Left ventricular structure and function and the link with oxidative stress in young adults: The African-PREDICT study

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Dissertation submitted in fulfilment of the requirements for the degree Masters of Heath Science in Cardiovascular Physiology at the North West University

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

PREFACE

This dissertation for the Master in Health Sciences (MHSc) study “Left ventricular structure and function and the link with oxidative stress in young adults: The African-PREDICT study” is submitted in fulfilment of the requirements for the degree MHSc in Cardiovascular Physiology at the North-West University. This dissertation is presented in article format (Chapter 3), which is an approved format of the North-West University as set out in the guidelines for postgraduate studies.

The chapter outline of this dissertation is as follows:

Chapter 1: Background, motivation, literature study, aims and hypotheses for the study

Chapter 2: Methodology

Chapter 3: Research article

Chapter 4: Concluding remarks and future recommendations

All relevant references are provided at the end of each chapter. The manuscript was prepared, according to the author guidelines of the journal *Heart, Lung and Circulation* (which are summarised before the manuscript). In order to ensure uniformity of the dissertation, the Vancouver reference style was used throughout, as this is the preferred style of the journal *Heart, Lung and Circulation*.

AUTHOR CONTRIBUTIONS

Miss LC Hawley: Responsible for conducting literature search, writing of the research proposal and ethics application, literature review, performing statistical analyses, interpretation of results and writing of all sections of this dissertation including the research article.

Prof R Kruger: Supervised writing of the research proposal, ethics application, literature review, statistical analyses, and guidance in interpretation of results, initial planning and design of the manuscript.

Prof Carina Mels: Co-supervised writing of the research proposal, ethics application, literature review, statistical analyses, and guidance in interpretation of results, initial planning and design of the manuscript.

Dr W Smith: Co-supervised writing of the research proposal, ethics application, literature review, statistical analyses, and guidance in interpretation of results, initial planning and design of the manuscript.

The following is a statement of the co-authors confirming their individual roles in the study and giving their permission that the manuscript may form part of this mini-dissertation.

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SUMMARY

Background

In developed counties, the contribution of cardiovascular diseases (CVDs) to morbidity and mortality rates is well documented. In a study conducted in South Africa (N=4506), it was found that 92% of the study population (mean age 53 years) were suffering from CVDs, 53.5% of which were hypertensive and 47.1% of which suffered from heart failure. Oxidative stress is a well-known contributor to cardiovascular diseases. However, previous studies that linked oxidative stress to cardiac structure and function were done in older individuals or experimental animal studies. Less is known about the link between cardiac structure and function and oxidative stress-related markers in young populations before the development of CVDs. This study, therefore, focused on young and healthy adults to uncover early associations of cardiac structure and function with oxidative stress-related markers.

Objectives

The objectives of this study were (i) to compare cardiac structure and oxidative stress-related markers and (ii) to explore independent associations of cardiac structure and function with oxidative stress-related markers.

Methods

This study formed part of the African Prospective Study on the Early Detection and Identification of Cardiovascular Disease and Hypertension (African-PREDICT). We included 361 individuals with complete oxidative stress data. The participants were from Potchefstroom and surrounding areas. They were aged between 20 and 30 years, had to have an office blood pressure of less than 140 mmHg systolic and 90 mmHg diastolic, were of self-reported black or white ethnicity and were apparently healthy. Standardised methods were used to determine anthropometric measurements. Biochemical measurements, including oxidative stress-related markers (gamma-glutamyl transferase (GGT), glutathione

peroxidase (GPx), total glutathione (tGSH); and total antioxidant status (TAS) and traditional CVD risk markers (such as total cholesterol, high-density lipoprotein cholesterol, C-reactive protein and cotinine levels). Echocardiographic measurements, including let ventricular mass index (LVMi), relative wall thickness (RWT), ejection fraction (EF) and fractional shortening (FS), were determined by standard transthoracic echocardiography.

Several interactions were identified for ethnicity and gender on the association between measures of LV structure and function markers and oxidative stress-related markers. The participants were therefore stratified according to gender and ethnicity. Independent t-Tests and Chi-square tests were performed to compare means and proportions among groups. Partial and multiple regression analyses were used to investigate the relationship between LV structure and function and oxidative stress-related markers while considering possible confounding factors.

Results and conclusions

We found that the LVMi was comparable among the men and women and the RWT was higher in both the black women ($p<0.001$) and the black men ($p=0.014$). Ejection fraction and FS were comparable among the women, but lower in the white men than in the black men ($p=0.013$). Oxidative stress-related markers revealed higher GGT in the black women than in the white women ($p<0.001$), whereas in the men, GGT was comparable. Glutathione peroxidase ($p<0.001$) and TAS ($p<0.001$) were lower, but tGSH ($p<0.001$) was higher among both the black groups. In multiple regression analyses, after adjusting for confounding factors, LVMi was independently associated with GPx in black women ($\beta=–0.286$; $p=0.010$) and white men ($\beta=0.329$; $p=0.004$). In the white men only, both EF ($\beta=–0.345$; $p=0.018$) and FS ($\beta=–0.335$; $p=0.019$) were inversely associated with GGT. These results indicate the importance of adequate antioxidant capacity to prevent the onset of early cardiac dysfunction. Further investigations are warranted to clarify the opposing associations between iLVM and GPx in the black women and white men.

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LIST OF ABBREVIATIONS

ABPM:	Ambulatory blood pressure monitoring
AEE:	Activity energy expenditure
African-PREDICT:	African Prospective Study on the Early Detection and Identification of Cardiovascular Disease and Hypertension
BH_4:	Tetrahydrobiopterin
BP:	Blood pressure
CAT:	Catalase
CI:	Confidence interval
cm:	Centimetres
CVD:	Cardiovascular disease
Cys-gly:	Cysteinyl-glycine
DBP:	Diastolic blood pressure
DNA:	Deoxyribonucleic acid
EF:	Ejection fraction
eNOS:	Endothelial nitric oxide synthase
FS:	Fractional shortening
g/m^2:	Grams per square metre
G-6-PDH:	Glucose-6-phosphate dehydrogenase
Gamma-glu aa:	Gamma-glutamyl amino acid
GGT:	Gamma-glutamyl transferase
GPx:	Glutathione peroxidase
GR:	Glutathione reductase
GSH:	Glutathione (reduced form)
GSSG:	Glutathione disulphide
H_2O_2:	Hydrogen peroxide
HART:	Hypertension in Africa Research Team

kCal/kg/day:	Kilocalories per kilogram per day
kg:	Kilograms
LV:	Left ventricle (ventricular)
LVH:	Left ventricular hypertrophy
LVMi:	Left ventricular mass index
µM:	Micromoles
m:	Metre
m^2:	Square metre
mg/L:	Milligrams per litre
mmHg:	Millimetres mercury
mmol/L:	Millimoles per litre
n:	Number of
$NADP^+$:	Nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH:	Nicotinamide adenine dinucleotide phosphate (reduced)
ng/L:	Nanograms per litre
$O_2^{\bullet-}$:	Superoxide
p:	Probability value
PWV:	Pulse wave velocity
r:	Regression coefficient
ROS:	Reactive oxygen species
RWT:	Relative wall thickness
RyR2:	Cardiac ryanodine receptor
SBP:	Systolic blood pressure
SD:	Standard deviation
SOD:	Superoxide dismutase
SR:	Sarcoplasmic reticulum
TAS:	Total anti-oxidant status
tGSH:	Total glutathione

U/L:	Units per litre
U/mL:	Units per millilitre
WC:	Waist circumference
WHO:	World Health Organization

CHAPTER 1

Literature study, motivation, aims and hypotheses of the study

LITERATURE STUDY

Background

Cardiovascular diseases (CVDs) are the primary cause of death globally, as reported by the World Health Organisation [1]. In the Heart of Soweto Study, with a population size of 4 506 South African individuals and mostly black Africans, it was found that 92% of the patients had an underlying CVD [2]. Hypertension is an important risk factor for CVD, and in 2015, sub-Saharan Africa was one of the countries in the world with the highest blood pressure levels [3]. In 2012, hypertensive heart disease was the fifth main cause of mortality in black South Africans; however, in white South Africans, hypertensive heart diseases were not among the top ten causes of mortality [4]. This indicates ethnic differences in the epidemiology of CVD, which should be investigated further. In a recent study involving young and healthy South Africans, masked hypertension was positively associated with the left ventricular mass index (LVMi), demonstrating that despite a young age, LVMi may already be altered [5]. Oxidative stress affects the progression of CVD [6-9]. Studies done in South Africa also showed significant differences in the oxidative stress profiles of black individuals compared to their white counterparts [10-12]. In addition it was also demonstrated in young black men that oxidative stress-related markers, such as lower glutathione peroxidase (GPx), are associated with higher pulse pressure, suggesting that oxidative stress may already influence the early phases of vascular disease development [13]. However, a large portion of the literature available on the relation of between oxidative stress (and oxidative stress-related markers) with CVD has been on research performed in older individuals with [14, 15] or without [16, 17] advanced CVD or in experimental animal models [18-27]. In the literature search, we identified a gap in the link between oxidative stress-related markers with cardiac structure and function markers in young, healthy individuals.

Reactive oxygen species, the antioxidant defence system and oxidative stress

In normal physiology, reactive oxygen species (ROS) are very tightly controlled by anti-oxidants and remain in very low concentrations, allowing ROS to act as second messengers in signal transduction pathways [28]. Oxidative stress is defined as an imbalance between oxidants and antioxidants, i.e. oxidants exceeding anti-oxidants [29]. Various factors contribute to redox imbalance, including lifestyle factors such as alcohol consumption [30] smoking [31], inflammation [32] or infection [33]. There are two types of free radicals in human physiology namely ROS and reactive nitrogen species (RNS) [34], with ROS being the most abundant [35].

The antioxidant defence system includes an enzyme system, including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and gamma-glutamyl transferase (GGT), and non-enzymatic molecules, such as total glutathione (tGSH) and vitamins A, E and C [36]. The antioxidant enzyme SOD is responsible for the reduction of superoxide ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) [37]. Hydrogen peroxide is then eliminated by the GPx enzyme, which leads to the oxidation of reduced glutathione (GSH) to form glutathione disulphide (GSSG) [14]. The enzyme GR is responsible for the reduction of GSSG to make GSH available again (**Figure 1**) [38]. The balance between the activities of SOD and GPx is important to prevent oxidative lipid, protein and DNA damage [39].

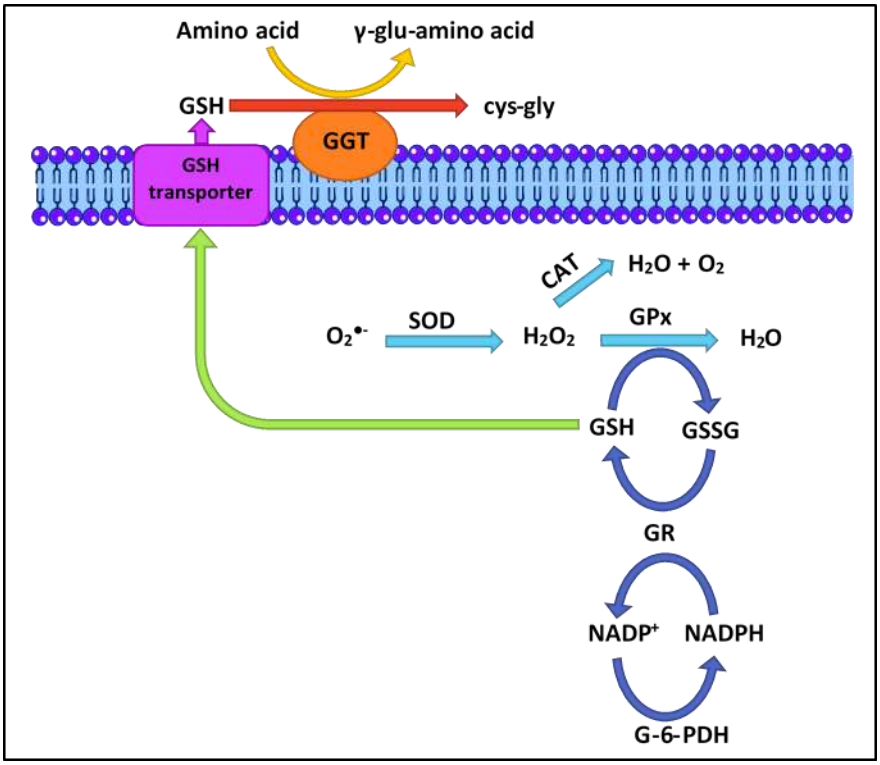

Figure 1: The enzymatic antioxidant system.

Abbreviations: CAT: Catalase; GPx: glutathione peroxidase; GR: glutathione reductase; SOD: superoxide dismutase; $O_2^{\bullet-}$: superoxide; H_2O_2: hydrogen peroxide; GSH: glutathione; GSSG: glutathione disulphide; $NADP^+$: Nicotinamide adenine dinucleotide phosphate (oxidised); NADPH: nicotinamide adenine dinucleotide phosphate (reduced); G-6-PDH: glucose-6-phosphate dehydrogenase; GGT: gamma-glutamyl transferase; Cys-Gly: cysteinyl-glycine; gamma-glu-amino acid: gamma-glutamyl amino acid [38].

Gamma-glutamyltransferase is a known marker of alcohol abuse, since elevated GGT can indicate liver damage [40-42]. Some studies suggest that elevated GGT may also be an early and sensitive marker for oxidative stress [40-42] independent of alcohol consumption [17, 43, 44]. Gamma-glutamyltransferase is responsible for the extracellular breakdown of glutathione, while transferring a glutamyl moiety to acceptor amino acids to be made available for intracellular resynthesis of GSH [45]. Experimental studies indicated that GGT increases the production of ROS [46-48]. Cysteinyl-glycine, one of the products of GGT action, is capable of reducing Fe^{3+} to Fe^{2+}, directly resulting in the production of free radical species [41].

Oxidative stress and cardiac structure and function

Reactive oxygen species plays an important role in the heart for cardiomyocytes produce ROS through NAD(P)H oxidase to regulate intracellular signalling cascades [49]. Oxidative stress is therefore also involved in alterations in left ventricular structure and function. Oxidative stress have several different effects on the heart, the most eminent being damage to macromolecules, membranes and enzymes involved in energy production, which may lead to energy deficiency and increased apoptosis [50].

Changes in cardiac structure and function due to oxidative stress can develop as a result of two mechanisms (direct and indirect). Indirectly, increased ROS (or oxidative stress) may contribute to the development of hypertension and arterial stiffness and this increases the workload on the heart through the increased afterload effect [51]. When the heart experiences a hemodynamic load, as is the case with hypertension and increased arterial stiffness, the following compensatory mechanisms can be implemented: (i) the Frank-Starling mechanism to increase cross-bridge formation or (ii) increasing muscle mass. The Frank-Starling law briefly states that the force of the contraction of the heart depends on the blood volume present in the ventricle at the end of diastole. The stretching of the muscle fibres alters the Ca^{2+} sensitivity of the myofibrils and then causes an increase in actin-myosin cross-bridges to form [52]. This mechanism is limited due to the limited muscle fibre length [53]. Therefore, increased left ventricular (LV) muscle mass assumes the key role in the compensatory mechanisms [53].

Oxidative stress and the vasculature

Reactive oxygen species have been implicated in numerous cardiovascular diseases, including hypertension, atherosclerosis, cardiac hypertrophy, heart failure and stroke [54-56]. In the vasculature, nitric oxide (NO) is responsible for endothelium-dependent vasorelaxation [57]. The enzyme responsible for NO production – endothelial nitric oxide synthase (eNOS) – requires tetrahydrobiopterin (BH_4), bound near its heme group, and L-arginine to form L-

citrulline and NO. Also, BH_4 is responsible for a balance between NO and $O_2^{\bullet-}$ [28]. Branched arteries are exposed to oscillatory shear stress, which leads to the continuous NAPDH-dependent production of $O_2^{\bullet-}$. Increased $O_2^{\bullet-}$ reacts with NO to form peroxynitrite ($ONOO^-$), which in turn oxidises BH_4, the eNOS-cofactor [58]. In the presence of oxidised BH_4, eNOS becomes uncoupled and produces more $O_2^{\bullet-}$ and H_2O_2 [7, 57, 59]. This leads to a vicious cycle of ROS-induced ROS production and ultimately leads to endothelial dysfunction [7, 58, 59]. Endothelial dysfunction in lesion-prone areas of the arterial vascular leads to circulating lipoprotein particles accumulating in the sub-endothelial space [60, 61]. This then leads to the gathering of circulating monocytes to the intima [60, 62], where they differentiate into macrophages and, finally, foam cells [60]. Foam cells in the intima are the first sign for the development of an atherosclerotic lesion (**Figure 2**)[63].

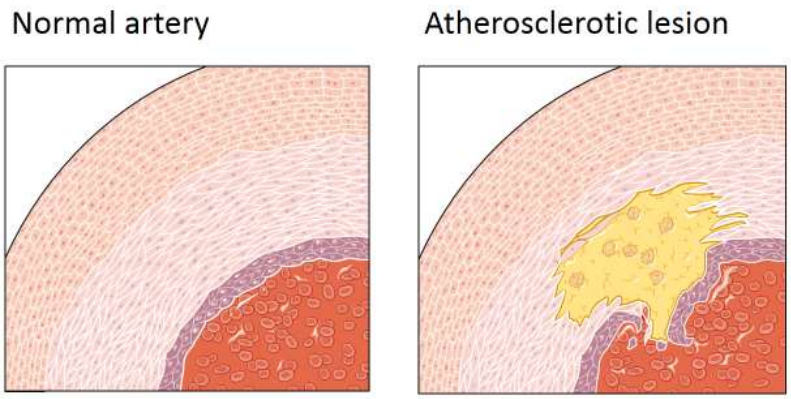

Figure 2: A normal arterial wall versus an arterial wall with an atherosclerotic lesion

Oxidative stress and hypertension

Several studies have demonstrated the link between elevated ROS and the development of hypertension, some including experimental hypertension models as well as human hypertension [64-66]. Studies have also revealed that antioxidant treatment can be effective for lowering high blood pressure [67]. It is believed that there is a feed-forward system whereby a pro-oxidant state promotes hypertension which, in turn, causes increased ROS formation [54]. Ultimately, chronic hypertension leads to compensatory hypertrophy to handle the increased workload on the heart [68]. Several studies have shown that the beneficial

effects of anti-hypertensive drugs, such as angiotensin I converting enzyme inhibitors, angiotensin-II receptor antagonists, b-adrenergic, and calcium channel blockers, may, in part, be mediated by decreasing vascular oxidative stress [69-72].

Reactive oxygen species directly influences the function of the heart as a mediator of excitation-contraction coupling [73]. Similarly, structure is influenced by ROS by stimulating a variety of hypertrophy-signalling kinases [73-75].

Oxidative stress and excitation-contraction coupling

Reactive oxygen species plays an important role in the heart, and oxidative stress is, therefore involved in alterations in LV structure and function. Oxidative stress has several different effects on the heart, the most eminent being damage to the macromolecules, membranes and enzymes involved in energy production, which may lead to energy deficiency and increased apoptosis [50]. The excitation-contraction coupling system, responsible for the coordination of the contractile function of the heart, is susceptible to oxidative modification [73].

The cardiac ryanodine receptor (RyR2), located on the sarcoplasmic reticulum (SR), acts as the main effector of calcium-induced calcium release in the excitation-contraction-coupling system [73, 76-78]. The RyR2 receptor is activated by Ca^{2+}, entering through voltage-gated Ca^{2+} channels [73, 76-78]. This induces the further release of Ca^{2+} from the SR, thereby activating the contraction system [73, 76-78]. Abnormal oxidative modification of RyR2s by ROS, presumably through disulphide oxidation, leads to sarcoplasmic reticulum Ca^{2+} loss [27]. Sarcoplasmic reticulum Ca^{2+} depletion, in turn, causes a negative inotropic effect in the heart (reduced contractile force) [73]. Studies have also shown that cardiac voltage-gated Ca^{2+} and Na^{+} channels, sarcoplasmic reticulum calcium ATPase, Na^{+}/Ca^{2+} exchanger, and other ion-handling proteins are subject to oxidative modifications, resulting in altered (beneficial or detrimental) cardiac contractile function [25, 73].

Oxidative stress and hypertrophy-signalling kinases

Cardiac hypertrophy are partially regulated by redox-dependent modifications [75, 79]. In a study done on neonatal rat cardiomyocytes, SOD was inhibited gradually by the copper chelator, diethyldithiocarbamic acid [80]. With a small increase in ROS due to inhibition of SOD, hypertrophy increases, leading to increased LVMi, while a further increase in ROS due to prolonged inhibition of SOD might result in apoptosis [75, 79-81]. Similar to superoxide, hydrogen peroxide can cause alterations in the heart structure through activation of hypertrophy signalling kinases such tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, mitogen-activated protein kinases and Jun-nuclear kinase [74, 75, 81]. As mentioned before, the GPx enzyme is responsible for elimination of hydrogen peroxide, and therefore decreased GPx may result in increased levels of hydrogen peroxide [14].

Gender and racial differences in oxidative stress-related markers and cardiac structure and function.

In a study done in 2013, it was found that black South Africans have higher serum peroxides and GGT levels as well as a more vulnerable cardiovascular profile, than their white counterparts [10]. Another study revealed that black South Africans might be more susceptible to early onset cardiac changes under conditions of higher SBP compared to white South Africans of a similar age [82]. The study population of these studies included black and white participants aged 20 to 70 years with a mean age of about 40 years, whereas our population sample will be of participants aged 20 to 30 years. Cardiovascular gender differences have been explored by several studies and may be due to sex hormone differences [83, 84] and differences distribution of abdominal visceral adipose tissue [85]. Adipose tissue, which secretes pro-inflammatory cytokines, such as tumour necrosis factor α, interleukin-1, and interleukin-6, are potential stimulators for the production reactive oxygen species and reactive nitrogen species by macrophages and monocytes [86, 87].

MOTIVATION

A strong link exists between the oxidant and anti-oxidant system and the development of cardiovascular disease [8, 88-90]. Previous studies on black South Africans linked oxidative stress-related markers with arterial wall remodelling, increased blood pressure, reduced compliance and increased vascular resistance, all which have detrimental effects on cardiac structure and function [12, 91]. However, these findings were observed in older participants, with some already hypertensive or at increased risk of cardiovascular disease. The associations between oxidative stress-related markers and LV structure and function markers in young and healthy adults have not yet been studied. On investigating whether oxidative stress-related markers associate with LV structure and function in this currently understudied population, we can help elucidate where age, hypertension status and other co-morbidities of the previous studies have [14-17] confounded the reported results.

AIMS

We explored whether cardiac structure and function are associated with oxidative stress-related markers in young and healthy South Africans, in groups stratified by gender and ethnicity.

OBJECTIVES

In a study population of young (20-30 years) healthy black and white men and women, our objectives are to:

i. To compare cardiac structure (left ventricular mass index (LVMi) and relative wall thickness (RWT)) and function (ejection fraction (EF) and fractional shortening (FS)), and oxidative stress-related markers (GGT, GPx, tGSH and total antioxidant status (TAS)) between groups stratified by gender and ethnicity.

ii. To explore associations of cardiac structure and function with oxidative stress-related markers between groups stratified by gender and ethnicity.

HYPOTHESIS

Regarding our first objective, we hypothesise that:

- ✓ markers of cardiac structure (LVM and RWT) will be higher in the black men and women compared to the white men and women;
- ✓ markers of systolic function (EF and FS) will be comparable between the black and white women and the black and white men respectively; and
- ✓ GGT will be higher in the black men and women, and GPx, GSH and TAS will be lower in the black men and women compared to the white men and women.

With regard to our second objective, we hypothesise that:

- ✓ markers of cardiac structure (LVM and RWT) will positively associate with GGT and negatively with GPx GSH and TAS in the black men and women only; and
- ✓ ejection fraction and FS will positively associate with GGT and negatively with GPx, GSH and TAS in the black men and women only.

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CHAPTER 2

Methodology

STUDY DESIGN AND POPULATION SAMPLE

Cross-sectional data from 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) were used. The African-PREDICT study is a longitudinal study focusing on the early stages of hypertension and CVD development in 1 202 young, healthy black and white individuals (aged 20-30 years) over a follow-up period of ten years. This ongoing study is performed in and around the Potchefstroom area. The population sample for this study required young and healthy individuals; therefore, the African-PREDICT study adhered to strict eligibility criteria, listed in **Table 1**.

Table 1: Eligibility criteria for the African-PREDICT study

Inclusion criteria	Exclusion criteria
1. Self-reported black or white ethnicity 2. Aged 20-30 years 3. Men and Women (equally distributed) 4. Apparently healthy 5. Normotensive or pre-hypertensive (SBP<140 and DBP<90mmHg) based on the average of 4 BP measures in one day	1. Self-reported Indian, Asian, mixed origin ethnicity 2. A not permanent resident of Potchefstroom or surrounding areas or not intending to return regularly to this area 3. Inability to read or understand English 4. Previously diagnosed with Type 1 or 2 Diabetes Mellitus 5. Elevated glucose >5.6 mmol/L (confirmed glycated haemoglobin (HbA1c) ≥ 6.5%) 6. HIV or another known infectious disease 7. Fever (ear temperature > 37.5°C on the research day) 8. Previously diagnosed liver disease, cancer, tuberculosis or renal disease 9. Microalbuminuria > 30 mg/ml in spot morning urine or proteinuria

	10. Medication use for chronic disease, i.e. antihypertensive, anti-diabetic, antiretroviral or anti-inflammatory medication 11. Self-reported pregnancy or women who breastfeed 12. Recent surgery or trauma (within the past three months) 13. Self-reported previous history of stroke, angina pectoris or myocardial infarction 14. Phobia for needles (used during blood sampling)

For this MHSc study, we included the first consecutive 426 individuals of the African PREDICT study since, at the time of commencement of this study, this was the number of participants for which the biochemical analyses were complete and available in the dataset. After excluding individuals with missing values for variables of interest (n=65), a total of 361 participants were included in this cross-sectional study. Both the African-PREDICT study (NWU-00001-12-A1) and this MHSc study (NWU-00047-17-A1) (Appendix A) had been approved by the Health Research Ethics Committee of the North-West University, with all procedures performed according to the principles as set out in the Declaration of Helsinki.

ORGANISATIONAL PROCEDURES

Recruitment for the African-PREDICT study took place from the beginning of 2012 until the baseline sample of 1 202 participants was reached. The recruited participants firstly entered into a screening phase, where the eligibility of the participants was assessed. All of the participants received feedback at the end of the screening day. The participants who met the eligibility criteria were invited to be part of the African-PREDICT study and given detailed information about the study and the measurements that would be taken. For those participants who were willing and able to participate, an appointment was made at the Hypertension Research and Training Clinic of the North-West University.

The screening took place at the Hypertension Research and Training Clinic and other locations, such as the participants' workplace, to increase accessibility for participants. Screening procedures included a general health and demographic questionnaire, fasting rapid blood analyses via a finger prick (to measure total cholesterol, blood glucose and an HIV test), brachial blood pressure, a dipstick spot urine test and anthropometric measurements. Prior to the brachial office blood pressure being taken, the individuals were requested not to smoke, exercise or eat for 30 minutes leading up to the measurement and they were asked to be seated in an upright position with the arm supported at heart level. The first measurement was taken on the left arm. After that, the blood pressure of the right arm was measured twice and, lastly, the left arm blood pressure was measured again. The HIV testing was done by a trained counsellor and the participants received pre- and post-counselling in a private room. All of the participants signed an informed consent form before the screening phase. Before taking part in the research study, the participants were also informed that they could withdraw at any time without penalty.

METHODOLOGY PERTAINING TO THIS MHSC STUDY

Questionnaire data

Each participant completed a general health questionnaire [1]. The data included demographic information, employment information, and information on alcohol and tobacco use, medication use, family history and socioeconomic status as well as basic data such as age, gender and ethnicity. The data obtained from the questionnaire would be used to obtain data on confounding factors such as alcohol, tobacco and medication use, especially contraception use in women.

Body composition and physical activity

Anthropometric measurements included body weight (SECA electronic scales, SECA, Birmingham, United Kingdom), height (m) (SECA stadiometer, SECA) and waist

circumference (cm) (Lufkin Steel Anthropometric tape, W606PM, Lufkin, Apex, United States of America). Body surface area was calculated by using the Mosteller equation [2], that is to say, body surface area (m^2) is equal to the square root of (height (cm) x weight (kg)/3600). All anthropometric measurements were performed according to the guidelines as described by the International Society for the Advancement of Kinanthropometry [3]. The anthropometric data are important to consider the effect of obesity on inflammation and oxidative stress [4, 5], as well as the effect on the heart structure and function [6]. All of the participants were fitted with an ActiHeart physical activity monitor, which was worn for a maximum of seven days (CamNtech Ltd., England, United Kingdom). The ActiHeart monitor records heart rate, inter-beat-interval and physical activity. The data were used to obtain the activity energy expenditure (AEE) [7], which is of importance because strenuous exercise has been reported to influence the redox balance [8] as well as effects on the heart [8].

Blood pressure measurements

Participants were fitted with a 24-hour ambulatory blood pressure (ABPM) and electrocardiography apparatus (Card(X)plore, Meditech, Budapest, Hungary, British Hypertension Society-validated). An appropriately sized cuff was fitted to each participant and the device was programmed to take recordings every 30 minutes during the day (6 am to 10 pm) and every hour during the night (10 pm to 6 am). The mean successful inflation rate of the participants over a 24-hour period was 87.5%. The ABPM was fitted to each participant at approximately the same time every day (late morning). The Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research recommends using 24-hour ambulatory blood pressure measurement for measuring blood pressure, since several prospective studies have revealed that it predicts the risk of morbid cardiovascular events better than office blood pressure [9].

Echocardiography

A standard transthoracic echocardiography procedure was followed by a trained technologist while each participant was in a partial left decubitus position with the head of the examining table slightly elevated. The echocardiography data were analysed using the EchoPAC software (GE, Version 10.8.1) to determine measures of LV structure and function. The General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway) was used (**Figure 1**), along with the 2.5 to 3.5 MHz transducer and a single electrocardiogram (ECG) lead for timing purposes. Standardised methods were used to obtain high-quality recordings, according to the guidelines of the European Association of Echocardiography and the American Society of Echocardiography [10, 11].

Figure 1: A photo taken of the General Electric Vivid E9 device in the HART Research and Training Lab for echocardiography data

Left ventricular mass was calculated by the corrected Devereux formula and indexed for body surface area [12], hence LV mass index (LVMi) [13]. Left ventricular mass needs to be normalized according to body size and according to the American Society of Echocardiography's guidelines [14]. Relative wall thickness (RWT) was calculated as twice the posterior wall thickness by LV diastole diameter [10]. Global LV systolic function was derived from linear measurements obtained from 2D images since the study population did not present with regional wall motion abnormalities or irregular heart rhythm [15]. Standard methods were used to determine endocardial fractional shortening (see **Formula 1**) (FS) [10]. Left ventricular ejection fraction (EF) was calculated from LV end-diastolic and end-systolic volume estimates derived from 2D images according to the biplane method (see **Formula 2**) [10].

Formula 1: Fractional shortening [16]

$$\text{Fractional shortening} = \frac{\text{End diastolic dimension} - \text{End systolic dimension}}{\text{End diastolic dimension}}$$

Formula 2: Ejection fraction [16]

$$\text{Ejection Fraction} = \frac{\text{End diastolic volume} - \text{End systolic volume}}{\text{End diastolic volume}}$$

Biochemical measurements

Blood samples from fasting participants were collected in the appropriate tubes and processed accordingly to yield serum and plasma fractions. Glucose was determined in plasma, prepared in sodium fluoride tubes, using a Cobas Integra 400 plus auto-analyser (Roche, Basel, Switzerland). Basic serum analysis was done, including total cholesterol, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein and GGT measured using the Cobas

Integra 400 plus (Roche, Basel, Switzerland). Total cholesterol and high-density lipoprotein was used as adjustments in the multiple regression analysis due to it being indicative of cardiovascular risk [17]. C-reactive protein is a marker for inflammation [18]. Cotinine, a metabolite of nicotine, was measured with a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany).

Glutathione peroxidase and TAS were measured using assay kits (Randox, Co., Antrim, United Kingdom) on the Cobas Integra 400 plus (Roche, Basel, Switzerland). Total Glutathione levels were determined with the use of the BIOXYTECH® GSH/GSSG-412TM kit (*Oxis*Research™, a division of Health Products, Foster City, California, United States of America) on Synergy H4 hybrid microplate reader (BioTek, Winooski, Vermont, United States of America). The African-PREDICT study protocol included the following panel of oxidative stress-related markers; glutathione reductase, TAS, reactive oxygen species (or serum peroxides, superoxide dismutase, glutathione peroxidase, and total glutathione. We excluded some of the oxidative stress-related markers that did not have any significant interactions or correlations with the cardiac measures.

In order to express the precision of the biochemical assays, we reported the intra- and inter-assay variability (**Table 2**), which is calculated by dividing the standard deviation of the set of measurements by the mean of that set, and then multiplying this by a hundred to yield a percentage.

Table 2: Summary of the intra-assay and inter-assay variability of the biochemical measures

Biochemical measurement	Intra-assay variability (%)	Inter-assay variability (%)
Gamma-Glutamyl transferase (U/mL)	1.80	1.80
Glutathione Peroxidase (U/L)	4.86	7.30
Glutathione (µM)	6.16	13.7
Total anti-oxidant status (mmol/L)	4.07	5.06
Glucose (mmol/L)	1.80	2.10
Total cholesterol (mmol/L)	0.51	1.90
High density lipoproteins (mmol/L)	1.13	1.00
Cotinine (ng/mL)	10.7 in low concentrations 5.5 in high concentrations*	
C-reactive protein (mg/L)	1.30	3.50

* Intra- and inter-assay variability was similar with cotinine however differs with low and high concentrations

Student participation in data collection

The African-PREDICT study is a longitudinal study and the data collection takes place over several years; therefore, it is not possible for a student to be part of every phase of the data collection. The biochemical analysis were done by the Laboratory Manager and the students were not able to obtain these skills during the course of a Master's degree. The cardiac measures were done by a trained clinical technologist, however I was present during several measurements with permission form the participant to observe the manner in which data were collected. My involvement in the data collection involved the screening of participants, specifically the rapid tests (blood glucose, total cholesterol, blood typing and urine analysis), blood pressure measurements, pulse wave analysis and biological sample preparation and aliquoting for long-term storage in the research laboratory.

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CHAPTER 3

Research Article

AUTHOR INSTRUCTIONS FOR HEART LUNG AND CIRCULATION

- The word limit for original articles is a maximum of 4500 words including title page, abstract, text, figure legends and references.
- Tables and figures may be presented with captions within the main body of the manuscript; if so, figures should additionally be uploaded as high-resolution files.
- Manuscripts in 11 point Arial or Times New Roman fonts are preferred.
- The text should be in single column format.
- Do not use the word processor's options to justify text or to hyphenate words, however, do use bold face, italics, subscripts, superscripts etc.
- When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns.
- Every submission, regardless of category, must include; Cover letter, Conflict of interest, Gene association studies, complete manuscript, Permission
- Arrange complete manuscript as follows: (1) title page, (2) abstract and keywords if required, (3) text, (4) acknowledgments, (5) disclosures if required, (6) references, (7) tables (each complete with title and footnotes) (8) figures and (9) figure legends.
- Number pages consecutively, beginning with the title page as page 1 and ending with the legend page.
- Divide your article into clearly defined sections: Introduction, Materials and methods, Results, Discussion (avoid extensive citations and discussion of published literature), and Conclusions (may stand-alone or form a subsection of a Discussion or Results and Discussion section), Appendices.
- Essential title page information; Title, Author names and affiliations, Corresponding author, Present/permanent address
- The abstract should state briefly the purpose of the research, the principal results and major conclusions.

- Immediately after the abstract, provide at least 2 keywords associated with their paper using British spelling.
- Tables should be double-spaced on separate pages (one to each page). Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.
- *Reference style:* Consecutive numbers in square brackets should be used to indicate references in the text, e.g., [1,2], as part of the text and not raised above it.
- The full reference should be cited in a numbered list essentially according to the Vancouver Uniform Requirements (see 5th ed., Ann Intern Med 1997;126(1):36-47).
- Journal References should contain the names of the first 6 authors (surnames followed by initials), followed by “et al."
- Example of reference

  1. Ordain TM, Shainoff JR, Lawrence SO, and Simpson-Haidaris PJ. Thrombin cleavage enhances exposure of the heparin-binding domain in the N-terminus of the fibrin beta chain. Blood 1996;88:2050-61.

  2. Copley AL. The endothelial fibrin lining. Thromb Res 1983;(SV):1-154. Book References should contain Author Name(s) in the same format as above: Title. Publisher's location: Name; Year of publication; Page range. Davies JT, Rideal EK. Interfacial Phenomena. New York-London: Academic Press; 1961. p. 110-30.

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Left ventricular structure and function and the link with oxidative stress-related markers in young adults: The African-PREDICT study

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Conflicts of interest

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Number of tables: 4

ABSTRACT

Background: Oxidative stress is associated with detrimental effects on the heart and vasculature, especially in older individuals. However, it is unclear whether oxidative stress-related markers are associated with cardiac structure and function in young, healthy adults. We, therefore, investigated the links between cardiac structure and function with oxidative stress-related markers in young, healthy adults.

Methods: A total of 361 black and white men and women (aged 20-30 years) were included in this cross-sectional study. Echocardiography measures included relative wall thickness (RWT), left ventricular mass index (LVMi), ejection fraction (EF) and fractional shortening (FS). The oxidative stress markers, gamma-glutamyl transferase (GGT), glutathione peroxidase (GPx), total glutathione (tGSH) and total antioxidant status (TAS) were also measured.

Results: When comparing the black and white groups, EF and FS were comparable between women but lower for the white men than the black men ($p=0.013$). LVMi was comparable among all of the black and white groups. When comparing oxidative stress markers, GGT was higher in the black women that the white women ($p<0.001$), while GPx was lower among both the black groups (all $p<0.05$). In the multiple regression analysis, LVMi was independently associated with GPx in black women ($\beta=–0.286$; $p=0.010$) and white men ($\beta=0.329$; $p=0.004$). In the white men only, both EF ($\beta=–0.345$; $p=0.018$) and FS ($\beta=–0.335$; $p=0.019$) were inversely associated with GGT.

Conclusions: In a young population, oxidative stress-related markers were associated with markers of cardiac function and structure. Future longitudinal investigations are needed to clarify the whether these associations are beneficial or adverse.

Keywords: Left ventricular mass, glutathione peroxidase, gamma-glutamyl transferase, ethnicity, young adults

INTRODUCTION

Reactive oxygen species (ROS) are involved in physiological signal transduction pathways, such as oxidative modification of regulatory and contractile proteins [1]. However, oxidative stress (an imbalance between oxidants and antioxidants) is associated with vascular remodelling, endothelial dysfunction and cardiomyopathy [1-3], which may consequently contribute to cardiac hypertrophy [4-6] and adverse cardiac function [1, 7].

In South Africa, previous studies revealed significant differences in oxidative stress and blood pressure [8, 9], carotid intima-media thickness [8] and arterial stiffness [9], in older black individuals with increased cardiovascular disease risk factors. Several previous studies focussed on blood pressure and hypertension and its association with oxidative stress-related markers in black individuals compared to their white counterparts, however the mean age of the participants in this studies were all above 40 years [8, 10, 11], while some investigated oxidative stress in relation with cardiac hypertrophy and the progression of heart failure [4, 6, 12-15]. Consequently, it is not known how markers of oxidative stress relates to cardiac structure and function in young healthy individuals. Previous results on such a population indicated that oxidative stress-related markers, such as lower glutathione peroxidase (GPx), is negatively associated with higher pulse pressure, suggesting that oxidative stress may be associated with early vascular changes [16]. We aimed to explore whether left ventricular structure and function associated with oxidative stress-related markers in young healthy black and white men and women.

METHODS

Study design and population sample

Cross-sectional data from the African prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) were used in this study. The first consecutive 426 individuals (aged 20-30 years) of the African-PREDICT study with complete oxidative stress data were included. The African-PREDICT study (NWU-00001-12-A1), as well as this sub-study (NWU-00047-17-A1), was approved by the North-West Province Department of Health in South Africa and the Health Research Ethics Committee of the North-West University. All procedures were performed according to the declaration of Helsinki. The procedures were fully explained to the participants of the study in their preferred language, after which the participants signed an informed consent form.

Participants were included in the study on the condition that they were normotensive (i.e. office blood pressure below 140/90 mmHg), had no history of cardiovascular diseases or other chronic diseases (or treatment thereof) and were not pregnant or breastfeeding. A total of 361 participants were included in this cross-sectional study after excluding individuals with missing values for variables of interest (n=64).

Questionnaire data

Basic data, such as age, gender and ethnicity, were collected via a general health questionnaire. The questionnaire also included data on hormonal contraception use in women, self-reported smoking and alcohol consumption.

Body composition and physical activity

Anthropometric measurements included body weight (SECA electronic scales, SECA, Birmingham, United Kingdom), height (m) (SECA stadiometer, SECA) and waist circumference (cm) (Lufkin Steel Anthropometric tape, W606PM, Lufkin, Apex, United States

of America). Body surface area was calculated using the Mosteller equation [17]. All anthropometric measurements were performed according to the guidelines as described by the International Society for the Advancement of Kinanthropometry [18]. To obtain activity energy expenditure (AEE), the participants were fitted with an ActiHeart physical activity monitor (CamNtech Ltd., England, United Kingdom), which was worn for a maximum of seven days.

Blood pressure measurements

The participants were fitted with a 24-hour ambulatory blood pressure (ABPM) and electrocardiography apparatus (Validated CardioXplore devices (CardioXplore, Meditech, Budapest, Hungary)), programmed to take recordings every 30 minutes during the day (6 am to 10 pm) and every hour during the night (10 pm to 6 am). The mean successful inflation rate of the participants over a 24-hour period was 87.5%. The ABPM was fitted to each participant at approximately the same time every day (late morning), using an appropriately sized cuff.

Echocardiography

A standard transthoracic echocardiography procedure was followed while each participant was in a partial left decubitus position with the head of the examining table slightly elevated. The echocardiography data were analysed using the EchoPAC software (GE, Version 10.8.1) to determine measures of LV structure and function. The General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway) was used, along with the 2.5 to 3.5 MHz transducer and a single electrocardiogram (ECG) lead for timing purposes. Standardised methods were used to obtain high-quality recordings, according to the guidelines of the European Association of Echocardiography and the American Society of Echocardiography [19, 20].

Left ventricular mass (LVM) was calculated by the corrected Devereux formula and indexed for body surface area [21], therefore, left ventricular mass index (LVMi) [22]. Relative wall thickness (RWT) was calculated as twice the posterior wall thickness by LV diastole diameter

[19]. Global LV systolic function was derived from linear measurements obtained from 2D images as the study population did not present with regional wall motion abnormalities or irregular heart rhythm [23]. Standard methods were used to determine endocardial fractional shortening (FS) [19]. Left ventricular ejection fraction (EF) was calculated from LV end-diastolic and end-systolic volume estimates derived from 2D images according to the biplane method [19].

Biochemical analyses

Blood samples from fasting participants were collected in the appropriate tubes and processed accordingly to yield serum and plasma fractions. Glucose was determined in plasma, prepared in sodium fluoride tubes, using the Cobas Integra 400 plus (Roche, Basel, Switzerland). Basic serum analysis was done, including total cholesterol, high-density lipoprotein cholesterol, C-reactive protein and gamma-glutamyl transferase (GGT) measured using the Cobas Integra 400 plus (Roche, Basel, Switzerland). Cotinine, a metabolite of nicotine, was measured with a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany).

Glutathione peroxidase (GPx) and total antioxidant status (TAS) were measured using assay kits (Randox, Co. Antrim, United Kingdom) on the Cobas Integra 400 plus (Roche, Basel, Switzerland). Total glutathione (tGSH) levels were determined with the use of the BIOXYTECH® GSH/GSSG-412TM kit (*Oxis*Research™ a division of Health Products, Foster City, California, United States of America) on Synergy H4 hybrid microplate reader (BioTek, Winooski, Vermont, United States of America).

Statistical analyses

All statistical analyses were performed with the IBM® SPSS® Statistics version 25 (IBM Corporation, Armonk, New York, United States of America). The normality of all variables was tested by assessing skewness and kurtosis. Those with a non-Gaussian distribution included AEE, total cholesterol, high-density lipoprotein cholesterol, triglycerides, and cotinine, C -

reactive protein, GGT and GPx. These skewed variables were logarithmically transformed. Interactions of ethnicity and gender were tested for all associations between LV structure and function markers with oxidative stress-related markers using analysis of covariance (ANCOVA). Independent T-tests were performed to compare means (with standard deviations or 95% percentile boundaries for logarithmically transformed variables) between groups. Chi-square tests were performed to assess proportions for categorical variables. Partial correlations (adjusting for age, waist circumference and systolic blood pressure) and multiple regression analyses were performed to investigate relationships of LV structure and function with markers related to oxidative stress. Variables that entered the multiple regression models included age, 24-hour ambulatory systolic blood pressure, AEE, waist circumference, total cholesterol and glucose. In the women, we additionally included contraceptive use in the models, since hormonal contraception has been shown to influence the redox balance [24, 25].

RESULTS

Significant interactions of both gender and ethnicity were found for several associations tested (Table 1), and the total group of participants was, therefore, stratified according to gender and ethnicity. The general characteristics of the study population are presented in Table 2. The LVMi was comparable in both women and men, whereas RWT was higher in the black men ($p=0.014$) and women ($p<0.001$) than their white counterparts. In the women, EF and FS were comparable, but higher in the black men than the white men (both $p=0.013$). GGT was higher in the black women than the white women ($p<0.001$), but comparable in the men. GPx activity and TAS were lower in the black groups than the white groups, whereas tGSH was higher in the black groups than the white groups (all p-values <0.05).

In the partial regression analysis (Table 3), after adjusting for age, systolic blood pressure and waist circumference, an inverse correlation was observed between LVMi and GPx ($r=–0.292$; $p=0.004$) in the black women, but a positive correlation was observed between LVMi and GPx ($r=0.343$; $p=0.004$) in white men. RWT correlated positively with GGT ($r=0.212$; $p=0.036$) in the black women, whereas in the black men, RWT correlated positively with GPx ($r=0.228$; $p=0.048$). Inverse correlations of EF ($r=–0.304$; $p=0.011$) and FS ($r=–0.317$; $p=0.008$) with GGT were found in the white men only.

In the multiple regression analysis (Table 4), an independent association was confirmed between LVMi and GPx in the black women ($\beta=–0.286$; $p=0.010$) and the white men ($\beta=0.329$; $p=0.004$). In Table 5, EF ($\beta=–0.345$; $p=0.018$) and FS ($\beta=–0.335$; $p=0.019$) correlated inversely with GGT in the white men only. No significant associations were found with RWT in the multiple regression analysis.

DISCUSSION

We aimed to explore whether markers of LV structure and function were associated with oxidative stress-related markers in young, healthy adults. We found an inverse association between LVMi and GPx in the black women and a positive association between LVMi and GPx in the white men, even though LVMi was comparable and GPx was higher in the white men and women compared to the black men and women. In addition, inverse associations of EF and FS with GGT were found in the white men only.

Our result of an inverse association between LVMi and lower GPx activity in the black women is in accordance with findings from experimental studies done in GPx knockout mice [4, 26]. In one of these studies, it was found that significantly reduced GPx activity promotes cardiac-specific hypertrophy [4], whereas in another study, it was indicated that the overexpression of GPx, after a myocardial infarction, prevented LV remodelling and heart failure in mice [26]. Our findings also add to previous findings from the African-PREDICT study indicating that lower GPx activity is associated with arterial stiffness [16]. Arterial stiffness increases the workload on the heart through the increased afterload effect and this may also effect the LV structure due to cardiomyocyte hypertrophy [27]. Our findings suggest that lower GPx activity may also facilitate early subclinical LV structural changes. The mechanism by which lower GPx activity may facility cardiac deterioration may involve the role of GPx in lowering hydrogen peroxides [28]. If hydrogen peroxide accumulates (when GPx is low), a variety of hypertrophy-signalling kinases, such as protein kinase C, Jun-nuclear kinase and Akt kinases, are activated [5, 6], which may lead to increased LVMi. Therefore, the link between LVMi and GPx may suggest that black women are at a higher risk of developing potentially unfavourable changes in LV structure that are related to lower GPx activity when compared to their white counterparts [4, 26, 29].

The positive association of LVMi with GPx in the white men is unexpected and in contradiction to the literature and what we have found in the black women. Although our study design does

not allow us to elucidate this finding, we propose the following mechanism. When comparing the white men to their black counterparts, the white men displayed a more favourable oxidative stress profile, with higher GPx activity and higher TAS, but iLVM was comparable among the men. The positive association found in the white men between LVMi and GPx may be as a result of sufficient GPx activity in maintaining physiological hydrogen peroxide levels and, therefore, may delay the onset of structural cardiac changes [4, 26]. A similar finding was previously reported where it was argued that sufficient glutathione reductase (GR) activity may play a role in delaying or preventing the onset of hypertension in the white men, black women and white women [16].

Our results also revealed inverse independent associations of EF and FS with GGT in the white men only. A study showed the potential role of high GGT (mean value of 39.0 U/L) in predicting LV dysfunction in patients during the early post-myocardial infarction period [30, 31]. Another study confirmed the presence of elevated GGT in young patients with pre-hypertension [32]. Gamma-glutamyltransferase can provide cysteine for *de novo* synthesis of glutathione by breaking down extracellular glutathione into its constitutive amino acids [33]. This maintains the *in vivo* homeostasis of GSH and cysteine [33]. However, when GGT is high, it may contribute to the production of ROS and low-density lipoprotein oxidation [34]. In a pro-oxidant state, ROS affects the contractile function of the heart by oxidation of proteins central to excitation-contraction coupling [1, 5-7]. This may lead to changes in the contractile properties of the myocardium and depressed cardiac function [1, 13, 28, 35, 36]. However, the GGT levels in the white men were within the normal ranges (≤ 50 U/L for men) therefore, in this group, the result may suggest that the normal function of GGT (maintaining adequate cysteine levels for the *de novo* synthesis of glutathione) may play a role in maintaining adequate EF and FS.

The findings of our study should be interpreted in the context of its limitations and strengths. This study is a cross-sectional study; therefore, causality cannot be determined. The study population consisted of participants from the Potchefstroom area and may not represent the

South African population. Although our results were consistent after multiple adjustments, we cannot exclude other unknown interactions that may also play a role in the link between oxidative stress-related markers and cardiac structure and function. However, our study was well designed and conducted under strict conditions in a fully equipped research facility.

In conclusion, the inverse association between LVMi and GPx found in the black women may suggest that black women are potentially subjected to the development of unfavourable changes in LV structure relating to lower GPx activity. With comparable GGT levels amongst black and white men, the inverse association with EF and FS in white men may indicate subclinical changes in cardiac function. However, further studies are encouraged to investigate this hypothesis and whether these associations are beneficial or adverse.

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Table 1: Interactions of sex and ethnicity on the associations of cardiac structure and function measures and oxidative stress measures

	Sex		Ethnicity	
	F	**P**	**F**	**P**
Relative wall thickness (cm)				
γ -Glutamyl transferase (U/L)	8.35	0.039	13.9	<0.001
Glutathione peroxidase (U/L)	–	–	11.9	<0.001
Glutathione (μM)	–	–	9.88	<0.001
Total anti-oxidant status (mmol/L)	–	–	12.5	0.001
Left ventricular mass index (g/m2)				
γ-Glutamyl transferase (U/L)	43.4	<0.001	6.28	0.017
Glutathione peroxidase (U/L)	47.8	<0.001	–	–
Glutathione (μM)	39.5	<0.001	–	–
Total anti-oxidant status (mmol/L)	–	–	–	–
Ejection fraction (%)				
γ-Glutamyl transferase (U/L)	6.88	<0.001	2.95	0.023
Glutathione peroxidase (U/L)	5.47	0.005	–	–
Glutathione (μM)	8.01	<0.001	–	–
Total anti-oxidant status (mmol/L)	8.80	<0.001	–	–
Fractional shortening (%)				
γ-Glutamyl transferase (U/L)	6.89	0.001	–	–
Glutathione peroxidase (U/L)	5.56	0.004	–	–
Glutathione (μM)	6.75	0.001	–	–
Total anti-oxidant status (mmol/L)	7.67	0.001	–	–

(–) Values omitted from the table were not significant

Table 2: General characteristics of the study population

	Black women n = 100	**White women** n = 109	**p-value**	**Black men** n = 80	**White men** n = 72	**p-value**
Age (years)	24.3 ± 3.45	25.4 ± 2.77	0.011	24.4 ± 3.3	25.4 ± 3.0	0.036
Anthropometry						
Height (m)	1.59 ± 0.064	1.67 ± 0.062	< 0.001	1.70 ± 0.062	1.79 ± 0.063	< 0.001
Weight (kg)	67.8 ± 14.7	68.2 ± 15.0	0.87	63.0 ± 10.4	90.6 ± 19.4	< 0.001
Body surface area (m^2)	1.72 ± 0.197	1.77 ± 0.192	0.075	1.72 ± 0.157	2.11 ± 0.229	< 0.001
Waist circumference (cm)	79.9 ± 11.7	75.6 ± 11.6	0.008	74.2 ± 8.67	91.9 ± 14.5	< 0.001
Cardiovascular measures						
Systolic blood pressure (mmHg)	114 ± 8	113 ± 8	0.85	121 ± 9	125 ± 7	0.002
Diastolic blood pressure (mmHg)	69 ± 5	68 ± 6	0.62	70 ± 6	71 ± 6	0.53
Left ventricular mass index (g/m^2)	62.9 ± 11.9	65.8 ± 15.0	0.12	77.2 ± 16.4	80.9 ± 13.1	0.12
Relative wall thickness (cm)	0.364 ± 0.070	0.326 ± 0.060	< 0.001	0.354 ± 0.071	0.329 ± 0.049	0.014
Ejection fraction (%)	69.1 ± 6.09	68.7 ± 6.87	0.60	67.8 ± 6.92	65.3 ± 5.57	0.013
Fractional shortening (%)	38.9 ± 4.97	39.0 ± 5.37	0.86	38.1 ± 5.42	36.1 ± 4.10	0.013
Biochemical measures						
γ-Glutamyl transferase (U/L)	23.3 (10.3; 58.4)	14.0 (6.8; 40.6)	< 0.001	29.1 (12.1; 107.1)	24.8 (10.4; 72.4)	0.12
Glutathione peroxidase (U/L)	18.5 (14.7; 20.8)	19.9 (18.0; 22.0)	< 0.001	18.4 (15.0; 20.5)	19.7 (16.9; 21.6)	< 0.001
Glutathione (μM)	1199 ± 280	941 ± 244	< 0.001	1268 ± 310	924 ± 245	< 0.001
Total anti-oxidant status (mmol/L)	1.26 ± 0.116	1.39 ± 0.116	< 0.001	1.39 ± 0.106	1.57 ± 0.126	< 0.001
Glucose (mmol/L)	3.92 ± 0.743	4.54 ± 0.802	< 0.001	3.81 ± 0.951	5.00 ± 0.734	< 0.001
Total cholesterol (mmol/L)	3.72 (2.66; 520)	4.69 (3.29; 6.71)	< 0.001	3.81 (2.80; 5.67)	4.62 (3.27; 6.14)	< 0.001
High density lipoproteins (mmol/L)	1.20 (0.789; 1.87)	1.58 (1.02; 2.45)	< 0.001	1.31 (0.841; 1.83)	1.08 (0.706; 1.74)	< 0.001
Cotinine (ng/mL)	2.04 (1.00; 159)	1.91 (1.00; 184)	0.78	14.25 (1.00; 395)	3.93 (1.00; 327)	< 0.001
C-reactive protein (mg/L)	2.23 (0.261; 13.2)	1.15 (0.100; 12.3)	< 0.001	0.720 (0.100; 6.22)	1.08 (0.182; 9.52)	0.046
Lifestyle measures						
AEE (kCal/kg/day)	6.53 (3.04; 12.6)	6.09 (2.47; 12.5)	0.29	5.95 (2.75; 11.3)	4.14 (1.47; 8.06)	< 0.001
Contraception, n (%)	52 (52.0)	45 (41.2)	0.063	–	–	–
Oral contraceptives, n (%)	21 (40.4)	44 (97.8)	0.005	–	–	–
Contraceptive injection, n (%)	31 (59.6)	1 (0.2)	< 0.001	–	–	–
Smoke, n (%)	11 (11.0)	11 (10.1)	0.83	44 (55.0)	17 (23.6)	< 0.001
Alcohol n (%)	54 (54.0)	69 (63.3)	0.27	61 (76.3)	50 (69.4)	0.28

Values are arithmetic mean ± SD, geometric mean (5th and 95th percentiles) or the number of participants. Abbreviations: AEE - Activity energy expenditure

Table 3: Partial correlations between cardiac structure and function and oxidative stress measures

	Left ventricular mass index (g/m2)				Relative wall thickness (cm)			
	Black women (n = 100)	**White women** (n = 109)	**Black men** (n = 80)	**White men** (n = 72)	**Black women** (n = 100)	**White women** (n = 109)	**Black men** (n = 80)	**White men** (n = 72)
γ-Glutamyl transferase (U/L)	*r*= 0.168 *p*= 0.099	*r*= –0.173 *p*= 0.075	*r*= 0.063 *p*= 0.71	*r*= –0.086 *p*= 0.48	*r*= 0.212 *p*= 0.036	*r*= 0.116 *p*= 0.23	*r*= –0.044 *p*= 0.70	*r*= –0.081 *p*= 0.51
Glutathione peroxidase (U/L)	*r*= –0.292 *p*= 0.004	*r*= 0.175 *p*= 0.072	*r*= 0.151 *p*= 0.19	*r*= 0.343 *p*= 0.004	*r*= –0.067 *p*= 0.51	*r*= –0.145 *p*= 0.14	*r*= 0.228 *p*= 0.048	*r*= –0.091 *p*= 0.45
Glutathione (μM)	*r*= 0.133 *p*= 0.19	*r*= 0.030 *p*= 0.76	*r*= –0.0013 *p*= 0.91	*r*= –0.007 *p*= 0.95	*r*= 0.037 *p*= 0.72	*r*= 0.012 *p*= 0.90	*r*= –0.086 *p*= 0.45	*r*= 0.112 *p*= 0.36
Total anti-oxidant status (mmol/L)	*r*= –0.104 *p*= 0.31	*r*= –0.099 *p*= 0.31	*r*= –0.107 *p*= 0.35	*r*= 0.023 *p*= 0.85	*r*= –0.109 *p*= 0.29	*r*= –0.149 *p*= 0.13	*r*= –0.063 *p*= 0.58	*r*= 0.051 *p*= 0.68
	Ejection Fraction (%)				**Fractional Shortening (%)**			
	Black women (n = 100)	**White women** (n = 109)	**Black men** (n = 80)	**White men** (n = 72)	**Black women** (n = 100)	**White women** (n = 109)	**Black men** (n = 80)	**White men** (n = 72)
γ-Glutamyl transferase (U/L)	*r*= 0.094 *p*= 0.36	*r*= 0.105 *p*= 0.28	*r*= –0.090 *p*= 0.43	*r*= –0.304 *p*= 0.011	*r*= 0.073 *p*= 0.48	*r*= 0.137 *p*= 0.16	*r*= –0.074 *p*= 0.52	*r*= –0.317 *p*= 0.008
Glutathione peroxidase (U/L)	*r*= 0.071 *p*= 0.49	*r*= 0.053 *p*= 0.59	*r*= –0.022 *p*= 0.85	*r*= 0.068 *p*= 0.57	*r*= 0.067 *p*= 0.51	*r*= 0.088 *p*= 0.37	*r*= –0.028 *p*= 0.80	*r*= 0.051 *p*= 0.68
Glutathione (μM)	*r*= 0.155 *p*= 0.13	*r*= 0.036 *p*= 0.71	*r*= 0.100 *p*= 0.38	*r*= 0.081 *p*= 0.50	*r*= 0.159 *p*= 0.12	*r*= –0.005 *p*= 0.96	*r*= 0.101 *p*= 0.38	*r*= 0.048 *p*= 0.69
Total anti-oxidant status (mmol/L)	*r*= 0.064 *p*= 0.53	*r*= -0.119 *p*= 0.22	*r*= -0.188 *p*= 0.097	*r*= 0.059 *p*= 0.63	*r*= 0.060 *p*= 0.56	*r*= –0.093 *p*= 0.34	*r*= –0.188 *p*= 0.098	*r*= 0.013 *p*= 0.90

Adjusted for age, waist circumference and systolic blood pressure.

Table 4: Multiple regression analysis between the left ventricular mass index and glutathione peroxidase

	Left ventricular mass index (g/m^2)			
	Black women n = 100		**White women n = 109**	
Adjusted R^2	0.074		0.063	
	Standardized β	**p-value**	**Standardized β**	**p-value**
Glutathione peroxidase (U/L)	–0.286 (–0.334; –0.048)	0.010	0.171 (–0.038; 0.456)	0.097
Age (years)	–0.069 (–0.211; 0.115)	0.561	0.203 (-0.004; 0.404)	0.055
Contraception (n)	0.078 (–0.107; 0.225)	0.481	0.200 (–0.006; 0.354)	0.059
Systolic blood pressure (mmHg)	0.030 (–0.173; 0.225)	0.797	0.020 (–0.214; 0.254)	0.868
AEE (kCal/kg/day)	–0.091 (–0.240; 0.098)	0.407	0.048 (–0.137; 0.226)	0.630
Waist circumference (cm)	–0.002 (–0.002; 0.211)	0.987	0.189 (–0.057; 0.432)	0.132
Cholesterol (mmol/L)	–0.167 (–0.315; 0.038)	0.122	–0.247 (–0.454; –0.046)	0.017
Glucose (mmol/L)	–0.120 (–0.697; 0.214)	0.294	–0.076 (–0.196; 0.085)	0.436
	Black men n = 100		**White men n = 109**	
Adjusted R^2	0.087		0.165	
	Standardized β	**p-value**	**Standardized β**	**p-value**
Glutathione peroxidase (U/L)	0.143 (–0.083; 0.365)	0.214	0.329 (0.121; 0.618)	0.004
Age (years)	0.008 (–0.234; 0.251)	0.945	–0.017 (-0.217; 0.187)	0.881
Systolic blood pressure (mmHg)	0.376 (0.141; 0.682)	0.003	0.272 (0.016; 0.620)	0.039
AEE (kCal/kg/day)	0.155 (–0.091; 0.45)	0.187	0.144 (–0.080; 0.324)	0.232
Waist Circumference (cm)	–0.195 (–0.764; 0.135)	0.167	–0.032 (–0.239; 0.190)	0.819
Cholesterol (mmol/L)	–0.097 (–0.406; 0.186)	0.461	–0.103 (–0.315; 0.120)	0.372
Glucose (mmol/L)	–0.017 (–0.222; 0.190)	0.880	0.215 (-0.005; 0.347)	0.057

Abbreviations: CI – Confidence interval; AEE – Activity Energy Expenditure

Table 5: Multiple regression analysis of ejection fraction and fractional shortening with gamma-glutamyl transferase in men

	Ejection fraction (%)			
	Black men n = 80		**White men n = 72**	
Adjusted R^2	–0.038		0.126	
	Standardized β	**p-value**	**Standardized β**	**p-value**
γ-Glutamyl transferase (U/L)	–0.059 (–0.533; 0.311)	0.679	–0.345 (–0.590; –0.059)	0.018
Age (years)	–0.087 (–0.347; 0.170)	0.498	–0.125 (–0.323; 0.098)	0.290
Systolic blood pressure (mmHg)	0.095 (–0.184; 0.394)	0.472	0.147 (–0.138; 0.488)	0.269
AEE (kCal/kg/day)	–0.118 (–0.437; 0.394)	0.345	–0.150 (–0.339; 0.081)	0.223
Waist circumference (cm)	–0.044 (–0.575; 0.433)	0.779	0.132 (–0.147; 0.353)	0.414
Cholesterol (mmol/L)	–0.134 (–0.478; 0.171)	0.349	0.036 (–0.200; 0.270)	0.769
Glucose (mmol/L)	0.026 (–0.200; 0.248)	0.830	–0.290 (–0.418; –0.051)	0.013
	Fractional shortening (%)			
	Black men n = 80		**White men n = 72**	
Adjusted R^2	–0.029		0.151	
	Standardized β	**p-value**	**Standardized β**	**p-value**
γ-Glutamyl transferase (U/L)	–0.038 (–0.331; 0.252)	0.788	–0.335 (–0.543; –0.050)	0.019
Age (years)	–0.087 (–0.346; 0.169)	0.496	–0.106 (–0.286; 0.105)	0.360
Systolic blood pressure (mmHg)	0.134 (–0.141; 0.435)	0.311	0.094 (–0.186; 0.395)	0.474
AEE (kCal/kg/day)	–0.126 (–0.444; 0.144)	0.313	–0.148 (–0.315; 0.074)	0.221
Waist Circumference (cm)	–0.078 (–0.629; 0.374)	0.614	0.195 (–0.089; 0.375)	0.222
Cholesterol (mmol/L)	–0.144 (–0.489; 0.158)	0.310	–0.024 (–0.239; 0.196)	0.844
Glucose (mmol/L)	0.023 (–0.201; 0.244)	0.847	–0.325 (–0.418; –0.077)	0.005

Abbreviations: CI – Confidence interval; AEE – Activity Energy Expenditure

CHAPTER 4

Concluding Remarks & Future Recommendations

INTRODUCTION

This chapter presents a summary of the findings reported in the research article (Chapter 3). The hypotheses, as set out in Chapter 1, will be accepted or rejected, based on our findings. The main findings of this study will also be compared to the existing literature. After that, recommendations will be made for future studies investigating the link between oxidative stress-related markers and LV structure and function.

SUMMARY OF MAIN FINDINGS AND REFLECTION ON HYPOTHESES

It is well known from the literature that a link exists between oxidative stress and CVD [1-6]. In the literature available on the link between oxidative stress (and oxidative stress-related markers) and CVD, the studies were performed in older individuals with [1-5, 7] or without [6] advanced CVD or in experimental animal models [8-15]. Some studies focused on hypertension and oxidative stress [16-22], while several studies investigated oxidative stress and cardiac hypertrophy or heart failure [8, 23-27]. One study from our research group found that only in the black men, the 24-hour pulse pressure associated negatively with GPx activity [28], which may suggest that lower GPx may accelerate vascular ageing. In spite of all of this information available, we identified a gap in the literature as none of these studies had focused on the link between oxidative stress-related markers and LV structure and function in young populations before the onset of CVD. This study can contribute to the understanding of the role of oxidative stress in LV structure and function.

Hypothesis 1 (a): *Markers of cardiac structure (LVM and RWT) will be higher in the black men and women compared to the white men and women.*

Relative wall thickness was higher in the black men and women compared to their white counterparts, as expected, but LVMi was comparable among all of the groups. One study found that the black population is more likely to have a higher cardiovascular risk profile, which includes a higher RWT [29], therefore, it is more likely for black individuals to have a higher

RWT than white individuals. The result of a higher RWT in the black groups is in accordance with this study. The result that LVMi was comparable amongst the groups, was not as we had hypothesized, but is still in accordance with a study that revealed that LVMi differ with ethnicity, but rather that RWT in the black race is associated with greater RWT [30]. That study population consisted of only male participants with mild to moderate hypertension [30]. The young age and healthy status of the study population can potentially have contributed to the comparable result of LVMi. This hypothesis is, therefore, only partially accepted.

Hypothesis 1 (b): Markers of systolic function (EF and FS) will be comparable between the black and white women and the black and white men respectively.

As expected, the EF and FS were comparable between the black and white women; however, the black men revealed a higher EF and FS that the white men. Although we did not hypothesize EF and FS to be higher in the black men compared to the white men, the values of the EF and FS are still well within normal ranges. In the previous studies that found black individuals had a worse cardiovascular risk profile that white individuals, with higher systolic and diastolic blood pressure [31, 32], the population sample had a higher mean age than our study, which may have contributed to their result. This result in the men warrants further investigating as most literature also demonstrates that the black race usually has a worse cardiovascular profile than the white race. The strict inclusion and exclusion criteria of the African-PREDICT study to ensure only apparently healthy individuals are included, may have had an influence on the unexpected result in the white men, as the values are all within normal ranges. This hypothesis is, therefore, only partially accepted.

Hypothesis 1 (c)*: GGT will be higher in the black men and women, and GPx, GSH and TAS will be lower in the black men and women compared to the white men and women.*

In the women, GGT was higher in the black than the white women, as expected, but in the men, the GGT levels were comparable between the black men and the white men. Also, as

expected, GPx and TAS were lower in the black men and women; however, tGSH revealed opposite results, demonstrating higher values in the black men and women than the white men and women. A previous study done in South Africa also found higher tGSH levels in the black men only, but the mean age of the population was higher (44.4 ± 8.3 years) than our population. We can postulate that the higher GGT, that is directly involved in the synthesis of GSH [33], could have influenced the higher GSH levels in the black men and women. This finding warrants further investigation as our study design does not allow us to confirm whether GGT is responsible for the higher GSH.

Hypothesis 2 (a): *Markers of cardiac structure (LVM and RWT) will positively associate with GGT in the black men and women only, and GPx, GSH and TAS will be negatively associated with LVM and RWT in the black men and women only.*

We confirmed an independent and negative association between LVMi and GPx in the black women; however, we also found an independent and positive association between LVMi and GPx in the white men. No associations with GSH and TAS were found. The result in the black women is in accordance with findings from experimental studies done in GPx knockout mice [8], where it was found that significantly reduced GPx activity promotes cardiac-specific hypertrophy. The GPx enzyme is responsible for breakdown of hydrogen peroxide, which acts as a second messenger in oxidative metabolism, and is linked with changes in cell physiology, such as insulin signalling and in several growth factor-induced signalling cascades [34]. When hydrogen peroxide accumulates it can cause cardiac specific hypertrophy through the activation of hypertrophy signalling kinases [25, 35]. This link may suggest that black women are at a higher risk of developing potentially unfavourable changes in LV structure that are related to lower GPx activity, than white women [8, 16, 36] . The positive association between LVMi and GPx in the white men are in contradiction to the literature and what we have found in the black women. We can suggest that this may be because of sufficient GPx activity in maintaining physiological hydrogen peroxide levels and, therefore, may delay the onset of structural cardiac changes [8, 36]. In a previous study, a similar finding was reported where it

was argued that sufficient GR activity may play a role in delaying or preventing the onset of hypertension [28]. However, our study design does not allow us to clarify this finding; therefore, further investigations is necessary. Our hypothesis is, therefore, only partially accepted.

Hypothesis 2 (b): *EF and FS will positively associate with GGT in the black men and women only, and GPx GSH and TAS will be negatively associated with EF and FS in the black men and women only.*

Our results revealed inverse independent associations of EF and FS with GGT in the white men only. Regarding associations with GPx, GSH and TAS, no significant results were found. We did not hypothesize that an association in the white men would be found; however, the GGT levels in white men were within the normal ranges (≤ 50 U/L for men) [37]. The result in the white men may, therefore, possibly indicate subclinical changes in cardiac function related to GGT activity. However, further studies are encouraged to investigate this hypothesis and whether these associations are beneficial or adverse

FUTURE STUDY RECOMMENDATIONS

Our study had a specific design and only included participants from Potchefstroom and surrounding areas. We, therefore, suggest that large population studies, including randomly selected individuals from all over South Africa, should be conducted to have an improved representation of South Africa. Large population-based studies can also evaluate ethnic differences, not only in black and white individuals, but also in individuals of other ethnicities.

Future studies should also include other markers involved in oxidative stress and inflammation, such as Glutathione reductase (GR), GSH (reduced form), glutathione disulphide (GSSG), and interleukin-6. Interleukin-6 is a marker of inflammation and because inflammation and oxidative stress are so closely linked this might influence some of the associations [38-40]. Glutathione reductase is the enzyme responsible for reducing a GSH to

GSSG and involving these markers will give a better overview of the oxidative stress profile [41].

Our study formed part of the African-PREDICT study, which aimed to have a follow-up after five years and then again after ten years to assess both incident hypertension and early changes in cardiovascular structure and function. We will be able to evaluate the progression of cardiac structure and function changes, as well as how oxidative stress relates to these changes over time.

CONCLUSION

In conclusion, the two main findings of this study were (i) the inverse association between LVMi and GPx found in the black women and (ii) the inverse association of EF and FS with GGT in the white men. The first finding may suggest that black women may potentially develop unfavourable changes in LV structure relating to lower GPx activity. The second finding may indicate the role of lower GGT in adequate LV function, although GGT was comparable and within normal ranges. In a young population, oxidative stress-related markers were associated with markers of cardiac function and structure. Our study highlighted that associations between oxidative stress-related markers and cardiac structure and function warrants further investigation by means of future longitudinal studies to elucidate the nature of these associations (beneficial or adverse).

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APPENDIX A

Approval from the Health Research Ethics Committee

NORTH-WEST UNIVERSITY
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ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by **Health Research Ethics Committee (HREC)** on **10/08/2017** after being reviewed at the meeting held on **17/05/2017**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: The interplay between adiposity, oxidative stress and cardiac structures and function: The African-PREDICT study
Study Leader/Supervisor: Prof R Kruger
Student: LC Hawley-24184462

Ethics number:

N	W	U	-	0	0	0	4	7	-	1	7	-	A	1
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single study
Commencement date: 2017-08-10 **Risk:** Minimal

Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special conditions of the approval (if applicable):

- Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

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

- *The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,*
    - *the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *HREC can be contacted for further information or any report templates via Ethics-HRECApply@nwu.ac.za or 018 299 1206.*

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

Yours sincerely

Prof LA Du Plessis

Digitally signed by Prof LA Du Plessis
Date: 2017.08.31 15:05:19 +02'00'

Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

APPENDIX B

Certificate of Language Editing

PROOF OF LANGUAGE EDITING

Dr. L. Hoffman

Kroonstad

BA, BA(Hons), MA, DLitt et Phil

Member of the South African Translators' Institute

Cell no: 079 193 5256 Email: larizahoffman@gmail.com

DECLARATION

To whom it may concern

I hereby confirm that I have proofread and edited the language of the following dissertation, including the bibliography.

Title of dissertation

Left ventricular structure and function and the link with oxidative stress in young adults: The African-PREDICT study

Candidate

Lee-Ann Hawley

Lariza Hoffman

Kroonstad

23 November 2018

APPENDIX C

Turn-it-in Report

24184462:FULL_dissertation_TURN_IT_IN_(4_desember).docx

ORIGINALITY REPORT

15%	4%	14%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Systems Biology of Free Radicals and Antioxidants", Springer Nature, 2014 Publication	5%
2	Caitlynd Myburgh, Hugo W. Huisman, Catharina M. C. Mels. "The relation of blood pressure and carotid intima-media thickness with the glutathione cycle in a young bi-ethnic population: the African-PREDICT study", Journal of Human Hypertension, 2018 Publication	3%
3	Johanna I. Kriel, Carla M. T. Fourie, Aletta E. Schutte. "Monocyte Chemoattractant Protein-1 and Large Artery Structure and Function in Young Individuals: The African-PREDICT Study", The Journal of Clinical Hypertension, 2017 Publication	1%
4	van Zyl, Caitlynd, Hugo W. Huisman, and Catharina M.C. Mels. "Antioxidant enzyme activity is associated with blood pressure and carotid intima media thickness in black men	1%

and women: The SABPA study", Atherosclerosis, 2016.
Publication

5	www.thieme-connect.com Internet Source	1%
6	Gontse Gratitude Mokwatsi, Aletta Elisabeth Schutte, Catharina Martha Cornelia Mels, Ruan Kruger. "Morning blood pressure surge in young black and white adults: The African-PREDICT Study", Journal of Human Hypertension, 2018 Publication	<1%
7	www.nature.com Internet Source	<1%
8	webmail.stuba.sk Internet Source	<1%
9	www.termedia.pl Internet Source	<1%
10	Verhelst, J., B. Velkeniers, D. Maiter, P. Haentjens, G. T'Sjoen, E. Rietzschel, B. Corvilain, P. Abrams, F. Nobels, R. Abs, and M. Bex. "Active acromegaly is associated with decreased hs-CRP and NT-proBNP serum levels : Insights from the Belgian registry of acromegaly", European Journal of Endocrinology, 2012. Publication	<1%

11	Ramos-Nino, Maria E.. "The Role of Chronic Inflammation in Obesity-Associated Cancers", ISRN Oncology, 2013. Publication	<1%
12	Jacob-Ferreira, Anna L., and Richard Schulz. "Activation of intracellular matrix metalloproteinase-2 by reactive oxygen–nitrogen species: Consequences and therapeutic strategies in the heart", Archives of Biochemistry and Biophysics, 2013. Publication	<1%
13	Žižek, Bogomir, and Pavel Poredoš. "Increased left ventricular mass and diastolic dysfunction are associated with endothelial dysfunction in normotensive offspring of subjects with essential hypertension", Blood Pressure, 2007. Publication	<1%
14	Botelho-Ono, M.S.. "Acute superoxide scavenging restores depressed baroreflex sensitivity in renovascular hypertensive rats", Autonomic Neuroscience: Basic and Clinical, 20110120 Publication	<1%
15	nopr.niscair.res.in Internet Source	<1%
16	"IPOS 9th World Congress Abstracts", Psycho-	<1%

Oncology, 09/2007
Publication

17	Orbach, A., T. Bassan-Levin, P. Dan, B. Hihinashvili, and S. G. Marx. "Utilizing Gsk-3Î² as a Marker for the Diagnosis of GVHD : 2484", Transplantation, 2012. Publication	<1%
18	Sedda, Valentina, Benedetta De Chiara, Marina Parolini, Raffaele Caruso, Jonica Campolo, Giuliana Cighetti, Renata De Maria, Aldo Sachero, Luigi Donato, and Oberdan Parodi. "Plasma glutathione levels are independently associated with γ-glutamyltransferase activity in subjects with cardiovascular risk factors", Free Radical Research, 2008. Publication	<1%
19	Marit G A van Vonderen, Yvo M Smulders, Coen D A Stehouwer, Sven A Danner et al. "Carotid Intima-Media Thickness and Arterial Stiffness in HIV-Infected Patients: The Role of HIV, Antiretroviral Therapy, and Lipodystrophy", JAIDS Journal of Acquired Immune Deficiency Syndromes, 2009 Publication	<1%
20	Clarice D. Brown. "Body Mass Index and the Prevalence of Hypertension and Dyslipidemia", Obesity, 12/2000 Publication	<1%

21	Lee, Duk-Hee Lim, Ji-Sun Song, Kyungeun . "Graded associations of blood lead and urinary cadmium concentrations with oxidative-stress-related m", Environmental Health Perspectives, March 2006 Issue Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

APPENDIX D

Copy of informed consent

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

HART
Hypertension in Africa Research Team

Health Research Ethics Committee
Faculty of Health Sciences
NORTH-WEST University
(Potchefstroom Campus)
2017 -08- 22
HREC Stamp

INFORMED CONSENT FORM FOR THE African-PREDICT STUDY (RESEARCH PHASE):

TITLE OF THE RESEARCH PROJECT: African **PR**ospective study on the **E**arly **D**etection and **I**dentification of **C**ardiovascular disease and Hyper**T**ension (African-PREDICT)

ETHICS REFERENCE NUMBER: NWU-00001-12-A1

PRINCIPAL INVESTIGATOR: Prof. Alta Schutte (PhD Physiology)

Prof. Schutte and the research team have the expertise and interest in Cardiovascular Physiology, namely to understand the biological processes in humans when high blood pressure and heart disease develop.

ADDRESS: NORTH-WEST UNIVERSITY (Potchefstroom Campus); Hypertension in Africa Research Team (HART); Hypertension Research and Training Clinic Building F11, Office 101.

CONTACT NUMBERS: 018 299 2444 / 018 285 2466 / 018 299 2780

You are invited to take part in the African-PREDICT research study. Please take some time to read the information presented here, which will explain the details of this study. Please ask the researcher or person explaining the research to you any questions about any part of this study that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research is about and how you might be involved. Also, your participation is **entirely voluntary** and you are free to say no to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part now.

This study has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00001-12-A1) and will be conducted according to the ethical guidelines and principles of Ethics in Health Research: Principles, Processes and Structures (DoH, 2015) and other international ethical guidelines applicable to this study. It might be necessary for the research ethics committee members or other relevant people to inspect the research records.

What is this research study all about?

You will know already from taking part in the screening phase of the study that heart disease and especially high blood pressure (or hypertension) is a big problem in South Africa. Also, many people are unaware of it, as it has no symptoms. High blood pressure is a very important risk factor which may result in heart disease, kidney disease and stroke. (*When blood stops flowing to the heart, this can cause a heart attack and part of the heart dies. A stroke is when there is a problem with the blood supply to the brain and a part of the brain is damaged.*) That is why many people in South Africa suffer from these diseases resulting in death.

Since heart disease is mostly seen in older people, the purpose of this study is to include and focus on young healthy people to understand how high blood pressure and heart disease develop. It is believed that our lifestyle (e.g. what we eat, drink, and do) may have an impact on whether we will develop high blood pressure and heart disease. Also, it is not well known whether there are perhaps certain measurements (e.g. in your blood or urine) that may predict whether you will develop heart disease when you are older.

The aim of this study is therefore to determine how high blood pressure and heart disease develop in a group of 1200 healthy young South Africans living in and around Potchefstroom, by tracking everyone over 5-20 years. It is therefore of great importance that we take detailed measurements of your lifestyle, and your current health (e.g. heart, blood vessels, eyes, blood and urine). These measurements will be made at the beginning of the study, but it will be most important to repeat these measurements in following visits every 5 years, to see how these health measurements have changed. We expect that some participants will remain healthy with normal blood pressures, and other will develop high blood pressure. Only by tracking the changes in blood pressures and other detailed measurements will be able to understand the influences of e.g. lifestyle on changes in blood pressure.

If your results show that a certain measurement predicts that high blood pressure will develop later in life, this information could help doctors and nurses to prevent more people in the local community having strokes and heart attacks in the future.

Why have you been invited to participate?

Your screening tests show that you are healthy and suitable to take part in this study. You are also in the most important age group of 20 to 30 years. As we would like to follow you over time it is ideal that you have indicated that you intend to stay in or around or visit Potchefstroom for the next 5 years at least.

It will also be very important for us to be able to keep in touch with you. We kindly ask that you **tell us immediately about any changes of your contact details** (address, telephone number, email address etc.).

Once we have performed all of the measurements as described below, we will have a much better understanding of your health status. If we are not able to obtain important measurements (such as 24-hour blood pressure measurements, or if we are unable to obtain a blood sample, or if we detect a serious health abnormality), you will most likely not be able to take further part in the research project. Once we have completed the measurements, we will discuss your results with you and the way forward.

What will be expected of you?

The research team will make an appointment with you, and if necessary, transport will be provided to bring you to the Hypertension Clinic (Building F12) on the Potchefstroom Campus of the North-West University. Such an appointment will be made for early in the morning, as the measurements will start at approximately 08:00, and in total will take about 5 hours to complete.

To make sure that your results are valid and useful, it is important to take note of the following:

1. The **evening before – Do not eat or drink anything except water after 10pm** or before you come to the clinic in the morning.
2. **On the study day, please wear comfortable clothing** such as trousers and a top that can be easily removed for the tests (please avoid wearing skirts, dresses or tights as we will need to access your bare foot and put a blood pressure cuff around your thigh over your trousers).
3. **Please bring with you**:
    - All medication you currently are taking
    - Your ID document & clinic card/book

- Some good quality sunglasses to protect your eyes after the measurements

4. Let us know if transport should be arranged for you.

If you are happy to participate, we will ask you to sign this consent form stating that you are volunteering to participate in this study and that you understand all the procedures that will be performed. You are free to contact us with any questions should there be any uncertainty about any of the information provided. Then we will take the measures listed in the table below. Tests will be done in the Hypertension Clinic and we will provide you with a meal during the day. You will not be able to bath or shower for 24 hours after your clinic appointment due to the equipment you will be wearing when you leave the clinic.

WHAT TESTS WILL BE DONE?

- **Body composition:** we will measure your height, weight, waist, hip and neck circumference in a private room, while you are wearing your underwear. In another room, while you are clothed and lying down on a bed, we will also measure your body fat percentage by using a device that connects with sensors on your hand and on your foot. This is a completely painless procedure. (the measurements should take about 20 minutes to complete)
- **Biological samples:** early in the morning while you are lying down on a bed, a research nurse will take a blood sample from a vein in your arm by using standard clinical procedures.(10-20 min) We will also ask you to provide a urine sample in the morning, in a private restroom. At the end of the day, we will kindly request that you collect your urine over the next 24 hours (we will give you the containers and detailed instructions for this). These urine and blood samples will be used to test for genetic and a detailed range of biochemical markers (biomarkers) related to high blood pressure, heart disease and diabetes, such as glucose, cholesterol and markers of inflammation. You are more likely to have high blood pressure if one of your parents or a close family member has high blood pressure. This is because high blood pressure can be caused by differences in our genes. Our genes are like a very complicated "manual" in each of our cells that tells the body how to work properly. When there are changes in the genes, it changes the "manual" and the body then does not work as well as it should for example causing high blood pressure. We share our genes with our family because half of the gene "manual" comes from your mother and half from your father. Therefore, if they have high blood pressure due to differences in their genes then it is likely that you will get the same changes in your genes and develop high blood pressure. We would like to find out what these differences are in order to better understand how they cause high blood pressure so that we can find ways to stop it happening.
  Take note that some of your samples may be stored for many years in freezers before we will analyse the samples. We may also need to ship some of your to other local or international expert laboratories for analyses.
- **Blood pressure:** while you are sitting down in a private room, we will measure blood pressure twice on both arms, by placing a cuff around your upper arm. (20 min) Another blood pressure measurement will also be done by placing a small blood pressure cuff around your finger, and upper arm, while you are lying on a bed. We will then test your blood pressure responses when you do a colour word reading test and when you place your hand in cold water for 1 minute. (30 min) At the end of the measurement day, we will fit a portable blood pressure monitor to you which will assess your blood pressure over the next 24 hours, thus over a day and when you are sleeping at night. It is important that the device is not removed during this time to ensure a reliable measurement.
- **Blood vessel & heart health:** in a private room we will again ask you to lie down comfortably on a bed. We will first test your blood pressure at your upper arm, with a device that will also measure the blood pressure at your heart. We will then test how stiff your blood vessels are by using a small pen-like device rested on your neck to register the pulse in your neck on a computer. At the same time another blood pressure cuff will be placed around your thigh. (15 min) Afterwards, in a semi-

dark room we will use a sonar device (usually used during pregnancy) to take some sonar pictures and video clips of the blood vessels in your neck and of your heart on the bare chest. We will provide a blanket or gown for cover. (20 min)

- **ECG** (Electrocardiography test) **for heart health:** while you are lying down on a bed in a private room, we will test the natural electrical activity of your heart by placing several stickers with sensors on your chest. We will take care to ensure your privacy. (10 min)
- **Eye Pressure:** a research nurse will put some eye drops in both eyes and then she will measure the pressure in your eyes with a device that rests lightly on your eye. (10 min) This test will inform us whether you have a condition called glaucoma, which means that the pressure within your eyes are quite high. If so, we will advise you and refer you for necessary treatment. If the pressure is normal, we will continue with the next eye test as described below.
- **Testing the small vessels of the eye:** a research nurse will put an eye drop in one eye, and a researcher will ask you to look into a special camera, named a fundoscope. This is the same device used by ophthalmologists (eye doctors). This camera will shine a light into your eye and we will take some pictures of the small blood vessels at the back of your eye (there will be a camera-like flash). We will also check how well your small blood vessels respond to light flickering, by doing a light-flicker test with this special camera. (20-30 min)
- **Physical activity:** at the end of the measurement day, a researcher will place a small monitor on your chest that will record your activity and movement levels for 7 days. No pain or discomfort is associated with this device, and you are kindly requested not to remove the device before the 7 day measurements were completed.
- **HIV test:** Although this test was done during the screening phase, we will test again for HIV with each follow-up visit every 5 years.
- **Questionnaires:** during the course of the morning, you will be asked to complete several questionnaires with the help of a researcher. These include a general health questionnaires (with questions about your age, family history of disease, education, occupation, lifestyle habits, 15 min), Berlin sleep questionnaire (asking questions about how well you sleep, 5 min), physical activity questionnaire (to report on how active your lifestyle is, 5 min), dietary questionnaire (with the help of a dietician you will be asked what you ate during the past day (30 min). Within the next week the dietician will contact you again on two occasions to complete the questionnaire again. This should give us the best reflection on your eating habits). Finally, a trained psychologist will help you to complete a number of questionnaires on your personal well-being (including questions on stress and how well you cope with stress , 30-45 min).

Will you gain anything from taking part in this research?

- You will receive direct feedback during each advanced measurement on your health status. All of these advanced clinic tests are provided to you at no cost (worth ±R3 000).
- Should any abnormalities be detected, we will refer you to doctors, clinics or hospitals for further tests or treatment and the test results may assist your doctor in making decisions about further treatment.
- Apart from this personal benefit, your research data will help biomedical health researchers to gain a better understanding on how high blood pressure and heart disease develops, and may help us to develop better programmes to prevent or treat these diseases in our community and elsewhere. The data may also be used to advise the Ministry of Health on changes to the health system that may benefit the broader South Africa.

Are there risks involved in you taking part in this research and what will be done to prevent them?

To help you with a better understanding of the potential risks, and what we are doing to prevent these, please refer to the table below:

Risks	Precautions
• Taking a blood sample at a vein in the upper arm, may cause some pain and discomfort;	• A trained registered research nurse perform all blood sampling and regularly undergo training on clinical measurements.
• Applying an eye drop may cause a slight burning sensation; • Performing the eye pressure test is slightly uncomfortable; • Performing a light flicker test may also be slightly uncomfortable. • After the eye measurement some discomfort may be experienced (similar to a visit to an eye doctor) while waiting for the pupil to constrict.	• She also performs the eye pressure test and apply the eye drop. To ensure correct procedures and minimum participant discomfort she has undergone training at an eye doctor to ensure that she use the safest techniques to make the measurement quickly and correctly. The light flicker test may cause discomfort but the researcher is highly experienced and ensures that the measurement is done quickly and accurately. It does not cause any long term harm and is comparable to standard eye doctor measures. Afterwards, when the pupil is dilated, the eye is sensitive to light. Therefore an eye patch is provided and all lights of the clinic turned off when these assessments start (at the end of the day's measurements). You are also encouraged to bring sunglasses for when you leave the clinic. We also provide transport to you after we are finished as you are not encouraged to drive if your eye has not yet returned to normal.
• Placing the hand in an ice water bucket for 1 minute may cause some pain in your hand.	• Placing the hand in ice water causes some pain due to the very cold water. The time is only for 1 minute to reduce discomfort to a minimum, and a small electric blanket or hot water bottle is provided afterwards to heat up the hand and ensure comfort.
• You may experience some discomfort when having to undress for the body measurements or heart sonar measurements.	• All measurements are done in private temperature controlled rooms. For sensitive measurements a female scientist is trained to perform measurements to ensure especially comfort of female participants. All staff are also trained in these aspects to be highly professional and discreet and to ensure maximum comfort and to avoid any embarrassment. For heart sonars, an expert clinical technologist has vast experience in performing the sonars in a semi-dark room and also provides a blanket should you require this.
• When you complete the psychological questionnaires you may feel uncomfortable when giving personal information, such as feeling depressed or stressed.	• For psychological questionnaires a psychologist is well trained to complete the questionnaires in a private area. All necessary aspects are adhered to to make sure it is done in a professional and comfortable manner. If any abnormality is detected, the psychologist informs the research nurse, who will then privately discuss the results with you.
• All health measurements may cause some anxiety when you are worried about the results of the tests.	• For other health measurements, such blood pressure, the results may be stressful. We will therefore provide you with the information privately and if we note something abnormal, we will ensure that you are referred appropriately for further tests or treatment.
• If a health abnormality is identified, others may become aware of this private information, e.g. diabetes.	• If any health abnormalities was identified, you will meet individually with the research nurse in a private room for a feedback session. She will explain your results to you and provide you with a letter of

• As measurements take place during the working week you may suffer from a loss of income, or may get into trouble for not being at work due to time spent in the project.	referral for further testing or treatment. This will also be placed in a sealed envelope. • If you will lose wages due to your participation in the study, you need to inform the research nurse, who will make sure that communication is taken up with your employer. We will normally discuss your participation with your employer beforehand to make sure there won't be any loss in income. Once your employer agrees that you can attend the study during normal working hours without having to take leave or lose any wages, you can join the study.

There are more gains for you in joining this study than there are risks.

How will we protect your confidentiality and who will see your findings?

Anonymity of your findings will be protected by all of the researchers involved. A number, and not your name, will be assigned to your research results, and all scientists using your data will only note this number, and not your name. Your privacy will be respected by making sure that all the measurements are taken in private rooms and performed by well-trained scientists. Your results will be kept confidential by storing hard copies of your documentation in a locked cupboard within the Hypertension Clinic, and only the Principal Investigator, Head of the Hypertension Clinic and Data Manager having direct access. Electronic files with data are stored and handled by the Data Manager in a password protected online database using the University web-network (with firewall and security features), as well as some backup files on external password protected hard drives. Only the researchers, their postgraduate students and local and international collaborators will be able to look at your findings – however, all findings will be anonymised using your unique participant number. As this is a long term project, your data will be stored for 20 years or longer.

What will happen with the findings or samples?

As indicated above, your research results are safely stored on electronic files, with some results on hard copies, and in the form of blood or urine samples in biofreezers. We will store your data and your blood and urine samples for at least 30 years. Over time the research team will make sure that all of this information is analysed in the utmost detail to create new knowledge on how high blood pressure, heart disease, and related diseases develop over time. It is important to store the data and samples for a long period, as new scientific discoveries on markers of high blood pressure will be made by other scientists or ourselves in the future. It will then allow us to test if these markers are also useful in your (the South African) samples, and whether these can be used throughout South Africa in the future.

Some of your biological samples (from urine and blood) will be analysed immediately, but others will be stored for many years before analyses are performed. Please note that we will perform the biochemical analyses in our laboratories on the Potchefstroom Campus. But we may need to ship some of your samples to other laboratories in South Africa or internationally, when we do not have the funds, skills or the equipment to perform the analyses locally. Samples will be shipped using courier services approved for handling biological samples, to ensure the safekeeping and protection of the samples during transit. We will also ensure that the appropriate approvals from the South African Department of Health (export permit) and the Health Research Ethics Committee are obtained prior to shipping the samples.

Apart from your samples, your anonymised data may also be shared with other national or international collaborators. It is therefore possible that your anonymised results will be reported as stand alone data as part

of the African-PREDICT study, or your data may be pooled into other datasets from the province, country or globally in further research studies on high blood pressure and related health status. Your data will therefore be used to analyse your original state of blood pressure and health – in South Africa and in comparison to other local and international populations – and to analyse how your health status changes over time.

If we were to share your anonymised data or samples with external groups, the external groups will sign confidentiality and data or material transfer agreements with us. This process is overseen by the Legal Services of the North-West University. This will ensure that your information is adequately handled and protected, and that your data is only used for the intended purpose as described in the agreement.

It is also possible that your data may be useful for other purposes apart from the aim of the present study. When the data is to be used for such purposes, new applications will be submitted to the Health Research Ethics Committee, where the Committee will stand in on your behalf.

Findings from the study will be published in scientific journals, and discussed locally and internationally with scientific experts and the Department of Health.

How will you know about the results of this research?

During the course of the day you will receive direct feedback from each research station on your health status and findings. As described earlier, if any abnormalities are detected, a detailed report within a referral letter will be compiled by the research nurse and you will be directed to the appropriate healthcare provider. If at any stage (also after you have visited the clinic) you wish to know any of your research results, you are welcome to contact the researchers at the Hypertension Clinic.

The research team also intends to publish the research findings of the larger study in scientific literature, but also in local media, and perhaps also national media. This will not include you as an individual, but the collective findings of all the research participants. Furthermore, as this is a longitudinal study, the research team may provide you with further results of the study when you return to the clinic during follow-up measurements. As the research team will contact you annually to ensure that your contact details are still correct, we will inform you if any important research findings became apparent that you need to take note of.

Will you be paid to take part in this study and are there any costs for you?

No, you will not be paid to take part in the study, but the research team will provide you with a R300 gift voucher as a token of appreciation for your participation. We hope that the results of the measurements will be useful to you to understand your own health status.

We will provide transport to all participants, and a meal will be served during the course of the morning after you have given a blood sample.

There will thus be no costs involved for you, if you do take part in this study.

To cover all of the research expenses, this study is funded by several local and international funding bodies, including the Department of Science and Technology (National Research Foundation), Medical Research Council of South Africa and the Medical Research Council of the United Kingdom, as well as scientific grants from industry (GlaxoSmithKline, Pfizer, Boehringer-Ingelheim, Medi-Clinic Hospital Group).

Note* What happens after the study day?

At the end of the study, you may have one eye covered so it is advisable not to drive until you see that your eye has recovered, due to a possible loss of depth perception. You will know that the eye is fully recovered when the black part of the treated eye (pupil) has been reduced to a similar size as the pupil of the untreated eye. In

the week following the study day, we will make **three short appointments** with you to collect the blood pressure monitor, your urine collection and the activity monitor and to do two more short interviews (20-30 minutes) about your diet. We will give you a diary sheet so you can keep track of these appointments and they will be arranged to suit your schedule.

Is there anything else that you should know or do?

- You can contact Sr. Adele Burger (or Prof. Alta Schutte) at 018 285 2261/2446 if you have any further questions or have any problems.
- You can also contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 1206 or carolien.vanzyl@nwu.ac.za if you have any concerns that were not answered about the research or if you have complaints about the research.
- You will receive a copy of this information and consent form for your own purposes.

Address: Building F11, Potchefstroom Campus, North-West University, Potchefstroom 2520
Tel: 018-285 2261 (Office hours Mon-Fri) Fax: 018-285 2260; Email:adele.burger@nwu.ac.za

Declaration by participant

By signing below, I ..... agree to take part in the research study titled: The African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT).

I declare that:

- I have read this information/it was explained to me by a trusted person in a language with which I am fluent and comfortable.
- The research was clearly explained to me.
- I have had a chance to ask questions to both the person getting the consent from me, as well as the researcher and all my questions have been answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be handled in a negative way if I do so.
- I may be asked to leave the study before it has finished, if the researcher feels it is in the best interest, or if I do not follow the study plan, as agreed to.

I agree that my blood or urine samples may be sent to laboratories in South Africa or in other countries for analyses (with my personal details removed, and only identifiable by an anonymous number).	**Yes**	**No**

Signed at (*place*) ..... on (*date*) ..... 20....

_____ _____

Signature of participant **Signature of witness**

Declaration by person obtaining consent

I *(name)* ..... declare that:

- I clearly and in detail explained the information in this document to .....
- I did not use an interpreter.
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I gave him/her time to discuss it with others if he/she wished to do so.

Signed at (*place*) ..... on (*date*) ..... 20....

_____ _____

Signature of person obtaining consent **Signature of witness**

Declaration by researcher

I, *Aletta E.Schutte*, declare that:

- I explained the information in this document to the Head of the Hypertension Clinic, Head of Screening, and research assistants.
- I did not use an interpreter.
- I encouraged them to ask questions and took adequate time to answer them.

And that I was available should they want to ask any further questions.

- The informed consent was obtained by an independent person.
- I am satisfied that she adequately understands all aspects of the research, as described above.
- I am satisfied that she had time to discuss it with others if she wished to do so.

Signed at (*place*) ..... on (*date*) ..... 20....

_____	_____
Signature of researcher	**Signature of witness**