various mechanisms including diminished antioxidant enzyme activity, diminished availability of non-enzymatic antioxidants, increased oxygen consumption, cellular damage (leading to cytokine production) and altered oxygen metabolism through a high fat diet.[171]

Furthermore, the oxidative stress associated with obesity may lead to endothelial dysfunction, as it has been shown to improve with the administration of intrabrachial vitamin C.[170]

Smoking

Cigarette smoking is an easily modifiable risk factor in the development of oxidative stress and cardiovascular disease.[172] Cigarette smoke contains more than 4000 harmful chemicals, including ROS such as hydroxyl radicals and hydrogen peroxide.[172-174]

The effects of cigarette smoke on oxidative stress initiation may be either direct or indirect. Directly, the components of cigarette smoke are able to increase ROS and deplete antioxidants, tipping the redox balance towards a pro-oxidant state that leads to the downstream consequences discussed in previous sections.[172,173,175] Research has shown that GSH levels are drastically reduced, both due to acute and chronic cigarette smoking.[175] This being a substrate to many other antioxidant enzymes, it may cause a cascade effect able to diminish the protective activity of GPx and GR in the system.[175] However, this process is reversible, since cessation of smoking has shown to restore GSH levels and lower ROS production, thus reducing systemic oxidative stress from cigarette exposure.[175]

Indirectly, cigarette smoke components are able to initiate two important processes of oxidative stress driven cardiovascular pathology.[175,176] The ROS in cigarette smoke are able to initiate inflammatory processes, further exacerbating oxidative stress and endothelial dysfunction and promoting atherosclerosis and cardiovascular disease development.[172,173]

Cigarette smoke is linked to endothelial dysfunction through various mechanisms. Firstly, the ROS within cigarette smoke can interact with LDL-C molecules, transforming them into their oxidized state, which is the initiating step in the development of atherosclerosis.[173] Secondly, ROS from cigarette smoke is known to deplete the levels of BH_4, the critical cofactor for eNOS activity within the vasculature.[176] Exposure to cigarette smoke has also reduced the transport of L-arginine in the endothelium, which is also needed for proper functioning of the eNOS system.[174] Lastly, since ROS are able to damage protein molecules, it is also suggested that cigarette smoke compounds are able to damage the enzymes responsible for BH_4 production and hence decreases nitric oxide production and eventually leads to the consequences of eNOS uncoupling.[176]

Alcohol abuse

Both acute and chronic alcohol use is able to directly contribute to the increased production of ROS through the action of hepatocytes, endothelial cells and Kupffer cells in the liver during exposure to ethanol.[177-179]

A possible mechanism behind the production of ROS is explained by ethanol being able to produce endotoxins which themselves activate NOX enzymes within the cells and lead to ROS production.[180] Not only is the production of ROS increased during alcohol usage, but the activity of antioxidant enzymes (such as SOD, GPx and CAT) as well as the levels of non-enzymatic antioxidants (such as GSH and vitamin E and C) are also reduced, once again favoring oxidative stress. [177,179,180]

Furthermore, alcohol is also able to promote both endothelial dysfunction and inflammation by promoting LDL-C oxidation and the production of inflammatory cytokines which are implicated in vascular pathology.[179,180] The negative impact of alcohol on hypertension development is due to the ability of alcohol to stimulate the release of AngII and ET-1 while decreasing nitric oxide in the vasculature and promoting vascular inflammation.[181]

Although alcohol and oxidative stress are associated with cardiovascular disease development, research has shown that the administration of antioxidants that improve GSH levels are able to blunt the toxicity of alcohol in the system.[180]

Ageing

One of the factors that play a pivotal role in both oxidative stress and hypertension is ageing. It is well known that vascular structural and functional changes associated with age enhance the development of hypertension, however hypertension may also enhance early vascular changes.[182,183]

With regard to oxidative stress, age is shown to contribute to a redox imbalance by both increasing ROS production, as well as decreasing antioxidant activity, which may also promote structural and functional damage of the cardiovascular system.[182,184-186]

Since nitric oxide bioavailability plays such a crucial role in vascular tone, the impaired vasodilation due to oxidative stress has been proposed as a foundational mechanism for hypertension development with advancing age and that age in itself is a risk factor for endothelial dysfunction and impaired vasodilation.[182] This is due to the assumption that age increases NOX, xanthine oxidase and mitochondrial electron transport chain activity, while also inhibiting antioxidant enzyme activities, promoting oxidative stress and its consequences.[183] While endothelial dysfunction can be both a cause and consequence of hypertension, the fact remains that a loss of proper endothelial function is an important step in atherosclerosis and cardiovascular disease development, all of which worsen with advancing age.[183]

Chronic and acute stress

Cardiovascular reactivity is a measure of stress-induced increases in blood pressure whilst administering acute emotional or physical stressors (in the form of either a color-word conflict test or a cold-pressor test).[187-189]

Chronic stress has been shown to promote oxidative stress, possibly involving heightened cortisol as a mediator.[190] It is suggested that cortisol, known to prepare the body for stress, may diminish resistance to oxidative damage.[190] An increase in the cardiovascular reactivity in response to an acute stressor has been implicated in the development of cardiovascular disease.[187-189,191] There are various mechanisms thought to be involved in this pathological role of increased cardiovascular reactivity in the development of hypertension and cardiovascular disease, one of which may be sympathetic activation.[187-189] Administration of a stressor leads to heightened sympathetic activation, resulting in vasoconstriction of the arterioles and consequent increases in blood pressure.[188,189]

A second possible mechanism involves age, since older subjects have been shown to exhibit increased cardiovascular reactivity to stressors than their younger counterparts.[188] The proposed mechanism is thought to be as a result of age induced central arterial stiffness.[188] The development of arterial stiffness has been shown to induce various structural changes to the vasculature (and vice versa), which inhibit proper buffering of blood pressure fluctuations, as is the case with stress.[188]

2.6 Oxidative stress and cardiovascular disease in the South African population

Previous studies done within the Hypertension in Africa Research Team indicated a positive association between ROS (measured as serum peroxides) and systolic blood pressure (SBP) and pulse pressure (PP) in black men, suggesting a possible role for oxidative stress in hypertension development and increased arterial stiffness in this population.[192] In hypertensive black men, total glutathione (tGSH) levels were negatively associated with cIMT, proposing a possible role of attenuated GSH levels in atherosclerosis development.[193] A significant and independent association between ROS and angiogenic growth factors, along with an unfavorable cardiovascular profile, has also been proposed to form part of the oxidative stress driven vascular deterioration exhibited in black South Africans.[194]

Additionally, the finding of a negative association of urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) (an oxidized nucleoside of DNA excreted in urine once DNA undergoes repair) with BP and PP in African men, suggests increased oxidative stress in this population and suggests a dose-response relationship where oxidative stress may up-regulate antioxidant defenses and DNA repair processes.[195]

Building on this, previous research from the PhD candidate elaborated on these results in which upregulated GR activity in black men was independently associated with carotid wall thickening while decreased GPx activity was independently associated with blood pressure in black women. These results suggest compensatory GR activity to prevent arterial remodeling in black men, but also highlight the possible role of oxidative stress in hypertension development in black women.[196]

Studies pertaining to cardiovascular reactivity in this South African cohort have also been investigated within our research team and have indicated an increase in cardiovascular reactivity in black South Africans. Here it was suggested to play a fundamental role in hypertension development and end-organ damage among these participants.[197-199] A common conclusion observed is the role of impaired baroreflex functioning during stress administration in the heightened stress perception among black participants.[198,199]

3. Problem statement and motivation

The general aim of this study was to investigate markers of oxidative stress and their association with cardiovascular function in young (participants of the African-PREDICT study) and older (participants of the SABPA study) black and white South Africans.

3.1 Motivation, aims, objectives and hypotheses for each manuscript

This study comprises three manuscripts for publication in peer reviewed research journals.
In this section, a short motivation, aims, objectives and hypotheses are outlined for each manuscript.

Chapter 3: Manuscript 1

The relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population: The African-PREDICT study.

Motivation and problem statement:

Oxidative stress plays a role in the development of atherosclerosis and hypertension.[24,90,200,201] With hypertension development ever increasing,[10,202,203] coupled with evidence of early vascular aging in black South Africans,[20,22,204-206] it is unclear what the effect of oxidative stress may be on cardiovascular structure and function in a young, healthy cohort not yet diagnosed with hypertension. The relationship of both glutathione peroxidase (GPx) and glutathione reductase (GR) with blood pressure and carotid wall thickness remain to be explored in a young, healthy cohort.

Aim:

To explore the relationship between antioxidant enzyme activity and cardiovascular function and structure in young (aged 20-30 years) black and white South Africans of the African-PREDICT study.

Objectives:

1. To compare antioxidant enzyme activity (GPx, GR and SOD) and cardiovascular function (ambulatory blood pressures) and structure (cIMT) between young black and white participants of the African-PREDICT study.
2. To determine whether any relationship exists between antioxidant enzyme activity (GPx, GR and SOD) and cardiovascular function (ambulatory blood pressure) and structure (cIMT) in young black and white participants of the African-PREDICT study.

Hypotheses:

1. Antioxidant enzyme activity (GPx, GR and SOD) will be lower, while blood pressure, pulse pressure and cIMT will be higher in the black participants of the African-PREDICT study when compared to their white counterparts.
2. A negative, unfavorable relationship will exist between antioxidant enzyme activities (GPx, GR and SOD) and cardiovascular variables (ambulatory blood pressure and cIMT) in black and white participants of the African-PREDICT study.

Chapter 4: Manuscript 2

Cardiovascular reactivity and oxidative stress in young and older adults: The African-PREDICT and SABPA studies.

Motivation and problem statement:

Literature has shown that black populations display heightened cardiovascular responses to acute stress, which in itself is a marker for cardiovascular disease development.[197-199,207] Although black South Africans are at risk for early vascular changes, [20,22,204-206] and previous results have linked elevated blood pressure in black South Africans to oxidative stress,[192,196] the relationship of heightened cardiovascular reactivity with oxidative stress in a black South African population is unclear. The possible influence of age on the link between heightened cardiovascular reactivity and oxidative stress also remains to be explored.

Aim:

To investigate whether a difference in cardiovascular reactivity (in response to stress) exists between black and white participants of the African-PREDICT (younger cohort) and SABPA (older cohort) studies. A further aim is to investigate associations of antioxidant enzyme activity with cardiovascular reactivity in these groups.

Objectives:

1. To compare oxidative stress markers (ROS, total GSH, GPx, GR, SOD and γ-GT) and cardiovascular reactivity in response to stress in younger and older black and white participants.
2. To determine whether relationships exist between oxidative stress markers (ROS, total GSH, GPx, GR, SOD and γ-GT) and cardiovascular reactivity in younger and older participants.
3. To determine if any existing relationships between oxidative stress markers (ROS, total GSH, GPx, GR, SOD and γ-GT) and cardiovascular reactivity differ in cohorts of different ages.

Hypotheses:

1. Oxidative stress profiles (ROS, total GSH, GPx, GR, SOD and γ-GT) will be worse and cardiovascular reactivity in response to stressors will be higher in the black participants than in the white participants.
2. Adverse relationships exist between oxidative stress and cardiovascular reactivity in younger and older participants.
3. Any existing relationships are dependent of age.

Chapter 5: Manuscript 3

Three-year change in oxidative stress markers is linked to target organ damage in black and white men: The SABPA study.

Motivation and problem statement:

Cardiovascular disease is a growing concern globally and is increasing among developing countries such as South Africa.[7,202] Existing knowledge in the black South African population points to increased hypertension development and early vascular aging, confirmed to be linked to oxidative stress.[20,203,208] Results in older cohorts have defined existing relationships of oxidative stress with blood pressure, arterial stiffness and thickening of the carotid wall.[192,193,196,205] However, it is unknown whether changes in oxidative stress over time are associated with deterioration in target organ damage over time.

Aim:

To investigate whether changes in oxidative stress markers after a 3-year follow up are related to follow-up target organ damage in black and white South Africans.

Objectives:

1. To investigate whether black and white participants exhibit changes in oxidative stress markers (ROS, total GSH, GPx, GR, SOD and γ–GT) and target organ damage (cIMT, cross-sectional wall area (CSWA) and estimated glomerular filtration rate (eGFR)) after a three-year time span.
2. To determine whether changes in oxidative stress markers (ROS, total GSH, GPx, GR, SOD and γ–GT) associate with measures of target organ damage (cIMT, CSWA and eGFR) in black and white participants after a three-year time span.

Hypotheses:

1. Oxidative stress profiles (ROS, total GSH, GPx, GR, SOD and γ–GT) will worsen and markers of target organ damage (cIMT, CSWA and eGFR) will deteriorate more in black participants when compared to their white counterparts.
2. Changes in oxidative stress markers (ROS, total GSH, GPx, GR, SOD and γ–GT) associate adversely with markers target organ damage (cIMT, CSWA and eGFR) in black and white participants over a three-year time span.

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CHAPTER 2:

Study protocol and methodology

1. Study design

The burden of cardiovascular disease is overwhelming,[1,2] with blood pressure being highest in black groups and abundant research alluding to the same profile in the black South African population.[2-8] Many factors may be attributing to the development of cardiovascular disease in black South Africans including urbanization and early vascular aging.[9-12] However, this study focused on the link between oxidative stress and the cardiovascular system.

This study made use of the **African PR**ospective study on the **E**arly **D**etection and **I**dentification of **C**ardiovascular **D**isease and Hyper**T**ension (African-PREDICT) study and the **S**ympathetic activity and **A**mbulatory **B**lood **P**ressure in **A**fricans (SABPA) study (Figure 1).

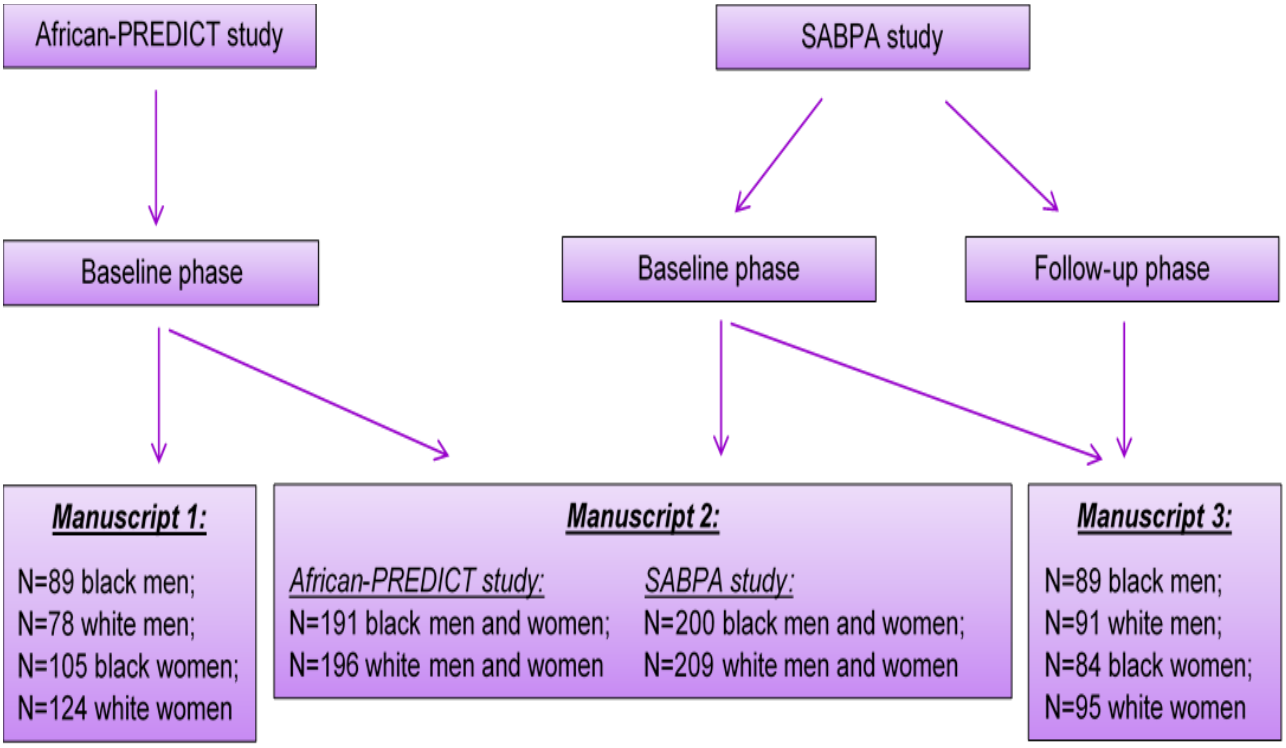

***Figure 1**: Study design for this thesis in which the study population of each manuscript is presented.*

The African-PREDICT study is a longitudinal study with the aim to contribute to the generation of knowledge behind cardiovascular disease development, especially in black South Africans, in order to identify novel markers to predict early cardiovascular disease development and implement prevention strategies from a younger age.[13] This study is conducted on 1202 young, healthy black South Africans over a period of 10-20 years, with the first phase having been conducted between 2013-2017. The five-year follow-up phase started in 2018, but in this study only data from the baseline phase will be analysed cross-sectionally.[13] A wide range of measurements were performed at baseline and those pertaining to this PhD study are discussed in this chapter.

The SABPA study was designed to investigate the impact of the apparent hyperactive nervous responses on cardiovascular disease development among older black and white South Africans.[14] Chronic stress, which is often evident among urban cohorts, has been associated with increased sympathetic nervous system activity and cardiovascular diseases. Therefore, the SABPA study is a prospective study investigating a hyperactive neural connection as a mechanism for cardiovascular pathophysiology and hopefully improve the morbidity and mortality associated with it.[14] Phase I of the SABPA study was conducted between February 2008 and May 2009 and phase II (follow-up) was conducted between 2011 and 2012, with protocols being designed to remain as similar as possible in the two phases of the study.[14]

Both of these studies were conducted by the Hypertension in Africa Research Team (HART) of the North-West University (Figure 2).

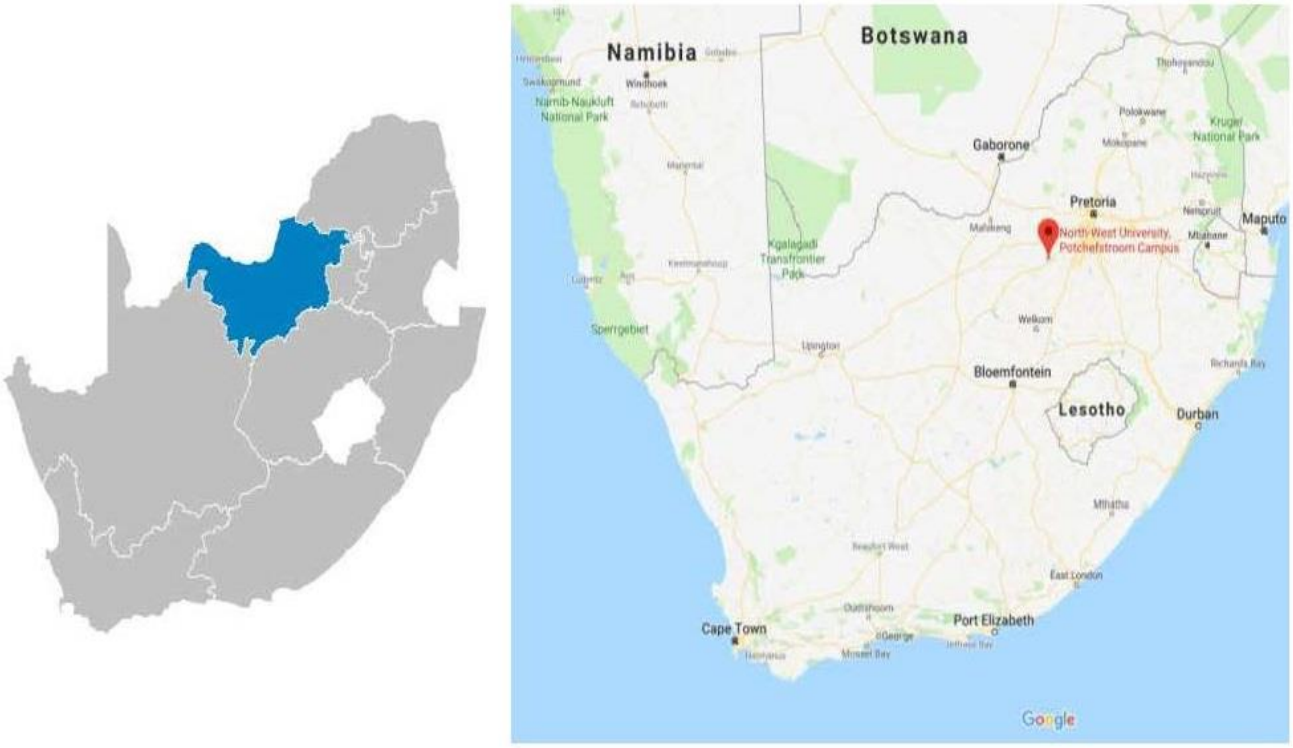

Figure 2*: Maps of South Africa highlighting the North-West province and the North-West University where the Hypertension in Africa Research Team (HART) is based.*

2. Recruitment processes

2.1 The African-PREDICT study

During the African-PREDICT study, participants in and around the Potchefstroom area were recruited by trained, active field workers by way of radio, noticeboards, newspaper advertisements or from their place of employment and subsequently grouped by socio-economic status into low-, medium- and high-level of employment, with equal sex distribution.[13]

Participant screening began in February 2013 and the African-PREDICT study was conducted in two phases, whereby the participants were first screened for eligibility before joining the advanced study phase (Figure 3). Once participants were selected, they were invited to participate in the screening phase of the study that provided an essential service to the community and was conducted by a registered nurse or trained staff member. Screening included general health questionnaires, rapid blood tests (cholesterol, blood glucose and HIV), spot urine sampling, office blood pressure and anthropometry.

Thereafter, selected participants who complied with the inclusion criteria were invited to participate in the advanced phase of the study within the Hypertension Research and Training Clinic of the North-West University. Here participants were subjected to a vast number of medical procedures including anthropometry, ambulatory blood pressure measurements, continual blood pressure and cardiovascular function analyses, electrocardiogram (ECG) recordings, pulse wave analyses, ultrasound carotid intima-media thickness (cIMT) analyses, cardiovascular reactivity testing, retinal microvascular analyses, physical activity monitoring, questionnaires and biological sampling. However, since not all of these are applicable to this sub-study, they are not discussed in detail further.

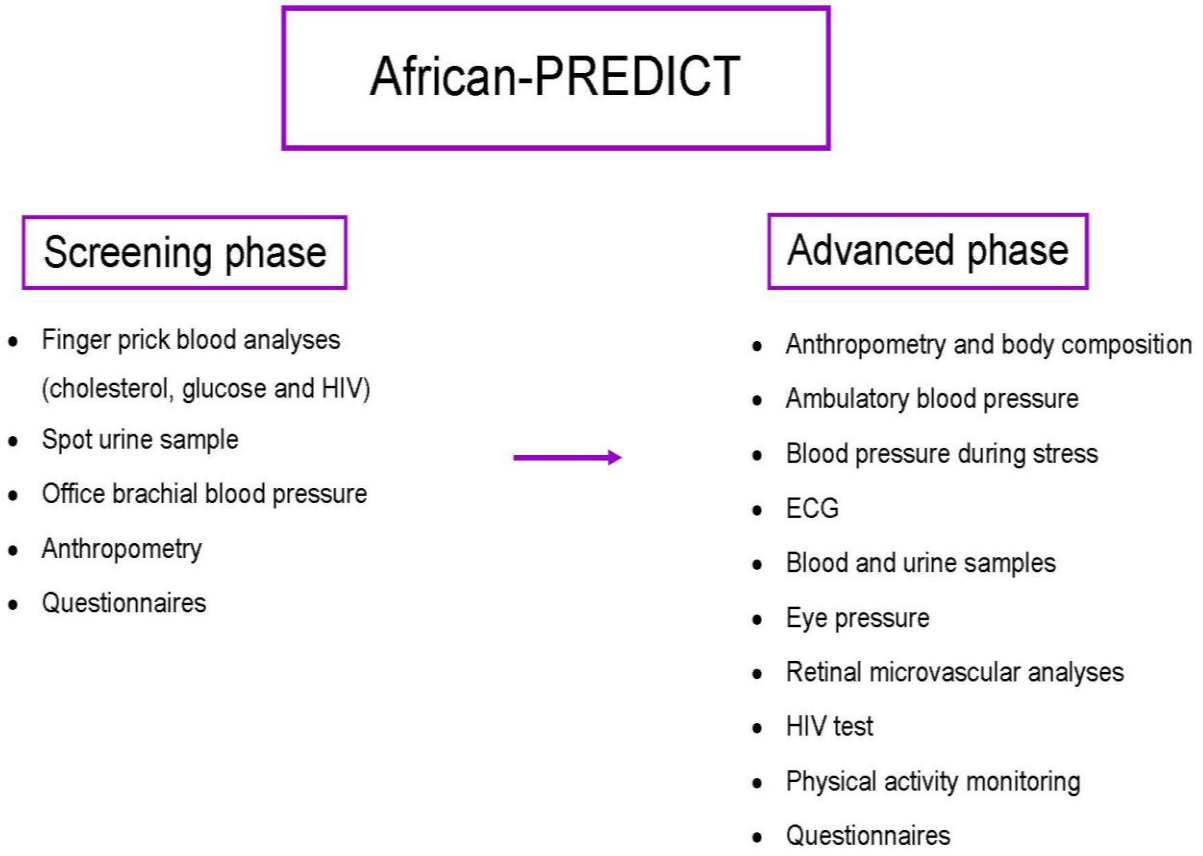

***Figure 3**: General outline of the screening and advanced legs of the African-PREDICT study.*

2.2 The SABPA study

A total of 2170 black and white teachers between the ages of 20 and 65 years were invited to participate in the SABPA study, all of which were enrolled in the 43 schools of the Dr. Kenneth Kaunda Education District (Klerksdorp and Potchefstroom) of the North-West Province South Africa.[14]

Recruitment was conducted 3 months prior to participation by the principal investigator and trained black African fieldworkers and once selected for participation in the study, participants were transported to the Metabolic Unit of the North-West University where they spent one night.

The study was conducted over a 2-day period and included anthropometry, ambulatory blood pressure measurements, continual blood pressure and cardiovascular function analyses, ECG recordings, ultrasound cIMT analyses, cardiovascular reactivity, physical activity monitoring, questionnaires, biological sampling and retinal microvascular analyses (only in phase II) (Figure 4).

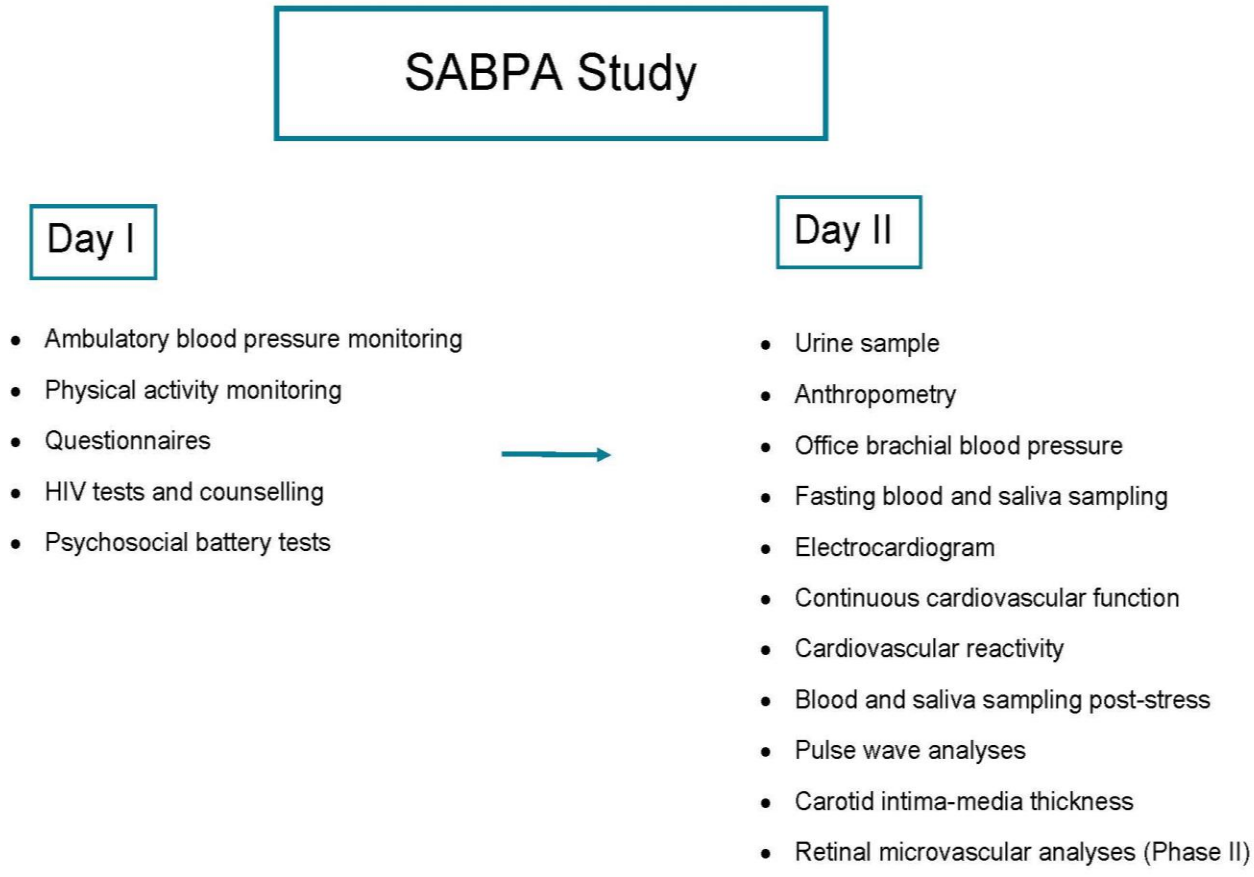

***Figure 4**: General outline of the 2-day study period during the SABPA study.*

Screening for eligibility resulted in a population size of 409 eligible participants, which were screened as part of Phase I of the study. Phase I (baseline) was conducted between 2008 and 2009 and comprised of 209 white (101 men and 108 women) and 200 black (99 men and 101 women) participants. A three-year successful follow-up rate of 87.8% provided a sample of 186 white (91 men and 95 women) and 173 black (89 men and 84 women) to participate in Phase II (follow-up) between 2011 and 2012.

3. Research methodology

3.1 Questionnaires

In both studies, a researcher assisted each participant in completing a general health questionnaire (Figure 5), either as hard copies or in the form of an online program that was used to obtain demographic data, medication use, socio-economic status (in the African-PREDICT study) and information on behavioral risk factors such as alcohol usage.

Figure 5*: Researcher of HART conducting a general health questionnaire in the African-PREDICT study.*

3.2 Anthropometric and physical activity measurements

In both studies, anthropometric measurements were performed in triplicate to the nearest 0.1cm and 0.1kg by an accredited anthropometrist (Figure 6) and these measurements complied with the International Standards for Anthropometric Assessment (International Society for the Advancement of Kinanthropometry).[15]

Anthropometric measurements of the African-PREDICT study included body height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany), body weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany) and waist circumference (which was measured wearing minimal clothing) (Lufkin Steel Anthropometric Tape (W606PM), Lufkin, Apex, USA) as measurements of health risk.[16] Similarly, anthropometric measurements of the SABPA study included height (Invicta Stadiometer IP 1465, Invicta, London, UK) weight (Precision Health Scale, A & D Company, Tokyo, Japan) and waist circumference (Holtain unstretchable flexible 7mm wide metal tape, Crosswell, Wales).

Body mass index (BMI) is used as an indirect indicator of obesity and was calculated from the obtained measurements in both studies using the following formula: BMI (kg/m^2) = weight (kg) / height (m^2) for both studies.[17,18]

Physical activity is an important risk factor for metabolic and cardiovascular diseases and various accelerometer devices were used in order to determine total energy expenditure (TEE), which was used a modifiable lifestyle risk factor in this PhD study.[19] The validated ActiHeart physical activity monitor (CamNtech Ltd., England, UK) was used to monitor TEE for a maximum of 7 days in the African-PREDICT study and in the follow-up phase of the SABPA study.[20,21] In the baseline phase of the SABPA study, TEE was measured over a 24 hour period using the Actical® omnidirectional accelerometer (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada) as another accurate measure of energy expenditure.[22]

***Figure 6**: Researcher of HART conducting anthropometry measurements in the African-PREDICT study.*

3.3 Cardiovascular measurements

Due to the nature of this study, various cardiovascular measurements were used to formulate the manuscripts of this PhD study. These included ambulatory blood pressure, cardiovascular reactivity and function and cIMT, which are discussed in detail below.

Ambulatory blood pressure measurements

Ambulatory blood pressure measurement (ABPM) is widely accepted as the gold standard method for predicting cardiovascular risk above that of conventional office blood pressure and during both studies, it was measured as a way of monitoring continuous 24-hour blood pressure.[23-26] A researcher fitted each participant with an ABMP device on his or her non-dominant arm making use of a correctly sized cuff as per the manufacturer's instructions (Figure 7).

This was achieved by using the Card(X)plore® device (CE0120, Meditech, Budapest, Hungary) in the African-PREDICT study and the Cardiotens® device (CE0120, Meditech, Budapest, Hungary) in the SABPA study, validated by the British Hypertension Society[27]. Blood pressure measurements were recorded at 30-minute intervals during the day (06:00 – 22:00) and one-hour intervals during the nighttime (22:00 – 06:00).

Participants were instructed on how to ensure successful inflations and were asked to continue with normal daily activities and record any abnormalities such as headache, nausea and stress on their ambulatory diary cards. Data obtained was entered into a database using the CardioVisions 1.19 Personal Edition (Meditech, Budapest, Hungary) software.

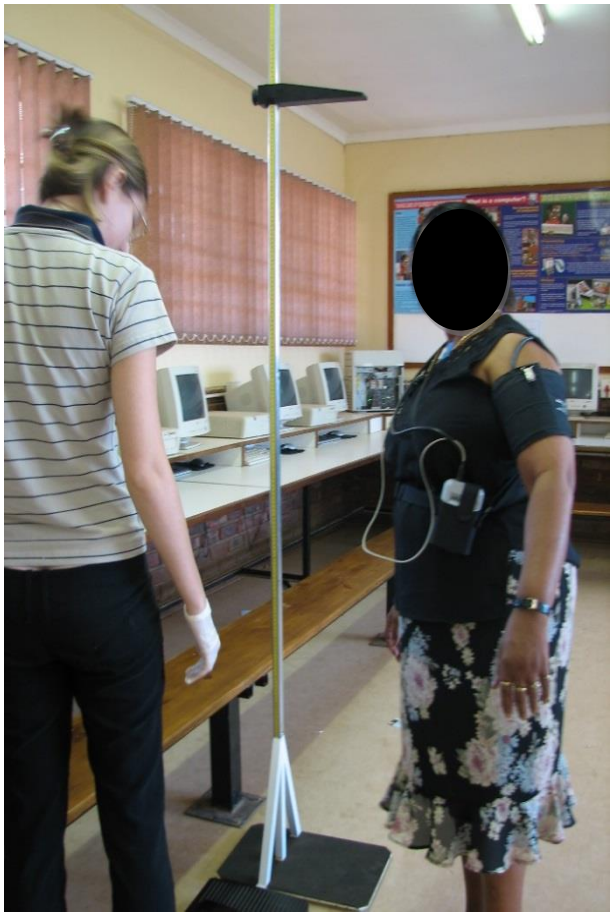

Figure 7*: Participant of the SABPA study wearing the ABPM Cardiotens® device getting ready for anthropometric measurements.*

Cardiovascular reactivity and cardiovascular function

Cardiovascular reactivity was monitored using the Stroop color-word conflict test, named after John Ridley Stroop who first described the protocol in 1935.[28] This is an acute mental stress test in which participants of the study were presented with a successive series of words and were requested to name the color of the ink instead of reading the incongruent colored word itself (Figure 8).

Figure 8*: An example of the Stroop color-word conflict test used as acute mental stressor when measuring cardiovascular reactivity in both studies.*

This interference provokes an autonomic nervous system response and affects cardiovascular function,[29,30] which was assessed in the study using the validated Finometer device (FMS, Finapres Medical Systems, Amsterdam, Netherlands).[31-33]

The finger cuff of the Finometer device was fitted on the left middle finger (Figure 9) and after a 10-minute resting period, a 5-minute continuous measurement of resting cardiovascular variables was recorded. During the recording, after 2 minutes, a return-to-flow systolic calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial artery pressure, which allows for the highest precision in cardiovascular measurements.[31]

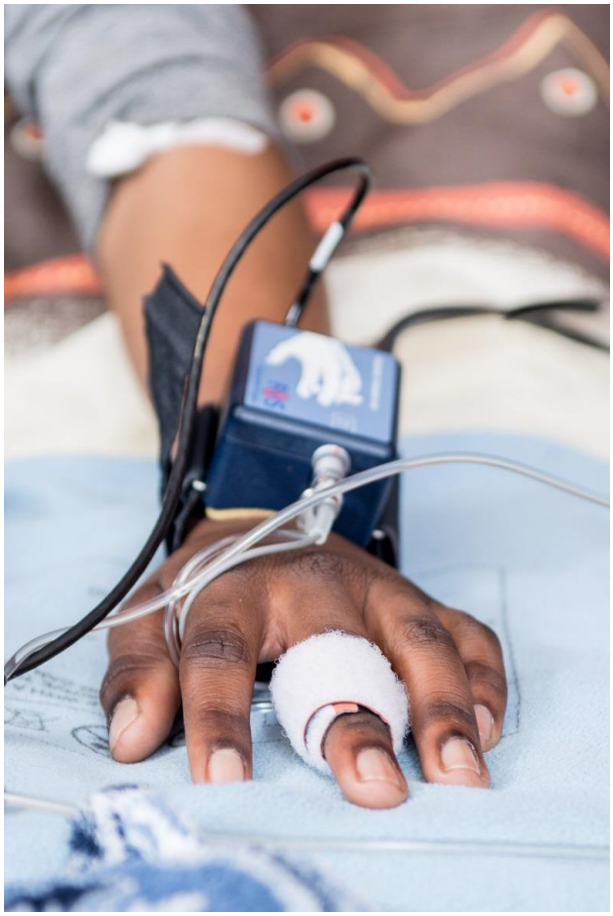

Figure 9*: An example of the finger cuff of the Finometer device that was used to measure cardiovascular functional variables both at rest and during exposure to acute stress.*

Resting measurements of cardiovascular function were performed by the Beatscope software and these included systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), stroke volume (SV), total peripheral resistance (TPR) and “Windkessel” arterial compliance (Cwk). All blood pressure measurements complied with the requirements of the Association for the Advancement of Medical Instrumentation (AAMI).[31]

Thereafter, the color-word conflict test was applied for one minute during which the above-mentioned cardiovascular variables were measured while being exposed to the stressor. The mean of the last minute of the resting values and the last 20 seconds during exposure was recorded. The cardiovascular reactivity was calculated as the percentage change from the resting values.[34]

Figure 10*: Finometer measurements of cardiovascular function being measured. On the left is a participant of the African-PREDICT study performing the Stroop color-word conflict test in order to obtain cardiovascular reactivity data. On the right is a participant of the SABPA study attached to the Finometer device.*

Carotid intima-media thickness

Participants' cIMT was used as a non-invasive measure of arterial wall alterations and cardiovascular disease development risk. This is an easily reproducible ultrasound technique and is considered a well-described method of choice for assessing atherosclerotic burden and predicting cardiovascular events.[35-38]

During the studies, cIMT measurements were made on the left and right common carotid artery, as well as the internal carotid according to the Mannheim Consensus (Figure 12 and Figure 13).[39] This was achieved by way of B-mode ultrasonography (General Electric Vivid E9, GE Vingmed Ultrasound A/S, Horten, Norway) in the African-PREDICT study and the high resolution SonoSite Micromaxx ultrasound system (SonoSite Inc., Bothell, WA, USA) and a 6-13 MHz linear array transducer in the SABPA study.

In order to determine a maximal 10mm segment of good image quality for use in analyses, images from at least two optimal angles were interpreted with the Artery Measurement Systems Software (Tomas Gustavsson, Gothenburg, Sweden). The semi-automated program performed approximately 100 measurements throughout the chosen segment of the media-adventitia and calculated the cIMT and diameter from the borders from the trailing edge of the near wall to the leading edge of the far wall. All measurements were performed and analyzed by a single experienced observer while participants were in a relaxed state,[39] and where applicable, the same optimal angles were used during baseline and follow-up phases of the SABPA study.

As a means to confirm structural (and not functional) changes in luminal diameter, carotid cross-sectional wall area (CSWA) was calculated using the formula $CSWA = \pi(d/2 + cIMT)^2 – \pi(d/2)^2$ where d denotes luminal diameter.[40,41]

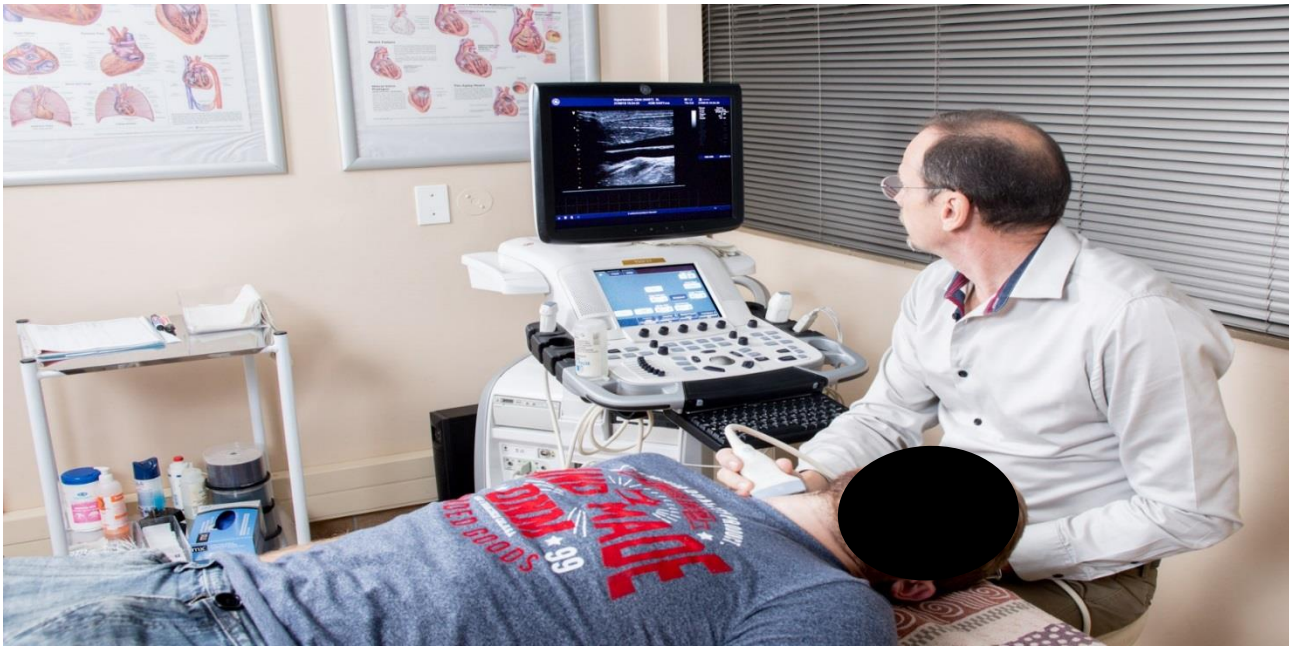

***Figure 11**: A researcher using the General Electric Vivid E9 ultrasound device to measure the carotid intima-media thickness of a participant in the African-PREDICT study.*

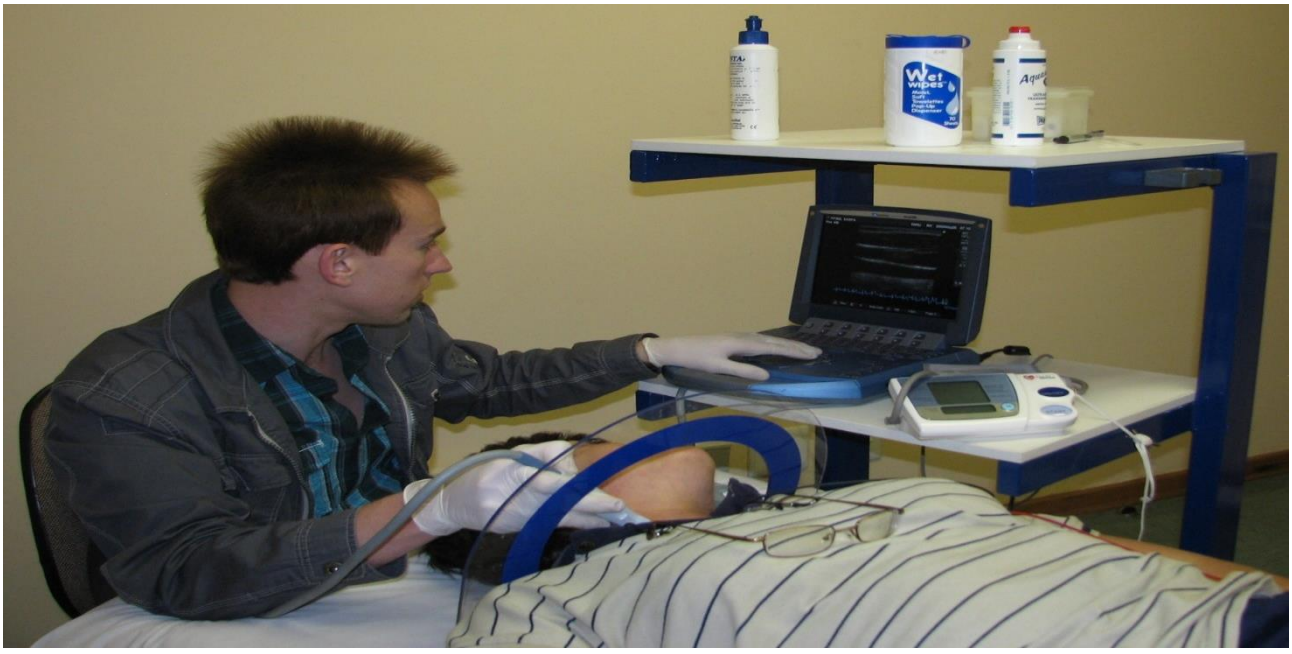

***Figure 12**: A researcher using the SonoSite Micromaxx ultrasound device to measure the carotid intima-media thickness of a participant in the SABPA study.*

3.4 Biological sampling

In both the African-PREDICT and SABPA studies, all biochemical measurements and analyses were independently performed by a trained researcher (Figure 13). A registered nurse collected a fasting blood sample from each participant from the antebrachial vein branches using a sterile winged infusion set. Spot urine and 24-hour urine samples were also collected from each participant.

All samples were immediately prepared and aliquoted into cryovials in the on-site laboratory. Samples were then stored in bio-freezers at -80°C until they were analyzed in order to maintain efficient long-term stability (Figure 14).[42]

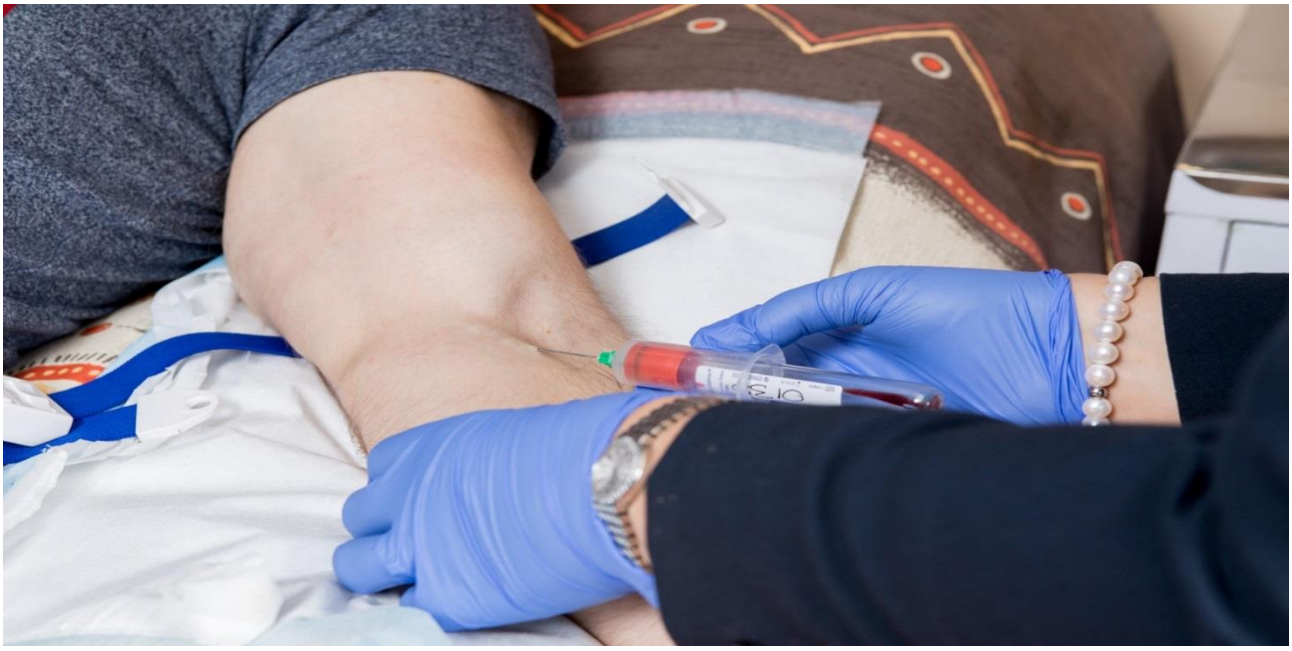

Figure 13*: Blood sampling being performed by the registered nurse of the African-PREDICT study.*

***Figure 14**: HART researchers storing cryovials of biological samples in biological freezers.*

3.5 Biochemical analyses

Oxidative stress markers were evaluated as main independent covariates in both studies. Reactive oxygen species were measured as serum peroxides (reported in units, where 1 mg H_2O_2/L is equivalent to one unit) using a high-throughput spectrophotometric assay and analyzed on a Synergy HT microplate reader (BioTek, Winooski, VT, USA) as described by Hayashi *et al.* in both studies.[43] Whole blood samples were used to measure total glutathione (tGSH) with a Synergy HT microplate reader (BioTek, Winooski, VT, USA) (Kit: BIOXYTECH GSH/GSSG-412) in both the SABPA study and the African-PREDICT study.

Antioxidant enzymes analysed in this study included glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD) which were measured using assay kits (Randox, Co. Antrim, United Kingdom) and the automated Cobas Integra 400 plus (Roche, Basel Switzerland) in the African-PREDICT study.

In both phases of the SABPA study, these antioxidant enzymes were measured using assay kits (Cayman Chemical Company, Ann Arbor, MI, USA) and a Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). In the African-PREDICT study, total antioxidant status was additionally measured using assay kits (Randox, Co. Antrim, United Kingdom) and the Cobas Integra 400 plus (Roche, Basel Switzerland). Another marker of oxidative stress which also serves as a marker of alcohol abuse is the enzyme γ-glutamyl transferase (γ-GT),[44-47] which was measured in serum using the using the Cobas Integra 400 plus (Roche, Basel, Switzerland) in both the African-PREDICT study and the follow-up phase of the SABPA study. The Unicel DXC 800 (Beckman and Coulter, Germany) and a Konelab™ 20I Sequential Multiple Analyzer Computer (Thermo Scientific, Vantaa, Finland) were used to measure γ-GT during the baseline phase of the SABPA study.

Variables that had been shown as confounding factors of the main dependent and independent variables were also measured and the reproducibility is shown as intra-assay variability and inter-assay variability in Table 1 below, along with that of the oxidative stress markers.

An enzymatic colorimetric method using the Cobas Integra 400 plus (Roche, Basel, Switzerland) was used to determine serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides and fluoride plasma glucose levels and an immunoturbidimetric method was performed on ethylenediaminetetraacetic acid whole blood in order to determine glycated hemoglobin (HbA1c) in the African-PREDICT study. In the SABPA study, serum total cholesterol and triglycerides were measured with the Unicel DXC 800 (Beckman and Coulter, Germany) and a Konelab™ 20I Sequential Multiple Analyzer Computer (Thermo Scientific, Vantaa, Finland) in the baseline phase and the Cobas Integra 400 plus in the follow-up phase. Serum HDL cholesterol and fluoride plasma glucose levels were measured using the Unicel DXC 800 (Beckman and Coulter, Germany) in the baseline phase and the Cobas Integra 400 plus (Roche, Basel, Switzerland) in the follow-up phase.

Serum creatinine was measured using an enzymatic colorimetric test analyzed on the Cobas Integra 400 plus (Roche, Basel, Switzerland) and was used in the calculation of the estimated glomerular filtration rate (eGFR) in ml/min/1.73m^2 according to the Modification of Diet in Renal Disease formula during the SABPA study.[48]

Interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) were measured as inflammatory markers using a high sensitivity Quantikine enzyme linked immunosorbent assay (ELISA) kit (R&D systems, Minneapolis, MN USA) analyzed on Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA) in both studies. High sensitivity C-reactive protein (CRP), which is also an inflammatory marker, was measured in serum, using the Cobas Integra 400 plus (Roche, Basel, Switzerland) during the African-PREDICT study and the follow-up phase of the SABPA study and the Unicel DXC 800, (Beckman and Coulter, Germany) during the baseline phase of the SABPA study.

A chemiluminescence method (Immulite, Siemens, Erlangen, Germany) was used to determine serum cotinine levels, a widely used biomarker for smoking,[49,50] in the African-PREDICT study and an immunoassay (Automated Modular, Roche, Basel, Switzerland) in the SABPA study. Cortisol was measured as a marker of sympathetic nervous activity by way of an electrochemiluminescence immunoassay (Elecsys 2010, Roche, Basel, Switzerland) in both studies.

Human Immunodeficiency Virus (HIV) status was determined using rapid antibody tests (First response kit, PMC Medical, India) in both studies. After testing positive, participants of the African-PREDICT study were excluded from the study population and referred to their local medical clinics for further evaluation and treatment. In the SABPA study, positive results were confirmed with the Pareekshak rapid test card (BHAT Biotech, India), where after they were included in the study and provided with appropriate counselling.

Table 1: Reproducibility of biochemical analyses of the African-PREDICT and SABPA studies.

	African-PREDICT study		SABPA study	
	Intra-AV (%CV)	**Inter-AV (%CV)**	**Intra-AV (%CV)**	**Inter-AV (%CV)**
Biochemical analyses				
Glucose	1.80	2.10	<10	<10
Total cholesterol	0.51	1.90	<10	<10
Triglycerides	1.60	1.90	<10	<10
Interleukin-6	7.80	9.60	5.90	18.9
Cortisol	7.10	12.7	7.10	12.7
Oxidative stress markers				
Reactive oxygen species	9.68	10.2	7.42	11.2
Total glutathione	6.16	13.7	<10	<10
Glutathione peroxidase	4.86	7.30	5.70	7.20
Glutathione reductase	4.84	6.55	3.70	9.30
Superoxide dismutase	4.64	7.07	3.20	3.70
γ-glutamyl transferase	1.80	1.80	<10	<10
Lifestyle				
Cotinine	9.60	12.2	<10	<10

Intra-AV, Intra-assay variability; Inter-AV, Inter-assay variability.

3.6 Statistical analyses

Statistical analyses used in this PhD study is discussed in broad terms below, as detailed descriptions are given in each manuscript.

Statistica version 13.2 (Dell, TX, USA) was used to perform the statistical analyses of this study and all results with $p<0.05$ were regarded as statistically significant. The GraphPad Prism software (GraphPad Software Inc., California, USA version 5.03) was used to represent data graphically.

Post hoc analyses were performed to calculate the achieved power (1-β) for multiple regression models using the program G*power v3.1.9.2.[51]

Interaction terms were used to determine the effect of race and sex on main dependent and independent variables using multiple regression analyses. Normal distribution of data was assessed based on visual inspection of the bell-shaped histogram curve. Descriptive statistics were performed on normally distributed variables and the central tendency and spread was expressed as the arithmetic mean and standard deviation. Abnormally distributed variables were logarithmically transformed and expressed as the geometric mean and the 5th and 95th percentiles.

Proportions between cohorts were compared using Chi-square tests while means of continuous variables were assessed using independent t-tests. Analyses of covariance (ANCOVA) were used to compare dependent variables across cohorts while adjusting for covariates. Mean differences between baseline and follow-up phases of the SABPA study were compared using dependent t-tests.

Unadjusted associations between cardiovascular variables and independent markers of oxidative stress were evaluated using single regression analyses, while partial correlations analyses were performed to evaluate the relationships between cardiovascular variables and oxidative stress markers while adjusting for confounders.

Multi-variate adjusted linear regression analyses were performed in order to investigate the independent associations between cardiovascular variables and oxidative stress markers while adjusting for confounding factors. The selection of covariates included in the models was based on literature or identified through exploratory unadjusted correlations of covariates with cardiovascular variables and oxidative stress markers. All independent variables entered into multiple regression models are listed in the footnotes and/or legends of each manuscript.

4. Ethical considerations

Applicable to both studies, participants signed written informed consent forms at all stages explaining procedures and protocols before joining the relevant study.

Participants received verbal and written informed consent, explaining every step of the study detail in their home language. Participants were provided an opportunity to ask questions and voice any concerns related to the studies. Participants were free to withdraw from either study at any stage without negative consequences, allowing free and voluntary participation in both studies.

Both the African-PREDICT study and the SABPA study complied with the Helsinki declaration of 1975 (and subsequent revisions) regarding investigations among human participants and were further approved by the Health Research Ethics Committee of the North-West University, Potchefstroom campus (African-PREDICT: NWU-00001-12-A1 and SABPA: NWU-00036-07-S6). The African-PREDICT study was also endorsed by the National Department of Health, under the Section Non-communicable Diseases. A letter of approval from the Health Research Ethics Committee (HREC) of the North-West University (Potchefstroom campus) for this study can be found in the annexures (Annexure A).

5. Involvement of the candidate in data collection and analyses

Due to the nature of the SABPA study, both phase I and phase II had been completed prior to the start of this PhD. However, the candidate was involved in the following data collection processes.

The candidate assisted with screening measurements in order to identify eligible participants for the advanced leg of the African-PREDICT study.

Screening activities took place in the F11 building of the Hypertension Research and Training Clinic of the North-West University, Potchefstroom Campus.

During the screening phase of the African-PREDICT study, the candidate assisted in the collection and analysis of spot urine samples while making use of Labstix urine test strips. This rapid dipstick urine test monitored specific gravity, pH, proteins, glucose, blood, ketones, nitrites and leukocyte levels in the sample.

The candidate was responsible for measuring office blood pressure during the screening phase of the African-PREDICT study. Brachial blood pressure was measured using the Dinamap Procare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA) in order to eliminate participants not aligned with the inclusion criteria of being normotensive or pre-hypertensive (blood pressure of <120/80 mmHg). Participants were seated in a resting state and fitted with an appropriately sized cuff on the left upper-arm. After a 5-minute resting period, blood pressure was taken which included systolic blood pressure, diastolic blood pressure and heart rate. The same set of measurements was then performed on the right upper-arm in duplicate, after which the final set of measurements was repeated on the left upper-arm.

As part of both the screening and advanced legs of the African-PREDICT study, the candidate was responsible for completing one-on-one questionnaires with the study participants. This included a general health questionnaire, a global physical activity questionnaire and the Berlin sleep apnea questionnaire, which covered a wide range of data including demographic, employment, lifestyle, family and physical activity information as well as sleep apnea risk.

During the advanced phase of the African-PREDICT study, the candidate was also involved in cardiovascular measurements and biochemical analyses. The candidate was trained in the use of the Sphygmocor device, which is the gold standard method for arterial stiffness assessment,[41,52] and was responsible for carotid-femoral pulse wave velocity measurements (Sphygmocor XCEL device, AtCor Medical Pty. Ltd., Sydney, Australia).

Participants were expected to remain in a supine position in a resting state for 5 minutes prior to the commencement of the measurement. An appropriately sized brachial cuff was placed on the right upper-arm while a femoral cuff was placed around the right thigh. Palpation was performed in order to locate the strongest pulse of the carotid artery of the participant and the distance from this point to the femoral artery was noted where after 80% of this distance was calculated and entered into the computer software program. The carotid pulse was evaluated with a tonometer while the Sphygmocor device calculated the central systolic blood pressure, augmentation index and pulse wave velocity in duplicate.

With regard to biochemical analyses, the candidate was involved in the preparation and processing of samples in the laboratory by way or centrifuging blood samples and aliquoting serum and urine into cryovials for long-term storage in -80°C biofreezers.

6. Acknowledgements

The figures in Chapter 1 of this thesis were downloaded from Servier Medical Art, while those in Chapter 2 were downloaded from Wikimedia Commons and Google Maps. Servier Medical Art is a licensed distributor of free-to-use medical images (Creative Commons Attribution 3.0 Unported License). Photographs were supplied by the relative principal investigators of the African-PREDICT and SABPA studies.

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CHAPTER 3:

The relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population: The African-PREDICT study

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ARTICLE

The relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population: the African-PREDICT study

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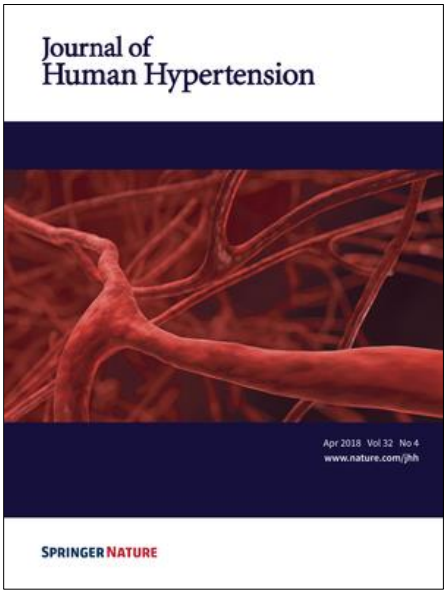
Abstract

Oxidative stress has been implicated in the development of hypertension, arterial stiffness and atherosclerosis. Optimal functioning of the enzymatic antioxidant system is central to prevent increased oxidative stress and its consequences. We aimed to investigate the relationships of ambulatory blood pressure and carotid intima-media thickness with enzyme activities of the glutathione cycle in 396 young, black and white South Africans of the African-PREDICT study. Ambulatory blood pressure and carotid intima-media thickness were measured and glutathione peroxidase and glutathione reductase activities were analyzed. Black participants had higher reactive oxygen species (men: $p = 0.019$; women: borderline $p = 0.064$) and total glutathione (both $p < 0.001$), but lower glutathione peroxidase activity and total antioxidant status (all $p < 0.001$). In black men, ambulatory pulse pressure was negatively associated with glutathione peroxidase activity ($R^2 = 0.19$; $\beta = -0.25$; $p = 0.06$). Black and white women displayed positive associations of ambulatory systolic blood pressure (black: $R^2 = 0.25$; $\beta = 0.21$; $p = 0.048$; white: $R^2 = 0.44$; $\beta = 0.18$; $p = 0.016$) with glutathione reductase activity, whereas white men displayed a positive association of ambulatory pulse pressure with glutathione reductase activity ($R^2 = 0.25$; $\beta = 0.29$; $p = 0.01$). The lower glutathione peroxidase activity and total antioxidant status, the higher reactive oxygen species, as well as the negative association between ambulatory pulse pressure and glutathione peroxidase activity in the black men suggest that oxidative stress may be associated with early vascular changes in this group. In the other three groups, the positive associations of blood pressure with glutathione reductase activity suggest a possible role for adequate glutathione reductase activity in preventing or delaying the development of hypertension.



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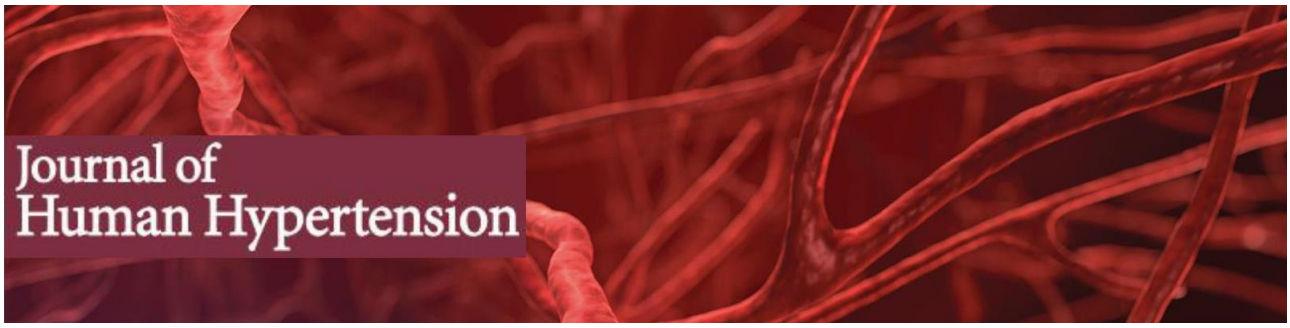
Please refer to Annexure D for the printed publication.



SUMMARY OF INSTRUCTIONS TO AUTHORS

Journal Details			
Title:	Journal of Human Hypertension.		
Impact factor;	2.433 (2017 Journal Citation Reports, Thomson Reuters, 2018).		
Aims and scope:	Journal of Human Hypertension is published monthly and is of interest to health care professionals who deal with hypertension (specialists, internists, primary care physicians) and public health workers. We believe that our patients benefit from robust scientific data that are based on well-conducted clinical trials. We also believe that basic sciences are the foundations on which we build our knowledge of clinical conditions and their management. Towards this end, although we are primarily a clinical based journal, we also welcome suitable basic sciences studies that promote our understanding of human hypertension. The journal aims to perform the dual role of increasing knowledge in the field of high blood pressure as well as improving the standard of care of patients. The editors will consider for publication all suitable papers dealing directly or indirectly with clinical aspects of hypertension, including but not limited to epidemiology, pathophysiology, therapeutics and basic sciences involving human subjects or tissues. We also consider papers from all specialties such as ophthalmology, cardiology, nephrology, obstetrics and stroke medicine that deal with the various aspects of hypertension and its complications.		
Publisher:	Nature Publishing Group.		
Journal Guidelines			
Author guidelines:	http://www.nature.com/article-assets/npg/jhh/jhh_new_gta.pdf	**Language:**	English.
Title:	Brief, informative, of 150 characters or less and should not make a statement or conclusion.	**Spacing:**	Double spaced.
Keywords:	-	**Font:**	-

Manuscript (words)	<4000 words excluding .abstract, references, figures/tables.	**Margins:**	Wide margin.
Abstract (words)	<250 words.	**Page numbers:**	All pages and lines are to be numbered.
Alignment:	-		
Conflict of interest statement:	Authors must declare whether or not there are any competing financial interests in relation to the work described. This information must be included at this stage and will be published as part of the paper. Conflict of interest should be noted in the cover letter.		
Acknowledgements:	These should be brief, and should include sources of support including sponsorship (e.g. university, charity, commercial organisation) and sources of material (e.g. novel drugs) not available commercially.		
Tables and figures:	Max 8 tables or figures. Tables should only be used to present essential data; they should not duplicate what is written in the text. Please make sure each table is cited within the text and in the correct order, e.g. (Table 3). Please save the files with extensions .xls / .xlsx / .ods / or .doc or .docx. Figures and images should be labelled sequentially and cited in the text. Figures should not be embedded within the text but rather uploaded as separate files. Use a coarse hatching pattern rather than shading for tints in graphs.		
References:	Only papers directly related to the article should be cited. Exhaustive lists should be avoided. References should follow the Vancouver format. In the text they should appear as numbers starting at one and at the end of the paper they should be listed (double-spaced) in numerical order corresponding to the order of citation in the text. E.g. Journal article, up to six authors: Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. Nat Immunology. 2005; 6: 353–360.		
Sections:	Articles must contain the following components: Cover letter; Title page (excluding acknowledgements); Abstract; Introduction; Materials (or Patients) and Methods; Results; Discussion; Acknowledgements; Conflict of Interest; References; Figure legends; Tables; Figures.		
Ethical considerations:	For experiments involving human subjects, authors must identify the committee approving the experiments, and include with their submission a statement confirming that informed consent was obtained from all subjects.		

*Please note: Slight adjustments in the format were made to ensure uniformity within the thesis.

Abstract

Oxidative stress has been implicated in the development of hypertension, arterial stiffness and atherosclerosis. Optimal functioning of the enzymatic antioxidant system is central to prevent increased oxidative stress and its consequences. We aimed to investigate the relationships of ambulatory blood pressure and carotid intima-media thickness with enzyme activities of the glutathione cycle in 396 young, black and white South Africans of the African-PREDICT study. Ambulatory blood pressure and carotid intima-media thickness were measured and glutathione peroxidase and glutathione reductase activities were analyzed. Black participants had higher reactive oxygen species (men: p=0.019; women: borderline p=0.064) and total glutathione (both p<0.001), but lower glutathione peroxidase activity and total antioxidant status (all p<0.001). In black men, ambulatory pulse pressure was negatively associated with glutathione peroxidase activity (R^2=0.19; β=-0.25; p=0.026). Black and white women displayed positive associations of ambulatory systolic blood pressure (black: R^2=0.25; β=0.21; p=0.048; white: R^2=0.44; β=0.18; p=0.016) with glutathione reductase activity, while white men displayed a positive association of ambulatory pulse pressure with glutathione reductase activity (R^2=0.25; β=0.29; p=0.012). The lower glutathione peroxidase activity and total antioxidant status, the higher reactive oxygen species, as well as the negative association between ambulatory pulse pressure and glutathione peroxidase activity in the black men suggest that oxidative stress may be associated with early vascular changes in this group. In the other three groups, the positive associations of blood pressure with glutathione reductase activity suggest a possible role for adequate glutathione reductase activity in preventing or delaying the development of hypertension.

Key words: arterial stiffness, carotid intima-media thickness, glutathione peroxidase, glutathione reductase, oxidative stress.

Introduction

Oxidative stress has been implicated in the development of hypertension,[1-3] which is well documented as an increasing epidemic, especially amongst developing countries.[4-6] South Africa is no exception, with urban black populations exhibiting an alarmingly high prevalence of hypertension.[6-8] In addition, black South Africans are more prone to early vascular changes when compared to their white counterparts.[9-11]

When oxidative stress increases, endothelial dysfunction may occur as a result of increased production of reactive oxygen species (ROS), decreased availability of antioxidants, decreased antioxidant enzyme activity (such as glutathione peroxidase and glutathione reductase) and the inactivation of nitric oxide.[12,13] This may in turn be linked with arterial stiffness, carotid wall thickening and cardiovascular disease development, all of which deteriorate with ageing.[12-14]

Previous results in black South Africans found that increased oxidative stress relates to elevated blood pressure,[15,16] vascular wall thickness[16,17] and arterial stiffness.[11,15] However, the aforementioned results were obtained in a population with an age range of 20 to 65 years and some participants already presented with hypertension. It is therefore not clear if these factors confounded the previous reported results and we therefore aimed to compare oxidative stress profiles and explore relationships of ambulatory blood pressure and carotid intima-media thickness (cIMT) with enzyme activities of the glutathione cycle – glutathione peroxidase (GPx) and glutathione reductase (GR) in young, healthy black and white South African men and women.

Methods

Study population

This study is embedded in the ongoing African PRospective study on the Early Detection and Identification of Cardiovascular disease and hyperTension (African-PREDICT). The aim of African-PREDICT is to recruit and follow participants over a period of 10-20 years in order to understand the pathological changes accompanying cardiovascular disease development and to identify novel markers related to early cardiovascular disease development in this population. Participants are continually being recruited from the Potchefstroom and surrounding areas of the North-West Province of South Africa by the use of active field workers, through their workplace, or by means of local newspaper and radio advertisements.

Participants of self-reported Indian, Asian or mixed origin ethnicity, who are not permanent residents of Potchefstroom or surrounding areas (or who do not plan regular trips to the area) and who are unable to read or understand English were excluded from the study. Participants who had elevated glucose levels (>5.6 mmol/L or confirmed glycated haemoglobin ≥6.5%), who were HIV infected and those who presented with a fever on the research day (ear temperature >37.5°C) were also excluded. Further exclusion criteria included being previously diagnosed with (or took medication for) type 1 or 2 diabetes mellitus, liver disease, cancer, tuberculosis, renal disease or cardiovascular diseases (hypertension, stroke, angina pectoris or myocardial infarction), who recently had surgery or trauma (within the past 3 months) and who was pregnant or breastfeeding.

Study procedures

After the initial screening, apparently healthy (in line with the exclusion criteria) normotensive or pre-hypertensive (systolic blood pressure (SBP) <140 and diastolic blood pressure (DBP) <90mmHg) black and white men and women between the ages of 20 and 30 years are invited to participate in the African-PREDICT study.

This study makes use of cross-sectional data obtained from the first 403 participants of the African-PREDICT study. Participants with incomplete antioxidant enzyme activity data (N=7) were excluded. In accordance with the aims of this study, the remaining 396 were divided into black men (N=89), white men (N=78), black women (N=105) and white women (N=124).

The African-PREDICT study complies with all the applicable requirements of the Declaration of Helsinki for the investigation of human participants. The study was also approved by the Health Research Ethics Committee of the North-West University. All procedures were explained to the participants and written informed consent was obtained before the measurements commenced.

Questionnaires

A researcher assisted each participant in completing a general health questionnaire in order to obtain information on socio-economic status as well as alcohol and tobacco usage. The socio-economic score was derived from three categories within the general health questionnaire, namely skills level, education and household income. Each category was awarded points in order to determine whether the participant fell into a low, middle or high socio-economic class.

Anthropometric and physical activity measurements

The anthropometric measurements were performed using standardized methods[18] and included body height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany), body weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany) and waist circumference (Lufkin Steel Anthropometric Tape (W606PM), Lufkin, Apex, MD, USA). Body mass index (BMI) was calculated as kg/m^2. Each participant was fitted with an ActiHeart physical activity monitor (CamNtech Ltd., England, UK) which recorded total energy expenditure (TEE) for a maximum of 7 days.

Cardiovascular measurements

Each participant was equipped with a CardioXplore® 24-hour ambulatory blood pressure monitoring (ABPM) apparatus (CE0120, Meditech, Budapest, Hungary) on the non-dominant arm. This device was programmed to record measurements every 30 minutes during the day (from 06:00 till 22:00) and every hour at night (from 22:00 till 06:00).

Carotid intima-media thickness (cIMT), as a measure of arterial wall alterations and cardiovascular disease development,[19] was measured using B-mode ultrasonography (General Electric Vivid E9, GE Vingmed Ultrasound A/S, Horten, Norway) according to the Mannheim Consensus.[19] A maximal 10mm segment with good image quality was chosen for offline analysis to assess the near and far wall. In this study the far wall measurements of cIMT were used.
All measurements were performed and analyzed by a single experienced observer using the Artery Measurement Systems Software (Tomas Gustavsson, Sweden) for dedicated analyses, and all participants were in a relaxed state during the measurements.

Biochemical measurements

Participants were required to fast for 8 hours prior to the commencement of measurements. A blood sample was obtained from each participant by a registered nurse from the antebrachial vein branches using a winged infusion set and syringes. Standard procedures were used for the preparation of serum and plasma, after which all samples were immediately aliquoted into cryovials in the on-site laboratory. Samples were then stored in bio-freezers at -80°C until they were analyzed, since antioxidant enzymes are shown to remain stable for long term storage at this temperature.[20]

Antioxidant enzymes including glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD), as well as total antioxidant status was measured using assay kits (Randox, Co. Antrim,United Kingdom) and the Cobas Integra 400 plus (Roche, Basel Switzerland). Total glutathione (tGSH) was measured using whole blood samples with a Synergy HT microplate reader (BioTek, Winooski, VT, USA) (Kit: BIOXYTECH GSH/GSSG-412, Oxis International Inc, CA, USA). Serum peroxides, as an indicator of ROS, were determined using a high-throughput spectrophotometric assay and analyzed on a Synergy HT microplate reader (BioTek, Winooski, VT, USA). ROS is reported in units, where 1 mg H_2O_2/L is equivalent to one unit.[21]

Triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), γ-glutamyl transferase (γ-GT) and uric acid were measured in serum, while glucose levels were measured in sodium fluoride plasma and 24 hour urinary sodium was measured in urine, using the Cobas Integra 400 plus (Roche, Basel, Switzerland). A chemiluminescence method (Immulite, Siemens, Erlangen, Germany) was used to determine serum cotinine levels. Serum interleukin-6 (IL-6) was measured using a high sensitivity Quantikine enzyme linked immunosorbent assay (R&D systems, Minneapolis, MN USA) analyzed on a Synergy HT hybrid microplate reader (BioTek, Winooski, VT, USA). White blood cells were measured in whole blood using the Coulter AcT5 diff OV Hematology analyzer (Beckman Coulter, Brea, CA, US).

Statistical analyses

Statistica version 13.2 (Dell, TX, USA) was used to perform the statistical analyses of this study. The central tendency and spread for normally distributed variables are expressed as arithmetic mean and standard deviation.

Normal distribution of data was analysed based on visual inspection of symmetry of the bell-shaped histogram curves and achieved by logarithmically transforming variables which were not normally distributed (triglycerides, IL-6, GPx, SOD and γ-GT). The central tendency and spread of logarithmically transformed variables were expressed as the geometric mean and the 5th and 95th percentile intervals. Continuous variables were compared using independent T-tests and the assumptions of homogeneity of variance were tested (**Table S1**). Mean values of cIMT were adjusted for 24hour SBP and comparisons were made with an analysis of covariance (ANCOVA). Single regression analyses were performed to evaluate unadjusted associations between cardiovascular variables and oxidative stress related variables. Thereafter, partial correlations were performed to evaluate the associations between variables while adjusting for BMI and TEE. Measurements of cIMT were additionally adjusted for 24hour SBP. Multiple regression analyses were performed in order to evaluate the independent associations between cardiovascular variables (blood pressure and cIMT) and oxidative stress markers (GPx and GR activity) in the four groups. Covariates entered in the models included the antioxidant enzyme activity, age, socio-economic score, BMI, TEE, γ-GT, cotinine, glucose, IL-6 and triglycerides. Models with cIMT as dependent variable were additionally adjusted for 24hour SBP. The selection of covariates included in the models were based on the strongest unadjusted correlations of covariates with cardiovascular variables and oxidative stress markers when considering the following: BMI, waist circumference, 24hour SBP, 24hour MAP, total cholesterol, HDL-C, LDL-C, triglycerides, C-reactive protein (CRP), IL-6, tumor necrosis factor-α (TNF-α), glucose, glycated haemoglobin (HbA1c), TEE, γ-GT and cotinine.

In a post hoc analyses the achieved power (1-β) were determined for multiple regression models with 10 variables, for a group with a sample size of n=89 for black men (1-β=0.99) and n=78 for white men (1-β=0.99) and n=105 for black women (1-β=0.99) and n=124 for white women (1-β=0.99) (G*power v3.1.9.2).[22]

Table S1: Variance analyses of the African-PREDICT study.

	Black and white men		Black and white women	
	Variance (F)	*P-variance*	*Variance (F)*	*P-variance*
Age (years)	1.20	0.424	1.52	0.027
Anthropometric measurements				
Body mass index (kg/m^2)	2.86	<0.001	1.17	0.414
Body height (cm)	1.01	0.978	1.12	0.545
Body weight (kg)	3.17	<0.001	1.09	0.661
Cardiovascular measurements				
24 hour systolic pressure(mmHg)	1.88	0.005	1.02	0.921
24 hour diastolic pressure(mmHg)	1.29	0.262	1.00	1.000
24 hour pulse pressure (mmHg)	1.04	0.872	1.03	0.883
24 hour mean arterial pressure (mmHg)	1.71	0.017	1.01	0.973
cIMT left far wall (mm)	1.41	0.127	1.45	0.049
Biochemical analyses				
Glucose (mmol/l)	1.83	0.008	1.36	0.113
Triglycerides (mmol/l)	1.00	1.000	1.96	0.001
HDL cholesterol (mmol/L)	1.67	0.027	1.50	0.045
LDL cholesterol (mmol/L)	1.58	0.047	1.05	0.815
Interleukin-6 (pg/ml)	1.14	0.554	1.28	0.198
Gamma glutamyl transferase (U/l)	1.33	0.210	1.06	0.760
24 hour urinary sodium (mmol/L)	1.15	0.582	1.07	0.746
White blood cells (x10^9/L)	1.12	0.620	1.02	0.903
Reactive oxygen species (Units)[1]	1.27	0.288	2.06	<0.001
Total glutathione (µM)	1.62	0.031	1.31	0.149
Glutathione peroxidase (nmol/min/ml)	2.36	<0.001	2.35	<0.001
Glutathione reductase (nmol/min/ml)	1.16	0.525	1.17	0.411
Superoxide dismutase (U/ml)	2.99	<0.001	1.58	0.015
Total antioxidant status (mmol/l)	1.21	0.404	1.07	0.732
Uric acid (µmol/L)	1.11	0.639	1.04	0.861
Lifestyle markers				
Cotinine (ng/ml)	1.79	0.010	1.24	0.258
Total energy expenditure (kcal/day)	2.46	<0.001	1.09	0.663

Socio-economic score (n)	1.13	0.597	1.27	0.211

cIMT, carotid intima-media thickness; HDL, high density lipoprotein; LDL, low density lipoprotein.

Results

Characteristics of the study population

In the black men the 24hour SBP (p=0.008) and 24hour PP (p=0.003) were slightly lower when compared to the white men (**Table 1**). No significant differences in cardiovascular measurements were noted when comparing black and white women. Biochemical analyses revealed significantly lower glucose and triglycerides in both black men and women (all p<0.001). In the black women IL-6 and γGT levels (both p<0.001) were significantly higher when compared to their white counterparts. Regarding the oxidative stress profile, black men and women had higher ROS (black men: p=0.019; black women: borderline p=0.064) and tGSH (both p<0.001), but lower GPx activity (both p<0.001), total antioxidant status (both p<0.001) and uric acid (both p<0.001) when compared to their white counterparts. Additionally, SOD activity (p=0.048) was higher in the black men than in the white men. Lifestyle biomarkers indicated that black men had higher cotinine levels and lower TEE (both p<0.001) when compared to white men. Additionally, both black men and women had significantly lower socio-economic scores (both p<0.001) when compared to the white groups.

Table 1: Characteristics of black and white men and women.

	Black men	White men	P-values	Black women	White women	P-values
N	89	78		105	124	
Age (years)	24 ± 3	26 ± 3	**0.015**	24 ± 4	26 ± 3	**0.003**
Anthropometric measurements						
Body mass index (kg/m^2)	21.8 ± 3.37	28.0 ± 5.71	**<0.001**	26.5 ± 5.85	24.7 ± 5.42	**0.015**
Body height (cm)	170 ± 6.17	179 ± 6.15	**<0.001**	159 ± 6.65	167 ± 6.28	**<0.001**
Body weight (kg)	63.3 ± 10.7	89.9 ± 19.0	**<0.001**	67.0 ± 15.1	69.0 ± 15.7	0.330
Cardiovascular measurements						
24 hour systolic pressure(mmHg)	121 ± 9.12	124 ± 6.66	**0.008**	114 ± 8.33	113 ± 8.41	0.624
24 hour diastolic pressure(mmHg)	70.6 ± 6.89	70.6 ± 6.08	0.934	69.0 ± 5.70	68.2 ± 5.71	0.288
24 hour pulse pressure (mmHg)	50.4 ± 6.88	53.6 ± 7.01	**0.003**	44.7 ± 5.60	45.0 ± 5.68	0.728
24 hour mean arterial pressure (mmHg)	87.4 ± 6.99	88.5 ± 5.34	0.231	83.9 ± 6.15	83.2 ± 6.17	0.380
cIMT left far wall (mm)[1]	0.45 ± 0.01	0.45 ± 0.01	0.653	0.43 ± 0.01	0.44 ± 0.01	0.209
Biochemical analyses						
Glucose (mmol/l)	3.82 ± 0.97	5.01 ± 0.71	**<0.001**	3.91 ± 0.74	4.53 ± 0.86	**<0.001**
Triglycerides (mmol/l)	0.84 (0.42; 1.90)	1.08 (0.57; 2.18)	**<0.001**	0.71 (0.40; 1.17)	0.90 (0.43; 2.13)	**<0.001**
HDL cholesterol (mmol/L)	1.35 ± 0.34	1.12 ± 0.27	**<0.001**	1.21 ± 0.32	1.61 ± 0.40	**<0.001**
LDL cholesterol (mmol/L)	2.36 ± 0.84	3.28 ± 1.05	**<0.001**	2.50 ± 0.81	2.90 ± 0.83	**<0.001**
Interleukin-6 (pg/ml)	0.80 (0.36; 3.21)	0.77 (0.27; 2.84)	0.729	1.17 (0.47; 3.43)	0.70 (0.27; 2.32)	**<0.001**
Gamma glutamyl transferase (U/l)	29.2 (13.0; 107)	24.9 (10.8; 65.4)	0.111	23.3 (10.5; 57.4)	14.0 (6.90; 38.4)	**<0.001**
24 hour urinary sodium (mmol/L)	120 ± 46.8	120 ± 43.6	0.983	96.3 ± 52.2	112 ± 50.4	0.054

White blood cells (x10^9/L)	5.12 ± 1.56	5.73 ± 1.65	**0.016**	5.68 ± 1.80	5.80 ± 1.82	0.632
Oxidative stress markers						
Reactive oxygen species (Units)[2]	156 ± 46.2	140 ± 40.9	**0.019**	230 ± 70.7	207 ± 101	0.064
Total glutathione (µM)	1258 ± 334	913 ± 263	**<0.001**	1205 ± 271	942 ± 236	**<0.001**
Glutathione peroxidase (nmol/min/ml)	18.3 (15.0; 20.5)	19.8 (17.4; 21.6)	**<0.001**	18.5 (14.8; 20.8)	19.9 (18.0; 21.9)	**<0.001**
Glutathione reductase (nmol/min/ml)	52.5 ± 17.2	57.1 ± 16.0	0.078	52.9 ± 17.2	53.7 ± 15.9	0.719
Superoxide dismutase (U/ml)	2.38 (1.58; 3.54)	2.12 (1.13; 3.72)	**0.048**	2.28 (1.02; 3.79)	2.24 (1.40; 3.89)	0.707
Total antioxidant status (mmol/l)	1.39 ± 0.11	1.57 ± 0.12	**<0.001**	1.27 ± 0.12	1.40 ± 0.12	**<0.001**
Uric acid (µmol/L)	331 ± 72.6	422 ± 76.6	**<0.001**	268 ± 62.2	300 ± 63.3	**<0.001**
Lifestyle markers						
Cotinine (ng/ml)	119 ± 143	49.7 ± 107	**<0.001**	20.3 ± 55.2	19.6 ± 61.5	0.936
Total energy expenditure (kcal/day)	2232 ± 273	2679 ± 428	**<0.001**	2132 ± 428	2190 ± 447	0.340
Socio-economic score (n)	17.2 ± 6.09	25.0 ± 5.74	**<0.001**	17.6 ± 4.98	25.4 ± 4.43	**<0.001**

Data expressed as arithmetic mean ± standard deviation or geometric mean with 5th and 95th percentile boundaries or n.
cIMT, carotid intima-media thickness; HDL, high density lipoprotein; LDL, low density lipoprotein.
[1] cIMT ANCOVA (adjusted for 24hr SBP) expressed as adjusted mean ± standard error.
[2] Reactive oxygen species measured as serum peroxides where 1 unit = 1.0 mg/L H_2O_2

Single, partial and multivariate analyses

Correlations between cardiovascular variables and antioxidant enzymes are depicted in **Table 2** (black and white men) and **Table 3** (black and white women).

In black men single and partial regression analyses revealed a negative association of 24hour PP (r=-0.30, p=0.006) with GPx activity, while a positive association was found between left far wall cIMT and GPx activity in white men (r=0.25, p=0.031). Both of these associations remained significant after full adjustments were made in subsequent multiple regression analyses: 24hour PP with GPx activity (R^2=0.19; β=-0.25; p=0.026) in black men (**Table 4**) and left far wall cIMT with GPx activity (R^2=0.06; β=0.24; p=0.044) in white men (data not shown). White men also displayed a positive association with 24hour PP (r=0.33, p=0.004) and a negative association of 24hour DBP (r=-0.30, p=0.010) with GR activity, both of which remained significant after full adjustments were made (**Figure 1**): 24hour DBP (R^2=0.26; β=-0.24; p=0.037), 24hour PP (R^2=0.25; β=0.29; p=0.012).

Single and partial regression analyses in both groups of women revealed positive associations of blood pressure measurements with GR activity. This includes 24hour SBP (black women: r=0.25, p=0.019; white women: r=0.20, p=0.033), 24hour DBP (black women: r=0.24, p=0.027; white women: r=0.17, p=0.08 (borderline)) and 24hour MAP (black women: r=0.27, p=0.013; white women: r=0.19, p=0.045). The independent positive associations of 24hour SBP (black women: R^2=0.25; β=0.21; p=0.048; white women: R^2=0.44; β=0.18; p=0.016) and 24hour MAP (black women: R^2=0.20; β=0.23; p=0.034; white women: R^2=0.32; β=0.19; p=0.026) with GR activity were established in multiple regression analyses (**Figure 2**). Meanwhile, the positive associations of 24hour DBP with GR activity were borderline significant in both the groups. Also in the black women, a positive association of left far wall cIMT with GPx activity (r=0.23, p=0.032) was confirmed after full adjustments (R^2=0.11; β=0.23; p=0.026) (data not shown).

Table 2: Single and partial regression analyses of cardiovascular variables with antioxidant enzyme activities in black and white men.

	Black men (n=89)						***White men (n=78)***					
	GR		*GPx*		*SOD*		*GR*		*GPx*		*SOD*	
	Single	Partial	Single	Partial	Single	Partial	Single	Partial	Single	Partial	Single	Partial
24-h SBP (mmHg)	r=0.10	r=0.11	r=-0.002	r=-0.05	r=-0.08	r=-0.01	r=0.21	r=0.09	r=0.02	r=0.02	r=0.10	r=0.01
	p=0.36	p=0.35	p=0.98	p=0.66	p=0.46	p=0.93	p=0.06	p=0.44	p=0.89	p=0.84	p=0.37	p=0.94
24-h DBP(mmHg)	r=0.14	r=0.15	**r=0.26**	**r=0.25**	r=-0.11	r=-0.07	r=-0.19	**r=-0.30**	r=-0.03	r=-0.02	r=-0.11	r=-0.16
	p=0.19	p=0.20	**p=0.013**	**p=0.026**	p=0.32	p=0.57	p=0.10	**p=0.010**	p=0.82	p=0.84	p=0.33	p=0.16
24-h PP(mmHg)	r=-0.01	r=-0.01	**r=-0.27**	**r=-0.30**	r=0.004	r=0.05	**r=0.36**	**r=0.33**	r=0.04	r=0.04	r=0.19	r=0.15
	p=0.92	p=0.91	**p=0.012**	**p=0.006**	p=0.97	p=0.65	**p=0.001**	**p=0.004**	p=0.74	p=0.72	p=0.09	p=0.21
24-h MAP (mmHg)	r=0.14	r=0.15	r=0.17	r=0.15	r=-0.11	r=-0.05	r=-0.06	r=-0.20	r=-0.01	r=-0.01	r=-0.04	r=-0.13
	p=0.21	p=0.21	p=0.11	p=0.19	p=0.33	p=0.67	p=0.63	p=0.08	p=0.91	p=0.94	p=0.70	p=0.28
cIMT left far wall (mm) [1]	r=0.10	r=0.10	r=-0.001	r=0.02	r=0.09	r=0.06	r=-0.14	r=-0.07	**r=0.25**	**r=0.25**	r=0.15	r=0.19
	p=0.37	p=0.40	p=0.99	p=0.86	p=0.42	p=0.62	p=0.24	p=0.55	**p=0.030**	**p=0.031**	p=0.19	p=0.10

Relationships adjusted for body mass index and total energy expenditure.
24-h, 24hour; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; cIMT, carotid intima-media thickness; GR, glutathione reductase, GPx, glutathione peroxidase; SOD, superoxide dismutase.
[1] Relationships additionally adjusted for ambulatory systolic blood pressure.

Table 3: Single and partial regression analyses of cardiovascular variables with antioxidant enzyme activities in black and white women.

	Black women (n=105)						***White women (n=124)***					
	GR		*GPx*		*SOD*		*GR*		*GPx*		*SOD*	
	Single	*Partial*	*Single*	*Partial*	*Single*	*Partial*	*Single*	*Partial*	*Single*	*Partial*	*Single*	*Partial*
24-h SBP (mmHg)	**r=0.28**	**r=0.25**	r=-0.08	r=-0.01	r=-0.06	r=-0.08	**r=0.24**	**r=0.20**	r=-0.10	r=-0.003	**r=-0.21**	r=-0.14
	p=0.006	**p=0.019**	p=0.41	p=0.95	p=0.57	p=0.47	**p=0.008**	**p=0.033**	p=0.25	p=0.97	**p=0.018**	p=0.12
24-h DBP(mmHg)	**r=0.25**	**r=0.24**	r=-0.06	r=-0.01	r=-0.16	r=-0.15	**r=0.21**	r=0.17	r=-0.12	r=-0.07	**r=-0.28**	**r=-0.24**
	p=0.014	**p=0.027**	p=0.57	p=0.95	p=0.12	p=0.16	**p=0.026**	p=0.08	p=0.19	p=0.43	**p=0.002**	**p=0.010**
24-h PP(mmHg)	r=0.17	r=0.12	r=-0.06	r=-0.002	r=0.07	r=0.04	r=0.15	r=0.09	r=-0.04	r=0.09	r=-0.04	r=0.08
	p=0.09	p=0.28	p=0.52	p=0.99	p=0.52	p=0.72	p=0.10	p=0.38	p=0.70	p=0.37	p=0.68	p=0.38
24-h MAP (mmHg)	**r=0.28**	**r=0.27**	r=-0.07	r=-0.01	r=-0.12	r=-0.13	**r=0.23**	**r=0.19**	r=-0.12	r=-0.05	**r=-0.27**	**r=-0.22**
	p=0.005	**p=0.013**	p=0.47	p=0.95	p=0.23	p=0.22	**p=0.011**	**p=0.045**	p=0.19	p=0.58	**p=0.003**	**p=0.019**
cIMT left far wall (mm)[1]	r=-0.003	r=-0.01	**r=0.23**	**r=0.23**	r=0.03	r=0.10	r=0.04	r=0.05	r=0.07	r=0.05	r=-0.08	r=-0.10
	p=0.98	p=0.94	**p=0.018**	**p=0.032**	p=0.76	p=0.34	p=0.70	p=0.61	p=0.43	p=0.61	p=0.39	p=0.29

Relationships adjusted for body mass index and total energy expenditure.

24-h, 24hour; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; cIMT, carotid intima-media thickness; GR, glutathione reductase, GPx, glutathione peroxidase; SOD, superoxide dismutase.

[1] Relationships additionally adjusted for ambulatory systolic blood pressure.

Table 4: Multiple regression analyses of 24hour pulse pressure and 24hour diastolic blood pressure with glutathione peroxidase activity in black and white men.

	24hour Pulse pressure			
	Black men (n=89)		**White men (n=78)**	
	Adjusted R^2: 0.19		Adjusted R^2: 0.18	
	β (95% CI)	p	β (95% CI)	p
GPx	**-0.25 (-0.47; -0.04)**	**0.026**	0.07 (-0.14; 0.29)	0.503
Age	-0.07 (-0.31; 0.18)	0.595	**-0.41 (-0.71; -0.11)**	**0.009**
Socio-economic score	-0.24 (-0.51; 0.03)	0.087	0.18(-0.12; 0.48)	0.250
BMI	0.19 (-0.09; 0.47)	0.195	0.33 (-0.07; 0.73)	0.114
TEE	**0.32 (0.07; 0.58)**	**0.015**	0.12 (-0.24; 0.47)	0.527
γ-GT	-0.14 (-0.41; 0.13)	0.322	0.05 (-0.22; 0.32)	0.708
Cotinine	-0.12 (-0.35; 0.11)	0.318	-0.14 (-0.37; 0.10)	0.261
Glucose	0.05 (-0.19; 0.28)	0.709	-0.06 (-0.27; 0.16)	0.600
IL-6	-0.21 (-0.43; 0.004)	0.058	-0.10 (-0.34; 0.13)	0.402
Triglycerides	-0.02 (-0.27; 0.24)	0.888	**-0.40 (-0.67; -0.14)**	**0.004**
	24hour Diastolic blood pressure			
	Black men (n=89)		**White men (n=78)**	
	Adjusted R^2: 0.31		Adjusted R^2: 0.21	
	β (95% CI)	p	β (95% CI)	p
GPx	0.11 (-0.09; 0.31)	0.269	-0.07 (-0.28; 0.14)	0.512
Age	0.15 (-0.07; 0.38)	0.184	**0.37 (0.07; 0.66)**	**0.018**
Socio-economic score	0.02 (-0.23; 0.27)	0.870	-0.19 (-0.48; 0.11)	0.222
BMI	-0.01 (-0.27; 0.25)	0.947	0.06 (-0.33; 0.46)	0.748
TEE	0.07 (-0.17; 0.30)	0.581	0.04 (-0.31; 0.39)	0.834
γ-GT	**0.26 (0.01; 0.51)**	**0.047**	0.08 (-0.18; 0.34)	0.538
Cotinine	**-0.22 (-0.43; -0.004)**	**0.050**	0.21 (-0.03; 0.44)	0.086
Glucose	0.15 (-0.07; 0.37)	0.177	0.10 (-0.11; 0.31)	0.342
IL-6	0.14 (-0.06; 0.35)	0.165	-0.07 (-0.30; 0.17)	0.579
Triglycerides	0.13 (-0.10; 0.37)	0.268	**0.32 (0.06; 0.58)**	**0.019**

GPx, glutathione peroxidase; BMI, body mass index; TEE, total energy expenditure; γ-GT, γ-glutamyl transferase; IL-6, interleukin-6.

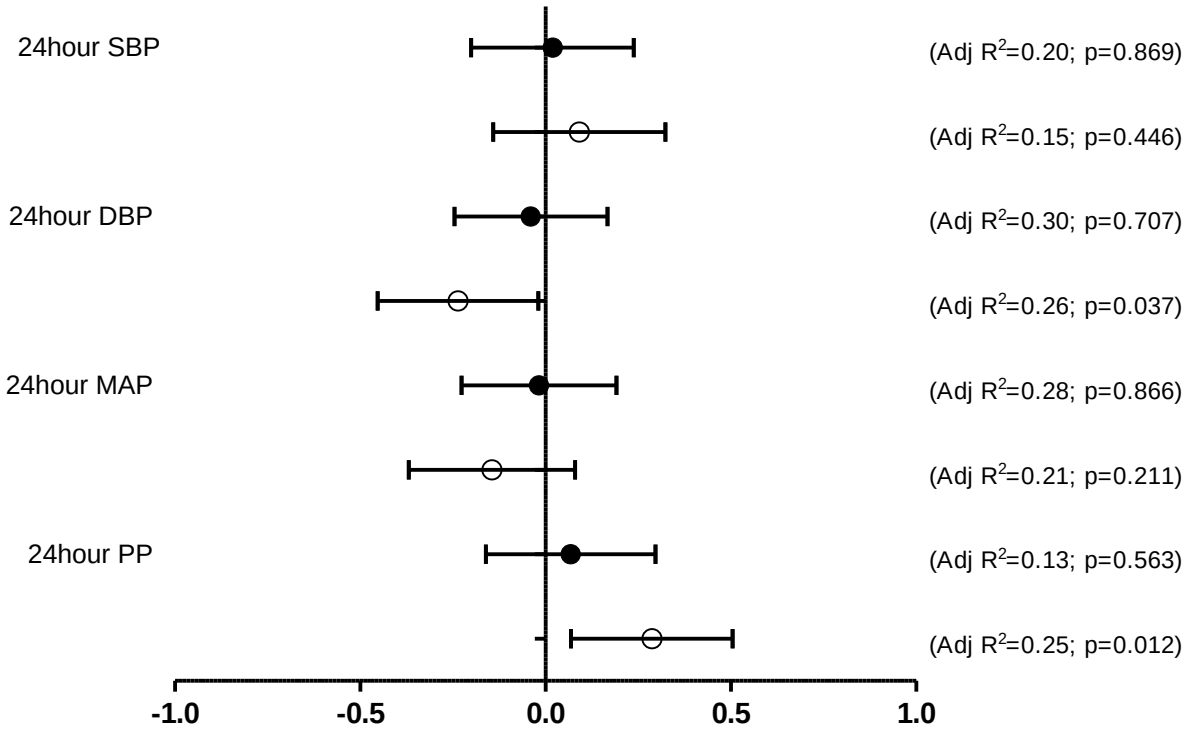


Figure 1: Multiple regression analysis of glutathione reductase activity in black and white men

24hour SBP, Ambulatory systolic blood pressure; 24hour DBP, Ambulatory diastolic blood pressure; 24hour MAP, Ambulatory mean arterial pressure; 24hour PP, Ambulatory pulse pressure.

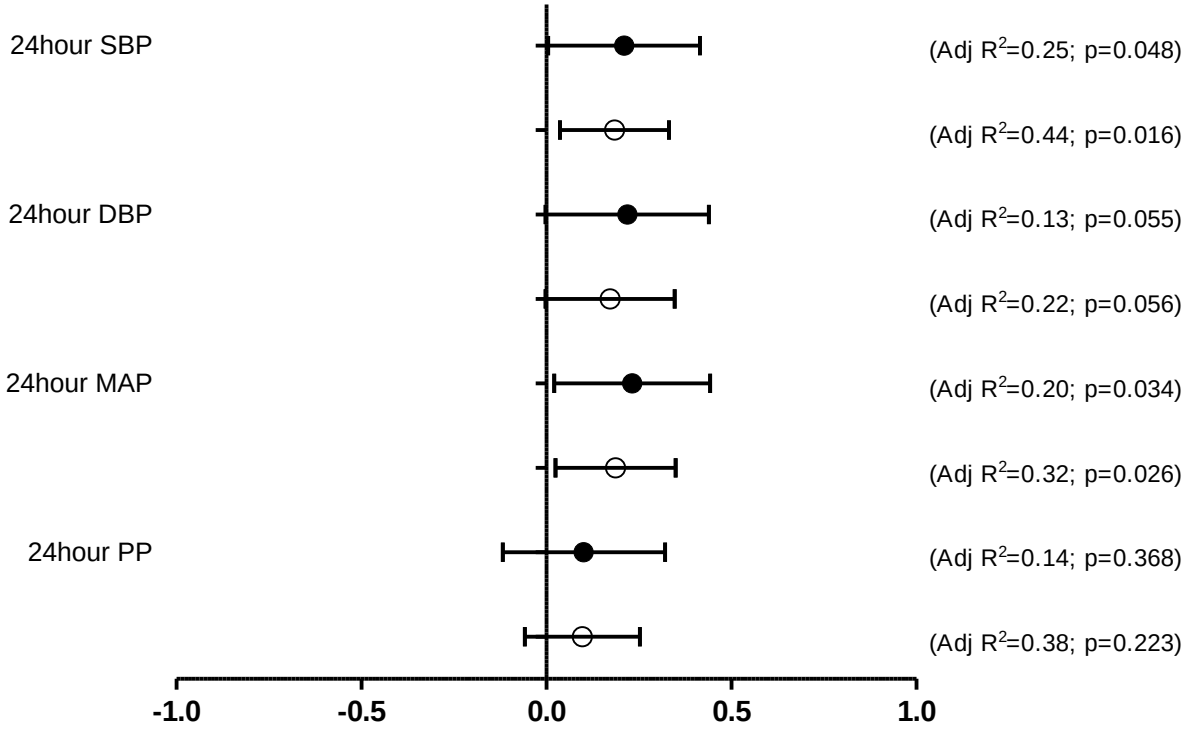


Figure 2: Multiple regression analysis of glutathione reductase activity in black and white women

● Black women ○ White women

24hour SBP, Ambulatory systolic blood pressure; 24hour DBP, Ambulatory diastolic blood pressure; 24hour MAP, Ambulatory mean arterial pressure; 24hour PP, Ambulatory pulse pressure.

Discussion

We compared oxidative stress profiles and explored relationships of ambulatory blood pressure and cIMT with enzyme activities of the glutathione cycle in young, healthy black and white South African men and women.

In the black groups, the oxidative stress profiles were worse (as indicated by higher ROS as well as lower total antioxidant status and lower activity of the glutathione cycle enzyme, GPx). The cardiovascular profiles were similar in the black and white groups, except for the slightly lower 24hour SBP and 24hour PP in the young black men. In the same group 24hour PP was inversely related with GPx activity, suggesting a possible role for oxidative stress in early vascular changes. An increase in pulse pressure, a measure of arterial stiffness, is associated with ageing and is also a predictor of future cardiovascular events.[23-25] Oxidative stress is prevented by the proper functioning of various antioxidant enzymes in order to balance ROS production,[26] and the association of pulse pressure with diminished GPx activity in the black men may indicate that GPx, especially plays an important regulatory role to balance oxidative stress and prevent early vascular changes and eventually arterial stiffness.[23] The involvement of oxidative stress in early vascular changes, before the onset of hypertension, was previously suggested in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, in which a marker of lipid oxidation was linked to increased total peripheral resistance and decreased arterial compliance in black men and women. However, the population group was older with a wide age spread (age 47.2 ± 7.97).[11]

Similarly, in a group of healthy subjects (age 42.6 ± 14 years) it was found that high cystine levels (as a marker of oxidative stress) were associated with arterial stiffness, again suggesting an important role for oxidative stress in the loss of arterial elasticity and early vascular changes.[27]

Our results further contribute to this, as we have shown that not only do these associations already exist in a young group of black men (24.3 ± 3.19 years), but they are independent of lifestyle risk factors such as adiposity, high alcohol intake (as indicated by γ-GT levels), smoking (cotinine) and even socio-economic status. Since this link was found only in the black men, these results suggest a degree of cardiovascular vulnerability for early vascular changes and arterial stiffness.[10,11]

In the three remaining groups (white men, black women and white women) associations of blood pressure with GR activity were indicated. In the white men, 24hour DBP was inversely related whereas 24hour PP was positively related to GR activity, while in both the black and white women 24hour SBP, 24hour DBP and 24hour MAP were positively associated with GR activity. In the antioxidant system, superoxide anions are scavenged by the SOD antioxidant enzyme; where after the by-product (hydrogen peroxide) is converted to water and oxygen by both GPx and catalase. The antioxidant enzyme GR is responsible to maintain redox balance and adequate availability of reduced glutathione, which is the substrate for the GPx enzyme.[26] When comparing these groups with their ethnic counterparts, the white men displayed a more favorable oxidative stress profile than the black men without any overt cardiovascular disease such as hypertension or atherosclerosis.[28,29] In the women the cardiovascular profile was similar in both groups with blood pressure measurements in the normal ranges,[29] but the black women had a slightly worse oxidative stress profile than the white women.

Thus, due to the young and apparently healthy study population without clear evidence of oxidative stress or blood pressure abnormalities, the results may indicate that the white men, black women and white women of our study are currently in a steady physiological state. The associations may indicate that sufficient protection against oxidative stress was maintained and all associations could be explained as a normal physiological profile.[30]

This does however warrant further research to evaluate the possibility of future deterioration of the cardiovascular profile in these groups.

An unexpected finding of our study is that cIMT was positively associated with GPx activity in white men and black women. This result differs from what is expected since GPx activity, and GPx overexpression,[31] seems to have vascular protective properties.[32-35] However, these results were found in mice, as well as older groups of different ethnicities, with already existing cardiovascular diseases or diabetes. This being a young, healthy cohort with normal cIMT, it could suggest that the activity of GPx may be sufficient to prevent vascular remodeling at this stage. To our knowledge, these results have not yet been evaluated in young, healthy black and white participants, and therefore further research is warranted in order to clarify this result.

This study has to be interpreted within the context of its strengths and limitations. Although our study population was based on participants from the Potchefstroom area in the North West Province of South Africa, it may not be indicative of the population as a whole. This was a cross-sectional study, and causality cannot be inferred. Although our results were consistent after multiple adjustments, we cannot exclude any unknown interactions which may play a role in the future development of arterial stiffness, carotid wall thickening or hypertension. The GSH:GSSG ratio within our study population is unknown, since total glutathione levels were measured in these cohorts. Future studies on this topic may include the GSH:GSSG ratio, or other markers of oxidative damage in order to improve oxidative stress analysis in these groups. However, our study was well planned and executed under strict conditions in a fully-equipped research facility, allowing us to dig deeper into the poorly researched effect of oxidative stress and antioxidant enzyme activity on cardiovascular disease development in our young South African population.

In conclusion, only in young black men 24hour PP associated negatively with GPx activity. This may suggest that lower GPx activity may accelerate vascular ageing in this group.

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Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF do not accept any liability in regard.

Declaration of interest

The authors have no conflicts of interest to disclose. Any opinions expressed and conclusions arrived at, are those of the authors alone.

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CHAPTER 4:

Cardiovascular reactivity and oxidative stress in young and older adults: The African-PREDICT and SABPA studies

Cardiovascular Reactivity and Oxidative Stress in Young and Older Adults: The African-PREDICT and SABPA Studies

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ORIGINAL ARTICLE

Cardiovascular reactivity and oxidative stress in young and older adults: the African-PREDICT and SABPA studies

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ABSTRACT

Background: Oxidative stress and increased cardiovascular reactivity are associated with endothelial dysfunction and cardiovascular disease development. These factors along with early vascular compromise are more pronounced in black populations. We aimed to compare cardiovascular reactivity and investigate associations thereof with oxidative stress in two bi-ethnic cohorts (younger: 25.0 ± 3.19yrs; older: 44.7 ± 9.61yrs).

Methods: Cardiovascular reactivity using the color-word conflict test was measured with the Finometer device. Oxidative stress markers included superoxide dismutase (SOD), γ-glutamyl transferase (γ-GT) and reactive oxygen species (ROS).

Results: Black groups displayed greater cardiovascular responses to stress than white groups. In younger white participants, diastolic blood pressure (DBP) ($\beta = 0.31$; $p = 0.001$) and mean arterial blood pressure (MAP) ($\beta = 0.28$; $p = 0.002$) associated with ROS. In older black participants, DBP ($\beta = 0.23$; $p = 0.009$), MAP ($\beta = 0.18$; $p = 0.033$), stroke volume ($\beta = -0.20$; $p = 0.023$) and arterial compliance ($\beta = -0.25$; $p = 0.005$) associated with γ-GT. In older white participants, systolic blood pressure ($\beta = -0.20$; $p = 0.006$) and MAP ($\beta = -0.19$; $p = 0.009$) associated with SOD.

Conclusions: In the older black group, cardiovascular reactivity associated with markers of glutathione metabolism, suggesting a possible compensatory up-regulation thereof in order to correct their heightened responses to stress. Independent of age, findings in the white groups support a regulatory role of ROS to maintain vascular tone during stress.

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Abstract

Oxidative stress and increased cardiovascular reactivity are associated with endothelial dysfunction and cardiovascular disease development. These factors along with early vascular compromise are more pronounced in black populations. We aimed to compare cardiovascular reactivity and investigate associations thereof with oxidative stress in two bi-ethnic cohorts (younger: 25.0 ± 3.19yrs; older: 44.7 ± 9.61yrs). Cardiovascular reactivity using the color-word conflict test was measured with the Finometer device. Oxidative stress markers included superoxide dismutase (SOD), γ-glutamyl transferase (γ-GT) and reactive oxygen species (ROS). Black groups displayed greater cardiovascular responses to stress than white groups. In younger white participants, diastolic blood pressure (DBP) (β=0.31; p=0.001) and mean arterial blood pressure (MAP) (β=0.28; p=0.002) associated with ROS. In older black participants, DBP (β=0.23; p=0.009), MAP (β=0.18; p=0.033), stroke volume (β=-0.20; p=0.023) and arterial compliance (β=-0.25; p=0.005) associated with γ-GT. In older white participants, systolic blood pressure (β=-0.20; p=0.006) and MAP (β=-0.19; p=0.009) associated with SOD. In the older black group, cardiovascular reactivity associated with markers of glutathione metabolism, suggesting a possible compensatory up-regulation thereof in order to correct their heightened responses to stress. Independent of age, findings in the white groups support a regulatory role of ROS to maintain vascular tone during stress.

Key words: acute stress; color-word conflict test; age; reactive oxygen species; glutathione metabolism

Background

Cardiovascular disease is a growing concern in the developing world,[1,2] with South Africa displaying an alarming increase in the incidence of hypertension, especially amongst urban black populations.[3-5] One of the key role players in the development of hypertension is oxidative stress, which occurs due to either an increased production of reactive oxygen species (ROS), or a decrease in ROS scavenging by the antioxidant system.[6] Physiologically, ROS play an important role in vascular biology and cell signaling as it is involved in control of vascular tone and endothelial function, cell growth, differentiation and apoptosis.[6-9] However, oxidative stress aggravates endothelial dysfunction, vascular remodeling and inflammation which may result in elevated blood pressure, peripheral resistance, arterial stiffness and atherosclerosis,[6,7,9] all of which worsen with aging.[10,11] Acute stress has also been associated with both endothelial dysfunction as well as oxidative stress,[12,13] and a heightened cardiovascular response to stress is a risk factor for the development of cardiovascular disease, including hypertension.[14-16] Upon acute stress, black populations have shown increased cardiovascular reactivity when compared to white populations.[17-19] Black South Africans are also more prone to early vascular changes,[20] and previous results linked increased blood pressure to oxidative stress.[21,22] However, it has not been previously investigated whether oxidative stress plays a role in a heightened cardiovascular response in black and white South Africans, and whether this link is age dependent. Therefore we aimed to compare oxidative stress and cardiovascular reactivity profiles between two age-stratified South African cohorts, and to determine whether relationships exist between cardiovascular reactivity and oxidative stress markers.

Methods

Study populations

This study is embedded in the baseline phases of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) study (mean age: 25.0 years) and the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study (mean age: 44.7 years).

After an initial screening phase (evaluating blood pressure, cholesterol, glucose levels, anthropometry, HIV status and a general health questionnaire) apparently healthy normotensive or pre-hypertensive (systolic blood pressure (SBP) <140 and diastolic blood pressure (DBP) <90mmHg) black and white men and women between 20 and 30 years of age were invited to participate in the African-PREDICT study.[23] Participants who are not permanent residents of Potchefstroom or surrounding areas (or plan regular trips to the area) and who are unable to read or understand English were excluded from the study. Participants with elevated glucose levels >5.6 mmol/L (or glycated haemoglobin ≥6.5%), who were HIV infected and those with ear temperature >37.5°C were also excluded. Further exclusion criteria included previous diagnoses of (or took medication for) type 1 or 2 diabetes mellitus, liver disease, cancer, tuberculosis, renal or cardiovascular diseases, who recently had surgery or trauma and who was pregnant or breastfeeding. This study included the first 387 participants with complete antioxidant enzyme activity data divided into black (N=191) and white groups (N=196).

Detail on the SABPA study was published elsewhere.[24] In summary, participants were between 20 and 65 years of age, and participants who were pregnant, lactating, exhibited ear temperature >37°C, used alpha and beta blockers, confirmed psychotropic substance abuse and those who donated blood or were vaccinated 3 months prior to their participation were excluded. We included 409 participants of the SABPA study divided into black (N=200) and white (N=209) groups.

Both studies complied with the Declaration of Helsinki regarding investigations among human participants, and were further approved by the Health Research Ethics Committee of the North-West University. The African-Predict study was registered as a clinical trial as required (ClinicalTrials.gov Identifier: NCT03292094). All participants were informed of each study in their home language, including stressor test protocols, and written informed consent was obtained before commencement.

Questionnaires

Participants completed a general health questionnaire to obtain demographic data and information on alcohol usage.

Anthropometric and physical activity measurements

All anthropometric measurements were performed using standardized methods.[25] These measurements included body height measured with a stadiometer (SECA 213 SECA, Hamburg, Germany and Invicta, IP 1465, Invicta, London, UK), body weight measured with an electronic scale (SECA 813, SECA, Hamburg, Germany and Precision Health Scale, A & D Company, Tokyo, Japan) and waist circumference measured with a metal tape (Lufkin Steel Anthropometric Tape, W606PM, Lufkin, Apex, MD, USA and Holtain unstretchable flexible 7mm wide metal tape, Crosswell, Wales). Body mass index (BMI) was calculated as kg/m^2 and body surface area was calculated as m^2 according to the Mosteller formula.[26]

Total energy expenditure (TEE) was monitored over 7 days in the African-PREDICT study (ActiHeart physical activity monitor, CamNtech Ltd., England, UK) and over 24 hours in the SABPA study (Actical® activity monitor, Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada).

Cardiovascular reactivity

Cardiovascular measurements were made non-invasively using the validated Finometer device (FMS, Finapres Medical Systems, Amsterdam, Netherlands). The Finometer device provides a beat-to-beat arterial blood pressure waveform after adjusting for hydrostatic height from the heart while computing additional cardiac parameters by way of the Modelflow method.[27] The finger cuff of the Finometer was fitted on the left middle-finger, and after a 10 minute resting period, a 5 minute continuous measurement of resting variables was recorded.

During the recording, after 2 minutes, a return-to-flow systolic calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial artery pressure, which allows for the highest precision in measurements. Resting measurements included SBP, DBP, mean arterial pressure (MAP), stroke volume (SV), total peripheral resistance (TPR) and Windkessel arterial compliance (Cwk). Thereafter the color-word conflict test was applied for 1 minute during which the cardiovascular variables were measured. The mean of the last minute of the resting values, and the last 20 seconds during exposure were used for the calculations. Cardiovascular reactivity was calculated as the percentage change from resting values.[28]

Biochemical measurements

Fasting blood samples were obtained from each participant by a registered nurse from the antebrachial vein. Serum and plasma samples were prepared using standardized procedures, aliquoted into cryovials and stored in bio-freezers at -80°C until analysis to maintain efficient long-term stability of antioxidant enzymes.[29] Antioxidant enzyme activities measured included glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD) and γ-glutamyl transferase (γ-GT). Additional oxidative stress markers included total glutathione (tGSH) and reactive oxygen species (ROS) (measured as serum peroxides and reported in units, where 1 mg H_2O_2/L equates to 1 unit).[30] In addition, fluoride plasma glucose and serum total cholesterol, triglycerides, cotinine, interleukin-6 (IL-6) and cortisol levels were determined. All apparatus used are summarized in **Table S1**.

Table S1: Apparatus used during the African PREDICT and SABPA studies.

	African-PREDICT study	Intra-AV (%CV)	Inter-AV (%CV)	SABPA study	Intra-AV (%CV)	Inter-AV (%CV)
Biochemical analyses						
Glucose	Cobas Integra 400 plus, Roche, Basel, Switzerland	1.80	2.10	Unicel DXC 800, Beckman and Coulter, Germany	<10	<10
Total cholesterol	Cobas Integra 400 plus, Roche, Basel, Switzerland	0.51	1.90	Unicel DXC 800, Beckman and Coulter, Germany and a Konelab™ 20I Sequential Multiple Analyzer Computer, Thermo Scientific, Vantaa, Finland	<10	<10
Triglycerides	Cobas Integra 400 plus, Roche, Basel, Switzerland	1.60	1.90	Unicel DXC 800, Beckman and Coulter, Germany and a Konelab™ 20I Sequential Multiple Analyzer Computer, Thermo Scientific, Vantaa, Finland	<10	<10
Interleukin-6	High sensitivity Quantikine ELISA kit (R&D systems, Minneapolis, MN, USA) analyzed on Synergy H4 hybrid microplate reader, BioTek, Winooski, VT, USA	7.80	9.60	High sensitivity Quantikine ELISA kit (R&D systems, Minneapolis, MN, USA) analyzed on Synergy H4 hybrid microplate reader, BioTek, Winooski, VT, USA	5.90	18.9
Cortisol	Electrochemiluminescence immunoassay (ECLIA) Elecsys 2010, Roche, Basel, Switzerland	7.10	12.7	Electrochemiluminescence immunoassay (ECLIA) e411, Roche, Basel, Switzerland	7.10	12.7

Oxidative stress markers

Reactive oxygen species (measured as serum peroxides)	A Synergy HT microplate reader, BioTek, Winooski, VT, USA	9.68	10.2	A Synergy HT microplate reader, BioTek, Winooski, VT, USA	7.42	11.2
Total glutathione	A Synergy HT microplate reader, BioTek, Winooski, VT, USA (Kit: BIOXYTECH GSH/GSSG-412, Oxis International Inc, CA, USA)	6.16	13.7	A Synergy HT microplate reader, BioTek, Winooski, VT, USA (Kit: BIOXYTECH GSH/GSSG-412, Oxis International Inc, CA, USA)	<10	<10
Glutathione peroxidase	Assay kits (Randox, Co. Antrim,United Kingdom) and the Cobas Integra 400 plus, Roche, Basel Switzerland	4.86	7.30	Assay kits (Cayman Chemical Company, Ann Arbor, MI, USA) and a Synergy H4 hybrid microplate reader, BioTek, Winooski, VT, USA	5.70	7.20
Glutathione reductase	Assay kits (Randox, Co. Antrim,United Kingdom) and the Cobas Integra 400 plus, Roche, Basel Switzerland	4.84	6.55	Assay kits (Cayman Chemical Company, Ann Arbor, MI, USA) and a Synergy H4 hybrid microplate reader, BioTek, Winooski, VT, USA	3.70	9.30
Superoxide dismutase	Assay kits (Randox, Co. Antrim,United Kingdom) and the Cobas Integra 400 plus, Roche, Basel Switzerland	4.64	7.07	Assay kits (Cayman Chemical Company, Ann Arbor, MI, USA) and a Synergy H4 hybrid microplate reader, BioTek, Winooski, VT, USA	3.20	3.70
γ-glutamyl transferase	Cobas Integra 400 plus, Roche, Basel, Switzerland	1.80	1.80	Unicel DXC 800, Beckman and Coulter, Germany and a Konelab™ 20I Sequential Multiple Analyzer Computer, Thermo Scientific, Vantaa, Finland	<10	<10

Lifestyle						
Cotinine	Chemiluminescence method, Immulite, Siemens, Erlangen, Germany	9.60	12.2	Immunoassay, Automated Modular, Roche, Basel, Switzerland	<10	<10

Intra-AV, Intra-assay variability; Inter-AV, Inter-assay variability.

Statistical analyses

Statistica version 13.2 (Dell, TX, USA) was used to perform the statistical analyses of this study. Normal distribution of data was assessed and the central tendency and spread for normally distributed variables were expressed as arithmetic mean and standard deviation. Skewed variables were logarithmically transformed (triglycerides, IL-6, total cholesterol, SOD and γ-GT in the African-PREDICT study and glucose, triglycerides, IL-6, GR, SOD and γ-GT in the SABPA study) and expressed as the geometric mean and the 5th and 95th percentile intervals.

Interactions of race and sex were tested on the relationships between main cardiovascular reactivity variables and oxidative stress markers using multiple regression analyses. Continuous variables were compared using independent T-tests, while proportions were compared using Chi-square tests. Single regression analyses were performed to evaluate unadjusted associations between cardiovascular variables and oxidative stress related variables. Thereafter, partial correlations were performed to evaluate the associations between variables while adjusting for age, sex and BMI. Multiple regression analyses were performed to evaluate the independent associations between cardiovascular reactivity variables and oxidative stress markers. Covariates entered into the models included the relative oxidative stress marker, age, sex, BMI, TEE, self-reported alcohol use, cotinine, glucose, IL-6, triglycerides and cortisol. Sensitivity analyses were performed by repeating the multiple regression analyses after including statin usage and anti-hypertension medication usage into the models.

In post hoc analyses the achieved power (1-β) was determined for multiple regression models with 11 covariates, with a group size of N=191 for black (1-β=0.97) and N=196 for white African-PREDICT (1-β=0.97), and N=200 for black (1-β=0.97) and N=209 for white SABPA (1-β=0.98) (G*power v3.1.9.2).[31]

Results

Characteristics of the study population

No interactions of sex on the relationships between cardiovascular reactivity and oxidative stress markers were found. Meanwhile, interactions of race were found on the association of Cwk with SOD (β=0.285; p=0.026) in the African-PREDICT study, and on the associations of SBP with SOD (β=-0.477; p=0.018), MAP with SOD (β=-0.578; p=0.004) and TPR with tGSH (β=0.635; p=0.015) in the SABPA study. Stratification was done accordingly to compare black and white groups.

Black participants of the African-PREDICT study were younger (24.4 ± 3.39; p<0.001) with a lower BMI (24.5 ± 5.41; p=0.013) than their white counterparts, while in the SABPA study the black participants displayed a higher BMI (30.1 ± 7.00; p<0.001) than the white group (Table 1).

Oxidative stress markers including ROS, tGSH, GPx and γ-GT revealed similar profiles in both studies with black participants having higher ROS (African-PREDICT: 197 ± 70.9; p=0.033; SABPA: 192 ± 63.0; p<0.001), tGSH (African-PREDICT: 1231 ± 295; p<0.001; SABPA: 895 ± 189; p<0.001) and γ-GT (African-PREDICT: 25.4 (10.6; 82.8); p<0.001; SABPA: 47.4 (20.1; 184; p<0.001) with lower GPx activity (African-PREDICT: 18.5 ± 1.76; p<0.010; SABPA: 33.3 ± 14.0; p=0.010) than their white counterparts. Although no differences were found in the African-PREDICT study, GR activity was higher in the black participants of the SABPA study (7.06 (2.55; 16.8); p<0.001).

Table 1: Characteristics of black and white groups.

	African-PREDICT study			SABPA study		
	Black	**White**	**P-values**	**Black**	**White**	**P-values**
N	191	196		200	209	
Sex, females (%)	56.0	60.7	0.349	50.5	48.3	0.660
Age (years)	24.4 ± 3.39	25.5 ± 2.87	**<0.001**	44.4 ± 8.11	45.0 ± 10.9	0.495
Anthropometry						
Body mass index (kg/m^2)	24.5 ± 5.41	25.9 ± 5.82	**0.013**	30.1 ± 7.00	27.6 ± 5.94	**<0.001**
Body surface area (m^2)	1.72 ± 0.18	1.91 ± 0.27	**<0.001**	1.92 ± 0.23	2.00 ± 0.28	**0.001**
Waist circumference (cm)	77.2 ± 10.9	82.3 ± 15.4	**<0.001**	93.6 ± 15.5	93.0 ± 16.1	0.707
Biochemical analyses						
Glucose (mmol/l)	3.87 ± 0.84	4.71 ± 0.85	**<0.001**	5.41 (4.04; 10.4)	5.62 (4.70; 6.90)	0.055
Total cholesterol (mmol/l)	3.78 (2.70; 5.48)	4.66 (3.30; 6.43)	**<0.001**	4.60 ± 1.19	5.54 ± 1.28	**<0.001**
Triglycerides (mmol/l)	0.77 (0.41; 1.56)	0.98 (0.45; 2.18)	**<0.001**	1.16 (0.49; 3.66)	1.01 (0.44; 2.79)	**0.024**
Interleuken-6 (pg/ml)	0.97 (0.40; 3.29)	0.74 (0.27; 2.55)	**<0.001**	1.13 (0.34; 3.07)	0.92 (0.30; 3.04)	**0.002**
Cortisol (nmol/l)	422 ± 186	489 ± 277	**0.009**	355 ± 152	384 ±160	0.063
Oxidative stress markers						
Reactive oxygen species (Units)[1]	197 ± 70.9	180 ± 89.3	**0.033**	192 ± 63.0	158 ± 58.2	**<0.001**
Total glutathione (µM)	1231 ± 295	931 ± 248	**<0.001**	895 ± 189	820 ± 173	**<0.001**
Glutathione peroxidase (nmol/min/ml)	18.5 ± 1.76	19.9 ± 1.34	**<0.001**	33.3 ± 14.0	36.2 ± 7.97	**0.010**
Glutathione reductase (nmol/min/ml)	52.9 ± 17.1	54.8 ± 16.1	0.263	7.06 (2.55; 16.8)	2.54 (0.25; 7.64)	**<0.001**

Superoxide dismutase (U/ml)	2.33 (1.27; 3.71)	2.19 (1.38; 3.83)	0.114	4.25 (0.83; 22.9)	4.13 (1.65; 7.92)	0.723
γ-glutamyl transferase (U/l)	25.4 (10.6; 82.8)	17.5 (7.40; 52.7)	**<0.001**	47.4 (20.1; 184)	19.3 (7.00; 76.0)	**<0.001**
Lifestyle						
Cotinine (ng/ml)	64.5 ± 115	33.7 ± 86.7	**0.003**	27.3 ± 60.9	22.7 ± 77.5	0.514
Total energy expenditure (kcal/day)	2183 ± 364	2383 ± 504	**<0.001**	2685 ± 796	3113 ± 1597	**0.001**
Self-reported alcohol use (% yes)	64.9	65.8	0.845	26.0	49.0	**<0.001**
Statin use (% yes)	0	0	-	1.00	4.31	**0.039**
Anti-hypertension medication use (% yes)	0	0	-	34.5	12.9	**<0.001**

Data expressed as arithmetic mean ± standard deviation or geometric mean with 5th and 95th percentile boundaries, % or n.
[1] Reactive oxygen species measured as serum peroxides where 1 unit = 1.0 mg/L H_2O_2

Cardiovascular reactivity

In the younger cohort, black participants displayed higher DBP, MAP and TPR but lower SV and Cwk at rest and after acute stress when compared to the younger white participants (**Figure 1**). Similarly, black participants of the older cohort also displayed higher DBP and MAP with lower Cwk at rest and after acute stress than the white participants.

Additionally, this group also displayed higher SBP at rest and after acute stress along with a higher TPR than their white counterparts after acute stress only.

In the younger black participants, a larger decrease in SV ($p<0.001$) and smaller decrease in TPR (<0.001) were noted in comparison to the younger white participants (**Figure 2**). In the older black group, cardiovascular reactivity after applying the stressor revealed greater increases in DBP ($p=0.002$), and MAP ($p=0.031$) than their white counterparts. When comparing change in TPR the older black group experienced an increase in TRP whereas the white group experienced a decrease in TPR, and these responses differed significantly between the two groups ($p=0.014$). The older black group also showed a significantly larger decrease in Cwk ($p=0.001$) and SV ($p<0.001$) when compared to their white counterparts.

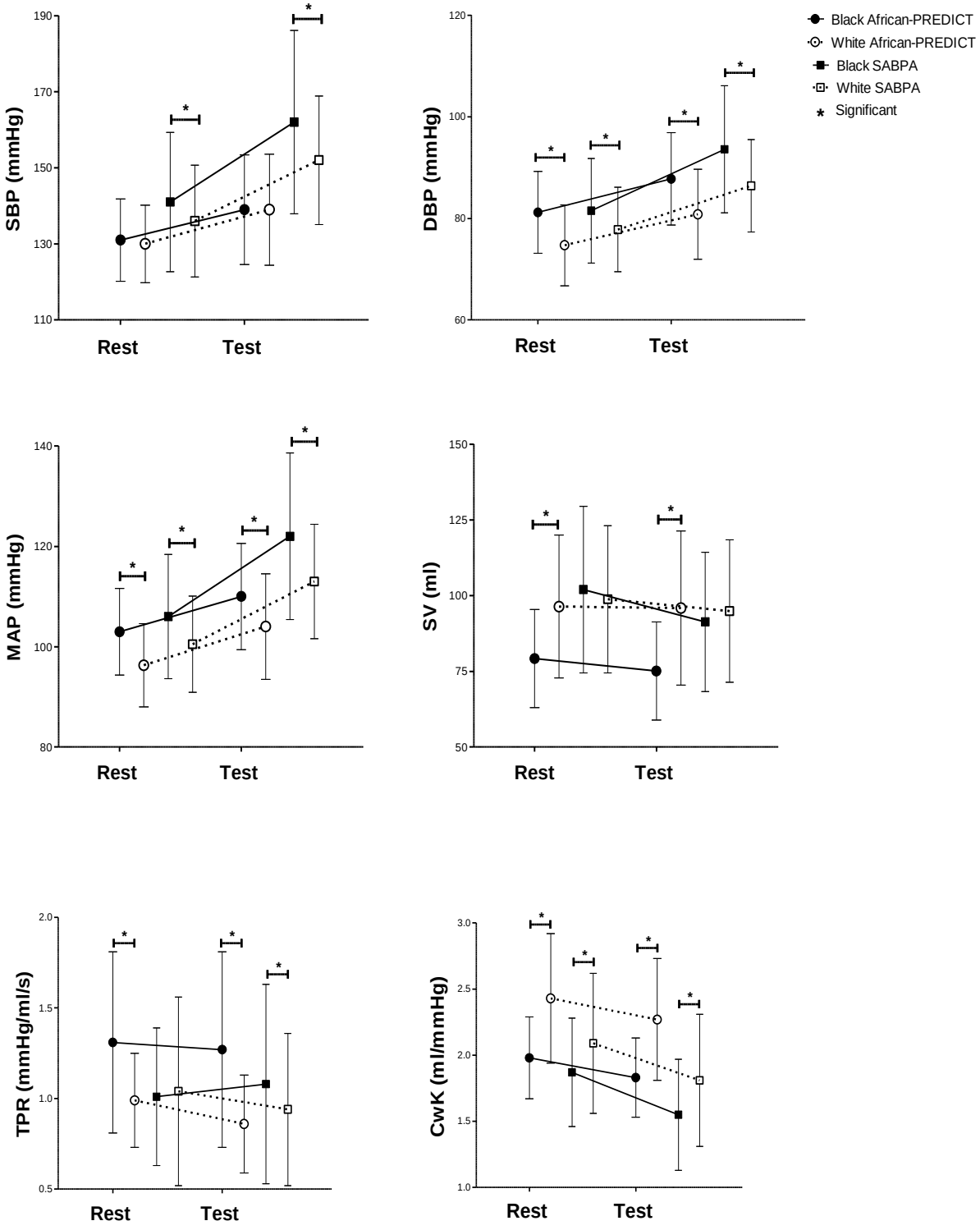

Figure 1: Rest and change in cardiovascular variables after the color-word conflict test showing significant differences between black and white groups of the African-PREDICT and SABPA studies. SBP, Systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SV, stroke volume; TPR, total peripheral resistance; Cwk, Windkessel arterial compliance.

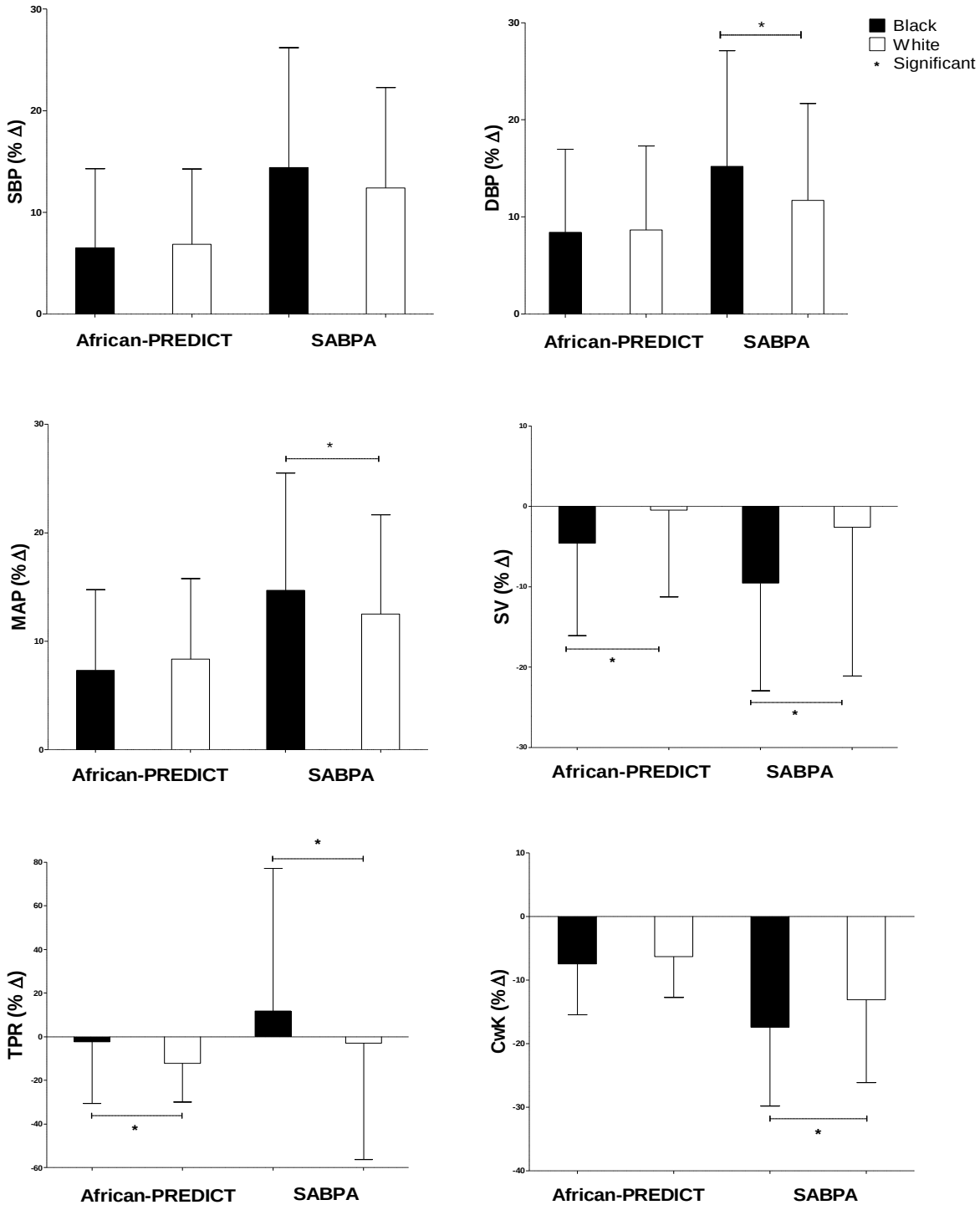

Figure 2: Cardiovascular reactivity following the color-word conflict test showing significant differences between black and white groups of the African-PREDICT and SABPA studies.
% Δ, percentage change; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SV, stroke volume; TPR, total peripheral resistance; Cwk, Windkessel arterial compliance.

Regression analyses

In single and partial regression analyses (**Table S2**), we found an association of change in TPR with SOD in the young black group, however, this lost significance after full adjustments were made (R^2=-0.004; β=-0.12; p=0.145). In the younger white group, changes in DBP (R^2=0.05; β=0.31; p=0.001) and MAP (R^2=0.10; β=0.28; p=0.002) associated positively with ROS, while change in Cwk associated positively with SOD activity (R^2=0.09; β=0.18; p=0.023) (**Table 2**).
In the older black group, single, partial (**Table S3**) and multiple regression analyses (**Table 2**) indicated an increase in DBP (R^2=0.03; β=0.23; p=0.009) and MAP (R^2=0.06; β=0.18; p=0.033) to be positively associated with γ-GT levels. Meanwhile, a decrease in SV (R^2=0.06; β=-0.20; p=0.023) and Cwk (R^2=0.07; β=-0.25; p=0.005) was negatively associated with γ-GT levels.

In the same group, an increase in TPR was borderline negatively associated with tGSH (R^2=0.04; β=-0.15; p=0.062). The borderline association of decreased SV with GPx in partial regression analyses (p=0.054) became significant after multiple regression analyses (R^2=0.06; β=0.16; p=0.032) in the older black group. In the older white group, increased SBP (R^2=0.04; β=-0.20; p=0.006), increased MAP (R^2=0.01; β=-0.19; p=0.009) were inversely associated with SOD. In the same group a decrease in SV was positively associated with tGSH (R^2=0.02; β=0.20; p=0.009) and negatively associated with SOD (R^2=0.0004; β=-0.14; p=0.052). All other associations lost significance after full adjustments.

Table S2: Single and partial regression analyses of cardiovascular reactivity variables with oxidative stress markers in black and white participants of the African-PREDICT study.

	GR		***GPx***		***SOD***		***tGSH***		***ROS***		***γ-GT***	
	Single	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**
African-PREDICT study: Black (n=191)												
SBP	r=-0.10	r=-0.09	r=-0.10	r=-0.08	r=-0.05	r=-0.07	r=0.10	r=0.07	r=-0.11	r=0.03	r=0.05	r=-0.002
	p=0.178	p=0.241	p=0.170	p=0.308	p=0.502	p=0.372	p=0.158	p=0.370	p=0.152	p=0.705	p=0.511	p=0.983
DBP	r=-0.06	r=-0.06	r=-0.06	r=-0.04	r=-0.07	r=-0.08	r=0.04	r=0.02	r=-0.03	r=0.07	r=0.02	r=-0.02
	p=0.393	p=0.450	p=0.424	p=0.567	p=0.345	p=0.283	p=0.544	p=0.786	p=0.667	p=0.363	p=0.822	p=0.779
MAP	r=-0.07	r=-0.06	r=-0.09	r=-0.07	r=-0.03	r=-0.04	r=0.07	r=0.04	r=-0.06	r=0.05	r=0.03	r=-0.01
	p=0.346	p=0.413	p=0.213	p=0.319	p=0.709	p=0.603	p=0.321	p=0.557	p=0.400	p=0.487	p=0.698	p=0.874
SV	r=-0.08	r=-0.07	r=-0.06	r=-0.05	r=0.05	r=0.04	r=0.11	r=0.11	r=-0.04	r=-0.02	r=0.01	r=-0.01
	p=0.304	p=0.369	p=0.402	p=0.511	p=0.535	p=0.596	p=0.115	p=0.154	p=0.594	p=0.800	p=0.934	p=0.950
TPR	r=-0.10	r=-0.09	r=0.01	r=0.03	r=-0.13	**r=-0.15**	r=0.11	r=0.09	r=-0.10	r=-0.06	r=-0.003	r=-0.03
	p=0.162	p=0.225	p=0.937	p=0.681	p=0.067	**p=0.046**	p=0.123	p=0.208	p=0.168	p=0.441	p=0.968	p=0.668
Cwk	r=0.03	r=0.03	r=0.03	r=0.02	r=-0.03	r=-0.02	r=-0.02	r=0.01	r=0.14	r=0.02	r=-0.07	r=-0.02
	p=0.650	p=0.690	p=0.639	p=0.802	p=0.702	p=0.787	p=0.815	p=0.861	p=0.052	p=0.785	p=0.346	p=0.764
African-PREDICT study: White (n=196)												
SBP	r=-0.03	r=-0.08	r=-0.07	r=-0.05	**r=-0.14**	r=-0.12	r=0.09	r=0.10	r=0.02	r=0.12	**r=0.22**	r=0.06
	p=0.729	p=0.259	p=0.352	p=0.473	**p=0.043**	p=0.093	p=0.219	p=0.171	p=0.799	p=0.101	**p=0.002**	p=0.383
DBP	r=-0.05	r=-0.05	r=0.01	r=0.01	r=0.01	r=0.02	r=-0.01	r=-0.01	**r=0.20**	**r=0.24**	r=0.07	r=0.05
	p=0.534	p=0.469	p=0.911	p=0.943	p=0.922	p=0.762	p=0.878	p=0.890	**p=0.007**	**p=0.001**	p=0.315	p=0.502
MAP	r=-0.03	r=-0.06	r=-0.01	r=-0.003	r=-0.08	r=-0.06	r=0.02	r=0.02	r=0.11	**r=0.19**	**r=0.16**	r=0.05
	p=0.731	p=0.401	p=0.882	p=0.997	p=0.263	p=0.424	p=0.825	p=0.773	p=0.148	**p=0.009**	**p=0.029**	p=0.491
SV	r=0.02	r=-0.03	r=-0.05	r=-0.04	r=0.01	r=0.03	r=0.10	r=0.11	r=-0.12	r=-0.05	**r=0.18**	r=0.05
	p=0.785	p=0.725	p=0.448	p=0.632	p=0.931	p=0.729	p=0.162	p=0.123	p=0.095	p=0.521	**p=0.010**	p=0.469
TPR	r=0.02	r=0.01	r=-0.09	r=-0.08	r=-0.06	r=-0.08	r=0.06	r=0.04	**r=0.21**	**r=0.16**	r=0.09	r=0.12
	p=0.787	p=0.933	p=0.217	p=0.257	p=0.374	p=0.303	p=0.418	p=0.561	**p=0.003**	**p=0.029**	p=0.226	p=0.103
Cwk	r=0.02	r=0.05	r=-0.01	r=-0.02	**r=0.22**	**r=0.22**	r=0.02	r=0.02	r=-0.03	r=-0.11	r=-0.10	r=0.01
	p=0.812	p=0.482	p=0.936	p=0.757	**p=0.002**	**p=0.003**	p=0.743	p=0.823	p=0.706	p=0.121	p=0.165	p=0.880

Relationships adjusted for age, sex and body mass index.

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SV, stroke volume; TPR, total peripheral resistance; Cwk, Windkessel arterial compliance.

Table 2: Summary of multiple regression analyses of cardiovascular reactivity with markers of oxidative stress in participants of the African-PREDICT and SABPA studies.

	African-PREDICT study					
	Black (n=191)			White (n=196)		
	Adjusted R^2	*β (95% CI)*	*p*	*Adjusted R^2*	*β (95% CI)*	*p*
			Diastolic blood pressure (% Δ)			
Reactive oxygen species	-0.02	0.05 (-0.16; 0.25)	0.643	0.05	0.31 (0.13; 0.49)	**0.001**
			Mean arterial pressure (% Δ)			
Reactive oxygen species	-0.004	0.01 (-0.19; 0.21)	0.899	0.10	0.28 (0.10; 0.45)	**0.002**
			Windkessel arterial compliance (% Δ)			
Superoxide dismutase	0.01	-0.02 (-0.18; 0.15)	0.860	0.09	0.18 (0.03; 0.32)	**0.023**

	SABPA study					
	Black (n=200)			White (n=209)		
	Adjusted R^2	*β (95% CI)*	*p*	*Adjusted R^2*	*β (95% CI)*	*p*
			Systolic blood pressure (% Δ)			
Superoxide dismutase	0.06	0.03 (-0.12; 0.17)	0.708	0.04	-0.20 (-0.33; -0.06)	**0.006**
			Diastolic blood pressure (% Δ)			
γ-glutamyl transferase	0.03	0.23 (0.06; 0.40)	**0.009**	-0.04	0.08 (-0.10; 0.26)	0.361
			Mean arterial pressure (% Δ)			
Superoxide dismutase	0.05	0.10 (-0.04; 0.25)	0.178	0.01	-0.19 (-0.33; -0.05)	**0.009**
γ-glutamyl transferase	0.06	0.18 (0.02; 0.35)	**0.033**	-0.02	0.11 (-0.07; 0.29)	0.223
			Stroke volume (% Δ)			
Glutathione peroxidase	0.06	0.16 (0.02; 0.31)	**0.032**	-0.02	0.07 (-0.08; 0.22)	0.376
Superoxide dismutase	0.04	-0.09 (-0.23; 0.06)	0.233	0.0004	-0.14 (-0.28; 0.00)	0.052
Total glutathione	0.05	0.12 (-0.03; 0.28)	0.116	0.02	0.20 (0.05; 0.34)	**0.009**
γ-glutamyl transferase	0.06	-0.20 (-0.36; -0.03)	**0.023**	-0.02	0.02 (-0.16; 0.20)	0.813
			Total peripheral resistance (% Δ)			
Total glutathione	0.04	-0.15 (-0.30; 0.01)	0.062	0.01	-0.01 (-0.15; 0.14)	0.920
			Windkessel arterial compliance (% Δ)			
γ-glutamyl transferase	0.07	-0.25 (-0.41; -0.08)	**0.005**	-0.02	-0.04 (-0.22; 0.14)	0.652

Covariates included in each model included the relative oxidative stress marker, age, sex, body mass index, total energy expenditure, self-reported alcohol use, cotinine, glucose, interleukin-6, triglycerides and cortisol.

Table S3: Single and partial regression analyses of cardiovascular reactivity variables with oxidative stress markers in black and white participants of the SABPA study.

	GR		***GPx***		***SOD***		***tGSH***		***ROS***		***γ-GT***	
	Single	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**
SABPA study: Black (n=200)												
SBP	r=-0.06	r=-0.05	r=-0.02	r=-0.04	r=0.002	r=-0.001	r=-0.04	r=-0.04	r=-0.01	r=0.02	r=0.10	r=0.13
	p=0.444	p=0.513	p=0.798	p=0.619	p=0.978	p=0.986	p=0.632	p=0.591	p=0.944	p=0.812	p=0.189	p=0.072
DBP	r=0.02	r=0.03	r=-0.12	r=-0.12	r=0.12	r=0.12	r=-0.10	r=-0.09	r=0.10	r=0.10	**r=0.16**	**r=0.22**
	p=0.737	p=0.690	p=0.113	p=0.104	p=0.109	p=0.125	p=0.177	p=0.244	p=0.184	p=0.189	**p=0.027**	**p=0.003**
MAP	r=-0.02	r=-0.02	r=-0.08	r=-0.09	r=0.08	r=0.08	r=-0.07	r=-0.06	r=0.04	r=0.04	r=0.14	**r=0.19**
	p=0.785	p=0.817	p=0.287	p=0.249	p=0.273	p=0.300	p=0.357	p=0.442	p=0.603	p=0.583	p=0.053	**p=0.009**
SV	r=-0.14	r=-0.12	**r=0.16**	r=0.14	r=-0.07	r=-0.08	**r=0.16**	r=0.12	**r=-0.15**	r=-0.14	**r=-0.15**	**r=-0.18**
	p=0.064	p=0.118	**p=0.030**	p=0.054	p=0.320	p=0.315	**p=0.033**	p=0.098	**p=0.037**	p=0.063	**p=0.039**	**p=0.016**
TPR	r=0.13	r=0.10	r=-0.14	r=-0.13	r=0.02	r=0.02	**r=-0.21**	**r=-0.20**	r=0.09	r=0.09	**r=0.14**	r=0.13
	p=0.089	p=0.161	p=0.056	p=0.087	p=0.826	p=0.778	**p=0.005**	**p=0.008**	p=0.199	p=0.216	**p=0.049**	p=0.070
Cwk	r=-0.07	r=-0.05	r=0.14	r=0.14	r=-0.06	r=-0.06	r=0.12	r=0.10	r=-0.06	r=-0.07	**r=-0.21**	**r=-0.24**
	p=0.355	p=0.520	p=0.064	p=0.067	p=0.439	p=0.428	p=0.096	p=0.178	p=0.414	p=0.361	**p=0.005**	**p=0.001**
SABPA study: White (n=209)												
SBP	r=0.02	r=0.02	r=0.08	r=0.08	**r=-0.16**	**r=-0.19**	r=-0.01	r=-0.01	r=-0.10	r=-0.05	r=0.09	r=0.09
	p=0.776	p=0.798	p=0.274	p=0.286	**p=0.025**	**p=0.008**	p=0.905	p=0.855	p=0.168	p=0.530	p=0.225	p=0.186
DBP	r=0.03	r=0.02	r=0.02	r=0.01	r=-0.10	r=-0.12	r=-0.04	r=-0.03	r=-0.03	r=-0.02	r=0.02	r=0.06
	p=0.696	p=0.773	p=0.767	p=0.865	p=0.151	p=0.099	p=0.590	p=0.705	p=0.688	p=0.732	p=0.780	p=0.406
MAP	r=0.03	r=0.02	r=0.04	r=0.03	**r=-0.17**	**r=-0.19**	r=-0.02	r=-0.02	r=-0.06	r=-0.03	r=0.07	r=0.09
	p=0.706	p=0.780	p=0.578	p=0.649	**p=0.019**	**p=0.008**	p=0.803	p=0.825	p=0.389	p=0.677	p=0.326	p=0.228
SV	r=0.01	r=0.04	r=0.04	r=0.07	**r=-0.14**	**r=-0.15**	**r=0.18**	**r=0.16**	r=-0.003	r=0.03	r=0.01	r=-0.01
	p=0.879	p=0.597	p=0.548	p=0.346	**p=0.041**	**p=0.036**	**p=0.011**	**p=0.020**	p=0.968	p=0.687	p=0.848	p=0.897
TPR	r=-0.04	r=-0.04	r=-0.04	r=-0.03	r=0.09	r=0.10	r=0.06	r=0.04	r=-0.03	r=-0.01	r=0.03	r=-0.05
	p=0.611	p=0.608	p=0.583	p=0.627	p=0.223	p=0.153	p=0.420	p=0.599	p=0.723	p=0.908	p=0.670	p=0.528
Cwk	r=-0.02	r=0.01	r=-0.02	r<0.001	r=0.09	r=0.09	r=0.06	r=0.05	r=0.05	r=0.05	r=-0.04	r=-0.05
	p=0.824	p=0.868	p=0.746	p=0.996	p=0.226	p=0.188	p=0.403	p=0.478	p=0.444	p=0.457	p=0.527	p=0.521

Relationships adjusted for age, sex and body mass index.

SBP; systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SV, stroke volume; TPR, total peripheral resistance; Cwk, Windkessel arterial compliance.

Sensitivity analyses

The African-PREDICT study excluded participants using statins and anti-hypertension medication. After adding these variables as covariates into multiple regression analyses in the SABPA study, only the borderline negative association of TPR with tGSH in the black group became significant after including anti-hypertension medication into the model (R^2=0.05; β=-0.17; p=0.039).

Multiple regression analyses of SV with oxidative stress markers in the older group were repeated after substituting stroke volume with stroke volume index (SVI) (calculated as stroke volume/body surface area) as the main dependent variable in the model. Similar results were obtained in sensitivity analyses showing a significant negative association of SVI with γ-GT (R2=0.06; β=-0.20; p=0.023) in black groups, and a positive association of SVI with tGSH (R2=0.02; β=0.20; p=0.009) in white groups. The negative association of SVI with SOD (R2=0.0004; β=-0.14; p=0.052) was borderline significant in the older white group after full adjustments.

Discussion

We found age-related disparities in the link between cardiovascular reactivity and markers of oxidative stress in black populations. In the young black group TPR decreased less and SV decreased more, but we found no link between cardiovascular reactivity and markers of oxidative stress. In the older black group with a heightened cardiovascular response to stress, increased TPR and decreased SV and Cwk was associated with markers of glutathione metabolism. In contrast, our findings in the white groups indicated that cardiovascular reactivity was associated with ROS and important determinants of ROS availability, namely SOD and tGSH, independent of age.

Our findings in the older black group confirm results from a previous study by our research team, in which older black men displayed increased TPR reactivity to acute stress.[18] Increased TPR reactivity may enhance the afterload and subsequently decrease SV, which related to end-organ damage in this cohort.[18] Similar findings were noted in the younger black adults described in the present study, where TPR decreased less and SV decreased more when compared to the white adults during stress. This is in agreement with previous findings in another young black group (29.9 ± 2.4 years) where it was suggested that increased TPR may play a role in the pathogenesis of hypertension in blacks.[19] Despite evidence of an altered cardiovascular response in the young black group, we were unable to link this to any oxidative stress markers investigated in this study. This finding may suggest that factors other than ROS, such as enhanced arterial stiffness and premature vascular aging, may play a more prominent role in cardiovascular reactivity in young black South Africans [20,32].

In the older black group, on the other hand, increased TPR and decreased SV and Cwk were associated with markers of glutathione metabolism. Glutathione, a powerful antioxidant, exists in either reduced (GSH) or oxidized (GSSG) form.[33] The synthesis of glutathione is dependent on the intracellular availability of its amino acid constituents. While γ-GT was originally considered a marker for alcohol abuse and liver dysfunction, the enzyme acts as an oxidative stress marker since it is involved in the breakdown of extracellular glutathione to increase the availability of amino acids for intracellular glutathione synthesis.[34]

In this regard γ-GT has a dual function since it is also able to produce ROS directly.[35-38] The link between heightened cardiovascular reactivity and markers of glutathione metabolism may be explained by two possible mechanisms; firstly that acute stress may induce increased shear stress which may promote oxidative stress,[12,39] and secondly that oxidative stress may leads to a deficiency in nitric oxide, thus further diminishing endothelial function.[6,7,40] During conditions of oxidative stress, glutathione may be consumed at a higher rate, leading to possible increased expression of γ-GT in an effort to increase intracellular glutathione levels.[36] The elevated γ-GT in this group may then also perhaps be explained as compensatory in an effort to stabilize the redox balance, which is further strengthened by the presence of higher tGSH in this group.

In contrast, the findings in the white cohorts indicated that cardiovascular reactivity was associated with ROS and important determinants of ROS availability, namely SOD and tGSH, independent of age. This was also indicated in an experimental study in which a role for ROS in the regulation of blood pressure during and after acute stress was confirmed. Here it was suggested that a lack of ROS is implicated in a greater MAP reactivity and a longer recovery period from acute stress.[41] This finding supports the important role of physiologically regulated ROS signaling in the maintenance of vascular tone during stress, while suggesting a possible role for an adequate antioxidant system, especially SOD activity, in the prevention of a heightened cardiovascular response to stress in white participants regardless of age.

This study has to be interpreted within the context of its strengths and limitations. Both our study populations included participants from the Potchefstroom area, and may not represent the South African population as a whole. This being a cross-sectional study, causality cannot be inferred, and although our results were consistent after multiple adjustments, we cannot exclude confounding of unknown factors. Measurements of ROS were made using an indirect biomarker and we cannot discern the sources of ROS generation in this study. Participants were not screened or excluded based on previous exposure to the color-word conflict test, which may be considered for future studies in this field.

However, to the best of our knowledge, this is the first study investigating the associations of oxidative stress with cardiovascular reactivity in 2 age-stratified cohorts of black and white South Africans. Both studies were well executed under controlled conditions in a fully-equipped research facility. This study offers further insight into the role of redox signaling in cardiovascular responses to acute stress.

In conclusion, age-related differences were found in the link between cardiovascular reactivity and markers of oxidative stress in the black cohorts. Heightened cardiovascular reactivity during the color-word conflict test associated with markers of glutathione metabolism in older black participants, suggesting a possible compensatory up-regulation of this system in order to correct their unfavorable cardiovascular responses to stress. Findings in the white groups support a possible regulatory role of ROS in the maintenance of vascular tone during stress, independent of age.

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Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF does not accept any liability in this regard.

Declaration of interest statement

The authors have no conflicts of interest to disclose. Any opinions expressed and conclusions arrived at, are those of the authors alone.

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CHAPTER 5:

Three-year change in oxidative stress markers is linked to target organ damage in black and white men: The SABPA study

Three-year change in oxidative stress markers is linked to target organ damage in black and white men: The SABPA study

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Abstract

Oxidative stress is implicated in the development of hypertension, carotid wall thickening and renal dysfunction. Links between oxidative stress and cardiovascular pathology are visible in black South Africans who display high prevalence of hypertension and early vascular ageing. However, there is limited longitudinal data relating change in oxidative stress with vascular and renal deterioration over time. We aimed to investigate whether changes in oxidative stress markers over a 3-year follow-up period are associated with markers of target organ damage in black (N=89) and white (N=91) men. Carotid intima-media thickness was measured using the SonoSite Micromaxx ultrasound system and cross-sectional wall area (CSWA) was calculated. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula. Changes over a three-year period in oxidative stress markers included percentage change (% Δ) in reactive oxygen species (ROS) and superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) activities. Over 3 years, black men revealed decreased ROS, SOD and GR activities, while white men revealed decreased SOD and GPx but increased GR activities. Black men displayed positive associations of CSWA with % Δ ROS (β=0.28; p=0.017) and % Δ SOD (β=0.24; p=0.047). White men displayed a negative association of CSWA with % Δ SOD (β=-0.22; p=0.042) and positive associations of eGFR with % Δ GPx (β=0.33; p=0.001) and % Δ GR (β=0.39; p<0.001). In white men, the link of CSWA with a decrease in SOD activity suggests a role for oxidative stress in macrovascular remodeling of the carotid arteries, while the link between stable eGFR with components of the glutathione system suggests a postponement of microvascular deterioration. In black men, associations of oxidative stress markers with CSWA suggests a protective role for a sufficiently functioning antioxidant system in delaying target organ damage in this group.

Key words: cross-sectional wall area, estimated glomerular filtration rate, glutathione peroxidase, longitudinal study, superoxide dismutase.

Introduction

Cardiovascular disease is one of the leading risk factors for mortality both globally and in developing countries.[1-4] In South Africa, and especially among the urban black population, the prevalence of hypertension is a mounting concern.[5-9] Oxidative stress is implicated in the development of hypertension,[10,11] and associations of oxidative stress and blood pressure have already been confirmed in black South Africans.[12,13] Under normal physiological conditions, ROS act as signaling molecules that maintain endothelial function and vascular tone.[14,15] However, excessive ROS production or ineffective functioning of the antioxidant system enhances inflammation, vascular remodeling and endothelial dysfunction, all of which may lead to cardiovascular disease and related co-morbidities such as renal disease, especially with advanced age.[14,16,17] Previous results within our South African population have linked oxidative stress with pulse pressure (as an indicator of early vascular changes),[18] arterial stiffness[12,19] and carotid wall thickening.[13,20] However, it is not clear if changes in oxidative stress over time are associated with target organ damage such as deterioration of vascular structure and renal function. We aimed to investigate the relationship between change in markers of oxidative stress over 3 years with measures of target organ damage (glomerular filtration rate and carotid wall thickening) in a black and white South African cohort.

Methods

Study population

This study is embedded in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study. This is a prospective cohort study of black and white teachers of similar socio-economic status from the Dr Kenneth Kuanda Education District in the North West Province of South Africa. Detail on the study population and protocol was reported elsewhere.[21] In summary, participants were between the ages of 20 and 65 years at baseline and participants who were pregnant, lactating, exhibited elevated ear temperature (>37°C), used alpha and beta blockers, confirmed psychotropic substance abuse and those with a history of blood donation or vaccination in the 3 months prior to commencement of the study were excluded. Phase I (baseline) of the SABPA study was conducted between 2008 and 2009 while phase II (follow-up) was conducted between 2011 and 2012. The protocols of Phase I and Phase II were designed to remain similar in both phases of the study. A three year successful follow-up rate of 87.8% provided a sample of 359 participants in the follow-up phase that were subsequently divided into black men (N=89), white men (N=91), black women (N=84) and white women (N=95).

The SABPA study adhered to all the requirements of the Declaration of Helsinki regarding investigations among human participants, and was further approved by the Health Research Ethics Committee of the North-West University (NWU-00036-07-S6). Participants were informed of the study in their home language both verbally and in a written manner. Written informed consent was acquired before commencement of the study.

Questionnaires

A general health questionnaire was completed by each participant with the help of a researcher in order to obtain demographic data and information on behavioral risk factors such as alcohol usage.

Anthropometric and physical activity measurements

All anthropometric measurements were performed using standardized methods once calibrated,[22] and included body height (Invicta Stadiometer, IP 1465, Invicta, London, UK), body weight (Precision Health Scale, A & D Company, Tokyo, Japan) and waist circumference (Holtain unstretchable flexible 7mm wide metal tape, Crosswell, Wales). Body mass index (BMI) was calculated as kg/m^2. Each participant was equipped with an Actical® omnidirectional accelerometer (Actical® activity monitor, Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada) for 24 hours in the baseline phase and an ActiHeart physical activity monitor (CamNtech Ltd., England, UK) for 7 days in the follow-up phase in order to monitor total energy expenditure (TEE).

Cardiovascular measurements

Ambulatory blood pressure measurements were taken for 24 hours at 30 minute intervals during the day (07:00 – 22:00), and at one hour intervals during the night (22:00 – 06:00) using the Cardiotens device (CE0120, Meditech, Budapest, Hungary). Ambulatory measurements included systolic blood pressure (24hr SBP), diastolic blood pressure (24hr DBP), pulse pressure (24hr PP) and mean arterial pressure (24hr MAP). The device was fitted with a correctly sized cuff on the non-dominant arm and participants continued with their normal daily activities while reporting any abnormalities (such as headaches, nausea or stress) on their ambulatory diary cards.

The carotid intima-media thickness (cIMT) of the left common carotid artery was measured using B-mode ultrasonography with the high resolution SonoSite Micromaxx ultrasound system (SonoSite Inc., Bothell, WA, USA) and a 6-13 MHz linear array transducer according to the Mannheim Consensus.[23] Still frame images from at least two optimal angles of clearest viewing of the left and right common carotid arteries were obtained at maximal dilation (as determined by a 3-lead ECG recording). Results were interpreted with a semi-automated program, namely the Artery Measurement Systems (AMS) II v1.139 (Gothenburg, Sweden) in order to obtain a maximal 10mm segment of good image quality proximal to the carotid bifurcation for use in offline analyses. The program automatically identifies the borders from the trailing edge of the media-adventitia at the near wall to the leading edge of the far wall media-adventitia, and calculates the cIMT and diameter using approximately 100 measurements throughout the chosen segment. The same optimal angles were used in both baseline and follow-up phase and in this study the far wall measurements of cIMT were used. The analysis of cIMT was performed by a single observer and the intra-observer variability was 0.04 mm between two measurements made four weeks apart (N=10). Carotid cross-sectional wall area (CSWA) was calculated to confirm structural changes in luminal diameter using the formula $CSWA = \pi(d/2 + cIMT)^2 – \pi(d/2)^2$, where d denotes luminal diameter.[24,25]

Biochemical measurements

A fasting blood sample was obtained from each participant by a registered nurse from the antebrachial vein branches using a winged infusion set and syringes.

Serum and plasma were prepared using standardized procedures, where after they were immediately aliquoted into cryovials in the on-site laboratory and stored in bio-freezers at -80°C until analysis in order to maintain efficient long-term stability of antioxidant enzymes.[26]

In both phases of the SABPA study, antioxidant enzyme activities including glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) were measured

using assay kits (Cayman Chemical Company, Ann Arbor, MI, USA) and a Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). We measured γ-glutamyl transferase (γ-GT) with the Unicel DXC 800 (Beckman and Coulter, Germany) and a Konelab™ 20I Sequential Multiple Analyzer Computer (Thermo Scientific, Vantaa, Finland) during baseline and the Cobas Integra 400 plus (Roche, Basel, Switzerland) during follow-up. Additional oxidative stress markers measured during both phases of the study include total glutathione (tGSH) (Kit: BIOXYTECH GSH/GSSG-412, Oxis International Inc, CA, USA) and ROS (Synergy HT microplate reader, BioTek, Winooski, VT, USA) which is measured as serum peroxides and reported in units, where 1 mg H_2O_2/L is equivalent to one unit.[27]

Serum total cholesterol and triglycerides were measured with the Unicel DXC 800 (Beckman and Coulter, Germany) and a Konelab™ 20I Sequential Multiple Analyzer Computer (Thermo Scientific, Vantaa, Finland) in the baseline phase, and the Cobas Integra 400 plus (Roche, Basel, Switzerland) in the follow-up phase. Serum high-density lipoprotein cholesterol (HDL) and fluoride plasma glucose levels were measured using the Unicel DXC 800 (Beckman and Coulter, Germany) in the baseline phase, and the Cobas Integra 400 plus (Roche, Basel, Switzerland) in the follow-up phase. Interleukin-6 (IL-6) was measured using a high sensitivity Quantikine ELISA kit (R&D systems, Minneapolis, MN, USA) analyzed on Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). An immunoassay (Automated Modular, Roche, Basel, Switzerland) was used to determine serum cotinine levels. Serum creatinine was measured with the Unicel DXC 800 (Beckman and Coulter, Germany) and a Konelab™ 20I Sequential Multiple Analyzer Computer (Thermo Scientific, Vantaa, Finland) in the baseline phase, with an enzymatic colorimetric test on the Cobas Integra 400 plus in the follow-up phase. Serum creatinine was used in the calculation of the estimated glomerular filtration rate (eGFR) according to the Modification of Diet in Renal Disease formula.[28]

Statistical analyses

Statistica version 13.2 (Dell, TX, USA) was used to perform the statistical analyses of this study. Normal distribution of data was analysed based on visual inspection of symmetry of the bell-shaped histogram curves. The central tendency and spread for normally distributed variables were expressed as arithmetic mean and standard deviation, while skewed variables were logarithmically transformed (glucose, triglycerides, IL-6, GR, SOD and γ-GT). The central tendency and spread of logarithmically transformed variables were expressed as the geometric mean and the 5th and 95th percentile intervals. Change in oxidative stress markers were calculated as the percentage change (% Δ) from baseline to follow-up phase.

Interactions of race and sex were tested on the relationships between main dependent variables (blood pressure, macrovascular and microvascular measurements) and oxidative stress markers using multiple regression analyses. Continuous variables were compared using dependent T-tests. Single regression analyses were performed to evaluate unadjusted associations between follow-up cardiovascular variables and % Δ in ROS, tGSH, GPx, GR, SOD and γ-GT from baseline to follow-up. Partial correlations were performed to evaluate the associations between variables while adjusting for % Δ in BMI. Associations of cIMT and CSWA were additionally adjusted for % Δ in 24hr SBP. Multiple regression analyses were performed to evaluate the independent associations between follow-up cardiovascular variables and 3-year change in oxidative stress markers in black and white men and women. Covariates entered into each model included % Δ in the oxidative stress marker, the follow-up cardiovascular variable, % Δ in age, % Δ in body mass index, % Δ in total energy expenditure, % Δ in cotinine, % Δ in glucose, % Δ in triglycerides, % Δ in interleukin-6 and change in self-reported alcohol use. Change in alcohol use was coded as no change in behavior, started using alcohol or stopped using alcohol from baseline to follow-up phase. Measurements of cIMT and CSWA were additionally adjusted for % Δ in 24hr SBP.

Results

Characteristics of the study population

Interactions of race were found on the association of CSWA with SOD (β=-0.884; p<0.001), DBP with GR (β=0.587; p=0.030), and MAP with GR (β=0.613; p=0.023). In accordance with the interactions found, as well as the aims of this study and previous results within our South African population, this study was stratified by both race and sex.[12,13] Descriptive analyses were performed in women and revealed similar characteristics as male cohorts, however no significant associations between follow-up cardiovascular variables and three year change in oxidative stress markers were found. (**Table S1 and S2**)

Table S1 Change in characteristics of black and white women after 3 years.

	Black women			White women		
	Baseline	**Follow-up**	**P-values**	**Baseline**	**Follow-up**	**P-values**
N	84	84	-	95	95	-
Age (years)	45.9 ± 7.96	48.9 ± 7.96	-	46.8 ± 9.62	49.8 ± 9.62	-
Anthropometric measurements						
Body mass index (kg/m^2)	32.6 ± 6.44	33.2 ± 6.92	**0.027**	26.3 ± 6.36	27.4 ± 6.89	**<0.001**
Biochemical analyses						
Glucose (mmol/l)	5.05 (3.91; 6.89)	5.10 (4.25; 7.58)	0.665	5.38 (4.60; 6.60)	4.07 (2.94; 5.09)	**<0.001**
HDL cholesterol (mmol/l)	1.20 ± 0.31	1.07 ± 0.29	**<0.001**	1.43 ± 0.42	1.26 ± 0.37	**<0.001**
Total cholesterol (mmol/l)	4.49 ± 1.19	4.48 ± 0.98	0.923	5.59 ± 1.37	4.44 ± 1.09	**<0.001**
Triglycerides (mmol/l)	0.89 (0.42; 2.30)	0.94 (0.46; 1.74)	0.133	0.81 (0.35; 2.22)	0.83 (0.38; 2.00)	0.572
Interleukin-6 (pg/ml)	1.19 (0.41; 2.97)	1.54 (0.59; 3.32)	**0.001**	0.97 (0.33; 3.04)	0.81 (0.23; 2.83)	**0.034**
Cardiovascular variables						
24hr SBP (mmHg)	128 ± 14.8	132 ± 18.5	**0.015**	121 ± 12.9	119 ± 11.0	**0.036**
24hr DBP (mmHg)	78.9 ± 8.60	80.0 ± 10.4	0.283	74.3 ± 7.90	71.9 ± 7.19	**<0.001**
24hr PP (mmHg)	49.3 ± 9.16	52.4 ± 10.7	**<0.001**	46.6 ± 7.80	47.1 ± 7.74	0.254
24hr MAP (mmHg)	95.3 ± 10.2	97.4 ± 12.7	0.085	89.9 ± 9.12	87.7 ± 7.85	**0.002**
Macrovascular measurements						
cIMT (mm)	0.67 ± 0.14	0.66 ± 0.12	0.427	0.63 ± 0.15	0.69 ± 0.13	**<0.001**
CSWA (mm)	12.6 ± 4.11	13.0 ± 3.43	0.239	11.8 ± 3.93	13.6 ± 3.42	**<0.001**
Microvascular measurements						
eGFR (ml/min/1.73m^2)	99.3 ± 21.3	231 ± 87.9	**<0.001**	94.5 ± 17.9	96.6 ± 25.4	0.263
Oxidative stress markers						
Reactive oxygen species (Units)[1]	107 ± 25.6	87.3 ± 30.5	**<0.001**	103 ± 31.1	85.4 ± 22.0	**<0.001**
Total glutathione (µM)	864 ± 177	824 ± 180	0.054	775 ± 162	804 ± 325	0.405

Glutathione peroxidase (nmol/min/ml)	32.8 ± 14.4	36.3 ± 10.9	0.078	37.3 ± 7.92	32.6 ± 6.78	**<0.001**
Glutathione reductase (nmol/min/ml)	6.22 (2.04; 14.5)	3.23 (0.25; 27.4)	**<0.001**	2.78 (0.25; 7.64)	2.30 (0.51; 50.4)	0.231
Superoxide dismutase (U/ml)	4.61 (0.85; 22.1)	1.85 (0.37; 5.81)	**<0.001**	3.95 (1.50; 11.3)	2.41 (0.35; 9.13)	**<0.001**
γ-glutamyl transferase (U/l)	34.5 (16.7; 95.0)	27.6 (11.1; 98.4)	**<0.001**	14.2 (6.00; 39.0)	14.1 (6.00; 39.6)	0.845
Lifestyle						
Cotinine (ng/ml)	20.7 ± 60.3	22.2 ± 72.5	0.831	14.3 ± 53.7	11.8 ± 43.6	0.231
Total energy expenditure (kcal/day)	2644 ± 816	3147 ± 1256	**0.002**	2591 ± 649	2898 ± 906	**0.003**

Data expressed as arithmetic mean ± standard deviation or geometric mean with 5th and 95th percentile boundaries, % or n.
[1] Reactive oxygen species measured as serum peroxides where 1 unit = 1.0 mg/L H_2O_2. ABPM, ambulatory blood pressure; 24hr, 24 hours; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; cIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; eGFR, estimated glomerular filtration rate.

Table S2: Single and partial regression analyses of percentage change in oxidative stress markers with cardiovascular variables after 3 years in black and white women.

	Black Women											
	% Δ ROS		***% Δ tGSH***		***% Δ GPx***		***% Δ GR***		***% Δ SOD***		***% Δ γ-GT***	
	Single	*Partial*	*Single*	*Partial*	*Single*	*Partial*	*Single*	*Partial*	*Single*	*Partial*	*Single*	*Partial*
24hr SBP	r=0.12 p=0.310	r=0.12 p=0.373	r=-0.002 p=0.985	r=-0.001 p=0.994	r=-0.06 p=0.595	r=-0.06 p=0.650	r=0.02 p=0.870	r=0.02 p=0.893	r=0.06 p=0.619	r=0.06 p=0.672	r=0.16 p=0.154	r=0.16 p=0.215
24hr DBP	r=0.14 p=0.205	r=0.16 p=0.227	r=0.08 p=0.482	r=0.07 p=0.576	r=-0.01 p=0.899	r=-0.01 p=0.913	r=0.08 p=0.495	r=0.08 p=0.540	r=-0.03 p=0.778	r=-0.04 p=0.800	r=0.19 p=0.098	r=0.20 p=0.131
24hr PP	r=0.06 p=0.627	r=0.04 p=0.746	r=-0.08 p=0.477	r=-0.07 p=0.585	r=-0.09 p=0.441	r=-0.09 p=0.511	r=-0.05 p=0.640	r=-0.06 p=0.658	r=0.12 p=0.293	r=0.13 p=0.361	r=0.10 p=0.400	r=0.09 p=0.514
24hr MAP	r=0.13 p=0.233	r=0.15 p=0.272	r=0.04 p=0.707	r=0.04 p=0.763	r=-0.04 p=0.745	r=-0.04 p=0.781	r=0.05 p=0.648	r=0.05 p=0.686	r=0.01 p=0.930	r=0.01 p=0.946	r=0.18 p=0.109	r=0.19 p=0.152
cIMT	r=0.17 p=0.134	r=0.19 p=0.107	r=-0.02 p=0.850	r=-0.03 p=0.798	r=0.10 p=0.396	r=0.10 p=0.383	r=0.07 p=0.525	r=0.08 p=0.503	**r=0.30** **p=0.008**	**r=0.30** **p=0.012**	r=-0.18 p=0.106	r=-0.19 p=0.096
CSWA	r=0.04 p=0.747	r=0.05 p=0.658	r=-0.02 p=0.846	r=-0.04 p=0.750	r=0.04 p=0.714	r=0.06 p=0.627	r=0.11 p=0.333	r=0.12 p=0.291	r=0.22 p=0.056	r=0.21 p=0.087	r=-0.11 p=0.335	r=-0.14 p=0.247
eGFR	r=-0.03 p=0.793	r=-0.01 p=0.926	**r=-0.22** **p=0.050**	r=-0.24 p=0.073	r=-0.16 p=0.147	r=-0.16 p=0.214	r=-0.02 p=0.846	r=-0.02 p=0.909	r=-0.04 p=0.743	r=-0.04 p=0.759	r=0.17 p=0.125	r=0.19 p=0.144
	White Women											
24hr SBP	r=-0.06 p=0.546	r=-0.08 p=0.487	r=0.02 p=0.886	r=-0.01 p=0.923	r=-0.01 p=0.928	r=0.02 p=0.880	r=0.08 p=0.431	r=0.08 p=0.482	r=-0.06 p=0.600	r=-0.04 p=0.729	r=-0.06 p=0.555	r=-0.12 p=0.262
24hr DBP	r=-0.02 p=0.853	r=-0.03 p=0.765	r=0.07 p=0.496	r=0.04 p=0.683	r=0.10 p=0.355	r=0.13 p=0.239	r=0.14 p=0.165	r=0.14 p=0.197	r=-0.20 p=0.077	r=-0.18 p=0.121	r=-0.08 p=0.433	r=-0.15 p=0.169
24hr PP	r=-0.07 p=0.491	r=-0.08 p=0.485	r=-0.05 p=0.664	r=-0.06 p=0.609	r=-0.10 p=0.322	r=-0.09 p=0.393	r=-0.02 p=0.865	r=-0.02 p=0.851	r=0.10 p=0.398	r=0.10 p=0.383	r=-0.01 p=0.911	r=-0.03 p=0.750
24hr MAP	r=-0.04 p=0.693	r=-0.06 p=0.611	r=0.05 p=0.628	r=0.02 p=0.837	r=0.05 p=0.602	r=0.09 p=0.429	r=0.13 p=0.223	r=0.12 p=0.262	r=-0.15 p=0.187	r=-0.13 p=0.268	r=-0.08 p=0.450	r=-0.15 p=0.170
cIMT	r=0.04 p=0.718	r=0.04 p=0.715	r=-0.11 p=0.274	r=-0.10 p=0.332	r=-0.02 p=0.811	r=0.0002 p=0.999	r=0.04 p=0.669	r=0.05 p=0.623	r=0.12 p=0.291	r=0.12 p=0.279	r=-0.01 p=0.955	r=-0.01 p=0.934
CSWA	r=0.06 p=0.599	r=0.06 p=0.584	r=-0.11 p=0.316	r=-0.08 p=0.427	r=-0.08 p=0.429	r=-0.05 p=0.672	r=0.10 p=0.322	r=0.12 p=0.262	r=0.12 p=0.283	r=0.13 p=0.259	r=0.05 p=0.656	r=0.05 p=0.651

eGFR	r=0.07 p=0.525	r=0.07 p=0.536	r=-0.07 p=0.493	r=-0.07 p=0.533	r=0.02 p=0.873	r=0.01 p=0.904	r=0.03 p=0.773	r=0.03 p=0.777	r=0.14 p=0.220	r=0.14 p=0.252	r=0.06 p=0.570	r=0.07 p=0.529

Partial regression analyses adjusted for percentage change in body mass index. Associations of cIMT and CSWA are additionally adjusted for percentage change in 24hr SBP.

% Δ, percentage change; SBP; systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; cIMT, carotid intima-media thickness; CSWA, cross sectional wall area; eGFR, estimated glomerular filtration rate; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; tGSH, total glutathione; γ-GT, γ-glutamyl transferase.

Change in characteristics from baseline to follow-up of the black and white men are summarized in **Table 1**. Both black and white men experienced significant increases in BMI ($p<0.001$) while black men displayed increases in IL-6 ($p=0.022$) and TEE ($p<0.001$). White men displayed a decline in lipid markers from baseline to follow-up, including HDL cholesterol ($p<0.001$), total cholesterol ($p<0.001$) and triglycerides ($p=0.008$), while black men also showed a decrease in HDL cholesterol over 3 years ($p<0.001$).

Changes in vascular structure and renal function markers over the 3-year study period in men were illustrated in **Figure 1**. An increase in cIMT and CSWA (all $p<0.001$) was noted in white men, while eGFR increased in the black men ($p<0.001$).

Oxidative stress markers in black men revealed decreases in ROS ($p<0.001$), tGSH ($p<0.001$), GR activity ($p<0.001$), SOD activity ($p=0.025$) and γ-GT activity ($p=0.051$) (borderline) over 3 years. In the white men, tGSH ($p<0.001$) and GR activity ($p=0.050$) increased while GPx activity and SOD activity (both $p<0.001$) decreased from baseline to follow-up (**Figure 2**).

Table 1 Change in characteristics of black and white men after 3 years.

	Black men			White men		
	Baseline	**Follow-up**	**P-values**	**Baseline**	**Follow-up**	**P-values**
N	89	89	-	91	91	-
Age (years)	43.2 ± 7.51	46.2 ± 7.51	-	46.4 ± 10.2	49.4 ± 10.2	-
Anthropometric measurements						
Body mass index (kg/m^2)	27.6 ± 5.59	28.3 ± 5.64	**<0.001**	29.3 ± 5.19	30.2 ± 5.12	**<0.001**
Biochemical analyses						
Glucose (mmol/l)	5.84 (4.50; 11.7)	5.81 (4.54; 11.4)	0.825	5.97 (5.00; 7.80)	4.56 (3.10; 7.24)	**<0.001**
HDL cholesterol (mmol/l)	1.06 ± 0.33	0.93 ± 0.34	**<0.001**	0.98 ± 0.28	0.85 ± 0.23	**<0.001**
Total cholesterol (mmol/l)	4.74 ± 1.19	4.63 ± 1.04	0.238	5.60 ± 1.21	4.21 ± 1.00	**<0.001**
Triglycerides (mmol/l)	1.48 (0.57; 3.67)	1.47 (0.63; 3.80)	0.876	1.36 (0.61; 3.16)	1.23 (0.62; 3.02)	**0.008**
Interleukin-6 (pg/ml)	1.04 (0.34; 3.57)	1.37 (0.50; 3.93)	**0.022**	0.88 (0.27; 2.90)	0.72 (0.24; 2.41)	0.063
Cardiovascular variables						
24hr SBP (mmHg)	138 ± 16.3	139 ± 17.6	0.298	129 ± 10.6	128 ± 10.7	0.398
24hr DBP (mmHg)	88.3 ± 10.8	87.6 ± 10.5	0.393	80.3 ± 7.18	79.3 ± 7.60	0.097
24hr PP (mmHg)	49.3 ± 8.28	51.3 ± 10.0	**0.003**	48.2 ± 6.94	48.4 ± 6.30	0.789
24hr MAP (mmHg)	105 ± 12.3	105 ± 12.4	0.994	96.4 ± 7.82	95.5 ± 8.23	0.153
Macrovascular measurements						
cIMT (mm)	0.71 ± 0.15	0.71 ± 0.14	0.983	0.70 ± 0.16	0.76 ± 0.17	**<0.001**
CSWA (mm)	14.7 ± 5.48	15.6 ± 3.77	0.056	15.4 ± 4.20	17.2 ± 4.60	**<0.001**
Microvascular measurements						
eGFR (ml/min/1.73m^2)	128 ± 27.2	187 ± 60.6	**<0.001**	93.8 ± 16.9	95.6 ± 23.1	0.382
Oxidative stress markers						
Reactive oxygen species (Units)[1]	83.7 ± 17.7	74.0 ± 21.5	**<0.001**	76.2 ± 15.4	75.0 ± 17.4	0.371
Total glutathione (µM)	937 ± 199	857 ± 211	**<0.001**	857 ± 180	1070 ± 363	**<0.001**

Glutathione peroxidase (nmol/min/ml)	35.2 ± 13.7	37.6 ± 17.1	0.375	35.1 ± 7.83	30.4 ± 6.71	**<0.001**
Glutathione reductase (nmol/min/ml)	7.68 (3.31; 16.8)	4.06 (0.76; 13.8)	**<0.001**	2.21 (0.22; 7.39)	3.17 (0.76; 27.5)	**0.050**
Superoxide dismutase (U/ml)	3.81 (0.62; 23.3)	2.65 (0.77; 7.74)	**0.025**	4.35 (2.12; 7.53)	2.11 (0.41; 5.67)	**<0.001**
γ-glutamyl transferase (U/l)	65.3 (27.5; 280)	58.1 (22.9; 242)	0.051	28.6 (11.0; 102)	27.3 (9.90; 121)	0.323
Lifestyle						
Cotinine (ng/ml)	32.6 ± 59.4	42.7 ± 92.3	0.123	34.3 ± 101	32.0 ± 99.1	0.592
Total energy expenditure (kcal/day)	2737 ± 829.27	3487 ± 1256.13	**<0.001**	3741 ± 2173	4066 ± 1990	0.307

Data expressed as arithmetic mean ± standard deviation or geometric mean with 5th and 95th percentile boundaries, % or n.

[1] Reactive oxygen species measured as serum peroxides where 1 unit = 1.0 mg/L H_2O_2. HDL, high-density lipoprotein; LDL, low-density lipoprotein; ABPM, ambulatory blood pressure; 24hr, 24 hours; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; cIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; eGFR, estimated glomerular filtration rate.

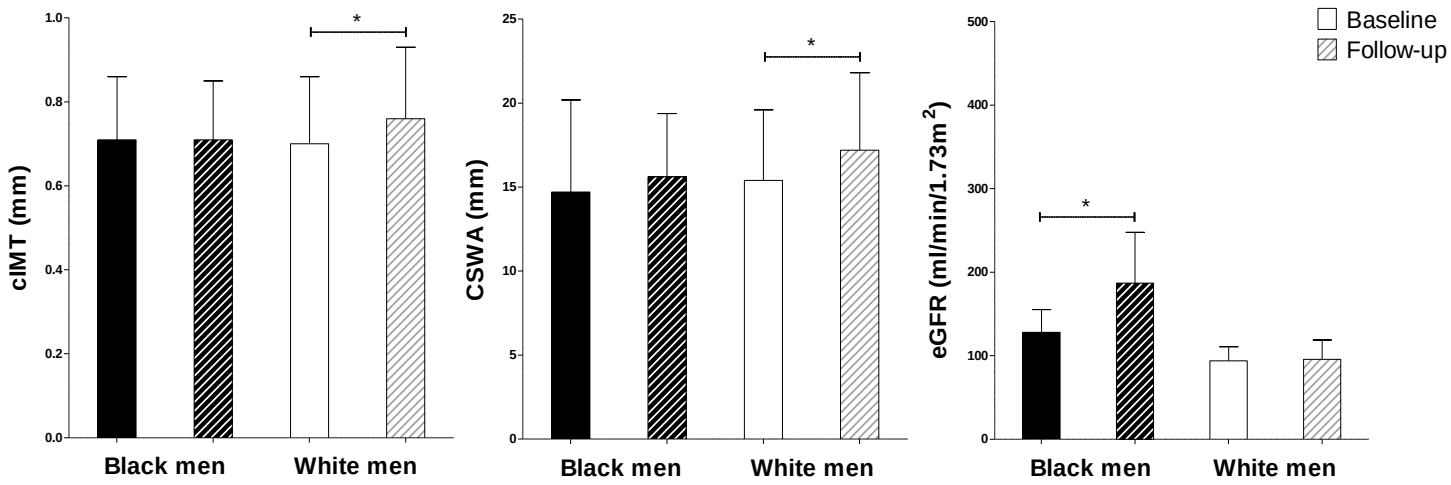

Figure 1: Baseline and 3 year follow-up markers of target organ damage in black and white men.
cIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; eGFR, estimated glomerular filtration rate.

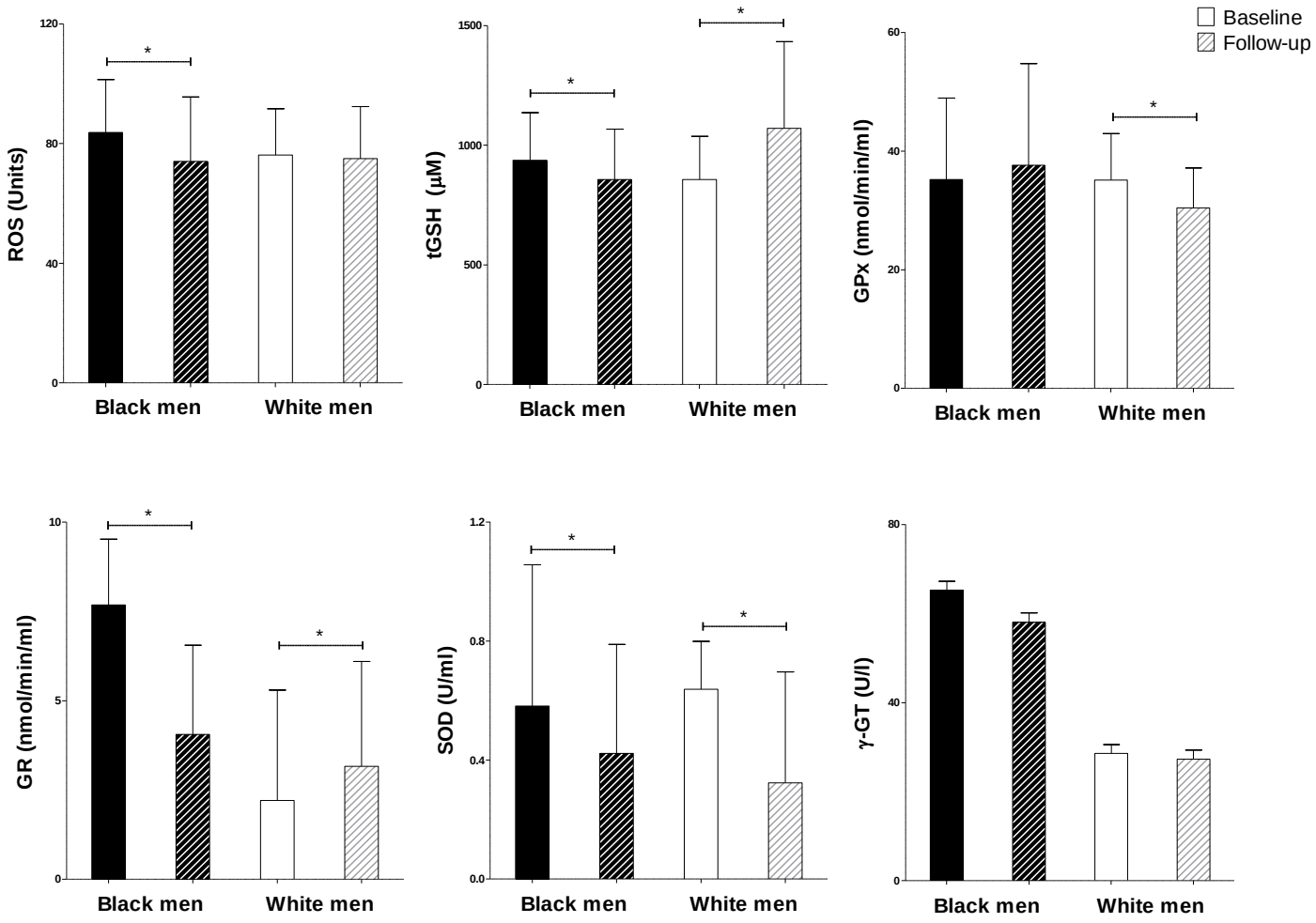

Figure 2: Baseline and 3 year follow-up oxidative stress markers in black and white men.
ROS, reactive oxygen species; tGSH, total glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; SOD, superoxide dismutase; γ-GT, γ-glutamyl transferase.

Regression analyses

Single regression analyses (**Figure 3**) revealed positive associations of cIMT and CSWA with change in ROS (CSWA: r=0.24, p=0.028) and SOD (borderline) (cIMT: r=0.20, p=0.076; CSWA: r=0.20, p=0.068) in black men. On the other hand, white men displayed negative associations of cIMT and CSWA with change in ROS (cIMT: r=-0.21, p=0.048; CSWA (borderline): r=-0.19, p=0.077). In white men these associations were not significant (cIMT: r=-0.12, p=0.299; CSWA: r=-0.13, p=0.228). However upon full adjustments in multiple regression analyses the association of CSWA and change in SOD reached significance (R^2=0.22; β=-0.22; p=0.042).

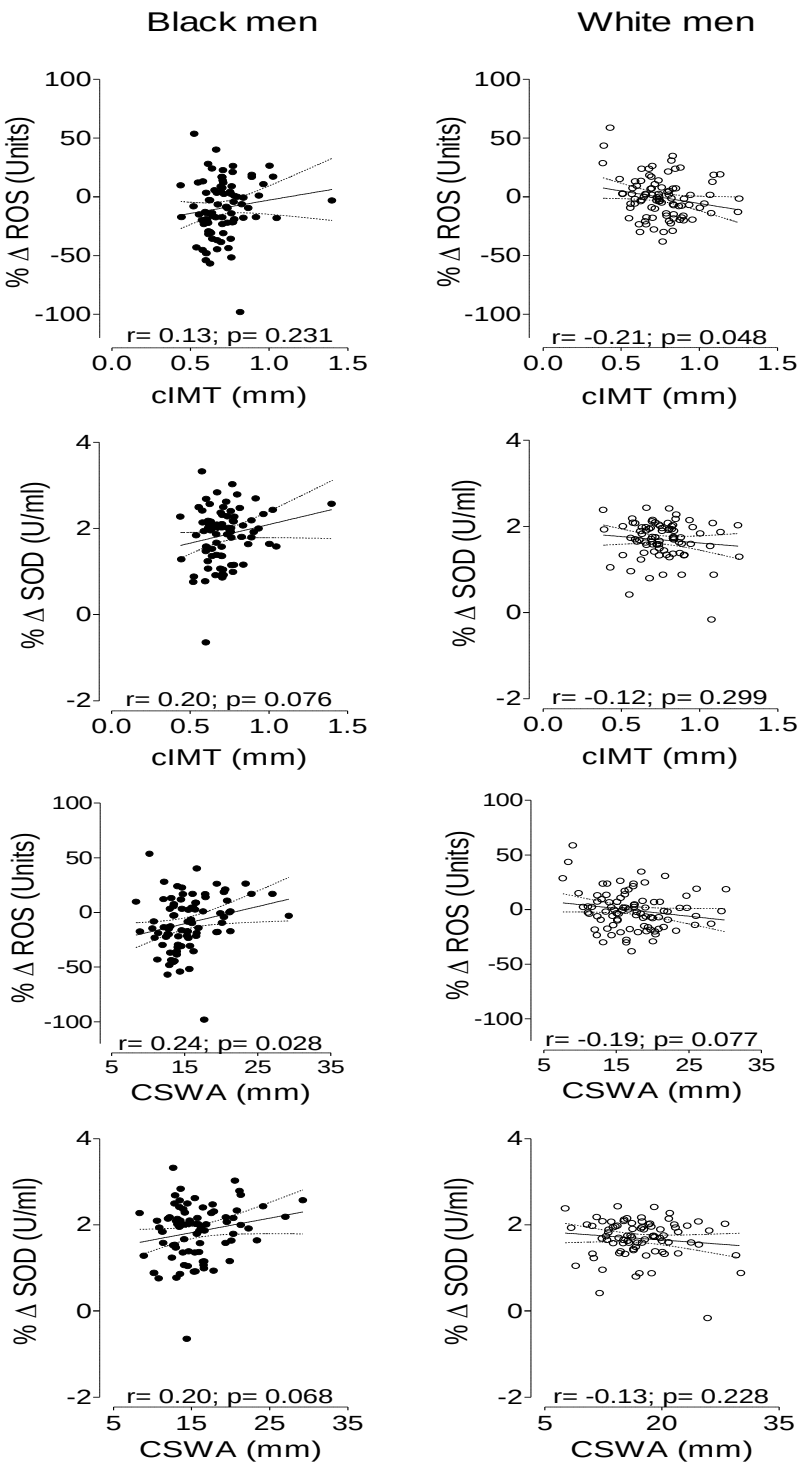

Figure 3: Unadjusted correlations of changes in ROS and SOD activity with cIMT and CSWA in black and white men.
% Δ, percentage change; ROS, reactive oxygen species; SOD, superoxide dismutase; cIMT, carotid intima-media thickness; CSWA, cross-sectional wall area.

Single (**Table S3**), partial (**Table 2**) and multiple regression analyses (**Table 3**) revealed positive associations of CSWA with change in ROS (R^2=0.04; β=0.28; p=0.017) and SOD activity (R^2=0.01; β=0.24; p=0.047) in black men. The negative association of CSWA with change in SOD activity (R^2=0.22; β=-0.22; p=0.042) became significant in white men after full adjustments were made.

In the white men, positive associations of eGFR were found in single, partial and multiple regression analyses with change in GPx activity (R^2=0.21; β=0.33; p=0.001) and change in GR activity (R^2=0.24; β=0.39; p<0.001) (**Table 4**).

Positive associations in the white men of 24hr DBP (R^2=0.07; β=0.32; p=0.006) and 24hr MAP (R^2=0.06; β=0.31; p=0.007) with increased GR activity were confirmed to be independent of various confounders (results not shown). All other associations indicated in single and partial regression analyses were no longer significant once full adjustments were made.

Sensitivity analyses

Multiple regression analyses of cIMT and CSWA were repeated after adding % Δ HDL cholesterol and total cholesterol separately as additional covariates into the models. Similar results were obtained in sensitivity analyses, while the additional lipid biomarkers did not significantly contribute to these models.

Table S3: Single regression analyses of percentage change in oxidative stress markers with cardiovascular variables after 3 years in black and white men.

	Black Men					
	% Δ ROS	***% Δ tGSH***	***% Δ GPx***	***% Δ GR***	***% Δ SOD***	***% Δ γ-GT***
24hr SBP	r=-0.10 p=0.378	r=0.08 p=0.466	r=0.06 p=0.565	r=-0.14 p=0.196	r=0.09 p=0.411	r=-0.10 p=0.343
24hr DBP	r=-0.05 p=0.622	r=0.14 p=0.197	r=0.04 p=0.720	r=-0.08 p=0.478	r=0.07 p=0.504	r=-0.17 p=0.109
24hr PP	r=-0.12 p=0.284	r=-0.003 p=0.976	r=0.07 p=0.527	r=-0.17 p=0.107	r=0.08 p=0.487	r=0.002 p=0.986
24hr MAP	r=-0.08 p=0.488	r=0.12 p=0.281	r=0.05 p=0.636	r=-0.11 p=0.312	r=0.09 p=0.444	r=-0.15 p=0.176
cIMT	r=0.13 p=0.231	r=-0.06 p=0.589	r=0.04 p=0.680	r=-0.14 p=0.180	r=0.20 p=0.076	r=0.04 p=0.715
CSWA	**r=0.24** **p=0.028**	r=-0.01 p=0.927	r=0.05 p=0.653	r=-0.18 p=0.097	r=0.20 p=0.068	r=-0.04 p=0.746
eGFR	r=0.07 p=0.550	r=-0.04 p=0.721	r=-0.06 p=0.559	r=-0.03 p=0.772	r=-0.19 p=0.085	r=0.0001 p=0.999
	White Men					
24hr SBP	r=-0.004 p=0.967	r=0.05 p=0.620	r=0.06 p=0.555	r=0.19 p=0.079	r=-0.09 p=0.410	r=0.03 p=0.777
24hr DBP	r=0.05 p=0.653	r=0.13 p=0.232	r=0.13 p=0.233	**r=0.27** **p=0.011**	r=0.01 p=0.912	r=0.01 p=0.930
24hr PP	r=-0.07 p=0.539	r=-0.06 p=0.550	r=-0.05 p=0.667	r=0.002 p=0.985	r=-0.17 p=0.115	r=0.04 p=0.707
24hr MAP	r=0.03 p=0.795	r=0.11 p=0.341	r=0.11 p=0.323	**r=0.25** **p=0.020**	r=-0.03 p=0.773	r=0.02 p=0.860
cIMT	**r=-0.21** **p=0.048**	r=-0.02 p=0.870	r=-0.03 p=0.744	r=-0.06 p=0.597	r=-0.12 p=0.299	r=-0.01 p=0.962
CSWA	r=-0.19 p=0.077	r=0.05 p=0.636	r=0.01 p=0.938	r=-0.05 p=0.622	r=-0.13 p=0.228	r=0.01 p=0.953
eGFR	r=-0.05 p=0.645	r=0.14 p=0.181	**r=0.33** **p=0.002**	**r=0.37** **p<0.001**	r=-0.07 p=0.543	r=-0.19 p=0.071

% Δ, percentage change; SBP; systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; cIMT, carotid intima-media thickness; CSWA, cross sectional wall area; eGFR, estimated glomerular filtration rate; ROS, reactive oxygen species; tGSH, total glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; SOD, superoxide dismutase; γ-GT, γ-glutamyl transferase.

Table 2: Partial regression analyses of percentage change in oxidative stress markers with cardiovascular variables after 3 years in black and white men.

	Black Men					
	% Δ ROS	***% Δ tGSH***	***% Δ GPx***	***% Δ GR***	***% Δ SOD***	***% Δ γ-GT***
24hr SBP	r=-0.14 p=0.244	r=0.07 p=0.544	r=0.05 p=0.646	r=-0.15 p=0.199	r=0.11 p=0.381	r=-0.07 p=0.568
24hr DBP	r=-0.11 p=0.370	r=0.13 p=0.260	r=0.03 p=0.813	r=-0.09 p=0.441	r=0.09 p=0.438	r=-0.13 p=0.267
24hr PP	r=-0.13 p=0.261	r=-0.01 p=0.955	r=0.06 p=0.581	r=-0.18 p=0.131	r=0.08 p=0.499	r=0.02 p=0.891
24hr MAP	r=-0.13 p=0.290	r=0.11 p=0.354	r=0.04 p=0.725	r=-0.12 p=0.296	r=0.10 p=0.393	r=-0.11 p=0.371
cIMT	r=0.12 p=0.285	r=-0.06 p=0.579	r=0.04 p=0.718	r=-0.13 p=0.216	r=0.21 p=0.065	r=0.05 p=0.633
CSWA	**r=0.22** **p=0.045**	r=-0.01 p=0.899	r=0.04 p=0.704	r=-0.17 p=0.117	**r=0.22** **p=0.053**	r=-0.02 p=0.889
eGFR	r=0.05 p=0.689	r=-0.05 p=0.643	r=-0.08 p=0.484	r=-0.04 p=0.695	r=-0.19 p=0.128	r=0.02 p=0.868
	White Men					
24hr SBP	r=0.01 p=0.896	r=0.05 p=0.629	r=0.07 p=0.547	r=0.19 p=0.086	r=-0.09 p=0.443	r=0.05 p=0.676
24hr DBP	r=0.08 p=0.451	r=0.13 p=0.243	r=0.14 p=0.223	**r=0.28** **p=0.012**	r=0.02 p=0.882	r=0.04 p=0.734
24hr PP	r=-0.08 p=0.497	r=-0.06 p=0.566	r=-0.05 p=0.669	r=0.001 p=0.991	r=-0.18 p=0.129	r=0.03 p=0.763
24hr MAP	r=0.06 p=0.604	r=0.10 p=0.354	r=0.11 p=0.314	**r=0.25** **p=0.023**	r=-0.03 p=0.809	r=0.04 p=0.696
cIMT	**r=-0.23** **p=0.034**	r=-0.02 p=0.868	r=-0.04 p=0.732	r=-0.06 p=0.595	r=-0.12 p=0.296	r=-0.02 p=0.893
CSWA	r=-0.20 p=0.063	r=0.05 p=0.642	r=0.01 p=0.947	r=-0.05 p=0.621	r=-0.14 p=0.227	r=-0.0001 p=0.999
eGFR	r=0.03 p=0.780	r=0.16 p=0.164	**r=0.37** **p=0.001**	**r=0.40** **p<0.001**	r=-0.06 p=0.608	r=-0.14 p=0.221

Partial regression analyses adjusted for percentage change in body mass index. Associations of cIMT and CSWA are additionally adjusted for % Δ 24hr SBP.

% Δ, percentage change; SBP; systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; cIMT, carotid intima-media thickness; CSWA, cross sectional wall area; eGFR, estimated glomerular filtration rate; ROS, reactive oxygen species; tGSH, total glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; SOD, superoxide dismutase; γ-GT, γ-glutamyl transferase.

Table 3: Summary of multiple regression analyses of follow-up CSWA with % Δ in oxidative stress markers.

	Black men (n=89)		**White men (n=91)**	
	CSWA (mm) β (95% CI)		*CSWA (mm) β (95% CI)*	
	Adjusted R^2=0.04	*P-values*	*Adjusted R^2=0.19*	*P-values*
% Δ ROS	**0.28 (0.06; 0.50)**	**0.017**	-0.10 (-0.31; 0.11)	0.349
% Δ Age	-0.12 (-0.33; 0.10)	0.303	**-0.44 (-0.64; -0.24)**	**<0.001**
% Δ BMI	0.10 (-0.16; 0.35)	0.453	0.08 (-0.13; 0.29)	0.460
% Δ TEE	-0.06 (-0.28; 0.17)	0.616	-0.01 (-0.21; 0.19)	0.907
% Δ Cotinine	-0.08 (-0.30; 0.15)	0.494	0.14 (-0.05; 0.34)	0.148
% Δ Glucose	0.09 (-0.15; 0.33)	0.471	-0.04 (-0.25; 0.17)	0.725
% Δ Triglycerides	-0.25 (-0.51; 0.01)	0.064	-0.14 (-0.35; 0.07)	0.183
% Δ Interleukin-6	-0.19 (-0.41; 0.04)	0.104	-0.03 (-0.23; 0.17)	0.776
% Δ 24hr SBP	-0.08 (-0.30; 0.14)	0.483	0.01 (-0.19; 0.21)	0.953
Δ SR alcohol use	0.11 (-0.12; 0.34)	0.355	0.01 (-0.18; 0.21)	0.908
	Adjusted R^2=0.01	*P-values*	*Adjusted R^2=0.22*	*P-values*
% Δ SOD	**0.24 (0.01; 0.46)**	**0.047**	**-0.22 (-0.42; -0.01)**	**0.042**
% Δ Age	-0.14 (-0.37; 0.09)	0.228	**-0.48 (-0.68; -0.28)**	**<0.001**
% Δ BMI	0.03 (-0.23; 0.29)	0.822	0.07 (-0.15; 0.28)	0.550
% Δ TEE	-0.03 (-0.26; 0.20)	0.804	-0.03 (-0.24; 0.17)	0.756
% Δ Cotinine	-0.03 (-0.26; 0.20)	0.810	0.14 (-0.06; 0.33)	0.181
% Δ Glucose	0.12 (-0.13; 0.37)	0.337	-0.06 (-0.28; 0.15)	0.571
% Δ Triglycerides	-0.23 (-0.49; 0.04)	0.106	-0.17 (-0.38; 0.04)	0.118
% Δ Interleukin-6	-0.16 (-0.38; 0.07)	0.175	-0.05 (-0.26; 0.15)	0.619
% Δ 24hr SBP	-0.14 (-0.36; 0.09)	0.234	0.04 (-0.17; 0.25)	0.704
Δ SR alcohol use	0.06 (-0.18; 0.29)	0.631	-0.01 (-0.21; 0.20)	0.960

Covariates included in each model included % Δ in the relevant oxidative stress marker, age, body mass index, total energy expenditure, cotinine, glucose, triglycerides, interleukin-6, 24hr systolic blood pressure and change in self-reported alcohol use.

% Δ, percentage change; Δ, change; CSWA, cross-sectional wall area; ROS, reactive oxygen species; SOD, superoxide dismutase; BMI, body mass index; TEE, total energy expenditure; 24hr, 24 hour; SBP, systolic blood pressure; SR, self-reported. .

Table 4: Summary of multiple regression analyses of follow-up eGFR with % Δ in oxidative stress markers.

	Black men (n=89)		**White men (n=91)**	
	eGFR (ml/min/1.73m²) β (95% CI)		*eGFR (ml/min/1.73m²) β (95% CI)*	
	Adjusted R^2=0.07	*P-values*	*Adjusted R^2=0.21*	*P-values*
% Δ GPx	-0.01 (-0.24; 0.21)	0.900	**0.33 (0.14; 0.52)**	**0.001**
% Δ Age	0.09 (-0.12; 0.30)	0.401	0.08 (-0.12; 0.27)	0.433
% Δ BMI	-0.17 (-0.42; 0.07)	0.168	**-0.33 (-0.53; -0.12)**	**0.002**
% Δ TEE	-0.04 (-0.26; 0.17)	0.697	-0.08 (-0.28; 0.12)	0.435
% Δ Cotinine	-0.10 (-0.32; 0.12)	0.372	0.03 (-0.16; 0.22)	0.767
% Δ Glucose	0.10 (-0.13; 0.33)	0.387	-0.09 (-0.3; 0.11)	0.381
% Δ Triglycerides	-0.18 (-0.43; 0.07)	0.167	-0.12 (-0.32; 0.08)	0.238
% Δ Interleukin-6	**-0.28 (-0.49; -0.07)**	**0.012**	-0.01 (-0.21; 0.19)	0.948
Δ SR alcohol use	0.08 (-0.15; 0.31)	0.494	0.18 (-0.01; 0.38)	0.066
	Adjusted R^2=0.08	*P-values*	*Adjusted R^2=0.24*	*P-values*
% Δ GR	0.11 (24.4; -24.1)	0.434	**0.39 (0.20; 0.59)**	**<0.001**
% Δ Age	0.11 (-9.38; 9.60)	0.324	0.08 (-0.11; 0.27)	0.413
% Δ BMI	0.12 (3.25; -3.00)	0.142	**-0.31 (-0.51; -0.11)**	**0.003**
% Δ TEE	0.11 (0.17; 0.05)	0.754	-0.13 (-0.33; 0.07)	0.202
% Δ Cotinine	0.11 (0.11; 0.11)	0.343	0.08 (-0.11; 0.27)	0.421
% Δ Glucose	0.12 (-146; 147)	0.395	-0.14 (-0.34; 0.06)	0.183
% Δ Triglycerides	0.13 (111; -111)	0.195	-0.11 (-0.31; 0.09)	0.293
% Δ Interleukin-6	**0.11 (70.8; -70.5)**	**0.009**	-0.06 (-0.26; 0.14)	0.571
Δ SR alcohol use	0.11 (-20.3; 20.5)	0.403	0.15 (-0.04; 0.34)	0.120

Covariates included in each model included % Δ in the relevant oxidative stress marker, age, body mass index, total energy expenditure, cotinine, glucose, triglycerides, interleukin-6 and change in self-reported alcohol use.
eGFR, estimated glomerular filtration rate; % Δ, percentage change; Δ, change; GPx, glutathione peroxidase; GR, glutathione reductase; BMI, body mass index; TEE, total energy expenditure; SR, self-reported.

Discussion

We aimed to investigate whether change in markers of oxidative stress over three years associate with follow-up measures of target organ damage in black and white South African participants.

In both black and white men, SOD activity (along with ROS in black men) decreased from baseline to follow-up, while CSWA remained similar in the black men but increased in the white men over time. Despite these similarities in oxidative stress profiles, CSWA was positively related to change in ROS and SOD in the black men, whereas an inverse association of CSWA with change in SOD was evident in the white men. The opposing results of CSWA with decreased SOD activity in the black and white groups of this study are surprising. Superoxide plays an important role in maintaining optimal nitric oxide availability, and in turn nitric oxide plays an important role within the vascular system to maintain vascular tone and to prevent the development of atherosclerosis.[10,29,30] Although there was a decrease in SOD in both groups, the decrease in the black group may not be to such an extent to affect CSWA. However, in the white group, SOD activity may have decreased to such an extent that it may result in insufficient inactivation of superoxide, lower nitric oxide availability and hence may in part explain the association with vascular deterioration (increased CSWA over time).[31,32] While a diminished SOD activity may play a role in carotid wall thickening, previous results in men have found that serum SOD activity increases in direct relation to the pathological progression of plaque formation as a marker of heightened oxidative stress in the carotid artery.[33] This suggests a need for SOD activity to be highly adaptable to superoxide levels in order to prevent vascular remodelling.

Despite no change in mean eGFR, a measure of renal microvascular health, eGFR at follow-up associated positively with GPx and GR activity in white men only. Studies have confirmed an inverse relationship between oxidative stress and eGFR, as shown by increased oxidative stress markers and decreased antioxidant enzyme activity with advancing stages of chronic kidney disease.[17,34-37] However, eGFR was maintained in white men over time despite diminished GPx activity.

This may suggest that this group may currently be in a steady physiological state, but positive associations of markers of the glutathione system with eGFR in white men may suggest that further fluctuations of the glutathione system may impact negatively on renal function in this group. Interestingly, the black men displayed a mean eGFR above 150 ml/min/1.73m^2 at follow-up phase,[34,38,39] which is a well-documented predictor for glomerular hypertension and albuminuria, and has also been shown to occur during states of oxidative stress.[38,40,41] However, the lack of associations between renal function and oxidative stress in the black men of our study uncover the possible role of alternative confounding factors, such as early vascular aging, in the deterioration of the renal microvascular function in this group.[42]

This study has to be interpreted within the context of its strengths and limitations. Although our study population was based on participants from the Potchefstroom area in the North West Province of South Africa, it may not be indicative of the population as a whole. While this was a prospective study, our results are based on associations and causality cannot be inferred. Although our results were consistent after multiple adjustments, we cannot exclude any unknown interactions that may play a role in the development of deteriorated cardiovascular function and structure. However, to the best of our knowledge, this is the first study investigating the associations of changes in oxidative stress with cardiovascular variables in black and white cohorts over a 3-year period. Our study was well planned and executed under strict conditions in a fully-equipped research facility, allowing us to dig deeper into the possible mechanisms involving oxidative stress in promoting target organ damage over time.

In conclusion, the inverse association of CSWA with SOD activity in white men highlights the atherogenic effects of oxidative stress (as reflected by diminished SOD activity) in the macrovasculature. Associations of CSWA with ROS and SOD activity in black men, and associations of eGFR with the glutathione system in white men suggest a role for adequate antioxidant enzyme activity in delaying target organ damage in these groups.

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Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF do not accept any liability in regard.

Declaration of interest

The authors have no conflicts of interest to disclose. Any opinions expressed and conclusions arrived at, are those of the authors alone.

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CHAPTER 6:

General findings and final conclusions

1. Introduction

This chapter presents a summarized interpretation of the main findings of the manuscripts within this study. The central aim of this study was to investigate the association between markers of oxidative stress and cardiovascular function and structure in the understudied black and white South Africans of various age groups. Our results shed light on the association of oxidative stress with cardiovascular risk in a vulnerable black South African population. A younger and older population, as well as a baseline and follow-up phase of the older population was used to ascertain the effect in various stages of life and to evaluate the effect of changes in oxidative stress on cardiovascular function and structure over time.

In this chapter, original hypotheses are discerned and results of the three manuscripts are interpreted and compared with existing literature. Conclusions are drawn based on the results and recommendations made for future research in this field.

2. Summary of main findings and comparison to relevant literature

The main findings within each manuscript of this study (Chapters 3, 4 and 5) are reported as follows:

2.1. Manuscript 1, published in the Journal of Human Hypertension:

The relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population: the African-PREDICT study

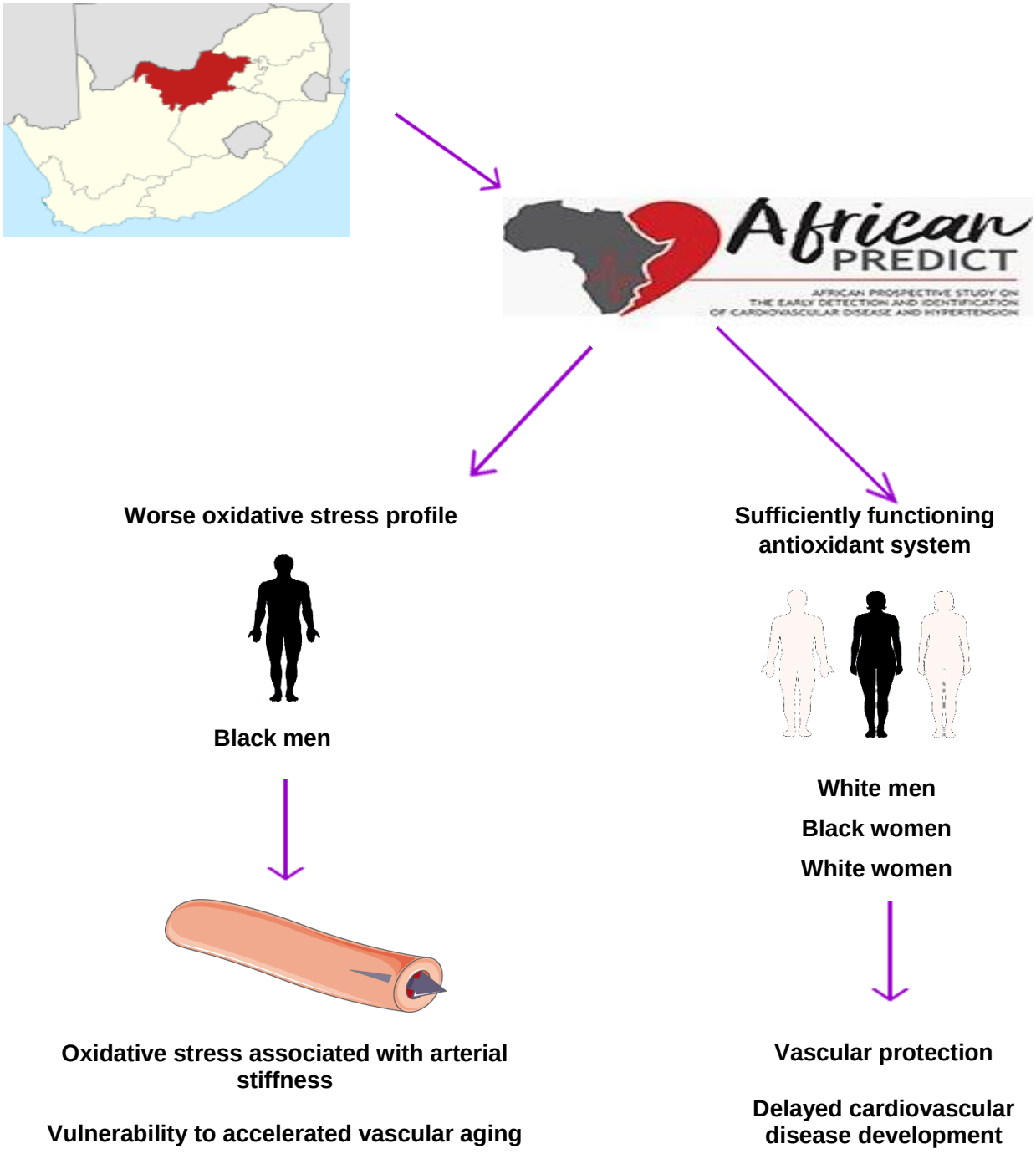


Figure 1: The relationship of cardiovascular structure and function with antioxidant enzyme activity in young, healthy black and white South Africans.

In the first manuscript, young (aged 20-30 years), apparently healthy black and white South Africans as part of the African-PREDICT study were examined. This was a cross-sectional study, which aimed to investigate the relationship of cardiovascular structure (carotid intima-media thickness (cIMT)) and function (ambulatory blood pressure) with antioxidant enzyme activity (glutathione peroxidase (GPx) and glutathione reductase (GR)) in 89 black men, 78 white men, 105 black women and 124 white women. In the first instance, antioxidant enzyme activity and cardiovascular function and structure were compared between racial groups of the African-PREDICT study. There after it was determined if associations between antioxidant enzyme activities and cardiovascular function and structure exist in these groups.

Hypothesis 1: *Antioxidant enzyme activities will be lower while measures of cIMT and ambulatory blood pressure will be higher in black compared to white participants.*

It is a well-accepted notion that South Africa is heavily burdened by hypertension and cardiovascular disease, especially in urban black groups that are found to be more vulnerable to advanced vascular compromise.[1-4] Studies examining racial differences in oxidative stress profiles confirmed higher levels of oxidative stress in African-Americans when compared to white Americans,[5] independent of metabolic syndrome, inflammation and cardiovascular disease risk factors.[6] Interestingly, another American study examining the genetic heritability of oxidative stress (using familial correlations) found black subjects had lower heritable hydrogen peroxide production than their white counterparts, suggesting a strong influence of alternate factors on their redox status.[7] While information in the South African population is limited, oxidative stress has been investigated in older cohorts, also confirming that black South Africans have worse oxidative stress profiles than white South Africans.[8-12]

In this study, worse oxidative stress profiles were visible in black groups, with higher reactive oxygen species (ROS), lower total antioxidant status and lower GPx antioxidant enzyme activity when compared to white groups. However, the only differences in cardiovascular measurements between black and white groups were slightly lower 24hour systolic blood pressure and 24hour pulse pressure in black men when compared to white men. These results may then be explained by their young, healthy nature since the inclusion criteria for the African-PREDICT study ensured that participants were between the ages of 20 and 30 years with a normotensive (<140/90 mmHg) clinic brachial blood pressure at time of the screening phase.[13] Even though brachial blood pressure is still a valid blood pressure assessment method, evidence has shown that central blood pressure and masked hypertension (defined as normotensive clinic but hypertensive out-of-office blood pressure) may be better predictors of cardiovascular risk.[13-17] Despite adhering to the inclusion criteria, this may not be a true reflection of the cardiovascular risk in black participants. Considering evidence of higher central SBP and a high prevalence of masked hypertension that has previously been found in black participants of this cohort, this may indicate a risk of developing cardiovascular disease from a younger age.[13,18-20]

The lower antioxidant enzyme activities but lack of worse cardiovascular measurements in black participants leads us to partially accept the first hypothesis of this manuscript.

Hypothesis 2: *Antioxidant enzyme activities will be inversely associated with cIMT and ambulatory blood pressure in both racial groups.*

With oxidative stress shown to play a distinct role in cardiovascular disease development,[21-23] it is not surprising then that numerous studies confirm the validity of diminished antioxidant capacity as predictors for cardiovascular events.[24-27]

Results in a European population with coronary artery disease showed an inverse association between GPx activity and risk for cardiovascular events.[24] Similarly, results in an American population with heart failure showed that a diminished antioxidant capacity (in the form of high-density lipoprotein arylesterase activity) predicts a heightened risk for cardiovascular events.[25] In American and European populations the impact of thiols on cardiovascular risk was also investigated, showing that diminished thiol antioxidants predict the development of atherosclerosis and complications from myocardial infarction.[26,27]

Increased pulse pressure, a measure of arterial stiffness, is associated with advancing age and is a useful predictor for cardiovascular events.[28-30] A study performed in an older American cohort revealed earlier carotid arterial stiffness in black Americans when compared to white Americans.[31] In the South African population, previous results in older black groups have linked their unfavorable oxidative stress profile with altered haemostasis,[8] early vascular aging (decreased Windkessel arterial compliance and increased total peripheral resistance),[32] hypertension development,[11,12] and atherosclerosis.[11,33] In this study we found an inverse association of ambulatory pulse pressure (24hr PP) with GPx activity in black men, which further highlights the possible role of oxidative stress in vascular aging in this group. White men showed positive associations of 24hr PP with GR activity and of carotid intima-media thickness with GPx activity, as well as a negative association of ambulatory diastolic blood pressure with GR activity. In women, both black and white groups showed positive associations of ambulatory systolic blood pressure and mean arterial pressure with GR activity. Black women also displayed a positive association of cIMT with GPx activity. The lack of evidence of oxidative stress or blood pressure abnormalities in these three remaining groups may point to a protective role of a currently sufficient antioxidant enzyme activity in the prevention of hypertension and atherosclerosis development.

Findings in our study were inconsistent, with cardiovascular measures associating with different oxidative stress markers across the various groups. However, the results in the black men of this study show a link between oxidative stress and 24hr PP, and therefore we can partially accept this hypothesis.

2.2. Manuscript 2, published in the journal Blood Pressure:

Cardiovascular reactivity and oxidative stress in young and older adults: The African-PREDICT and SABPA studies.

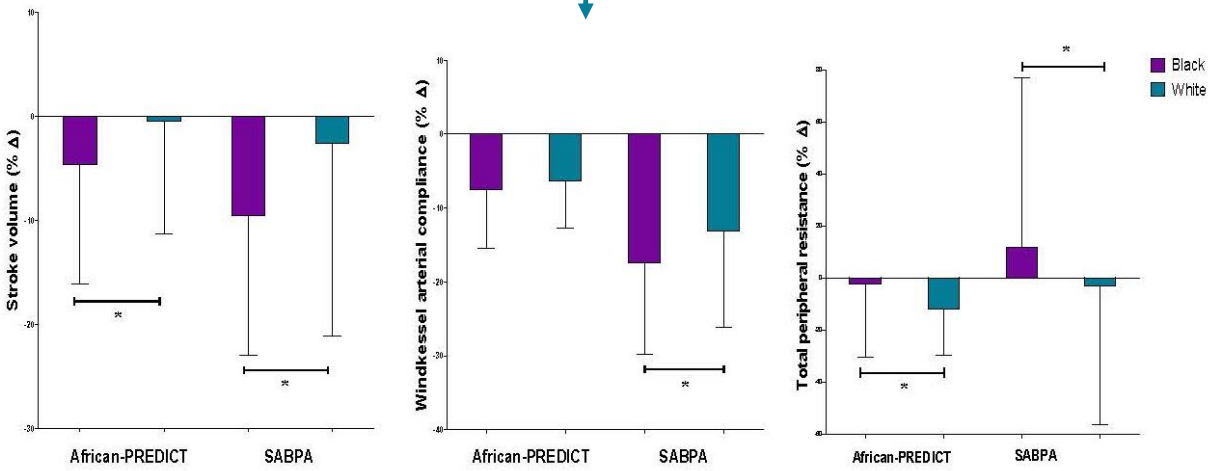
African-PREDICT

SABPA

Red
Green
Blue

Acute stress:
Color - word conflict test

Black groups:
⬆response to stress



Black:
Oxidative stress associated with worse cardiovascular reactivity

Upregulation of the glutathione system to correct unfavorable cardiovascular responses

White:
Cardiovascular reactivity associated with ROS and its determinants

Regulatory role of ROS and SOD to maintain vascular tone during stress

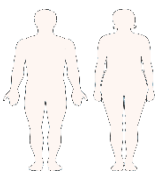


Figure 2: The link between cardiovascular reactivity and oxidative stress markers during the color-word conflict test in older and younger cohorts.

In the second manuscript, we examined young (mean age of 25.0 years) and older (mean age of 44.7 years) black and white South Africans as part of the African-PREDICT (191 black and 196 white participants) and the SABPA studies (200 black and 209 white participants). This was a cross-sectional study, which aimed to investigate differences in cardiovascular responses to the color-word conflict test between younger and older racial groups. We also aimed to investigate whether any relationships exist between oxidative stress markers and cardiovascular reactivity in these groups. In the first instance, we compared oxidative stress markers (GPx, GR, superoxide dismutase (SOD), γ-glutamyl transferase (γ-GT), total glutathione (tGSH) and ROS) and cardiovascular reactivity to acute stress in younger and older racial groups. Secondly, we investigated relationships between oxidative stress markers and change in cardiovascular reactivity to stress in these cohorts. Lastly, we wanted to evaluate if these relationships were age specific.

Hypothesis 1: *Black participants will have worse oxidative stress profiles and higher cardiovascular responses to stress than white participants.*

The color-word conflict test is a form of acute stress that involves exposing participants to a color-word written in an incongruent color to the word itself, thus eliciting a sympathetic nervous response.[34,35] There is mounting evidence showing a relation between endothelial dysfunction, oxidative stress and acute stress.[34,36] Whether this is through impaired vascular homeostasis, increased shear stress or elevated inflammation, these mechanisms provide a possible link between acute stress, atherosclerosis and cardiovascular disease such as hypertension.[34,36]

Older and younger black groups of our study showed higher ROS, γ-GT activity and GR activity (only in the SABPA study) with lower GPx activity along with a greater cardiovascular reactivity during the color-word conflict test than their white counterparts.

In the younger black participants, a larger decrease in stroke volume and smaller decrease in total peripheral resistance was noted compared to their white counterparts. The older black participants experienced greater increases in diastolic blood pressure, mean arterial pressure and total peripheral resistance along with larger decreases in Windkessel arterial compliance and stroke volume than older white participants.

With South Africans having a high prevalence of hypertension and cardiovascular disease risk,[2] and younger black Africans already predisposed to vascular compromise,[3] the possible influence of oxidative stress on cardiovascular reactivity is of vital importance. This is due to the fact that the daily psychological distress, as one would expect with urbanization, has been shown to enhance sympathetic activation and play a role in future hypertension development.[17,37] Research related to cardiovascular reactivity has shown that black participants both in South Africa[38-40] and elsewhere[41,42] have exacerbated stress responses when compared to white participants, which is in itself a risk factor for cardiovascular disease development.[43-45]

The first hypothesis pertaining to this manuscript is accepted as black participants of both the African-PREDICT and the SABPA studies had worse oxidative stress profiles and cardiovascular responses to acute stress than their white counterparts.

Hypothesis 2: *Adverse relationships exist between oxidative stress and cardiovascular reactivity in younger and older groups.*

While acute stress is able to hamper endothelial function and promote oxidative stress, oxidative stress in turn is also able to further diminish endothelial function.[23,34,46-48] Both the endothelial dysfunction as well as an elevated cardiovascular response accompanying acute stress have been shown as risk factors for cardiovascular disease development.[49-52]

No associations were found in young black participants of this study. In young white participants, positive associations of ROS with increases in blood pressure were found, as well as positive associations of superoxide dismutase activity with decreased Windkessel arterial compliance after acute stress administration. In the older black group, γ-GT associated positively with increases in blood pressure, but negatively with decreases in stroke volume (SV) and Windkessel arterial compliance. In the same group, an increase in total peripheral resistance was borderline negatively associated with tGSH, while decreased SV associated positively with GPx activity. In the older white group, increased blood pressures were inversely associated with SOD, while a decrease in SV associated positively with tGSH but negatively with SOD activity.

Findings in this study reveal significant links between oxidative stress and unfavorable cardiovascular responses to stress in older black groups, with results in white groups pointing to a regulatory role for ROS in normal vascular responsiveness to stress.

Based on these results, hypothesis 2 is only partially accepted since there were no associations present in young black groups, and physiological vascular responsiveness in white groups.

Hypothesis 3: *All relationships are dependent of age.*

Age, oxidative stress and cardiovascular disease form a vicious cycle, with age-related increases in ROS production and decreases in antioxidant enzyme activity worsening cardiovascular function and structure, which is also exacerbated by advancing age.[53,54] The heightened oxidative stress, arterial stiffness and an elevated sympathetic nervous system activity accompanying advancing age is also known to enhance one's cardiovascular reactivity in response to stress.[50,55,56]

Even though older individuals may be better equipped at handling daily emotional stress, evidence still points to an elevated cardiovascular reactivity to acute stress in older individuals.[55,57-59] Prospective results in an elderly (63 years), predominantly white Scottish population showed that over a period of 16 years, participants with heightened systolic and diastolic blood pressure reactivity to acute psychological stress are at greater risk for cardiovascular related mortality.[60] Results from an American research team also confirmed an age-related increase in systolic blood pressure reactivity to acute stress,[56] probably due to age-related increases in cardiac output and total peripheral resistance.[61]

The absence of associations between oxidative stress and cardiovascular reactivity in young black participants versus the confirmed associations of heightened cardiovascular responses to stress with a worse oxidative stress profile in older black groups suggests an age-related difference within the black population. However, associations of cardiovascular reactivity with ROS and its important regulating enzyme SOD were found in both white groups independent of age.

Based on our results, the third hypothesis is partially accepted since there is a clear age-related disparity in associations of oxidative stress markers with cardiovascular reactivity in black populations, but not in white populations.

2.3. Manuscript 3, submitted to the journal Hypertension Research:

Three-year change in oxidative stress markers is linked to target organ damage in black and white men: The SABPA study.

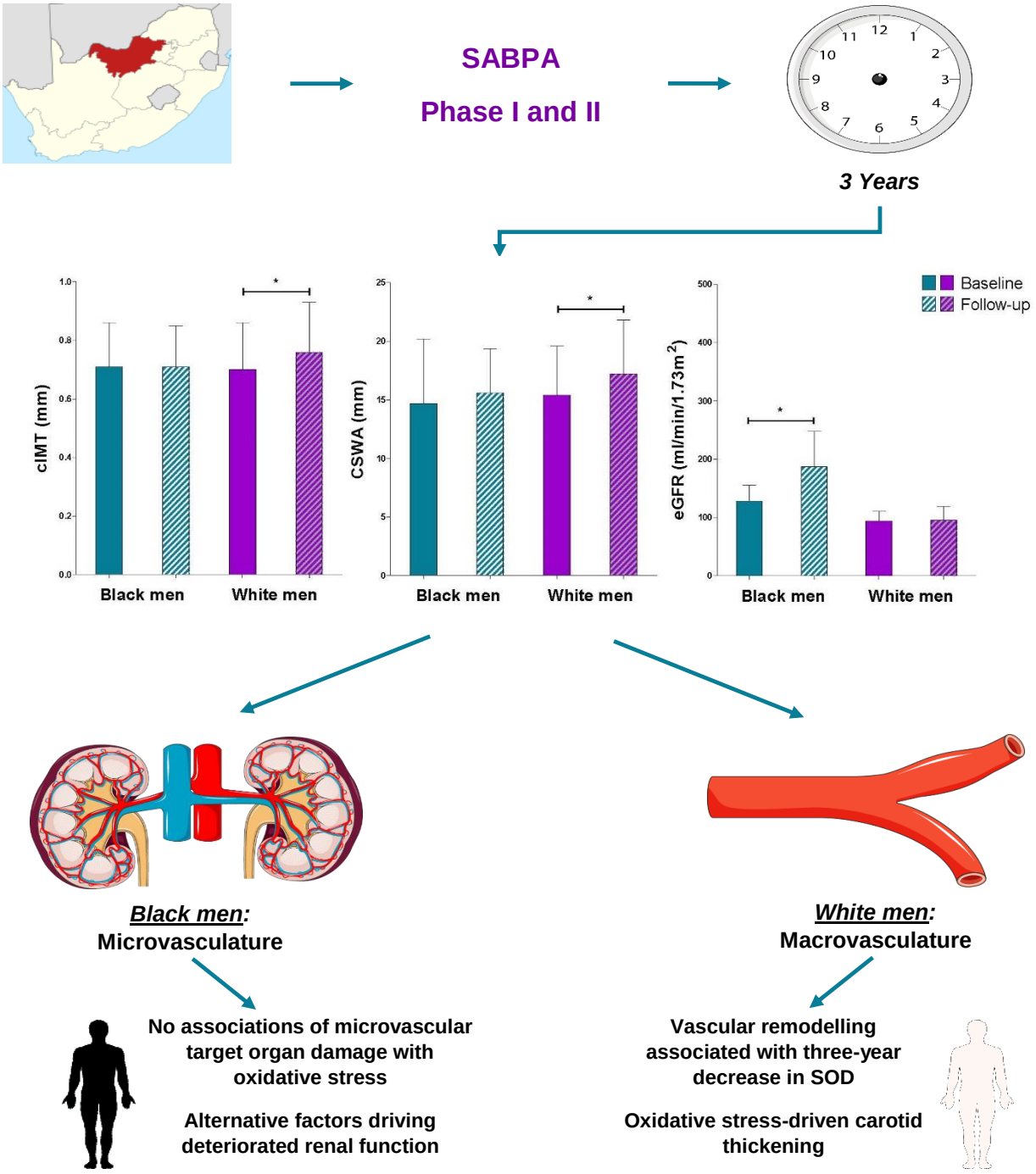


Figure 3: The prospective associations of 3-year changes in oxidative stress with target organ damage in black and white men.

The final manuscript was based on a longitudinal study in older (aged 20-65 years) black and white South African men and women over 3 years as part of the SABPA study. We aimed to investigate whether 3-year changes in oxidative stress related to target organ damage in 89 black men, 91 white men, 84 black women and 95 white women that participated in both phases of the SABPA study. In the first instance, we investigated changes in oxidative stress and cardiovascular markers over the 3-year period in black and white participants. We also investigated whether changes related to follow-up measures of target organ damage (vascular and renal deterioration) in black and white cohorts.

Hypothesis 1: *Black participants have a worse oxidative stress profile and more advanced deterioration in target organ damage (cIMT, cross-sectional wall area (CSWA) and estimated glomerular filtration rate) than white participants.*

Previous results from the SABPA study indicated higher blood pressure, carotid thickening and arterial stiffness in black participants when compared to whites.[8,12,32,33,62] Oxidative stress in this population has also been investigated, showing worse oxidative stress profiles in black groups.[8,10-12,63] While limited, one longitudinal study in this population showed greater increases in systolic and diastolic blood pressure, greater use of hypertensive medication and a greater prevalence of diagnosed heart disease in black groups after 3 years.[64]

Both black men and women of this study showed similar oxidative stress profiles with a decrease in ROS, tGSH, GR activity, SOD activity and γ-GT activity over 3 years. Changes in target organ damage revealed an increase in cIMT and CSWA in both white groups and an increase in glomerular filtration rate in both black groups. Based on these results we can partially accept the first hypothesis of this manuscript, since ROS decreased while macrovascular measures (cIMT and CSWA) remained similar over the study period in black groups.

Hypothesis 2: *Three-year change in oxidative stress markers associate adversely with target organ damage in both racial groups.*

Oxidative stress has been shown to play an active role in cardiovascular pathologies such as atherosclerosis, hypertension, coronary artery disease and renal dysfunction.[23,65-68] There are numerous studies describing relationships between increased oxidative stress and target organ damage.

In European populations, it was found that oxidative stress progressively increases with advancing stages of chronic kidney disease,[69] while data on diabetic patients suggested a role for diminished GPx activity in the susceptibility to renal disease development in black groups.[70] Cross-sectional results on another European population highlighted the role of increased xanthine oxidase activity (a ROS producing enzyme) and diminished SOD activity in endothelial dysfunction accompanying heart failure.[71] Prospective results in patients with coronary artery disease have shown that those presenting with high oxidative stress are at risk for cardiovascular events,[72] and twice as likely to die within 5 years.[73] This finding was independent of age and other cardiovascular risk factors.[73] With cross-sectional associations already found between oxidative stress and worse cardiovascular function and structure in this black South African cohort,[11,12] it is important to evaluate the prospective association of changes in oxidative stress with target organ damage in these groups.

No associations between follow-up cardiovascular measurements and three-year change in oxidative stress were found in women. While it is commonly known that estrogen performs a cardio-protective role in women, it is also shown that women are less susceptible to oxidative stress than males, likely due to the antioxidant effect of estrogen.[74]

While lower glomerular filtration rate has already been well established as a risk factor for renal disease and cardiovascular events, evidence has shown that glomerular hyperfiltration is an equally important predictor for adverse cardiovascular outcomes. [75]

Glomerular hyperfiltration, defined as an estimated glomerular filtration rate above 150 ml/min/1.73m^2, has been shown to develop during a combination of risk factors including pre-hypertension, oxidative stress and sympathetic nervous system activation.[76]

Interestingly, no associations of changes in oxidative stress with the elevated glomerular filtration rate in black men were found in our study. However, many participants were already hypertensive and the possibility of other confounding factors such early vascular aging and sympathetic nervous system activation may explain these results. The increased thickening of the carotid wall in white men associated negatively with three-year change in SOD activity, suggesting a role for oxidative stress in macrovascular remodeling of the carotid arteries in this group.

Although links between oxidative stress and target organ damage were only suggested in white groups, the absence of any associations with the apparent renal dysfunction in black men leads us to partially reject this hypothesis.

3. Strengths, limitations, chance and confounding

It is also vital to evaluate the factors that may have confounded our results throughout this study. Relevant issues are detailed as follows.

We made use of baseline data of the African-PREDICT and SABPA studies for manuscript one and two and hence due to the cross-sectional design, causality cannot be inferred from the results obtained in these populations.

Longitudinal data was used to formulate manuscript three and while this was a prospective study, our results are based on associations and causality cannot be inferred. However, this study does provide the first glance at longitudinal changes in oxidative stress and the associations with the cardiovascular system in a black and white South African population.

Both the African-PREDICT and the SABPA studies were well planned and conducted under strictly controlled conditions in a fully equipped research facility.

Both the African-PREDICT and the SABPA study recruited participants from the North-West Province of South Africa, thus our results cannot be representative of the oxidative stress and cardiovascular profiles of the entire South African population. With the African-PREDICT study including healthy young participants of various socio-economic backgrounds and the SABPA study including older participants of similar socio-economic statuses but already presenting with hypertension, direct comparisons between these studies were not made. During our study, the reduced vs. oxidized glutathione (GSH:GSSG) ratio was unknown, since total glutathione levels were measured. Measurements of ROS throughout all three manuscripts were made using an indirect biomarker and we cannot discern the sources of ROS generation in this study. While it may be challenging to measure clinical ROS accurately,[77] our study was strengthened by the use of numerous oxidative stress related markers throughout all three manuscripts.

It is also of vital importance to take the possibility of chance findings into account. Although our results across all three manuscripts were consistent after multiple adjustments with different multivariable regression models, we cannot exclude any unknown interactions that may play a role in these findings.

Since the SABPA study did not exclude patients using some forms of medication, sensitivity analyses were performed in order to confirm associations while eliminating the effect thereof on our results (as in manuscript two). In the same cohort, multiple regression analyses of SV with oxidative stress markers were repeated after substituting stroke volume with stroke volume index to take body surface area into account. Also in the SABPA study (manuscript 3), the confounding effect of cholesterol on carotid plaque formation was added as additional covariates into multiple regression models in sensitivity analyses.

All sensitivity analyses showed unchanged results, with the exception of a borderline significant association of TPR with tGSH in the black group of manuscript two becoming significant after including anti-hypertension medication into the model, possibly explained by the ability of antihypertensive medication to increase the availability of tGSH.[78]

Adjustments for potential confounding factors including age, sex, socio-economic score, body mass index, total energy expenditure, self-reported alcohol use, γ-GT, cotinine, glucose, interleukin-6, triglycerides, cortisol and 24hour SBP could have under- or overestimated the associations between oxidative stress and cardiovascular parameters.

The potential influence of factors that were not measured in either of the studies may have on our results cannot be ignored, including genetic components and undiagnosed infections and diseases. While the impact of dietary influences goes beyond the scope of this study, the fact that participants of the SABPA study received the same standardized meals and the African-PREDICT study collected data of participants' diet allows for further research in this regard. Lastly, the presence of a statistically significant result cannot conclusively be interpreted as physiologically significant and vice versa.

This study afforded the opportunity to evaluate cross-sectional and longitudinal associations of oxidative stress with cardiovascular structure and function across age, sex and racial groups. Both studies consisted of homogenous samples of relatively large size. The biggest strength of this study is a bridge in the gap of knowledge into the impact of oxidative stress on an already vulnerable cardiovascular system in our unique South African context.

4. Recommendations

Oxidative stress remains an important topic, both globally and in the understudied South African population. The following are recommendations for future research in the field of oxidative stress and cardiovascular function and structure:

- The ability of oxidative stress to predict cardiovascular disease development needs to be established in black and white South Africans.
- Intervention studies evaluating baseline and follow-up changes in oxidative stress in response to antioxidant administration are needed to confirm causality in cardiovascular deterioration of younger and older South African populations.
- Whether associations of oxidative stress with ambulatory blood pressure and cIMT manifest as cardiovascular disease in the young black and white South African populations remain to be confirmed in longitudinal studies and in future, this may be possible in the African-PREDICT study.
- Additional markers of oxidative stress (such as the GSH:GSSG ratio), target organ damage (such as left ventricular mass) and additional methods of ROS measurements (such as those described by Griendling *et al.*[77] including electron paramagnetic resonance spectroscopy, redox-active biosensors or the Amplex Red assay) should be investigated to provide a more comprehensive picture of oxidative stress status and the effect thereof on the cardiovascular system.
- Additional confounding factors not investigated in this study should be investigated with regards to cardiovascular function and structure in black groups, such as heightened sympathetic nervous system activation, arterial stiffness and masked hypertension.

- The lack of association between oxidative stress and renal dysfunction in the older black populations remains to be explained. Despite showing signs of renal hyperfiltration, the cause thereof is unknown at this stage.
- The lack of associations between changes in oxidative stress and target organ damage in women needs to be examined in further longitudinal studies.
- Since our participants were obtained solely from the North-West Province, research is needed in a larger representation of the entire population in order to confirm the relevance of our results in the full South African context.

5. Final conclusions

This study aimed to investigate the association between markers of oxidative stress and cardiovascular function and structure in the understudied black and white South Africans of various age groups. Black South Africans are already predisposed to cardiovascular risks from a younger age than their white counterparts. Considering that oxidative stress is a well-established role player in various mechanisms involved in the development of cardiovascular pathologies, it is of vital importance to understand the impact thereof in the vulnerable black population.

Collectively, the results of this study show a strong link between the diminished antioxidant capacity and enhanced ROS production that accompanies oxidative stress in the development of arterial stiffness, exaggerated stress responses and target organ damage. This study emphasizes that oxidative stress and the adverse effects it may have on cardiovascular function and structure, could increase the risk of cardiovascular disease development, both in younger and older South Africans. This is especially important in the black population which is already predisposed to early vascular compromise and cardiovascular risk.

In order to address the impact of oxidative stress on the current burden of cardiovascular disease in our country, research is needed to identify possible strategies, such as individualised antioxidant therapies, that may assist in maintaining a stable and efficiently functioning antioxidant system, especially among black populations.

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ANNEXURES


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Annexure A: Letter of approval from the Health Research Ethics Committee

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Web: http://www.nwu.ac.za

Faculty of Health Sciences Ethics Office for Research, Training and Support

Tel: 018 299 2092
Email:minrie.greeff@nwu.ac.za

Prof CMC Mels
HART

31 October 2018

Dear Prof Mels

FEEDBACK ON HREC ANNUAL MONITORING REPORT: NWU-00036-07-A6

We would like to thank you for submitting the annual monitoring report for your project entitled, ***"OLD: The relationship between markers of oxidative stress, inflammation and oxidative stress in bi-ethnic population NEW: Exploring the link between oxidative stress and the vasculature in a bi-ethnic population"***, to the Health Research Ethics Committee (HREC) in a timely manner. Please find below the decision of the HREC committee regarding the continuation of your project.

Classification	Mark with X	Comment	
Clarification			
Completion (Final report)			
Suspended			
Continuation	X	**Date of next monitoring report:**	31 October 2019
Termination			

Should you have any further queries, please feel free to contact Ms Jamey Henry at your earliest convenience (E-mail: Ethics-HRECMonitoring@nwu.ac.za; Tel: 018 299 2266). We wish you well in your future endeavours.

Yours sincerely

Prof Minrie Greeff
Head of Health Sciences Ethics
Office for Research, Training and Support

Prof Wayne Towers
Chairperson: HREC

Current details: (20536690) G:\My Drive\9. Research and Postgraduate Education\9.1.5.5 HREC Monitoring\NWU-000036-07-A6\9.1.5.5.4_Cont_NWU-00036-07-A6_31-10-2018.docm
31 October 2018

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Annexure B: Declaration of language editing

DECLARATION

I, C Vorster (ID: 710924 0034 084), Language editor and Translator and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of a thesis (for the qualification PhD in Cardiovascular Physiology), written by Ms C Myburgh from the North-West University (student number 22286233).

Title of the thesis: Exploring the link between oxidative stress and the vasculature in a bi-ethnic population

C Vorster — 3 April 2019

C Vorster — Date

cvlanguage.editing@gmail.com

Annexure C: Turnitin report

12076341:V5_-_Exploring_the_link_between_oxidative_stress_and_the_vas..ethnic_population_-_Turn-it-in.docx

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27 Aletta E Schutte. "Blood pressure variability is significantly associated with ECG left ventricular mass in normotensive Africans: The SABPA Study", Hypertension Research, 10/2011 <1%
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28 Cynthia A. van Deventer, Jeremie Z. Lindeque, Peet J. Jansen van Rensburg, Leoné Malan et al. "Use of metabolomics to elucidate the metabolic perturbation associated with hypertension in a black South African male cohort: the SABPA study", Journal of the American Society of Hypertension, 2015 <1%
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Annexure D: Published version of manuscript 1

Journal of Human Hypertension
https://doi.org/10.1038/s41371-018-0044-3

ARTICLE

The relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population: the African-PREDICT study

Caitlynd Myburgh[1,2] · Hugo W. Huisman[1,3] · Catharina M. C. Mels[1,3]

Received: 8 November 2017 / Revised: 24 January 2018 / Accepted: 5 February 2018

Abstract

Oxidative stress has been implicated in the development of hypertension, arterial stiffness and atherosclerosis. Optimal functioning of the enzymatic antioxidant system is central to prevent increased oxidative stress and its consequences. We aimed to investigate the relationships of ambulatory blood pressure and carotid intima-media thickness with enzyme activities of the glutathione cycle in 396 young, black and white South Africans of the African-PREDICT study. Ambulatory blood pressure and carotid intima-media thickness were measured and glutathione peroxidase and glutathione reductase activities were analyzed. Black participants had higher reactive oxygen species (men: $p = 0.019$; women: borderline $p = 0.064$) and total glutathione (both $p < 0.001$), but lower glutathione peroxidase activity and total antioxidant status (all $p < 0.001$). In black men, ambulatory pulse pressure was negatively associated with glutathione peroxidase activity ($R^2 = 0.19$; $\beta = -0.25$; $p = 0.06$). Black and white women displayed positive associations of ambulatory systolic blood pressure (black: $R^2 = 0.25$; $\beta = 0.21$; $p = 0.048$; white: $R^2 = 0.44$; $\beta = 0.18$; $p = 0.016$) with glutathione reductase activity, whereas white men displayed a positive association of ambulatory pulse pressure with glutathione reductase activity ($R^2 = 0.25$; $\beta = 0.29$; $p = 0.01$). The lower glutathione peroxidase activity and total antioxidant status, the higher reactive oxygen species, as well as the negative association between ambulatory pulse pressure and glutathione peroxidase activity in the black men suggest that oxidative stress may be associated with early vascular changes in this group. In the other three groups, the positive associations of blood pressure with glutathione reductase activity suggest a possible role for adequate glutathione reductase activity in preventing or delaying the development of hypertension.

Introduction

Oxidative stress has been implicated in the development of hypertension [1–3], which is well documented as an increasing epidemic, especially among developing countries [4–6]. South Africa is no exception, with urban black populations exhibiting an alarmingly high prevalence of hypertension [6–8]. In addition, black South Africans are more prone to early vascular changes when compared with their white counterparts [9–11].

When oxidative stress increases, endothelial dysfunction may occur as a result of increased production of reactive oxygen species (ROS), decreased availability of anti-oxidants, decreased antioxidant enzyme activity (such as glutathione peroxidase (GPx) and glutathione reductase (GR)) and the inactivation of nitric oxide (NO) [12, 13]. This may in turn be linked with arterial stiffness, carotid wall thickening and cardiovascular disease development, all of which deteriorate with ageing [12–14].

Previous results in black South Africans found that increased oxidative stress relates to elevated blood pressure [15, 16], vascular wall thickness [16, 17] and arterial stiff-ness [11, 15]. However, the aforementioned results were obtained in a population with an age range of 20–65 years and some participants already presented with hypertension. It is therefore not clear if these factors confounded the

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41371-018-0044-3) contains supplementary material, which is available to authorized users.

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Published online: 12 March 2018

previous reported results and we therefore aimed to compare oxidative stress profiles and explore relationships of ambulatory blood pressure and carotid intima-media thickness (cIMT) with enzyme activities of the glutathione cycle —GPx and GR in young, healthy black and white South African men and women.

Methods

Study population

This study is embedded in the ongoing African PRospective study on the Early Detection and Identification of Cardiovascular disease and hyperTension (African-PREDICT). More detail on the study population and study procedures are indicated in Appendix A. The aim of African-PREDICT is to recruit and follow participants over a period of 10–20 years in order to understand the pathological changes accompanying cardiovascular disease development and to identify novel markers related to early cardiovascular disease development in this population. Participants are continually being recruited from the Potchefstroom and surrounding areas of the North-West Province of South Africa by the use of active field workers, through their workplace, or by means of local newspaper and radio advertisements.

Participants of self-reported Indian, Asian or mixed origin ethnicity, who are not permanent residents of Potchefstroom or surrounding areas (or who do not plan regular trips to the area) and who are unable to read or understand English were excluded from the study. Participants who had elevated glucose levels (>5.6 mmol/L or confirmed glycated hemoglobin ≥6.5%), who were HIV infected and those who presented with a fever on the research day (ear temperature >37.5 °C) were also excluded. Further exclusion criteria included being previously diagnosed with (or took medication for) type 1 or 2 diabetes mellitus, liver disease, cancer, tuberculosis, renal disease or cardiovascular diseases (hypertension, stroke, angina pectoris or myocardial infarction), who recently had surgery or trauma (within the past 3 months) and who was pregnant or breastfeeding.

Study procedures

After the initial screening, apparently healthy (in line with the exclusion criteria) normotensive or pre-hypertensive (systolic blood pressure (SBP) <140 and diastolic blood pressure (DBP) <90 mmHg) black and white men and women between the ages of 20 and 30 years are invited to participate in the African-PREDICT study.

This study makes use of cross-sectional data obtained from the first 403 participants of the African-PREDICT study. Participants with incomplete antioxidant enzyme activity data ($N = 7$) were excluded. In accordance with the aims of this study, the remaining 396 were divided into black men ($N = 89$), white men ($N = 78$), black women ($N = 105$) and white women ($N = 124$).

The African-PREDICT study complies with all the applicable requirements of the Declaration of Helsinki for the investigation of human participants. The study was also approved by the Health Research Ethics Committee of the North-West University. All procedures were explained to the participants and written informed consent was obtained before the measurements commenced.

Questionnaires

A researcher assisted each participant in completing a general health questionnaire in order to obtain information on socioeconomic status, as well as alcohol and tobacco usage. The socioeconomic score was derived from three categories within the general health questionnaire, namely skills level, education and household income. Each category was awarded points in order to determine whether the participant fell into a low, middle or high socioeconomic class.

Anthropometric and physical activity measurements

The anthropometric measurements were performed using standardized methods [18] and included body height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany), body weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany) and waist circumference (Lufkin Steel Anthropometric Tape (W606PM), Lufkin, Apex, MD, USA). Body mass index (BMI) was calculated as kg/m^2. Each participant was fitted with an ActiHeart physical activity monitor (CamNtech Ltd, England, UK), which recorded total energy expenditure (TEE) for a maximum of 7 days.

Cardiovascular measurements

Each participant was equipped with a CardioXplore® 24-h ambulatory blood pressure monitoring (ABPM) apparatus (CE0120, Meditech, Budapest, Hungary) on the non-dominant arm. This device was programmed to record measurements every 30 min during the day (from 0600 till 2200 hours) and every hour at night (from 2200 till 0600 hours).

Carotid intima media thickness, as a measure of arterial wall alterations and cardiovascular disease development [19], was measured using B-mode ultrasonography (General Electric Vivid E9, GE Vingmed Ultrasound A/S, Horten, Norway) according to the Mannheim Consensus [19]. A maximal 10 mm segment with good image quality was chosen for offline analysis to assess the near and far

wall. In this study, the far wall measurements of cIMT were used. All measurements were performed and analyzed by a single experienced observer using the Artery Measurement Systems Software (Tomas Gustavsson, Sweden) for dedicated analyses, and all participants were in a relaxed state during the measurements.

Biochemical measurements

Participants were required to fast for 8 h prior to the commencement of measurements. A blood sample was obtained from each participant by a registered nurse from the antebrachial vein branches using a winged infusion set and syringes. Standard procedures were used for the preparation of serum and plasma, after which all samples were immediately aliquoted into cryovials in the on-site laboratory. Samples were then stored in bio-freezers at −80 °C until they were analyzed, as antioxidant enzymes are shown to remain stable for long-term storage at this temperature [20].

Antioxidant enzymes including GR, GPx and superoxide dismutase (SOD), as well as total antioxidant status was measured using assay kits (Randox Co., Antrim, United Kingdom) and the Cobas Integra 400 plus (Roche, Basel Switzerland). Total glutathione (tGSH) was measured using whole blood samples with a Synergy HT microplate reader (BioTek, Winooski, VT, USA) (Kit: BIOXYTECH GSH/GSSG-412, Oxis International Inc., CA, USA). Serum peroxides, as an indicator of ROS, were determined using a high-throughput spectrophotometric assay and analyzed on a Synergy HT microplate reader (BioTek, Winooski, VT, USA). ROS is reported in units, where 1 mg H_2O_2/L is equivalent to one unit [21].

Triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), γ-glutamyl transferase (γ-GT) and uric acid were measured in serum, whereas glucose levels were measured in sodium fluoride plasma and 24-h urinary sodium was measured in urine, using the Cobas Integra 400 plus (Roche, Basel, Switzerland). A chemiluminescence method (Immulite, Siemens, Erlangen, Germany) was used to determine serum cotinine levels. Serum interleukin-6 (IL-6) was measured using a high sensitivity Quantikine enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN USA) analyzed on a Synergy HT hybrid microplate reader (BioTek, Winooski, VT, USA). White blood cells were measured in whole blood using the Coulter AcT5 diff OV Hematology analyzer (Beckman Coulter, Brea, CA, US).

Statistical analyses

Statistica version 13.2 (Dell, TX, USA) was used to perform the statistical analyses of this study. The central tendency and spread for normally distributed variables are expressed as arithmetic mean and standard deviation. Normal distribution of data was analyzed based on visual inspection of symmetry of the bell-shaped histogram curves and achieved by logarithmically transforming variables, which were not normally distributed (triglycerides, IL-6, GPx, SOD and γ-GT). The central tendency and spread of logarithmically transformed variables were expressed as the geometric mean and the 5th and 95th percentile intervals. Continuous variables were compared using independent *T*-tests and the assumptions of homogeneity of variance were tested (Table S1). Mean values of cIMT were adjusted for 24-h SBP and comparisons were made with an analysis of covariance (ANCOVA). Single regression analyses were performed to evaluate unadjusted associations between cardiovascular variables and oxidative stress-related variables. Thereafter, partial correlations were performed to evaluate the associations between variables while adjusting for BMI and TEE. Measurements of cIMT were additionally adjusted for 24-h SBP. Multiple regression analyses were performed in order to evaluate the independent associations between cardiovascular variables (blood pressure and cIMT) and oxidative stress markers (GPx and GR activity) in the four groups. Covariates entered in the models included the antioxidant enzyme activity, age, socioeconomic score, BMI, TEE, γ-GT, cotinine, glucose, IL-6 and triglycerides. Models with cIMT as dependent variable were additionally adjusted for 24-h SBP. The selection of covariates included in the models were based on the strongest unadjusted correlations of covariates with cardiovascular variables and oxidative stress markers when considering the following: BMI, waist circumference, 24-h SBP, 24-h mean arterial pressure (MAP), total cholesterol, HDL-C, LDL-C, triglycerides, C-reactive protein (CRP), IL-6, tumor necrosis factor-α (TNF-α), glucose, glycated hemoglobin (HbA1c), TEE, γ-GT and cotinine.

In a post hoc analyses, the achieved power (1-β) were determined for multiple regression models with 10 variables, for a group with a sample size of $n = 89$ for black men (1-β = 0.99) and $n = 78$ for white men (1-β = 0.99) and $n = 105$ for black women (1-β = 0.99) and $n = 124$ for white women (1-β = 0.99) (G*power v3.1.9.2) [22].

Results

Characteristics of the study population

In the black men, the 24-h SBP ($p = 0.008$) and 24-h pulse pressure (PP; $p = 0.003$) were slightly lower when compared with the white men (Table 1). No significant differences in cardiovascular measurements were noted when comparing black and white women. Biochemical analyses revealed significantly lower glucose and triglycerides in

Table 1 Characteristics of black and white men and women

	Black men	White men	*p*-Values	Black women	White women	*p*-Values
N	89	78		105	124	
Age (years)	24 ± 3	26 ± 3	0.015	24 ± 4	26 ± 3	0.003
Anthropometric measurements						
Body mass index (kg/m^2)	21.8 ± 3.37	28.0 ± 5.71	<0.001	26.5 ± 5.85	24.7 ± 5.42	0.015
Body height (cm)	170 ± 6.17	179 ± 6.15	<0.001	159 ± 6.65	167 ± 6.28	<0.001
Body weight (kg)	63.3 ± 10.7	89.9 ± 19.0	<0.001	67.0 ± 15.1	69.0 ± 15.7	0.330
Cardiovascular measurements						
24-h systolic pressure(mmHg)	121 ± 9.12	124 ± 6.66	0.008	114 ± 8.33	113 ± 8.41	0.624
24-h diastolic pressure(mmHg)	70.6 ± 6.89	70.6 ± 6.08	0.934	69.0 ± 5.70	68.2 ± 5.71	0.288
24-h pulse pressure (mmHg)	50.4 ± 6.88	53.6 ± 7.01	0.003	44.7 ± 5.60	45.0 ± 5.68	0.728
24-h mean arterial pressure (mmHg)	87.4 ± 6.99	88.5 ± 5.34	0.231	83.9 ± 6.15	83.2 ± 6.17	0.380
cIMT left far wall (mm)[a]	0.45 ± 0.01	0.45 ± 0.01	0.653	0.43 ± 0.01	0.44 ± 0.01	0.209
Biochemical analyses						
Glucose (mmol/l)	3.82 ± 0.97	5.01 ± 0.71	<0.001	3.91 ± 0.74	4.53 ± 0.86	<0.001
Triglycerides (mmol/l)	0.84 (0.42; 1.90)	1.08 (0.57; 2.18)	<0.001	0.71 (0.40; 1.17)	0.90 (0.43; 2.13)	<0.001
HDL cholesterol (mmol/L)	1.35 ± 0.34	1.12 ± 0.27	<0.001	1.21 ± 0.32	1.61 ± 0.40	<0.001
LDL cholesterol (mmol/L)	2.36 ± 0.84	3.28 ± 1.05	<0.001	2.50 ± 0.81	2.90 ± 0.83	<0.001
Interleukin-6 (pg/ml)	0.80 (0.36; 3.21)	0.77 (0.27; 2.84)	0.729	1.17 (0.47; 3.43)	0.70 (0.27; 2.32)	<0.001
Gamma glutamyl transferase (U/l)	29.2 (13.0; 107)	24.9 (10.8; 65.4)	0.111	23.3 (10.5; 57.4)	14.0 (6.90; 38.4)	<0.001
24-h urinary sodium (mmol/L)	120 ± 46.8	120 ± 43.6	0.983	96.3 ± 52.2	112 ± 50.4	0.054
White blood cells (×10^9/L)	5.12 ± 1.56	5.73 ± 1.65	0.016	5.68 ± 1.80	5.80 ± 1.82	0.632
Oxidative stress markers						
Reactive oxygen species (units)[b]	156 ± 46.2	140 ± 40.9	0.019	230 ± 70.7	207 ± 101	0.064
Total glutathione (μM)	1258 ± 334	913 ± 263	<0.001	1205 ± 271	942 ± 236	<0.001
Glutathione peroxidase (nmol/min/ml)	18.3 (15.0; 20.5)	19.8 (17.4; 21.6)	<0.001	18.5 (14.8; 20.8)	19.9 (18.0; 21.9)	<0.001
Glutathione reductase (nmol/min/ml)	52.5 ± 17.2	57.1 ± 16.0	0.078	52.9 ± 17.2	53.7 ± 15.9	0.719
Superoxide dismutase (U/ml)	2.38 (1.58; 3.54)	2.12 (1.13; 3.72)	0.048	2.28 (1.02; 3.79)	2.24 (1.40; 3.89)	0.707
Total antioxidant status (mmol/l)	1.39 ± 0.11	1.57 ± 0.12	<0.001	1.27 ± 0.12	1.40 ± 0.12	<0.001
Uric acid (μmol/L)	331 ± 72.6	422 ± 76.6	<0.001	268 ± 62.2	300 ± 63.3	<0.001
Lifestyle markers						
Cotinine (ng/ml)	119 ± 143	49.7 ± 107	<0.001	20.3 ± 55.2	19.6 ± 61.5	0.936
Total energy expenditure (kcal/day)	2232 ± 273	2679 ± 428	<0.001	2132 ± 428	2190 ± 447	0.340
Socioeconomic score (*n*)	17.2 ± 6.09	25.0 ± 5.74	<0.001	17.6 ± 4.98	25.4 ± 4.43	<0.001

Data expressed as arithmetic mean ± standard deviation or geometric mean with 5th and 95th percentile boundaries or n

cIMT carotid intima-media thickness, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

[a] cIMT ANCOVA (adjusted for 24-h SBP) expressed as adjusted mean ± standard error

[b] Reactive oxygen species measured as serum peroxides where 1 unit = 1.0 mg/L H_2O_2

both black men and women (all $p < 0.001$). In the black women, IL-6 and γGT levels (both $p < 0.001$) were significantly higher when compared with their white counterparts. Regarding the oxidative stress profile, black men and women had higher ROS (black men: $p = 0.019$; black women: borderline $p = 0.064$) and tGSH (both $p < 0.001$), but lower GPx activity (both $p < 0.001$), total antioxidant status (both $p < 0.001$) and uric acid (both $p < 0.001$) when compared with their white counterparts. Additionally, SOD activity ($p = 0.048$) was higher in the black men than in the white men. Lifestyle biomarkers indicated that black men had higher cotinine levels and lower TEE (both $p < 0.001$) when compared with white men. Additionally, both black men and women had significantly lower socioeconomic scores (both $p < 0.001$) when compared with the white groups.

Single, partial and multivariate analyses

Correlations between cardiovascular variables and antioxidant enzymes are depicted in Table 2 (black and white men) and Table 3 (black and white women).

In black men, single and partial regression analyses revealed a negative association of 24-h PP ($r = -0.30$, $p = 0.006$) with GPx activity, whereas a positive association was found between left far wall cIMT and GPx activity in white men ($r = 0.25$, $p = 0.031$). Both of these associations remained significant after full adjustments were made in subsequent multiple regression analyses: 24-h PP with GPx activity ($R^2 = 0.19$; $\beta = -0.25$; $p = 0.026$) in black men (Table 4) and left far wall cIMT with GPx activity ($R^2 = 0.06$; $\beta = 0.24$; $p = 0.044$) in white men (data not shown). White men also displayed a positive association with 24-h PP ($r = 0.33$, $p = 0.004$) and a negative association of 24-h DBP ($r = -0.30$, $p = 0.010$) with GR activity, both of which remained significant after full adjustments were made (Fig. 1): 24-h DBP ($R^2 = 0.26$; $\beta = -0.24$; $p = 0.037$), 24-h PP ($R^2 = 0.25$; $\beta = 0.29$; $p = 0.012$).

Single and partial regression analyses in both groups of women revealed positive associations of blood pressure measurements with GR activity. This includes 24-h SBP (black women: $r = 0.25$, $p = 0.019$; white women: $r = 0.20$, $p = 0.033$), 24-h DBP (black women: $r = 0.24$, $p = 0.027$; white women: $r = 0.17$, $p = 0.08$ (borderline)) and 24-h MAP (black women: $r = 0.27$, $p = 0.013$; white women: $r = 0.19$, $p = 0.045$). The independent positive associations of 24-h SBP (black women: $R^2 = 0.25$; $\beta = 0.21$; $p = 0.048$; white women: $R^2 = 0.44$; $\beta = 0.18$; $p = 0.016$) and 24-h MAP (black women: $R^2 = 0.20$; $\beta = 0.23$; $p = 0.034$; white women: $R^2 = 0.32$; $\beta = 0.19$; $p = 0.026$) with GR activity were established in multiple regression analyses (Fig. 2). Meanwhile, the positive associations of 24-h DBP with GR activity were borderline significant in both the groups. Also in the black women, a positive association of left far wall cIMT with GPx activity ($r = 0.23$, $p = 0.032$) was confirmed after full adjustments ($R^2 = 0.11$; $\beta = 0.23$; $p = 0.026$) (data not shown).

Discussion

We compared oxidative stress profiles and explored relationships of ambulatory blood pressure and cIMT with enzyme activities of the glutathione cycle in young, healthy black and white South African men and women.

In the black groups, the oxidative stress profiles were worse (as indicated by higher ROS, as well as lower total antioxidant status and lower activity of the glutathione cycle enzyme, GPx). The cardiovascular profiles were similar in the black and white groups, except for the slightly lower 24-h SBP and 24-h PP in the young black men. In the same group, 24-h PP was inversely related with GPx activity, suggesting a possible role for oxidative stress in early vascular changes. An increase in PP, a measure of arterial stiffness, is associated with ageing and is also a predictor of future cardiovascular events [23–25] Oxidative stress is prevented by the proper functioning of various antioxidant enzymes in order to balance ROS production [26], and the association of PP with diminished GPx activity in the black men may indicate that GPx, especially plays an important regulatory role to balance oxidative stress and prevent early vascular changes and eventually arterial stiffness [23]. The involvement of oxidative stress in early vascular changes, before the onset of hypertension, was previously suggested in the sympathetic activity and ambulatory blood pressure in Africans (SABPA) study, in which a marker of lipid oxidation was linked to increased total peripheral resistance and decreased arterial compliance in black men and women. However, the population group was older with a wide age spread (age 47.2 ± 7.97) [11]. Similarly, in a group of healthy subjects (age 42.6 ± 14 years) it was found that high cystine levels (as a marker of oxidative stress) were associated with arterial stiffness, again suggesting an important role for oxidative stress in the loss of arterial elasticity and early vascular changes [27]. Our results further contribute to this, as we have shown that not only do these associations already exist in a young group of black men (24.3 ± 3.19 years), but they are independent of lifestyle risk factors such as adiposity, high alcohol intake (as indicated by γ-GT levels), smoking (cotinine) and even socioeconomic status. As this link was found only in the black men, these results suggest a degree of cardiovascular vulnerability for early vascular changes and arterial stiffness [10, 11].

In the three remaining groups (white men, black women and white women), associations of blood pressure with GR activity were indicated. In the white men, 24-h DBP was inversely related whereas 24-h PP was positively related to GR activity, whereas in both the black and white women 24-h SBP, 24-h DBP and 24-h MAP were positively associated with GR activity. In the antioxidant system, superoxide anions are scavenged by the SOD antioxidant enzyme; where after the by-product (hydrogen peroxide) is converted to water and oxygen by both GPx and catalase. The antioxidant enzyme GR is responsible to maintain redox balance and adequate availability of reduced glutathione, which is the substrate for the GPx enzyme [26]. When comparing these groups with their ethnic counterparts, the white men displayed a more favorable oxidative stress profile than the black men without any overt cardiovascular disease such as hypertension or atherosclerosis [28, 29]. In the women, the cardiovascular profile was similar in both groups with blood pressure measurements in the normal ranges [29], but the black women had a slightly worse oxidative stress profile than the white women. Thus, due to

Table 2 Single and partial regression analyses of cardiovascular variables with antioxidant enzyme activities in black and white men

	Black men ($n = 89$)						White men ($n = 78$)					
	GR		GPx		SOD		GR		GPx		SOD	
	Single	Partial	Single	Partial	Single	Partial	Single	Partial	Single	Partial	Single	Partial
24-h SBP (mmHg)	$r = 0.10$ $p = 0.36$	$r = 0.11$ $p = 0.35$	$r = -0.002$ $p = 0.98$	$r = -0.05$ $p = 0.66$	$r = -0.08$ $p = 0.46$	$r = -0.01$ $p = 0.93$	$r = 0.21$ $p = 0.06$	$r = 0.09$ $p = 0.44$	$r = 0.02$ $p = 0.89$	$r = 0.02$ $p = 0.84$	$r = 0.10$ $p = 0.37$	$r = 0.01$ $p = 0.94$
24-h DBP (mmHg)	$r = 0.14$ $p = 0.19$	$r = 0.15$ $p = 0.20$	**$r = 0.26$** **$p = 0.013$**	**$r = 0.25$** **$p = 0.026$**	$r = -0.11$ $p = 0.32$	$r = -0.07$ $p = 0.57$	$r = -0.19$ $p = 0.10$	**$r = -0.30$** **$p = 0.010$**	$r = -0.03$ $p = 0.82$	$r = -0.02$ $p = 0.84$	$r = -0.11$ $p = 0.33$	$r = -0.16$ $p = 0.16$
24-h PP (mmHg)	$r = -0.01$ $p = 0.92$	$r = -0.01$ $p = 0.91$	**$r = -0.27$** **$p = 0.012$**	**$r = -0.30$** **$p = 0.006$**	$r = 0.004$ $p = 0.97$	$r = 0.05$ $p = 0.65$	**$r = 0.36$** **$p = 0.001$**	**$r = 0.33$** **$p = 0.004$**	$r = 0.04$ $p = 0.74$	$r = 0.04$ $p = 0.72$	$r = 0.19$ $p = 0.09$	$r = 0.15$ $p = 0.21$
24-h MAP (mmHg)	$r = 0.14$ $p = 0.21$	$r = 0.15$ $p = 0.21$	$r = 0.17$ $p = 0.11$	$r = 0.15$ $p = 0.19$	$r = -0.11$ $p = 0.33$	$r = -0.05$ $p = 0.67$	$r = -0.06$ $p = 0.63$	$r = -0.20$ $p = 0.08$	$r = -0.01$ $p = 0.91$	$r = -0.01$ $p = 0.94$	$r = -0.04$ $p = 0.70$	$r = -0.13$ $p = 0.28$
cIMT left far wall (mm)[a]	$r = 0.10$ $p = 0.37$	$r = 0.10$ $p = 0.40$	$r = -0.001$ $p = 0.99$	$r = 0.02$ $p = 0.86$	$r = 0.09$ $p = 0.42$	$r = 0.06$ $p = 0.62$	$r = -0.14$ $p = 0.24$	$r = -0.07$ $p = 0.55$	**$r = 0.25$** **$p = 0.030$**	**$r = 0.25$** **$p = 0.031$**	$r = 0.15$ $p = 0.19$	$r = 0.19$ $p = 0.10$

Relationships adjusted for body mass index and total energy expenditure

Significant associations are indicated in bold text

SBP systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *MAP* mean arterial pressure, *cIMT* carotid intima-media thickness, *GR* glutathione reductase, *GPx* glutathione peroxidase, *SOD* superoxide dismutase

[a]Relationships additionally adjusted for ambulatory systolic blood pressure

Table 3 Single and partial regression analyses of cardiovascular variables with antioxidant enzyme activities in black and white women

	Black women ($n = 105$)						White women ($n = 124$)					
	GR		GPx		SOD		GR		GPx		SOD	
	Single	Partial	Single	Partial	Single	Partial	Single	Partial	Single	Partial	Single	Partial
24-h SBP (mmHg)	**$r = 0.28$** **$p = 0.006$**	**$r = 0.25$** **$p = 0.019$**	$r = -0.08$ $p = 0.41$	$r = -0.01$ $p = 0.95$	$r = -0.06$ $p = 0.57$	$r = -0.08$ $p = 0.47$	**$r = 0.24$** **$p = 0.008$**	**$r = 0.20$** **$p = 0.033$**	$r = -0.10$ $p = 0.25$	$r = -0.003$ $p = 0.97$	**$r = -0.21$** **$p = 0.018$**	$r = -0.14$ $p = 0.12$
24-h DBP (mmHg)	**$r = 0.25$** **$p = 0.014$**	**$r = 0.24$** **$p = 0.027$**	$r = -0.06$ $p = 0.57$	$r = -0.01$ $p = 0.95$	$r = -0.16$ $p = 0.12$	$r = -0.15$ $p = 0.16$	**$r = 0.21$** **$p = 0.026$**	$r = 0.17$ $p = 0.08$	$r = -0.12$ $p = 0.19$	$r = -0.07$ $p = 0.43$	**$r = -0.28$** **$p = 0.002$**	**$r = -0.24$** **$p = 0.010$**
24-h PP (mmHg)	$r = 0.17$ $p = 0.09$	$r = 0.12$ $p = 0.28$	$r = -0.06$ $p = 0.52$	$r = -0.002$ $p = 0.99$	$r = 0.07$ $p = 0.52$	$r = 0.04$ $p = 0.72$	$r = 0.15$ $p = 0.10$	$r = 0.09$ $p = 0.38$	$r = -0.04$ $p = 0.70$	$r = 0.09$ $p = 0.37$	$r = -0.04$ $p = 0.68$	$r = 0.08$ $p = 0.38$
24-h MAP (mmHg)	**$r = 0.28$** **$p = 0.005$**	**$r = 0.27$** **$p = 0.013$**	$r = -0.07$ $p = 0.47$	$r = -0.01$ $p = 0.95$	$r = -0.12$ $p = 0.23$	$r = -0.13$ $p = 0.22$	**$r = 0.23$** **$p = 0.011$**	**$r = 0.19$** **$p = 0.045$**	$r = -0.12$ $p = 0.19$	$r = -0.05$ $p = 0.58$	**$r = -0.27$** **$p = 0.003$**	**$r = -0.22$** **$p = 0.019$**
cIMT left far wall (mm)[a]	$r = -0.003$ $p = 0.98$	$r = -0.01$ $p = 0.94$	**$r = 0.23$** **$p = 0.018$**	**$r = 0.23$** **$p = 0.032$**	$r = 0.03$ $p = 0.76$	$r = 0.10$ $p = 0.34$	$r = 0.04$ $p = 0.70$	$r = 0.05$ $p = 0.61$	$r = 0.07$ $p = 0.43$	$r = 0.05$ $p = 0.61$	$r = -0.08$ $p = 0.39$	$r = -0.10$ $p = 0.29$

Relationships adjusted for body mass index and total energy expenditure

Significant associations are indicated in bold text

SBP systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *MAP* mean arterial pressure, *cIMT* carotid intima-media thickness, *GR* glutathione reductase, *GPx* glutathione peroxidase; *SOD* superoxide dismutase

[a] Relationships additionally adjusted for ambulatory systolic blood pressure

Table 4 Multiple regression analyses of 24-h pulse pressure and 24-h diastolic blood pressure with glutathione peroxidase activity in black and white men

	24-h pulse pressure			
	Black men ($n = 89$)		White men ($n = 78$)	
	Adjusted R^2: 0.19		Adjusted R^2: 0.18	
	β (95% CI)	p-Value	β (95% CI)	p-Value
GPx	−0.25 (−0.47; -0.04)	0.026	0.07 (−0.14; 0.29)	0.503
Age	−0.07 (−0.31; 0.18)	0.595	−0.41 (−0.71; −0.11)	0.009
Socioeconomic score	−0.24 (−0.51; 0.03)	0.087	0.18 (−0.12; 0.48)	0.250
BMI	0.19 (−0.09; 0.47)	0.195	0.33 (−0.07; 0.73)	0.114
TEE	0.32 (0.07; 0.58)	0.015	0.12 (−0.24; 0.47)	0.527
γ-GT	−0.14 (−0.41; 0.13)	0.322	0.05 (−0.22; 0.32)	0.708
Cotinine	−0.12 (−0.35; 0.11)	0.318	−0.14 (−0.37; 0.10)	0.261
Glucose	0.05 (−0.19; 0.28)	0.709	−0.06 (−0.27; 0.16)	0.600
IL-6	−0.21 (−0.43; 0.004)	0.058	−0.10 (−0.34; 0.13)	0.402
Triglycerides	−0.02 (−0.27; 0.24)	0.888	−0.40 (−0.67; −0.14)	0.004
	24-h diastolic blood pressure			
	Black men ($n = 89$)		White men ($n = 78$)	
	Adjusted R^2: 0.31		Adjusted R^2: 0.21	
	β (95% CI)	p-Value	β (95% CI)	p-Value
GPx	0.11 (−0.09; 0.31)	0.269	−0.07 (−0.28; 0.14)	0.512
Age	0.15 (-0.07; 0.38)	0.184	0.37 (0.07; 0.66)	0.018
Socioeconomic score	0.02 (−0.23; 0.27)	0.870	−0.19 (−0.48; 0.11)	0.222
BMI	−0.01 (−0.27; 0.25)	0.947	0.06 (−0.33; 0.46)	0.748
TEE	0.07 (−0.17; 0.30)	0.581	0.04 (−0.31; 0.39)	0.834
γ-GT	0.26 (0.01; 0.51)	0.047	0.08 (−0.18; 0.34)	0.538
Cotinine	−0.22 (−0.43; −0.004)	0.050	0.21 (−0.03; 0.44)	0.086
Glucose	0.15 (−0.07; 0.37)	0.177	0.10 (−0.11; 0.31)	0.342
IL-6	0.14 (−0.06; 0.35)	0.165	−0.07 (−0.30; 0.17)	0.579
Triglycerides	0.13 (−0.10; 0.37)	0.268	0.32 (0.06; 0.58)	0.019

GPx glutathione peroxidase, *BMI* body mass index, *TEE* total energy expenditure, *γ-GT* γ-glutamyl transferase, *IL-6* interleukin-6

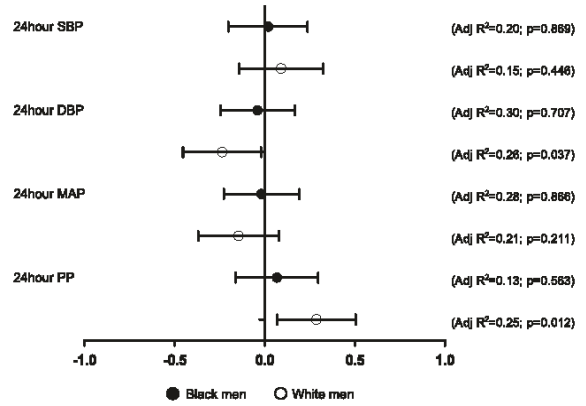

Fig. 1 Multiple regression analysis of glutathione reductase activity in black and white men. 24-h SBP ambulatory systolic blood pressure, 24-h DBP ambulatory diastolic blood pressure, 24-h MAP ambulatory mean arterial pressure, 24-h PP ambulatory pulse pressure

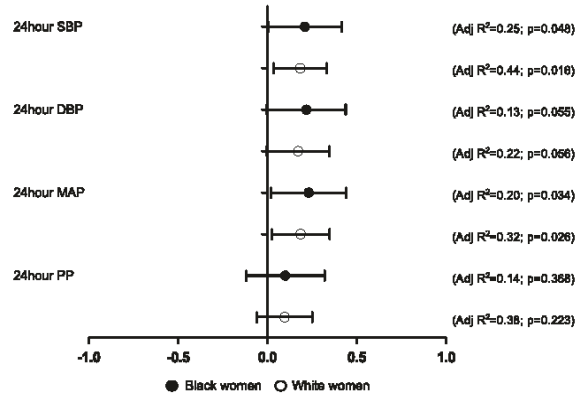

Fig. 2 Multiple regression analysis of glutathione reductase activity in black and white women. 24-h SBP ambulatory systolic blood pressure, 24-h DBP ambulatory diastolic blood pressure, 24-h MAP ambulatory mean arterial pressure, 24-h PP ambulatory pulse pressure

the young and apparently healthy study population without clear evidence of oxidative stress or blood pressure abnormalities, the results may indicate that the white men, black women and white women of our study are currently in a steady physiological state. The associations may indicate that sufficient protection against oxidative stress was maintained and all associations could be explained as a normal physiological profile [30]. This does, however, warrant further research to evaluate the possibility of future deterioration of the cardiovascular profile in these groups.

An unexpected finding of our study is that cIMT was positively associated with GPx activity in white men and black women. This result differs from what is expected since GPx activity, and GPx overexpression [31], seems to have vascular protective properties [32–35]. However, these results were found in mice, as well as older groups of different ethnicities, with already existing cardiovascular diseases or diabetes. This being a young, healthy cohort with normal cIMT, it could suggest that the activity of GPx may be sufficient to prevent vascular remodeling at this stage. To our knowledge, these results have not yet been evaluated in young, healthy black and white participants, and therefore further research is warranted in order to clarify this result.

This study has to be interpreted within the context of its strengths and limitations. Although our study population was based on participants from the Potchefstroom area in the North-West Province of South Africa, it may not be indicative of the population as a whole. This was a cross-sectional study, and causality cannot be inferred. Although our results were consistent after multiple adjustments, we cannot exclude any unknown interactions, which may play a role in the future development of arterial stiffness, carotid wall thickening or hypertension. The GSH:GSSG ratio within our study population is unknown, as tGSH levels were measured in these cohorts. Future studies on this topic may include the GSH:GSSG ratio, or other markers of oxidative damage in order to improve oxidative stress analysis in these groups. However, our study was well planned and executed under strict conditions in a fully equipped research facility, allowing us to dig deeper into the poorly researched effect of oxidative stress and antioxidant enzyme activity on cardiovascular disease development in our young South African population.

In conclusion, only in young black men 24-h PP associated negatively with GPx activity. This may suggest that lower GPx activity may accelerate vascular ageing in this group.

Summary Table

What is known about topic

- Oxidative stress has been implicated in the development of hypertension, arterial stiffness and atherosclerosis.
- South Africa is plagued by an alarmingly high prevalence of hypertension.
- Black South Africans are shown to exhibit relationships between increased oxidative stress and elevated blood pressure, vascular wall thickness and arterial stiffness. They are also more prone to early vascular changes when compared with white South Africans.

What this study adds

- We explored relationships of ambulatory blood pressure and cIMT with enzyme activities of the glutathione cycle—GPx and GR in young, healthy black and white South African men and women.
- The negative association discovered in black men between ambulatory PP and GPx activity may suggest

that oxidative stress may be associated with early vascular changes in this group.

- The positive associations discovered in the other groups between blood pressure and GR activity suggest a possible role for adequate GR activity in preventing or delaying the development of hypertension.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Cardiovascular reactivity and oxidative stress in young and older adults: the African-PREDICT and SABPA studies

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ABSTRACT

Background: Oxidative stress and increased cardiovascular reactivity are associated with endothelial dysfunction and cardiovascular disease development. These factors along with early vascular compromise are more pronounced in black populations. We aimed to compare cardiovascular reactivity and investigate associations thereof with oxidative stress in two bi-ethnic cohorts (younger: 25.0 ± 3.19yrs; older: 44.7 ± 9.61yrs).

Methods: Cardiovascular reactivity using the color-word conflict test was measured with the Finometer device. Oxidative stress markers included superoxide dismutase (SOD), γ-glutamyl transferase (γ-GT) and reactive oxygen species (ROS).

Results: Black groups displayed greater cardiovascular responses to stress than white groups. In younger white participants, diastolic blood pressure (DBP) ($\beta = 0.31$; $p = 0.001$) and mean arterial blood pressure (MAP) ($\beta = 0.28$; $p = 0.002$) associated with ROS. In older black participants, DBP ($\beta = 0.23$; $p = 0.009$), MAP ($\beta = 0.18$; $p = 0.033$), stroke volume ($\beta = -0.20$; $p = 0.023$) and arterial compliance ($\beta = -0.25$; $p = 0.005$) associated with γ-GT. In older white participants, systolic blood pressure ($\beta = -0.20$; $p = 0.006$) and MAP ($\beta = -0.19$; $p = 0.009$) associated with SOD.

Conclusions: In the older black group, cardiovascular reactivity associated with markers of glutathione metabolism, suggesting a possible compensatory up-regulation thereof in order to correct their heightened responses to stress. Independent of age, findings in the white groups support a regulatory role of ROS to maintain vascular tone during stress.

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KEYWORDS
Acute stress; color-word conflict test; age; reactive oxygen species; glutathione metabolism

Background

Cardiovascular disease is a growing concern in the developing world [1,2], with South Africa displaying an alarming increase in the incidence of hypertension, especially amongst urban black populations [3–5]. One of the key role players in the development of hypertension is oxidative stress, which occurs due to either an increased production of reactive oxygen species (ROS), or a decrease in ROS scavenging by the antioxidant system [6]. Physiologically, ROS play an important role in vascular biology and cell signaling as it is involved in control of vascular tone and endothelial function, cell growth, differentiation and apoptosis [6–9]. However, oxidative stress aggravates endothelial dysfunction, vascular remodeling and inflammation which may result in elevated blood pressure, peripheral resistance, arterial stiffness and atherosclerosis [6,7,9], all of which worsen with aging [10,11]. Acute stress has also been associated with both endothelial dysfunction as well as oxidative stress [12,13], and a heightened cardiovascular response to stress is a risk factor for the development of cardiovascular disease, including hypertension [14–16]. Upon acute stress, black populations have shown increased cardiovascular reactivity when compared to white populations [17–19]. Black South Africans are also more prone to early vascular changes [20], and previous results linked increased blood pressure to oxidative stress [21,22]. However, it has not been previously investigated whether oxidative stress plays a role in a heightened cardiovascular response in black and white South Africans, and whether this link is age dependent. Therefore we aimed to compare oxidative stress and cardiovascular reactivity profiles between two age-stratified South African cohorts, and to determine whether relationships exist between cardiovascular reactivity and oxidative stress markers.

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Supplemental data for this article can be accessed here.

Methods

Study populations

This study is embedded in the baseline phases of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) study (mean age: 25.0 years) and the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study (mean age: 44.7 years).

After an initial screening phase (evaluating blood pressure, cholesterol, glucose levels, anthropometry, HIV status and a general health questionnaire) apparently healthy normotensive or pre-hypertensive (systolic blood pressure (SBP) <140 and diastolic blood pressure (DBP) <90mmHg) black and white men and women between 20 and 30 years of age were invited to participate in the African-PREDICT study [23]. Participants who are not permanent residents of Potchefstroom or surrounding areas (or plan regular trips to the area) and who are unable to read or understand English were excluded from the study. Participants with elevated glucose levels >5.6 mmol/L (or glycated haemoglobin ≥6.5%), who were HIV infected and those with ear temperature >37.5 °C were also excluded. Further exclusion criteria included previous diagnoses of (or took medication for) type 1 or 2 diabetes mellitus, liver disease, cancer, tuberculosis, renal or cardiovascular diseases, who recently had surgery or trauma and who was pregnant or breastfeeding. This study included the first 387 participants with complete antioxidant enzyme activity data divided into black ($N = 191$) and white groups ($N = 196$).

Detail on the SABPA study was published elsewhere [24]. In summary, participants were between 20 and 65 years of age, and participants who were pregnant, lactating, exhibited ear temperature >37 °C, used alpha and beta blockers, confirmed psychotropic substance abuse and those who donated blood or were vaccinated 3 months prior to their participation were excluded. We included 409 participants of the SABPA study divided into black ($N = 200$) and white ($N = 209$) groups.

Both studies complied with the Declaration of Helsinki regarding investigations among human participants, and were further approved by the Health Research Ethics Committee of the North-West University. The African-Predict study was registered as a clinical trial as required (ClinicalTrials.gov Identifier: NCT03292094). All participants were informed of each study in their home language, including stressor test protocols, and written informed consent was obtained before commencement.

Questionnaires

Participants completed a general health questionnaire to obtain demographic data and information on alcohol usage.

Anthropometric and physical activity measurements

All anthropometric measurements were performed using standardized methods [25]. These measurements included body height measured with a stadiometer (SECA 213 SECA, Hamburg, Germany and Invicta, IP 1465, Invicta, London, UK), body weight measured with an electronic scale (SECA 813, SECA, Hamburg, Germany and Precision Health Scale, A & D Company, Tokyo, Japan) and waist circumference measured with a metal tape (Lufkin Steel Anthropometric Tape, W606PM, Lufkin, Apex, MD, USA and Holtain unstretchable flexible 7mm wide metal tape, Crosswell, Wales). Body mass index (BMI) was calculated as kg/m^2 and body surface area was calculated as m^2 according to the Mosteller formula [26]. Total energy expenditure (TEE) was monitored over 7 days in the African-PREDICT study (ActiHeart physical activity monitor, CamNtech Ltd., England, UK) and over 24 hours in the SABPA study (Actical® activity monitor, Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada).

Cardiovascular reactivity

Cardiovascular measurements were made non-invasively using the validated Finometer device (FMS, Finapres Medical Systems, Amsterdam, Netherlands). The Finometer device provides a beat-to-beat arterial blood pressure waveform after adjusting for hydrostatic height from the heart while computing additional cardiac parameters by way of the Modelflow method [27]. The finger cuff of the Finometer was fitted on the left middle-finger, and after a 10 minute resting period, a 5 minute continuous measurement of resting variables was recorded.

During the recording, after 2 minutes, a return-to-flow systolic calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial artery pressure, which allows for the highest precision in measurements. Resting measurements included SBP, DBP, mean

arterial pressure (MAP), stroke volume (SV), total peripheral resistance (TPR) and Windkessel arterial compliance (Cwk). Thereafter the color-word conflict test was applied for 1 minute during which the cardiovascular variables were measured. The mean of the last minute of the resting values, and the last 20 seconds during exposure were used for the calculations. Cardiovascular reactivity was calculated as the percentage change from resting values [28].

Biochemical measurements

Fasting blood samples were obtained from each participant by a registered nurse from the antebrachial vein. Serum and plasma samples were prepared using standardized procedures, aliquoted into cryovials and stored in bio-freezers at −80 °C until analysis to maintain efficient long-term stability of antioxidant enzymes [29]. Antioxidant enzyme activities measured included glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD) and γ-glutamyl transferase (γ-GT). Additional oxidative stress markers included total glutathione (tGSH) and reactive oxygen species (ROS) (measured as serum peroxides and reported in units, where 1 mg H_2O_2/L equates to 1 unit)[30]. In addition, fluoride plasma glucose and serum total cholesterol, triglycerides, cotinine, interleukin-6 (IL-6) and cortisol levels were determined. All apparatus used are summarized in Table S1.

Statistical analyses

Statistica version 13.2 (Dell, TX, USA) was used to perform the statistical analyses of this study. Normal distribution of data was assessed and the central tendency and spread for normally distributed variables were expressed as arithmetic mean and standard deviation. Skewed variables were logarithmically transformed (triglycerides, IL-6, total cholesterol, SOD and γ-GT in the African-PREDICT study and glucose, triglycerides, IL-6, GR, SOD and γ-GT in the SABPA study) and expressed as the geometric mean and the 5th and 95th percentile intervals.

Interactions of race and sex were tested on the relationships between main cardiovascular reactivity variables and oxidative stress markers using multiple regression analyses. Continuous variables were compared using independent T-tests, while proportions were compared using Chi-square tests. Single regression analyses were performed to evaluate unadjusted associations between cardiovascular variables and oxidative stress related variables. Thereafter, partial correlations were performed to evaluate the associations between variables while adjusting for age, sex and BMI. Multiple regression analyses were performed to evaluate the independent associations between cardiovascular reactivity variables and oxidative stress markers. Covariates entered into the models included the relative oxidative stress marker, age, sex, BMI, TEE, self-reported alcohol use, cotinine, glucose, IL-6, triglycerides and cortisol. Sensitivity analyses were performed by repeating the multiple regression analyses after including statin usage and anti-hypertension medication usage into the models.

In post hoc analyses the achieved power ($1-\beta$) was determined for multiple regression models with 11 covariates, with a group size of $N=191$ for black ($1-\beta=0.97$) and $N=196$ for white African-PREDICT ($1-\beta=0.97$), and $N=200$ for black ($1-\beta=0.97$) and $N=209$ for white SABPA ($1-\beta=0.98$) (G*power v3.1.9.2) [31].

Results

Characteristics of the study population

No interactions of sex on the relationships between cardiovascular reactivity and oxidative stress markers were found. Meanwhile, interactions of race were found on the association of Cwk with SOD ($\beta=0.285$; $p=0.026$) in the African-PREDICT study, and on the associations of SBP with SOD ($\beta=-0.477$; $p=0.018$), MAP with SOD ($\beta=-0.578$; $p=0.004$) and TPR with tGSH ($\beta=0.635$; $p=0.015$) in the SABPA study. Stratification was done accordingly to compare black and white groups.

Black participants of the African-PREDICT study were younger (24.4 ± 3.39; $p<0.001$) with a lower BMI (24.5 ± 5.41; $p=0.013$) than their white counterparts, while in the SABPA study the black participants displayed a higher BMI (30.1 ± 7.00; $p<0.001$) than the white group (Table 1).

Oxidative stress markers including ROS, tGSH, GPx and γ-GT revealed similar profiles in both studies with black participants having higher ROS (African-PREDICT: 197 ± 70.9; $p=0.033$; SABPA: 192 ± 63.0; $p<0.001$), tGSH (African-PREDICT: 1231 ± 295; $p<0.001$; SABPA: 895 ± 189; $p<0.001$) and γ-GT (African-PREDICT: 25.4 (10.6; 82.8); $p<0.001$; SABPA: 47.4 (20.1; 184; $p<0.001$) with lower GPx activity (African-PREDICT: 18.5 ± 1.76; $p<0.010$; SABPA: 33.3 ± 14.0; $p=0.010$) than their white counterparts. Although no differences were found in the African-PREDICT study, GR activity

Table 1. Characteristics of black and white groups.

	African-PREDICT study			SABPA study		
	Black	White	*p* values	Black	White	*p* values
N	191	196		200	209	
Sex, females (%)	56.0	60.7	0.349	50.5	48.3	0.660
Age (years)	24.4 ± 3.39	25.5 ± 2.87	**<0.001**	44.4 ± 8.11	45.0 ± 10.9	0.495
Anthropometric measurements						
Body mass index (kg/m^2)	24.5 ± 5.41	25.9 ± 5.82	**0.013**	30.1 ± 7.00	27.6 ± 5.94	**<0.001**
Body surface area (m^2)	1.72 ± 0.18	1.91 ± 0.27	**<0.001**	1.92 ± 0.23	2.00 ± 0.28	**0.001**
Waist circumference (cm)	77.2 ± 10.9	82.3 ± 15.4	**<0.001**	93.6 ± 15.5	93.0 ± 16.1	0.707
Biochemical analyses						
Glucose (mmol/l)	3.87 ± 0.84	4.71 ± 0.85	**<0.001**	5.41 (4.04; 10.4)	5.62 (4.70; 6.90)	0.055
Total cholesterol (mmol/l)	3.78 (2.70; 5.48)	4.66 (3.30; 6.43)	**<0.001**	4.60 ± 1.19	5.54 ± 1.28	**<0.001**
Triglycerides (mmol/l)	0.77 (0.41; 1.56)	0.98 (0.45; 2.18)	**<0.001**	1.16 (0.49; 3.66)	1.01 (0.44; 2.79)	**0.024**
Interleuken-6 (pg/ml)	0.97 (0.40; 3.29)	0.74 (0.27; 2.55)	**<0.001**	1.13 (0.34; 3.07)	0.92 (0.30; 3.04)	**0.002**
Cortisol (nmol/l)	422 ± 186	489 ± 277	**0.009**	355 ± 152	384 ± 160	0.063
Oxidative stress markers						
Reactive oxygen species (Units)[1]	197 ± 70.9	180 ± 89.3	**0.033**	192 ± 63.0	158 ± 58.2	**<0.001**
Total glutathione (μM)	1231 ± 295	931 ± 248	**<0.001**	895 ± 189	820 ± 173	**<0.001**
Glutathione peroxidase (nmol/min/ml)	18.5 ± 1.76	19.9 ± 1.34	**<0.001**	33.3 ± 14.0	36.2 ± 7.97	**0.010**
Glutathione reductase (nmol/min/ml)	52.9 ± 17.1	54.8 ± 16.1	0.263	7.06 (2.55; 16.8)	2.54 (0.25; 7.64)	**<0.001**
Superoxide dismutase (U/ml)	2.33 (1.27; 3.71)	2.19 (1.38; 3.83)	0.114	4.25 (0.83; 22.9)	4.13 (1.65; 7.92)	0.723
γ-glutamyl transferase (U/l)	25.4 (10.6; 82.8)	17.5 (7.40; 52.7)	**<0.001**	47.4 (20.1; 184)	19.3 (7.00; 76.0)	**<0.001**
Lifestyle						
Cotinine (ng/ml)	64.5 ± 115	33.7 ± 86.7	**0.003**	27.3 ± 60.9	22.7 ± 77.5	0.514
Total energy expenditure (kcal/day)	2183 ± 364	2383 ± 504	**<0.001**	2685 ± 796	3113 ± 1597	**0.001**
Self-reported alcohol use (% yes)	64.9	65.8	0.845	26.0	49.0	**<0.001**
Statin use (% yes)	0	0	–	1.00	4.31	**0.039**
Anti-hypertension medication use (% yes)	0	0	–	34.5	12.9	**<0.001**

Data expressed as arithmetic mean ± standard deviation or geometric mean with 5th and 95th percentile boundaries, % or *n*.
Bold text indicate statistical significance.
[1]Reactive oxygen species measured as serum peroxides where 1 unit = 1.0 mg/L H_2O_2.

was higher in the black participants of the SABPA study (7.06 (2.55; 16.8); $p < 0.001$).

Cardiovascular reactivity

In the younger cohort, black participants displayed higher DBP, MAP and TPR but lower SV and Cwk at rest and after acute stress when compared to the younger white participants (Figure 1). Similarly, black participants of the older cohort also displayed higher DBP and MAP with lower Cwk at rest and after acute stress than the white participants. Additionally, this group also displayed higher SBP at rest and after acute stress along with a higher TPR than their white counterparts after acute stress only.

In the younger black participants, a larger decrease in SV ($p < 0.001$) and smaller decrease in TPR (<0.001) were noted in comparison to the younger white participants (Figure 2). In the older black group, cardiovascular reactivity after applying the stressor revealed greater increases in DBP ($p = 0.002$), and MAP ($p = 0.031$) than their white counterparts. When comparing change in TPR the older black group experienced an increase in TRP whereas the white group experienced a decrease in TPR, and these responses differed significantly between the two groups ($p = 0.014$). The older black group also showed a significantly larger decrease in Cwk ($p = 0.001$) and SV ($p < 0.001$) when compared to their white counterparts.

Regression analyses

In single and partial regression analyses (Table S2), we found an association of change in TPR with SOD in the young black group, however, this lost significance after full adjustments were made ($R^2 = -0.004$; $\beta = -0.12$; $p = 0.145$). In the younger white group, changes in DBP ($R^2 = 0.05$; $\beta = 0.31$; $p = 0.001$) and MAP ($R^2 = 0.10$; $\beta = 0.28$; $p = 0.002$) associated positively with ROS, while change in Cwk associated positively with SOD activity ($R^2 = 0.09$; $\beta = 0.18$; $p = 0.023$) (Table 2).

In the older black group, single, partial (Table S3) and multiple regression analyses (Table 2) indicated an increase in DBP ($R^2 = 0.03$; $\beta = 0.23$; $p = 0.009$) and MAP ($R^2 = 0.06$; $\beta = 0.18$; $p = 0.033$) to be positively associated with γ-GT levels. Meanwhile, a decrease in SV ($R^2 = 0.06$; $\beta = -0.20$; $p = 0.023$) and Cwk ($R^2 = 0.07$; $\beta = -0.25$; $p = 0.005$) was negatively associated with γ-GT levels. In the same group, an increase in TPR was borderline negatively associated with tGSH ($R^2 = 0.04$; $\beta = -0.15$; $p = 0.062$). The borderline association of decreased SV with GPx in partial regression analyses ($p = 0.054$) became significant after multiple regression analyses ($R^2 = 0.06$; $\beta = 0.16$; $p = 0.032$) in the older black group. In the older white group, increased SBP

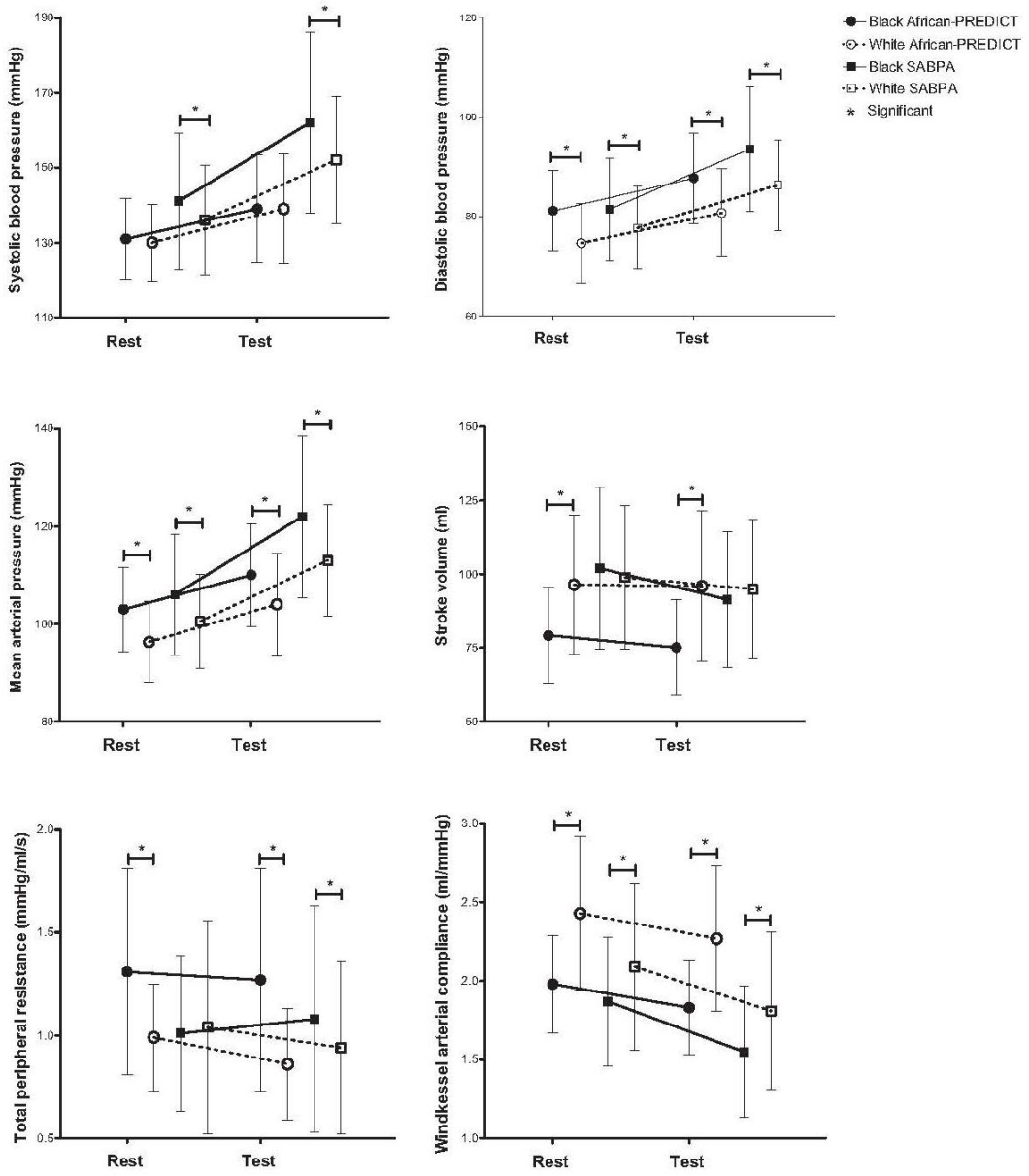

Figure 1. Rest and change in cardiovascular variables after the color-word conflict test showing significant differences between black and white groups of the African-PREDICT and SABPA studies.

($R^2 = 0.04$; $\beta = -0.20$; $p = 0.006$), increased MAP ($R^2 = 0.01$; $\beta = -0.19$; $p = 0.009$) were inversely associated with SOD. In the same group a decrease in SV was positively associated with tGSH ($R^2 = 0.02$; $\beta = 0.20$; $p = 0.009$) and negatively associated with SOD ($R^2 = 0.0004$; $\beta = -0.14$; $p = 0.052$). All other associations lost significance after full adjustments.

Sensitivity analyses

The African-PREDICT study excluded participants using statins and anti-hypertension medication. After adding these variables as covariates into multiple regression analyses in the SABPA study, only the borderline negative association of TPR with tGSH in the black group became significant after including anti-hypertension medication into the model ($R^2 = 0.05$; $\beta = -0.17$; $p = 0.039$).

Multiple regression analyses of SV with oxidative stress markers in the older group were repeated after substituting stroke volume with stroke volume index (SVI) (calculated as stroke volume/body surface area) as the main dependent variable in the model. Similar results were obtained in sensitivity analyses showing a significant negative association of SVI with γ-GT

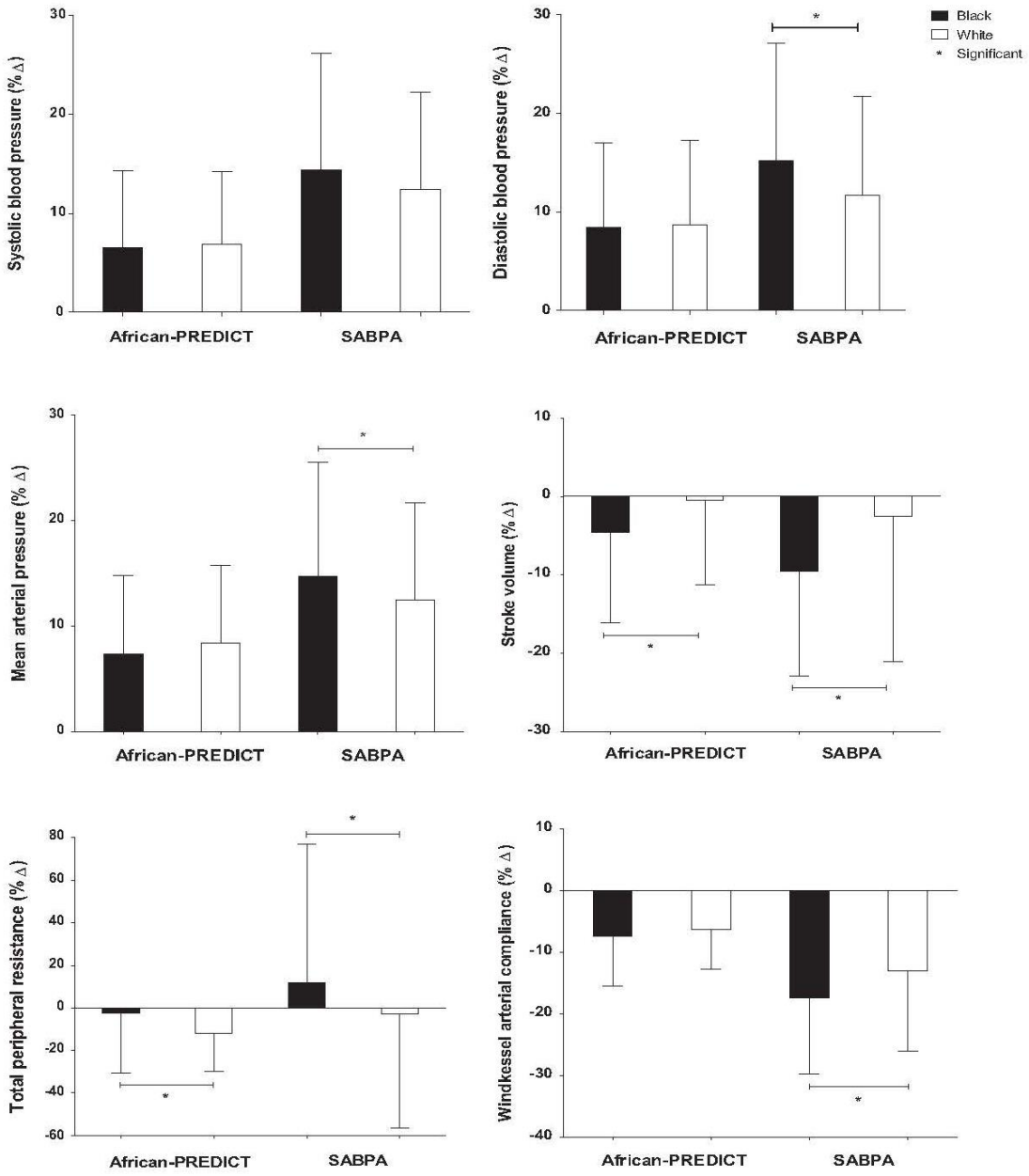

Figure 2. Cardiovascular reactivity following the color-word conflict test showing significant differences between black and white groups of the African-PREDICT and SABPA studies. % Δ, Percentage change.

($R^2 = 0.06$; $\beta = -0.20$; $p = 0.023$) in black groups, and a positive association of SVI with tGSH ($R^2 = 0.02$; $\beta = 0.20$; $p = 0.009$) in white groups. The negative association of SVI with SOD ($R^2 = 0.0004$; $\beta = -0.14$; $p = 0.052$) was borderline significant in the older white group after full adjustments.

Discussion

We found age-related disparities in the link between cardiovascular reactivity and markers of oxidative stress in black populations. In the young black group TPR decreased less and SV decreased more, but we found no link between cardiovascular reactivity and markers of oxidative stress. In the older black group with a heightened cardiovascular response to stress, increased TPR and decreased SV and Cwk was associated with markers of glutathione metabolism. In contrast, our findings in the white groups indicated that cardiovascular reactivity was associated with ROS and important determinants of ROS availability, namely SOD and tGSH, independent of age.

Table 2. Summary of multiple regression analyses of cardiovascular reactivity with markers of oxidative stress in participants of the African-PREDICT and SABPA studies.

	African-PREDICT study					
	Black ($n = 191$)			White ($n = 196$)		
	Adjusted R^2	*β (95% CI)*	*p*	*Adjusted* R^2	*β (95% CI)*	*p*
Diastolic blood pressure (% Δ)						
Reactive oxygen species	−0.02	0.05 (−0.16; 0.25)	0.643	0.05	0.31 (0.13; 0.49)	**0.001**
Mean arterial pressure (% Δ)						
Reactive oxygen species	−0.004	0.01 (−0.19; 0.21)	0.899	0.10	0.28 (0.10; 0.45)	**0.002**
Windkessel arterial compliance (% Δ)						
Superoxide dismutase	0.01	−0.02 (−0.18; 0.15)	0.860	0.09	0.18 (0.03; 0.32)	**0.023**
	SABPA study					
	Black ($n = 200$)			White ($n = 209$)		
	Adjusted R^2	*β (95% CI)*	*p*	*Adjusted* R^2	*β (95% CI)*	*p*
Systolic blood pressure (% Δ)						
Superoxide dismutase	0.06	0.03 (−0.12; 0.17)	0.708	0.04	−0.20 (−0.33; −0.06)	**0.006**
Diastolic blood pressure (% Δ)						
γ-glutamyl transferase	0.03	0.23 (0.06; 0.40)	**0.009**	−0.04	0.08 (−0.10; 0.26)	0.361
Mean arterial pressure (% Δ)						
Superoxide dismutase	0.05	0.10 (−0.04; 0.25)	0.178	0.01	−0.19 (−0.33; −0.05)	**0.009**
γ-glutamyl transferase	0.06	0.18 (0.02; 0.35)	**0.033**	−0.02	0.11 (−0.07; 0.29)	0.223
Stroke volume (% Δ)						
Glutathione peroxidase	0.06	0.16 (0.02; 0.31)	**0.032**	−0.02	0.07 (−0.08; 0.22)	0.376
Superoxide dismutase	0.04	−0.09 (−0.23; 0.06)	0.233	0.0004	−0.14 (−0.28; 0.00)	0.052
Total glutathione	0.05	0.12 (−0.03; 0.28)	0.116	0.02	0.20 (0.05; 0.34)	**0.009**
γ-glutamyl transferase	0.06	−0.20 (−0.36; −0.03)	**0.023**	−0.02	0.02 (−0.16; 0.20)	0.813
Total peripheral resistance (% Δ)						
Total glutathione	0.04	−0.15 (−0.30; 0.01)	0.062	0.01	−0.01 (−0.15; 0.14)	0.920
Windkessel arterial compliance (% Δ)						
γ-glutamyl transferase	0.07	−0.25 (−0.41; −0.08)	**0.005**	−0.02	−0.04 (−0.22; 0.14)	0.652

Covariates included in each model included the relative oxidative stress marker, age, sex, body mass index, total energy expenditure, self-reported alcohol use, cotinine, glucose, interleukin-6, triglycerides and cortisol.
Bold text indicate statistical significance.

Our findings in the older black group confirm results from a previous study by our research team, in which older black men displayed increased TPR reactivity to acute stress [18]. Increased TPR reactivity may enhance the afterload and subsequently decrease SV, which related to end-organ damage in this cohort [18]. Similar findings were noted in the younger black adults described in the present study, where TPR decreased less and SV decreased more when compared to the white adults during stress. This is in agreement with previous findings in another young black group (29.9 ± 2.4 years) where it was suggested that increased TPR may play a role in the pathogenesis of hypertension in blacks [19]. Despite evidence of an altered cardiovascular response in the young black group, we were unable to link this to any oxidative stress markers investigated in this study. This finding may suggest that factors other than ROS, such as enhanced arterial stiffness and premature vascular aging, may play a more prominent role in cardiovascular reactivity in young black South Africans [20,32].

In the older black group, on the other hand, increased TPR and decreased SV and Cwk were associated with markers of glutathione metabolism. Glutathione, a powerful antioxidant, exists in either reduced (GSH) or oxidized (GSSG) form [33]. The synthesis of glutathione is dependent on the intracellular availability of its amino acid constituents. While γ-GT was originally considered a marker for alcohol abuse and liver dysfunction, the enzyme acts as an oxidative stress marker since it is involved in the breakdown of extracellular glutathione to increase the availability of amino acids for intracellular glutathione synthesis [34]. In this regard γ-GT has a dual function since it is also able to produce ROS directly [35–38]. The link between heightened cardiovascular reactivity and markers of glutathione metabolism may be explained by two possible mechanisms; firstly that acute stress may induce increased shear stress which may promote oxidative stress [12,39], and secondly that oxidative stress may leads to a deficiency in nitric oxide, thus further diminishing endothelial function [6,7,40]. During conditions of oxidative stress, glutathione may be consumed at a higher rate, leading to possible increased expression of γ-GT in an effort to increase intracellular glutathione levels [36]. The elevated γ-GT in this group may then also perhaps be explained as compensatory in an effort to stabilize the redox balance, which is further strengthened by the presence of higher tGSH in this group.

In contrast, the findings in the white cohorts indicated that cardiovascular reactivity was associated with ROS and important determinants of ROS availability, namely SOD and tGSH, independent of age. This was also indicated in an experimental study in which a role for ROS in the regulation of blood pressure during and after acute stress was confirmed. Here it was suggested that a lack of ROS is implicated in a greater MAP reactivity and a longer recovery period from acute stress [41]. This finding supports the important role of physiologically regulated ROS signaling in the maintenance of vascular tone during stress, while suggesting a possible role for an adequate antioxidant system, especially SOD activity, in the prevention of a heightened cardiovascular response to stress in white participants regardless of age.

This study has to be interpreted within the context of its strengths and limitations. Both our study populations included participants from the Potchefstroom area, and may not represent the South African population as a whole. This being a cross-sectional study, causality cannot be inferred, and although our results were consistent after multiple adjustments, we cannot exclude confounding of unknown factors. Measurements of ROS were made using an indirect biomarker and we cannot discern the sources of ROS generation in this study. Participants were not screened or excluded based on previous exposure to the color-word conflict test, which may be considered for future studies in this field. However, to the best of our knowledge, this is the first study investigating the associations of oxidative stress with cardiovascular reactivity in 2 age-stratified cohorts of black and white South Africans. Both studies were well executed under controlled conditions in a fully-equipped research facility. This study offers further insight into the role of redox signaling in cardiovascular responses to acute stress.

In conclusion, age-related differences were found in the link between cardiovascular reactivity and markers of oxidative stress in the black cohorts. Heightened cardiovascular reactivity during the color-word conflict test associated with markers of glutathione metabolism in older black participants, suggesting a possible compensatory up-regulation of this system in order to correct their unfavourable cardiovascular responses to stress. Findings in the white groups support a possible regulatory role of ROS in the maintenance of vascular tone during stress, independent of age.

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