The African-PREDICT Study

Study Protocol

The African Prospective study on the Early Detection and Identification of Cardiovascular Disease and Hypertension

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Original Version: 13 August 2018

Revised 31 May 2022

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1. Study Title

African PRospective study on the **E**arly **D**etection and **I**dentification of **C**ardiovascular **D**isease and Hyper**T**ension (**African-PREDICT**)

2. Executive Summary

Background and Problem Statement: Recent global analyses have indicated that the highest blood pressures worldwide are recorded in black populations. The vulnerable cardiovascular profile of Africans is believed to result from a combination of factors such as rapid urbanisation, abnormal sodium handling, elevated vascular resistance and arterial stiffness. The frequent underdiagnoses and ineffective treatment of hypertension in general but especially in Africans, result in severe complications, such as stroke, heart and kidney disease. Since diagnosis and treatment are generally unsuccessful in black populations – especially in low and middle-income countries – prevention is key to curb the rapidly increasing incidence of death and disability from cardiovascular disease. Cardiovascular risk prediction in black populations worldwide is inadequate since we do not have a clear understanding of the complex mechanisms underlying the development of cardiovascular disease. **Main Aim:** In the "African PRospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension" (African-PREDICT) we aim to identify early markers or predictors for the development of cardiovascular disease in black South Africans. Only by understanding the early pathophysiology of disease development, and by identifying markers as potential screening indicators, predictors or targets for intervention, will we be able to implement successful prevention programmes in Africans at younger ages. We therefore aim to track and monitor change in young, normotensive black and white individuals (aged 20-30 years) over 10-20 years. To achieve this we will perform detailed cardiovascular and novel biomarker measurements, as well as behavioural and biopsychosocial assessments every 4-5 years in order to identify and understand early changes in cardiovascular function, and specific predictors contributing to the development of hypertension and target organ damage. **Methodology and Measurements:** From 2013-2017 we recruited, screened and assessed of 1202 apparently healthy participants (black N=606 and white N=596) with equal sex distribution. Five-year follow-up take place from 2018-2022. A wide range of basic and advanced measurements are taken within a Hypertension Clinic to get a highly detailed profile of the participants at each visit. We obtain: (1) relevant questionnaire data including medical history, lifestyle, social status, traditional risk factors (age, gender, smoking, alcohol intake) and validated questionnaires on dietary intake, personality and psychosocial profile; (2) Biological samples for biomarker analyses (serum, plasma, spot urine and 24-hr urine) are taken and preserved for the short and long-term at -80°C. We will assess a wide range of traditional and novel biomarkers related to hypertension and cardiovascular disease (including amongst others, lipid profile, glucose, glycated hemoglobin, C-reactive protein, interleukin-6, vitamin D, full blood count, sodium, potassium, creatinine, renin, aldosterone, angiotensin II, markers of oxidative stress and nitric oxide bio-availability, cortisol, sex hormones, insulin, C-peptide, leptin and other adipokines, angiogenic markers, the insulin-like growth factor-axis, soluble urokinase plasminogen activator receptor, Nt-proBNP, fibulin-1 and novel markers not yet identified). These samples will also be analysed in an attempt to identify bio-signatures in terms of the –omics sciences (genomic, metabolomic and proteomic profiles) as predictors of cardiovascular deterioration; (3) anthropometric measurements, bio-electrical impedance measurement of body fat and lean mass,

and 7-day physical activity monitoring; (4) A range of cardiovascular assessments: 24-hour blood pressure, central arterial pressure, and cardiovascular stress reactivity tests with continuous finger blood pressure; and (5) Assessments of early target organ damage including urinary albumin-to-creatinine ratio, carotid intima-media thickness, ECG, echocardiography, pulse wave velocity, and retinal microvascular calibre and dilation during provocation with a light flicker test. **Timetable:** The project was approved and is endorsed by the National and Provincial Department of Health, and was approved by the Ethics Committee of the North-West University in 2012 (NWU-00001-12-A1). Screening commenced in November 2012, and research participants started entering the study from 6 February 2013. The Health Research Ethics Committee approved continuation of the study on an annual basis.. **Anticipated Outcomes:** This project will increase our understanding of the complex mechanisms involved in the aetiology of early cardiovascular changes in relatively young individuals from African and European ancestry, which will (1) improve our ability to identify individuals at risk before the development of cardiovascular diseases (CVD), and (2) predict the development of future hypertension and related CVD. Both outcomes will make it possible to develop better individualised and population-based prevention of CVD contributing to better quality of life. These results will not only be applicable to Sub-Saharan Africa but are expected to have a broader impact with regards to white and black populations globally. Results are expected to have significant scientific impact by means of publications in high-impact journals; and also to translate directly into novel preventive healthcare policy and practices – translating into preventive measures regarding the development of CVDs in clinics throughout South Africa.

3. Problem Statement and Motivation

A recent systematic analysis undertaken in 5.4 million participants indicated that blood pressure (BP) has on average decreased worldwide since 1980. However, region-specific inspection of this data shows that in Africa, the mean SBP actually increased. In addition, the highest mean BPs recorded worldwide were in African countries.[1] The WHO's Study on Global Ageing and Adult Health including adults (aged > 50 yrs) in low and middle income countries, confirmed this by reporting that South Africa (SA) presented with the highest prevalence of hypertension ever reported in a nationally representative survey, with nearly 4 in 5 participants presenting with hypertension.[2]

Unfortunately, current practices to treat hypertension in Africa are overwhelmingly ineffective, evidenced by appallingly low control rates: 7.8% in SA,[2] 4.1% in Ghana[2] and 3.1% in Mozambique.[3] These failing practices are possibly due to poor health systems, education or current antihypertensive treatment not being as effective in black populations.[4] Despite calls to increase awareness and education regarding hypertension in Africa, awareness does not translate into control, with awareness figures as low as 38% in South Africa,[2] 23% in Ghana[2] and 14.8% in Mozambique.[3]

Hypertension additionally enforces an immense economic burden upon SA. The SA Demographic and Health Survey[5,6] states that from all participants taking chronic disease drugs, the most frequent condition being treated was hypertension. Some of the main consequences of hypertension are hypertensive heart disease and stroke. Apart from the additional economic burden of caring for stroke survivors, hypertension – especially in poverty-stricken communities – clearly results in lower quality of life. Due to the frequent underdiagnosis, the poor control, and the immense economic burden[7,8] regarding the *treatment of hypertension* in Africa, the status quo cannot be maintained in

Africa concerning current practices to manage hypertension. It may have significantly greater impact if tailored population- and individual targeted *prevention strategies* are employed to early detect, prevent and delay hypertension onset. This should not only relieve the financial implications of treatment, but more importantly, dramatically improve the quality of life of black populations.

South Africa, like several other developing countries, has been highlighted as experiencing a unique demographic moment to focus on introducing policies that will reduce the future impact of chronic disease, and to minimise the rise in CVD in particular.[9] Hypertension is largely preventable. Less than 100 years ago it was nearly impossible to find a single patient with hypertension in a hospital in Kenya.[10] Today, the health behaviours consequential to urbanisation reveal the susceptibility of black populations to rapidly develop hypertension. We have demonstrated this in a black SA population in transition, where nearly 1 in 4 of black participants with optimal BP (≤120 and 80 mmHg) developed hypertension over 5 years.[11]

Among the reasons for the vulnerability of black individuals to develop hypertension are several social (socio-economic status, health disparities limiting access to care, experience of stress) and pathophysiological reasons (salt sensitivity, suppressed renin-angiotensin system, sympathetic nerve activity),[12,13] but there is consensus amongst scientists that there is *a severe lack of longitudinal data in Africa*,[14] and especially a lack of knowledge on the early pathophysiological development of hypertension of young black individuals.[13] A better understanding of pathophysiological disease mechanisms involved in cardiovascular (CV) deterioration *prior* to hypertension development, will allow the identification of novel approaches to prevent and delay hypertension onset, and may identify new ethnic-specific targets for treatment.

4. Research Aims, Objectives and Hypotheses

4.1 Aims

The African-PREDICT study aims to

(i) generate new knowledge on the early pathophysiology accompanying hypertension development in black South Africans; and

(ii) to identify early novel markers or predictors for the development of hypertension and cardiovascular outcome. By employing also in Africa the latest cutting-edge scientific technologies to measure single and multiple biomarkers proven to predict hypertension and cardiovascular outcome (such as multiplex analyses, proteomics[15-18] and metabolomics[19-23]), precision medicine may have the potential to lead to novel strategies in preventing and treating hypertension in Africa.

4.2 Objectives

Short-term objectives (based on baseline data): To assess, compare and describe young, normotensive and apparently healthy black and white individuals in terms of

a) traditional CV risk assessment techniques (office BP and conventional risk scores, such as age, sex, waist circumference);
b) using advanced technologies to measure CV parameters of microvascular and macrovascular structure and function (retinal, brachial, carotid, aortic);

c) behavioural and biopsychosocial measures (tobacco, alcohol and dietary intake, 24-h sodium excretion, physical activity; body composition, personality and psychological well-being);
d) conventional and novel biomarkers from biological samples (single, multiple biomarkers, including multiplex analyses and the – omics);
e) subclinical organ damage (echocardiography, carotid intima-media thickness, arterial stiffness, renal function; retinal calibres and peak dilation);
f) to integrate conventional and advanced biomarkers to establish and evaluate ethnic-specific risk scores.

Medium to long term objectives:

a) To track young black and white individuals (mean age 25 yrs) and perform similar detailed assessments (identical to baseline) after 5 and 10 years to identify both incident hypertension and early changes in cardiovascular structure and function;
b) and whether specific predictors are apparent when these individuals were still in a normotensive and healthy condition – when compared to those who remained normotensive;
c) to test and evaluate the additive predictive utility of novel biomarkers against existing low-cost non-laboratory and laboratory-based risk scores;
d) once detailed analyses are done with available data in the total sample, attempt to validate new findings in other African settings to determine the wider applicability and scalability of the findings.

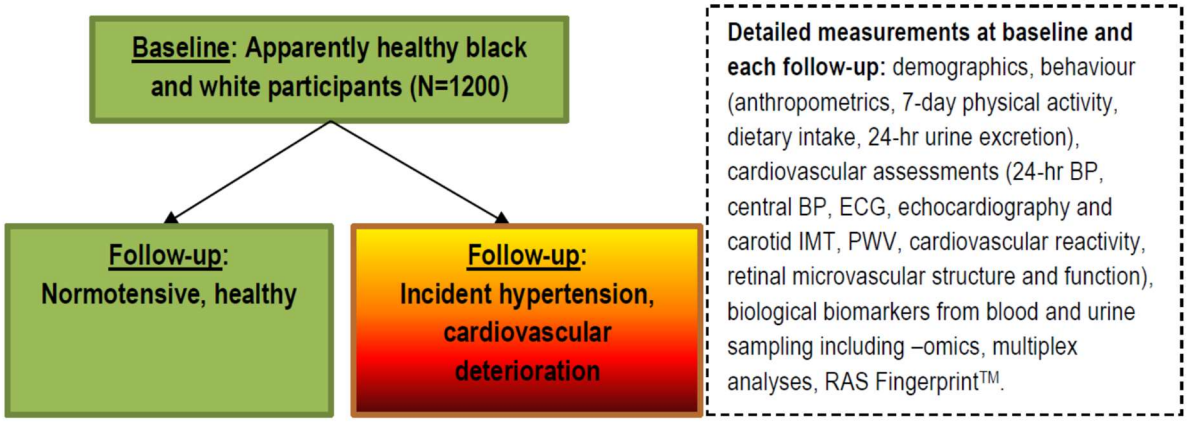
4.3 Hypotheses

a) At baseline young healthy black and white groups will exhibit similar brachial, central and 24-h BP as well as level of end-organ functioning (retina, heart, arteries, kidneys);
b) Health behaviours, and the biochemical biomarker profile (including markers of inflammation and nitric oxide (NO) bioavailability, as well as microvascular structure and function) will be more favourable in white than black participants;
c) During follow-up, black individuals will develop higher 24-h BP and arterial stiffness at younger ages, accompanied by a more pro-arteriogenic (arterial stiffness) biochemical profile as reflected by an enhanced inflammatory state, altered NO metabolism, extracellular matrix alterations, attenuated glucose handling, but a favourable lipid profile compared to the white group;
d) Biomarkers and biosignatures of the pro-arteriogenic biochemical profile (including biomarker and –omic profiles of increased elastin degradation, increased collagen production, endothelial dysfunction and inflammation) will be evident in black individuals and predict the development of hypertension, and associated end-organ damage.

5. Research Design

The **African Pr**ospective study on the **E**arly **D**etection and **I**dentification of **C**ardiovascular disease and Hyper**t**ension (**African-PREDICT**) employs a prospective study design to longitudinally characterise and monitor the early stages of hypertension development in 1200 young healthy black and white individuals (aged 20-30 years) over a follow-up period of 10 years. Healthy is defined here as brachial BP <140 and 90 mmHg; HIV uninfected; no previous diagnosis or medication for chronic

disease; not pregnant or breastfeeding. Our goal is to identify and understand early pathophysiological changes in CV function, and specific predictors contributing to the development of hypertension and target organ damage. To achieve this, we will use the latest technology in performing detailed CV and novel biomarker measurements (including multiplex measurements and –omics), as well as behavioural and biopsychosocial assessments every 5 years. The study is registered on ClinicalTrials.gov with identifier number NCT03292094 (https://clinicaltrials.gov/ct2/show/NCT03292094).



6. Target Population and Endpoints

To balance out the known confounding effects of age, sex, ethnicity and socio-economic status (SES)[2,4] on hypertension development, we employ a stratified study design that takes age, sex, ethnicity and SES into account. We used the stratification in **Table 1** as a participant selection approach. The ***inclusion criteria*** are: aged 20-30 years; apparently healthy; equal distribution of men/women, black/white and socio-economic status (low/mid/high); brachial blood pressure < 140 and 90 mmHg; HIV uninfected; no previous diagnosis of any chronic disease; not using any medication for chronic diseases; not pregnant or breastfeeding. Based on this stratification, we will recruit and follow-up for 10+ years a total of 1200 individuals. Participants will be contacted periodically to update contact details and any changes in residential address.

Endpoints/Outcomes: Since the prospective study sample comprises of participants in their twenties, we anticipate few hard endpoints (e.g. stroke) in the 10 years of follow-up. However, in preliminary analyses we found that 60% of black and 50% of white individuals of the first 403 participants, already present prehypertension (SBP>120 and/or DBP>80 mmHg).[4] As ***primary endpoint,*** we thus expect a substantial proportion with *incident hypertension as endpoint* during the first 5-year follow-up, and an even higher proportion at 10-year follow-up. As ***secondary endpoints***, the focus will also be on 'soft' surrogate outcomes such as intra-individual changes from baseline in CV measures, biomarker profiles and early target organ damage.

Due to the sensitivity of detailed CV measurements we expect to be able to detect statistically significant changes from baseline as part of the (early) vascular aging process. All CV, biochemical and metabolic changes will be closely monitored, and as appropriate % change will be used in statistical analyses (e.g. % change in SBP or % change in urinary albumin-to-creatinine ratio, and shift-tables for categorical measures will be generated).

Table 1: Participant stratification.

Low socio-economic status	**Sex**	**20-30 years**
Black	Men	100
	Women	100
White	Men	100
	Women	100
Mid socio-economic status		
Black	Men	100
	Women	100
White	Men	100
	Women	100
High socio-economic status ES		
Black	Men	100
	Women	100
White	Men	100
	Women	100

A participant diagnosed with hypertension or CVD at follow-up will continue to be followed, but pharmacologic treatment will be recorded. At each visit, identical clinical and laboratory evaluations will be completed in order to allow within-individual statistical analyses.

7. Sample Size and Power Calculations

Sample size and Power calculations were performed in collaboration with the biostatistician and collaborator of the African-PREDICT study (A/Prof. Phil Gona, Boston). Our ethnicity/age/sex/SES stratified sampling design will be a hybrid one with two distinct components. The first one is a cross-sectional design to evaluate and compare the detailed characteristics and CV profiles of apparently healthy 1200 black and white men and women (aged 20-30 yrs). The second aspect of the design includes longitudinal monitoring, every 5 years for 10+ years of systematic follow-up of the same detailed characteristics of participants. The sample size for the groups is set to 200 black and 200 white in each of the three SES categories of the 20-30 year old age group (for a total of 600 black and 600 white participants, **Table 1** above). Cross-sectional data analysis will focus on frequencies, proportions $\mathbf{p_1}$ and $\mathbf{p_2}$, means, and other basic statistical measures by ethnicity.

Hypothesis Testing Approach: Table 2 shows exact statistical power of two-sided tests for comparing two binomial proportions $\mathbf{p_1}$ and $\mathbf{p_2}$ with an alpha level of 0.05 and a sample size of 100

subjects per stratum for estimating proportions ranging from 10% to 50%. Power calculations were performed using a two-group Z test with pooled variance with a two-sided significance level of 0.05, using PASS 11 software. Highlighted cells are underpowered. Cells not highlighted indicate sufficient, i.e., >80% power. Power improves with increasing stratum size. Power also is higher when the distance $\mathbf{p_1}$ - $\mathbf{p_2}$ is large, and power is reduced when the distance $\mathbf{p_1}$ - $\mathbf{p_2}$ is small. If the prevalence of a condition in white participants is rare (2%, 4% or 6%), we will have adequate power to detect proportion differences larger than 18%, 16%, and 14%, respectively when n=100. Hypotheses which collapse across strata (e.g., comparing all black to all white participants, n=200 in each group) will have higher power to detect smaller differences in prevalence $\mathbf{p_1}$ and $\mathbf{p_2}$.

Table 2: Exact power of two-sided tests for comparing two binomial proportions $\mathbf{p_1}$ and $\mathbf{p_2}$ with an alpha level of 0.05 and a sample size of 100 in each stratum.

White group, % abnormality, p_2, N=100		**Black group,% Abnormality p_1, N=100**						
		10%	20%	30%	40%	50%	60%	70%
Rare	**2%**	0.69	1.00	1.00	1.00	1.00	1.00	1.00
	4%	0.39	0.96	1.00	1.00	1.00	1.00	1.00
	6%	0.18	0.86	1.00	1.00	1.00	1.00	1.00
Medium	**10%**	-	0.52	0.96	1.00	1.00	1.00	1.00
	15%	0.19	0.16	0.73	0.98	1.00	1.00	1.00
	20%	0.52	-	0.37	0.88	1.00	1.00	1.00
High	**30%**	0.96	0.37	-	0.32	0.83	0.99	1.00
	40%	1.00	0.88	0.32	-	0.31	0.83	0.99
	50%	1.00	1.00	0.83	0.31	-	0.31	0.83
	60%	1.00	1.00	0.99	0.83	0.31	-	0.32

For example if the prevalence in whites is rare (2%, 4% or 6%), we will have power to detect proportion differences larger than 8%, 16%, and 14%, respectively when n=200. This will of course further increase if all black and white (n=600 each) are collapsed. Therefore, we will have high confidence that the sample sizes will have sufficient power (>80%) to address the hypotheses. In most instances more sensitive continuous outcome variables (which yield more statistical power than binary outcome variables) will be used when comparing characteristics, as well as in cross-sectional and longitudinal regression models. Finally, published studies employing proteomics and metabolomics included total samples sizes ranging from N=25,[22] N=49,[24] N=60[15] and N=623[16] reporting significant and useful findings.

8. Human Research Participants

The research programme in itself entails detailed monitoring of human participants over many years.

8.1 Approval Health Research Ethics Committee (HREC)

Prior to commencement of data collection in November 2012, the project was approved by the Ethics Committee of the North-West University in 2012 (NWU-00001-12-A1). After screening commenced in 2012, eligible research participants started entering the study from 6 February 2013. As procedures in terms of approval and review of ethics applications were improved at the North-West University, a newly formed Health Research Ethics Committee (HREC) again reviewed the project and approved continuation of the study annually for continuation. A more detailed application for the full study was submitted again to the HREC in April 2016 and in 2017, to ensure fully that all of the latest ethics requirements are met.

The running of the full study is done mainly by trained research staff under the supervision of the Head of the Hypertension Clinic, who is a registered research nurse and acts as a mediator between the Principal Investigator and research participants. The risks to participants within this monitoring process falls within the category of medium risk, and participants are more likely to derive benefit from participation since measurement results are immediately made available to them. Should a health problem be identified, action is taken to immediately refer the participant for further medical treatment. Furthermore, the benefit to society from the long-term results of this study may have wide-ranging impact that may also benefit these individuals.

Risks:

Physical:

- **Physical discomfort:** venepuncture during blood sampling; performing eye pressure test with a tonometer, applying an eye drop may cause a slight burning sensation, and performing light flicker test may be slightly uncomfortable. After the eye measurement some discomfort may be experienced (similar to a visit to an ophtalmologist) while waiting for the pupil to dilate. Placing the hand in an ice water bucket for 1 minute may cause some physical discomfort.

Psychological:

- **Inconvenience, embarrassment, emotional discomfort**: participants may experience some emotional discomfort when having to undress for anthropometric measurements, ECG and echocardiography/heart sonar measurements. Furthermore as part of psychological questionnaires may experience some self-disclosure e.g. as part of depression information, or stress. HIV testing as part of screening procedures may cause anxiety or fear for participants anticipating or worried about the outcome of the test. This is also partly applicable to other health outcomes that are disclosed to participants during all assessments, e.g. indication of hypertension or diabetes.

Social harm:

- **Stigmitization:** as HIV testing is part of the test measurements there is a risk that the outcome of the test may become known to other participants or researchers involved.

Economic harm:

- **Loss of income not being on the job, time spent in the research:** as measurements take place during the working week (Monday to Friday), participants getting daily wages may suffer loss of income, or may get into trouble for not being at work due to time spent in the research.

Precautions by the research team to address the above risks are described below:

Physical:

- A trained, SANC registered research nurse perform all blood sampling and regularly undergo GCP training. She also performs the eye pressure test and apply the eye drop. To ensure correct procedures and minimum participant discomfort she undergone training at an Ophtalmologist to ensure correct procedures are undertaken to make it a swift procedure. 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 ophtalmologist measures. As the pupil is dilated, 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). Furthermore, participants are informed of these measurements beforehand and are also encouraged to bring sunglasses. Transport is also provided afterwards and participants discouraged to drive prior to the pupil returning to normal. Placing the hand in ice water causes physical discomfort and 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 provided afterwards to heat up the hand and ensure comfort.

- **Psychological**: Having to undress for some measurements may be experienced as uncomfortable – hence private temperature controlled rooms are available for every measurement. For sensitive measurements a female scientist is trained to perform measurements to ensure especially comfort of female participants, e.g. only female scientists perform ECG, ambulatory blood pressures and anthropometric measurements. Echocardiography is performed by a medical technologist with registration by the Health Professions Council of South Africa, in a semi-dark room. For heart sonars, the clinical technologist has vast experience in performing the sonars (also perform echocardiography on a daily basis in hospitals in Potchefstroom and Klerksdorp) and also provides a blanket should the participant require this. All staff are also trained in these aspects to be highly professional and discreet and to ensure maximum comfort of participants and to avoid any embarrassment. For psychological questionnaires a psychologist is trained by a registered clinical psychologist to complete the questionnaires in a private area. All necessary aspects are adhered to make sure it is done in a professional and comfortable manner. If any abnormality is detected, the psychologist informs the Head of the Hypertension Clinic, who will then refer the participant for consultation at the Potchefstroom Campus Institute for Psychology and Wellbeing (Dr. Annelize Bonthuys) at no cost – or to the participant's preferred psychologist. HIV testing is done by trained counsellors in a private room, and

include pre and post counselling. During the informed consent stage all participants are also very clearly informed that HIV testing forms part of the screening procedures and they are therefore prepared beforehand. Pre and post-counselling, and also support by the research nurse afterwards are done in positive cases, to ensure that the participant receives treatment immediately (reference, and follow-up done). For other health outcomes, such as giving feedback that the young participant is hypertensive may be quite stressful. Thus all participants with an identified abnormality individually receive their health feedback information, and are supported to make sure that they receive appropriate medical care and are taken care of.

- **Social harm:** With HIV stigmatisation being a reality in South Africa, specific care is taken to ensure highly private testing and counselling before and after testing in a private room.

- **Economic harm:** If a participant is losing wages due to his/her participation in the study, direct communication from the Principal Investigator or Project Leader is made with the employer. Generally the employer is contacted before participants are screened to get his/her support, namely to provide a service to give health information to his/her employees as part of the research project (results are not disclosed to the employer, but only to the participant). Once an employer agrees that the participant can attend the study during normal working hours without having to take leave or lose any wages, the participant will join the study.

Benefits to participants: The most significant benefit to participants is receiving high-level health measurements at no cost, with immediate feedback on their health status.

After selection of the participants, they are invited to attend the screening phase of the study, and if they comply to the inclusion criteria they will also be invited to take part in the advanced research project. In both instances, participation is voluntary and participants may withdraw at any time. They are also fully informed about the study prior to participation, after which they complete an informed consent form.

8.2 Recruitment and Eligibility Criteria

Recruitment:

Active participant recruitment takes place on a continuous basis until the full baseline sample of 1200 individuals have been included. Participants are invited from Potchefstroom and surrounding areas in South Africa via different routes. Centrally a research nurse was appointed to manage the recruitment of participants, and act as gatekeeper. As this is the central task of this nurse, she is not directly involved in the further research project measurements. The different routes of recruitment are described below in more detail:

a) Active contact via field workers,
   Field workers are appointed, and trained by the research nurse on the requirements and eligibility criteria of the project. This takes place on an ad hoc basis, when the study population is reviewed and when it is noticed that participants from a particular group should be recruited. They are further trained on how to approach possible candidates, and to target these specific groups of participants that would be suitable for the study, especially

as the study aims to include individuals from low, medium and high socio-economic status. Field workers access potential research participants within an area where they are known and knowledgeable, clearly explaining what will be required from them, and that participation will be voluntary. Those individuals expressing interest to undergo screening are then contacted, and an appointment is made. Transport to and from the Hypertension Clinic is provided.

b) Access through the workplace,
The research nurse may also target specific workplaces for recruitment, also in light of the attempt to include participants from low to high socio economic status. An appointment is made with the employer to gain permission to speak to employees. Once permission was obtained, it is arranged with the employer to make an appointment to discuss the project with a large group of employees. During this engagement, details of the screening and research project are discussed, and interested individuals are invited to contact the nurse should they be interested to participate. Once they contact the research team/nurse, an appointment is scheduled for screening. In many instances, and if agreed by the employer, the screening phase of the study is conducted at the workplace within a private area made available by the employer. This ensures that employees are only briefly absent from their workplace, as the screening procedures takes about 30 minutes. The process also differs depending on the specific workplace. In some instances, individuals from a particular employer were contacted by a field worker (see section (a) above), and then notifies the research nurse that there may be other colleagues interested to participate. Such individuals are then sometimes contacted directly by the field worker or research nurse, and not via the employer.

c) Advertisements by means of radio, noticeboards and local newspapers,
Advertisements, originally approved by the Health Research Ethics Committee, are placed in the local newspapers, on noticeboards in the city or using radio stations. The recruitment team also performs health screening in public places, where potential participants are invited should they adhere to the study eligibility criteria. Contact details of the research team are provided and interested individuals are invited to contact the research nurse should they wish to participate. Once contact was established, an appointment for screening is made.

Eligibility:

Participants that are recruited firstly undergo screening to determine eligibility for participation in the African-PREDICT study (Table 3). The participants therefore constitute a convenience or availability sample, stratified into different ethnic (black and white), sex and socio-economic class groups (low, mid, high).

Table 3: Eligibility criteria for the African-PREDICT study.

Inclusion criteria:	Exclusion criteria:
1. Self-reported black or white ethnicity	1. Self-reported Indian, Asian, mixed origin ethnicity
2. Aged 20-30 years	2. Not permanent resident of Potchefstroom or surrounding areas or not intending to return regularly to this area
3. Men and Women (equally distributed)	3. Inability to read or understand English
	4. Previously diagnosed with Type 1 or 2 Diabetes Mellitus

4. Apparently healthy 5. Normotensive or pre-hypertensive (SBP<140 and DBP<90mmHg) based on the average of 4 BP measures in one day	5. Elevated glucose >5.6 mmol/L (confirmed glycated haemoglobin (HbA1c) ≥ 6.5%) 6. HIV or other 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)

All participants receive feedback on their health measures and some basic health education at the end of the screening day. When eligible, participants are invited to join the research project or where screening results indicate necessary, participants are given onward referral for medical care.

Those participants meeting the eligibility criteria for the research project are invited to join the study and given the detailed participant information leaflet detailing what is involved. If they are willing to participate, an appointment is made for them in the Research Clinic (Building F12).

No additional follow-up visits are required for screening participants not meeting eligibility criteria or eligible participants who decline to participate, and referrals are made as appropriate by the Research Nurse.

8.3 Screening for Eligibility to Participate

Health screening takes place continuously and in parallel with the research project in order to identify eligible participants for the research study. This will take place until all participants for baseline measures are included. In addition, the health screening provides a valuable and much needed service to the local community.

Health screening will continue throughout the recruitment phase of the research project, continuing to provide a resource to the local community and a potential opportunity to follow up participants not eligible for research studies. The purpose is primarily for the selection of participants (based on inclusion/exclusion criteria) for the research protocol while providing appropriate care for screen failures. Screening takes place by a research nurse or permanent qualified research staff member in the Hypertension Clinic (F11) of the Potchefstroom Campus of the North-West University and also externally at other locations (e.g. participant's workplace) to increase accessibility for participants. The measures done as part of screening are detailed in Table 4. The research support staff of the Hypertension Clinic have all undergone training to act as HIV counsellors (VCT training). With regards to HIV testing, the testing is done by trained counsellors in a private room, and include pre and post counselling, using a specific VCT informed consent form. During the recruitment and information sessions (and when informed consent is done) all participants are also very clearly informed that HIV testing forms part of the screening procedures and they are therefore prepared beforehand. Pre and post-counselling, and also support by the research nurse after the screening is done (follow-up contact) to ensure that participants receive treatment.

Once screening was performed, each participant will meet individually with the research nurse in a private room for a feedback session. He/she will receive their feedback on their health measurements orally, and on a report that is placed in a sealed envelope. Also discussed is their eligibility to continue for the measurements of the larger study. If an individual was found not to be eligible for the study, due to any of the exclusion criteria, this is explained to them. If they have any health abnormalities they are referred for further testing or treatment and the referral letter is also included in the sealed envelope. If a participant is eligible for participation in the larger study, the details of the larger study are discussed and the informed consent form provided to them. Each item of the informed consent form is also discussed in detail, and this takes place at least 48 hours to 1 week before the participation in the larger study.

Table 4 Screening measures (normal range & referral range indicated)

Measure:	Optimal Range:	Referral indicated if:
Clinical Biochemistry:		
Fasted Blood (finger prick)		
*Total cholesterol[20]	<5.2 mmol/l	>6 mmol/l
Fasting Blood glucose	4-5.6 mmol/l	>6 mmol/l
HbA1c[#]	<6%	>7%
HIV test		Positive result
Urine (spot sample)		
Specific gravity & pH	1.002-1.030, pH5-7	
Protein	negative-trace	1+ (>20mg/dl)
Glucose	negative	1+ (& blood glucose >5.6mmol/l)
Ketone	negative	
Blood	Negative	Repeat measurement first; if 1+ (>5ery/ul if not menstruating)
Nitrite	negative	1+ (if leukocytes elevated)
Leukocyte	negative	3+ (>10/ul = significant Pyuria)
Physiological measures:		
Brachial Blood Pressure	<120/80 mmHg	>140/90 mmHg
Anthropometry:		
Height cm		
Weight kg		
Waist circumference cm[19]		
South Asian male	<89.9 cm	
South Asian female	<79.9 cm	
All other males	<93.9 cm	
All other females	<79.9 cm	
Hip circumference cm		
Neck circumference cm		
BMI (weight kg/height m^2)	18.5-25	

*Friedwald equation cannot be used at TG >4.5 mmol/l, Framingham risk score cannot be calculated at TG >5 mmol/l.

#HbA1c is only measured if participant has a fasting blood glucose of >5.6 mmol/l

8.4 Informed Consent

To protect the safety and rights of participants, it is critical that informed consent be obtained appropriately. This applies to both the screening and the African-PREDICT research study with two separate informed consent forms. Furthermore, informed consent for African-PREDICT will be obtained from each participant at baseline, and during each phase of follow-up.

Once a specific workplace, individual or area for recruitment is identified, the research nurse make contact with the participant before screening, either telephonically, sending an email, or meeting in person. The community worker acts as independent mediator between the Principal Investigator and the participant. During this contact session a detailed oral explanation is given on the study, all measurements and the eligibility criteria for participation. Once an individual has then voluntarily expressed interest to participate in screening (according to the Recruitment procedures, Section 8.2), an appointment is made for the screening procedures on a different day. When a participant meets with the research nurse and her assistants for the screening, the information contained in the informed consent form are discussed again in detail, the form is handed to the participant and they are given as much time as needed to decide on participation, and given sufficient opportunity to ask questions prior to deciding whether they wish to participate.

As part of the exclusion criteria of the study, is the inability to read or understand English – this is due to several questionnaires, dietary, psychological and other questionnaires forming part of the larger study. Prospective participants are also ensured that participation is voluntary and that they can withdraw at any stage. If they voluntarily and verbally express their intent to participate – also after questions were answered – the form is signed by the participant. Also, the research nurse responsible for explaining the screening process and taking informed consent (and the witness to the consent) also signs the form after the participant signs, as well as another witness who is not part of the study team. No study specific measures are taken before the consent is signed by all parties. The informed consent covers all measures and procedures to be taken and these are outlined in the participant information sheet and explained to the participant before consent is requested. This must also include any sub-studies that are planned in future. The whole process of informed consent is performed in a private area dedicated for the study – whether the Hypertension Clinic or in a dedicated study area off site - with privacy given to the individual to ask questions.

If the participant is found eligible (according to the inclusion/exclusion criteria) for the *African-PREDICT study*, they are invited to participate. This information is provided in a private feedback session after screening, as described in detail in Section 8.3. If they express interest, the detailed informed consent form for the research study is provided to them. They therefore have the opportunity to review this information over a few days. An appointment is scheduled, and transport provided (if required) to come to the Hypertension Clinic on the Potchefstroom Campus of the NWU (Building F12). Upon arrival at the Clinic, similar procedures as with screening are followed by the mediator (a research nurse). In addition, a video describing all study information and procedures are shown to research participants thereby providing them with visual clips on what each study procedure entails. This video is available in English and Setswana:
https://www.youtube.com/edit?o=U&video_id=_wTIu64tcLQ (English)

https://www.youtube.com/edit?o=U&video_id=bire3RdOa5s (Setswana)

9. Detailed Research Methodology

With reference to the aim and objectives of the study, a range of detailed measurements will be taken at baseline, and will be repeated every 4-5 years in order to track small changes in biomarkers, biopsychosocial measures, cardiovascular structure and function, and subclinical organ damage development.

9.1 Data Collection for the African-PREDICT study

Data collection takes place within the Hypertension Clinic (Building F12) of the Potchefstroom Campus of the North-West University under the supervision of a registered Research Nurse.

The Hypertension Clinic is equipped with an Emergency Kit in Building (with First Line Emergency Drugs). Also available in both Clinic buildings is an Automated External Defibrillator.

Participants arrive at the Clinic at 8:00 in the morning and are familiarised with the surroundings. The procedures of the measurements are again explained to the participants and details captured in the informed consent form discussed. Participants also have the opportunity to ask questions. After written informed consent is obtained, the measurements commence. Measurements are done in temperature controlled private rooms to take into consideration the privacy of the participant. A maximum of four participants are accommodated per day to ensure detailed and quality measurements. A summary of the measurements are provided below, starting with biological sampling. After blood sampling, anthropometry, bio-impedance and a set of cardiovascular measurements are done, the participants are provided with a light meal (excluding caffeine). When all measurements are completed at approximately 13:00, transport is provided to all participants to go home.

Table 4: Research measurements

Measure:
Vitals:
Ear temperature
Biological Sampling:
Fasted Blood Sample (100ml)
Urine (spot sample)
Urine (24-hour sample + additional spot sample on a separate day)
Cardiovascular Measures:
Brachial blood pressure
Pulse wave analyses (central BP, AI)
Carotid-femoral pulse wave velocity
ECG
Echocardiography
Carotid intima-media thickness and distensibility
Cardiovascular reactivity during stress testing (cold pressor test and Stroop test)
Retinal microvascular calibre and responses to a light flicker stimulus
24-hour Ambulatory BP and ECG monitoring
Anthropometry:
Height & Weight
Waist, hip and neck circumference
Body mass index

Bio-electrical impedance (body fat, lean body mass)
Physical Activity Monitoring:
7-day Accelerometry
Questionnaires:
General Health Questionnaire
GPAQ & Godin physical activity
Psychological questionnaire battery
24-hour dietary recall and salt frequency intake (on site and 2 within 7 days)
Berlin Sleep Questionnaire

In the detection of any abnormalities during measurements, participants are immediately referred.

9.2 Questionnaire Data

Questionnaires are completed with the help of a research nurse, trained research assistant or trained postgraduate students, and are done one-on-one in the Clinic. Depending on the nature of the questionnaires, some are done in a private room (e.g. psychological questionnaires), whereas others are performed in a quiet area within the Clinic. Dietary questionnaires are completed by a trained dietician, whereas psychological questionnaires are completed by a trained psychologist or intern psychologist.

General Health Questionnaire – completed online on a web-based program and involves demographic information, employment information, alcohol and tobacco use, medication use, and family history (15 min).

Berlin Sleep Apnea Questionnaire – to assess or identify the risk of patients for developing the sleep apnea syndrome (5 min).

Global Physical Activity Questionnaire (GPAQ) – This questionnaire was developed by the World Health Organization to collect information on physical activity participation in different countries. (5 min)

24-hour Dietary Recall Questionnaire and Salt Frequency Intake – dieticians administer this questionnaires on day 1 and on two further days within a week, using food models and photo books to estimate portion-size. Macro- and micronutrient intake are calculated in the appropriate units, using an algorithm developed by the Medical Research Council (South African Food Composition Tables). Factor analysis will be used to develop dietary clusters for use in statistical analysis. (30 min)

Psychosocial Questionnaire Battery – For psychological questionnaires a psychologist is trained by a registered clinical psychologist 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. Once questionnaires have been completed, the psychologist can detect any abnormalities on the same day. If any abnormality is detected, the psychology intern informs the research nurse, who will then privately debrief the participant (within her scope of practice as nurse with training in psychiatric nursing). If necessary, she will refer the participant for consultation at NWU Wellness (for NWU employees), INGRYP (for NWU students). All other participants are referred to their chosen clinic or psychologist.

Psychologists obtain information on positive mental health (Keyes et al. 2002; 2008), stress overload (Amirkhan, 2012), personality (Taylor & De Bruin, 2005), depression (Kroenke, Spitzer & Williams,

2001), psychological distress (Goldberg & Hillier, 1979), and coping responses (Amirkhan, 1990) using validated psychological questionnaires. (30-45 min) Once the questionnaires are completed in hard copy, the data is entered by a statistical consultant on an anonymous database, and hard copies of the questionnaires are stored in privately locked room at the Subject Group Psychology, under the supervision of Dr. Werner de Klerk.

	Scale	Abbreviation and number of items	Authors / developers	The measured construct
1.	Mental Health Continuum Short-Form*	MHC-SF (14 items)	Keyes (2002) Keyes et al. (2008)	Positive mental health (flourishing)
2.	Stress Overload Scale *	SOS (30 items)	Amirkhan (2012)	Stress overload: Event load and personal vulnerability
3.	Basic Traits Inventory (Short Form: Research Version)	BTI-S (77 items)	Taylor & de Bruin (2005)	Personality
4.	Patient Health Questionnaire – 9 *	PHQ-9 (10 items)	Kroenke, Spitzer & Williams (2001)	Depression and it severity
5.	Subjective Vitality Scale	SVS (7 items)	Ryan & Frederick (1997) Bostic, McGartland-Rubio & Hood (2000)	Subjective vitality
6.	Meaning in life Questionnaire*	MLQ (10 items)	Steger, Frazier, Oishi & Kaler (2006).	Meaning in life: Presence; search
7.	Coping Strategy Indicator*	CSI (33 items)	Amirkhan (1990)	Coping responses: Problem-solving; Seeking social support; Avoidance
8.	General Health Questionnaire*	GHQ (28 items)	Goldberg & Hillier (1979)	Psychological distress: Anxiety and insomnia; depression; social dysfunction; somatic symptoms
9.	New General Self-efficacy scale*	NGSES (8 items)	Chen, Gully, & Eden (2001)	Self-efficacy across life-domains
10.	Health-specific Self-efficacy Scales	N-SES (5 items) PE-SES (5 items) AR-SES (3 items)	Schwartzer & Renner (nd)	Self-efficacy for health-related behaviours: nutrition; physical exercise; alcohol resistance

*For each follow-up phase, only these questionnaires will be repeated since the other questionnaires measure stable traits or include duplicate measures of the same aspect.

9.3 Cardiovascular Measurements

Measurement of the **brachial blood pressure** is conducted using the Dinamap Procare 100 Vital Signs Monitor/Dinamap CARESCAPE V100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA) with appropriately sized GE Critikon latex-free Dura-Cuffs. Prior to the measurement being performed, participants are requested to not have smoked, exercised or eaten at least 30 minutes

beforehand and were required to be in a seated resting state with the arm supported at heart level. The first measurement is taken on the left arm after the participant was seated calmly for 5 minutes. Thereafter blood pressure is taken on the right arm in duplicate. A final measurement is made on the left upper-arm. Systolic blood pressure, diastolic blood pressure and heart rate are captured for each measurement.

24hr Ambulatory blood pressure (ABPM) is measured using a 24hr ABPM and electrocardiogram (ECG) apparatus (Card(X)plore, Meditech, Budapest, Hungary, British Hypertension Society (BHS) validated). An appropriate sized cuff is fitted to the participant's non-dominant arm and instructions given to participants on how to ensure successful inflations across the 24hr time period. The ABPM apparatus is programmed to measure blood pressure in 30 minute intervals during the day and hourly during the night, while the ECG records measurements continuously. An ambulatory diary card is distributed and completed by participants during the 24hr duration of the measurements.

Cardiovascular reactivity is tested by making use of the validated[25-27] Finometer device (FMS, Finapres Measurement Systems, Amsterdam, The Netherlands). The participant is requested to lie in the Fowler's position with their arm at heart level. A finger cuff is placed on the middle phalanx of the left hand's middle finger, and a brachial cuff is connected to the upper arm. A 2-minute calibration is performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial arterial pressure.[26] The highest precision in cardiovascular measurement can be achieved only after this calibration,[26] and blood pressure measurements complied with the requirements of the Association for the Advancement of Medical Instrumentation (AAMI).[26] Continuous measurement of resting cardiovascular variables is performed for a 5-minute period, after which the participant is exposed to the cold pressor test for 1 minute. It is performed by immersing the participant's right hand in ice water with a temperature of 4°C for 1 minute. After a 3-min resting period, another baseline measurement is recorded after which the Stroop test commences. The Beatscope® software is used to calculate systolic (SBP) and diastolic blood pressure (DBP), heart rate (HR) and computed stroke volume (SV), total peripheral resistance (TPR), and "Windkessel" compliance of the arterial system (Cwk).[28] Cwk is the "Windkessel" compliance of the arterial system also referred to as Windkessel compliance, or buffer compliance. As part of the nonlinear three-element model (aortic characteristic impedance, arterial compliance, and systemic vascular resistance), it is computed from an age-dependent, aortic pressure–area relationship and represents the lumped compliance of the entire arterial system.[28]. Cardiovascular reactivity is calculated for each participant as the percentage change from the resting value to the stressor value. All measurements are performed in a temperature controlled room.

Arterial stiffness is assessed according to the manufacturer's instructions to determine carotid-femoral pulse wave velocity (PWV) non-invasively using the Sphygmocor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia). Participants are requested to be in a supine position and in a relaxed state for approximately 5 minutes before commencement of the measurement. The device is firstly used to estimate **central systolic blood pressure** and the **augmentation index** by performing pulse wave analysis (PWA). A brachial cuff is placed on the right upper-arm. By using a general transfer function the device estimates central systolic pressure, and the measurement is performed in duplicate. Afterwards, the participant's right carotid artery is located by means of palpation to identify the strongest pulse point. The carotid pulse is measured using a tonometer while the femoral pulse is measured by a femoral cuff placed around the thigh of the participant. The direct

distance between the carotid pulse point and upper femoral cuff is noted (transit-distance method), and 80% of the distance calculated and entered. The PWV is therefore measured along the descending thoracic-abdominal aorta, using the foot-to-foot velocity method. Measurements are taken in duplicate.

9.4 Body Composition and Physical Activity Assessments

Anthropometric measurements: A trained female researcher uses standard procedures to obtain height (m) determined by the SECA 213 Portable Stadiometer (SECA, Hamburg, Germany), weight (kg) using the SECA 813 Electronic Scales with weighing capacity up to 200kg (SECA, Hamburg, Germany) and waist circumference (cm)(Lufkin Steel Anthropometric Tape; W606PM;Lufkin, Apex, USA). The body mass index (BMI) (weight (kg) / height (m_2)) is then calculated. All anthropometric measurements are performed according to guidelines as described by ISAK (International Society for the Advancement of Kinanthropometry). Privacy of the participant is taken into consideration by doing the measurements in a private room, with temperature control. **Bio-electrical impedance assessment (BIA)** is applied to assess lean body mass and body fat percentage, using a Bodystat 1500MDD dual-frequency analyser (Bodystat, Douglas, UK). Bodystat®1500MDD is a lightweight, battery operated bio-impedance analyser which is a non-invasive device, which measures the impedance value of the body providing quick and effective analysis of body composition.

After being fitted with the ambulatory blood pressure apparatus, participants will also be fitted with an **ActiHeart physical activity monitor** (CamNtech Ltd., England, UK). This is a compact, chest-worn monitoring device that records heart rate, inter-beat-interval and physical activity in one combined unit. It is designed for capturing heart rate variability data and for calculating and measuring activity energy expenditure. The ActiHeart device is worn for a maximum of 7 days.

9.5 Assessment of Target Organ Damage (TOD)

Electrocardiography (ECG) is performed by a trained female researcher registering a standard 12 lead ECG (Norav Medical Ltd, PC 1200, v5.030, Israel), with the participant in a supine position. At least six cardiac cycles are recorded. We use standard formules, such as the Cornell Voltage Product to provide an estimation of left ventricular wall thickness.[29-31]

Echocardiography: A standard transthoracic echocardiography procedure is followed by a medical clinical technologist (registered with the HPCSA) while each participant is in a partial left decubitus position with the head of the examining table modestly elevated. The General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway) is used along with the 2.5 to 3.5 MHz transducer and a single ECG-lead for timing purposes. Standardised methods are employed to obtain high quality recordings according to the current recommendations as outlined in the guidelines of the European Association of Echocardiography and the American Society of Echocardiography.[32,33] Two trained sonographers obtain the data and the data is analysed by one experienced registered clinical technician.

Prognostically validated gender-specific cut-off values are used to identify LV hypertrophy.[34] LV mass is calculated by a standard formula[32,35] and is normalized for both body surface area and height (meter$^{2.7}$).[34] Inappropriate LV mass is estimated using an equation previously developed in a separate population of normal-weight, normotensive adults between the ages of 18 to 85 years.[34,36] LV end-diastolic and end-systolic volumes are calculated using Teichholz's formula.[37] Stroke volume

is generated (mL/beat) and normalised for height in the power of 2.04 as the stroke volume index (SVi).[38,39] Stroke work (in gram-meters/beat) is computed as previously described.[34] Cardiac output is obtained by multiplying stroke volume with heart rate. The ratio between pulse pressure and SVi is used as a measure of arterial stiffness.[40]

Global LV systolic function is derived from linear measurements obtained from 2D images, since the study population did not present with regional wall motion abnormalities or irregular heart rhythm.[41] Standard methods are used to determine endocardial fractional shortening (fractional shortening).[42,43] The mid-wall fractional shortening (mFS) is alternatively calculated by a validated method previously described by De Simone, et al.[44] LV ejection fraction (EF) is calculated from LV end-diastolic and end-systolic volume estimates derived from 2D images according to the biplane method. LV EFs of <52% for men and <54% for women are suggestive of abnormal LV systolic function.[45]

A pulsed-wave (PW) Doppler is performed in the apical 4-chamber view to obtain mitral inflow velocities for assessing LV filling.[46] A 1-mm to 3-mm sample volume is then placed between the tips of the mitral valve leaflets with parallel alignment with the inflow. Parameters of left ventricular diastolic function include: peak velocities of both early (E) and atrial (A) diastolic filling; the E/A ratio and E-wave deceleration time (DT). We additionally perform Tissue Doppler imaging to calculate the velocity of myocardial tissue movement in relation to mitral valve blood flow. This is a clinically robust index of diastolic function in combining the assessment of transmitral flow and mitral annulus velocity. The E/E' ratio most confidently separates normal filling pressures (<8) from elevated filling pressures (>12).[47]

Carotid intima media thickness (cIMT) and plaque scores are made on the left and right common carotid artery, as well as the internal carotid (General Electric Vivid E9, GE Vingmed Ultrasound A/S, Horten, Norway). Various optimal angles on each side will be measured and recorded for similar future assessments. The images will be digitised and imported in the Artery Measurement Systems software for dedicated analyses (Gustavvson, Sweden).[48] Carotid cross-sectional wall area (CSWA) is calculated to confirm structural and not functional changes in luminal diameter ($CSWA = \pi(d/2 + CIMT)^2 - \pi(d/2)^2$, where d denotes luminal diameter). A videoclip will also be taken to determine carotid distensibility.

Retinal microvascular calibre, oxygen saturation and responses to flicker induced provocation: Retinal photography and the dynamic retinal vessel assessment of vessel responses following light flicker provocation is performed using the Dynamic Retinal Vessel Analyzer (Imedos, Jena, Germany) fitted with a Zeiss Fundus camera FF-450. No intake of food or fluid is permitted 1 hour prior to the measurement. Participants also refrained from smoking and exercise during this time. Prior to the measurement the research nurse determines the intraocular pressure in each eye with the Tonometer - Tonopen Avia (Reichert technologies) and Ocu-film sleeve. A local anaesthetic, Novesin Wander (0.4% eye drop) is instilled before this measurement can be made. If the eye pressure exceeds 24 mmHg no further ocular measurements are performed due to the possiblity of glaucoma. Fifteen to thirty minutes prior to the measurement a drop of Tropicamide (1% Alcon) is administered in the right eye to induce mydriatic conditions. To avoid inducing angle-closure glaucoma, an estimation of the depth of the angle of the anterior chamber is done by the research nurse prior to administering the Tropicamide. Should the participant indicate any adverse reactions from the

Tropicamide, Amazid (Acetazolamide 250 mg tablet) is kept on hand. The research nurse ensures that all substances (Novesin Wander, Tropicamide and Amazid) are kept within their expiry dates. All these procedures falls within the scope of practice of the research nurse (Sr. Adele Burger), and she was trained by Dr. Geldenhuys (ophthalmologist) to perform all procedures.

The Dynamic Retinal Vessel Analyzer is used to obtain static retinal images. These images can be used to to evaluate retinal artery and vein calibres, , estimations of vessel oxygen saturations and signs of retinopathy . In addition, a dynamic measurement is performed where the device records data for 6 minutes in which time a light flicker provocation is used to elicit retinal artery and vein reactivity/responses.

Monochrome and colour retinal images are captured (using Visualis 2.81 software) at a 50° camera angle. The monochrome or colour image underwent vessel analysis using VesselMap2 software. Briefly all vessels located between 0.5 and 2 optic disc diameters from the outer margin of the optic disc are marked either as artery or vein. The central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) are subsequently calculated using the Knudtson formula, where only the six largest artery and 6 largest vein segments were included in the calculation.[49] The arteriolar-to-venular ratio was calculated as CRAE/CRVE. Signs of retinopathy will also be quantified from this image. Both CRAE and CRVE were measured in measuring units (MU) where 1MU is equivalent to 1µM if the dimensions of the eye are similar to the normal Gullstrand eye. The reproducibility of the analysis was computed previously for a randomly selected cohort with a correlation coefficient of 0.84. The intra-class correlation (ICC) analysis involved a mixed-model framework, whereby random effects were assumed for participants and fixed effects were assumed for the graders. The Cronbach's alpha-reliability index for the AVR was 0.91 for this randomized cohort. The VesselMap2 software further enables the determination of estimated artery and vein oxygen saturation. Within the same measuring area as described above, artery and vein branches are selected, and the software determines the oxygen saturation of each vessel branch. At the end of vessel selection, the software averages are determined to yield an oxygen saturation value for arteries and veins.

For the dynamic measurement, two to 6 segments from each of an artery and vein branch are selected. The fundus camera is set at an angle of 30°. A light flicker stimulus is induced after a 50 second baseline phase, for 20 seconds followed by an 80 second recovery phase. The protocol is automatically repeated for a total of 3 cycles. Following the measurement, the software automatically generates a summary curve of the 3 cycles. From this curve, maximum flicker induced vessel (artery and vein) dilation and maximum arteriolar constriction is manually determined. Alternatively, the data is exported into an excel sheet with built in macros to determine the previously mentioned dilation and constriction parameters, but additionally determines time dependent parameters and information regarding the area under the curve to better describe these vessel dynamic respones.[50] The raw data can also be processed further to obtain information on retinal vessel pulsations and pulse wave velocity.[51]

Renal function: Urinary albumin-to-creatinine ratio is determined using spot urine samples and obtained early in the morning when participants arrive at the research facilities. It is also determined from 24-hour urine samples. Additionally the blood serum levels are used to determine Cystatin-C and creatinine concentrations, which are used to calculate the estimated creatinine clearance and estimated glomerular filtration rate (GFR). Methodology on estimation of urinary albumin and

creatinine, and serum Cystatin-C and creatinine are described in the Biochemical Measurements section.

9.6 Biological Sampling and Biochemical Measurements

The inner ear temperature is measured to establish that the participant is not suffering from an underlying infection resulting in an increase of body temperature (fever). Biological sampling entails the collection and preparation of different blood and urine samples. Planning for these storage procedures were done based on (a) the ability to analyse known markers of hypertension and related conditions, but also in line with the study aims (b) to store samples for the longer term and to measure novel biomarkers that may only be discovered after data collection were completed. In the early morning fasting blood samples are taken by a registered research nurse, and participants provide a spot urine sample. The following samples are collected:

- early morning spot urine sampling, to be stored as 7 x 1.5 ml cryovials and 3 x 500 µL cryovials;
- 24-hour urine sampling, to be stored as 4 x 1.5 ml cryovials and 1 x 2 ml cryovial;
- blood sampling (fasted), to be sampled as 5 x 9 ml red-top serum, 2 x 9 ml purple-top EDTA plasma, 3 x 4 ml purple-top EDTA full blood; 2 x 4.5 ml light-blue top citrate, 1 x 2ml gray-top sodium fluoride, 1 x 4.5 ml Stabilyte®;
- blood samples on absorbant paper Guthrie cards.

In the event that a blood sample cannot be obtained, the research nurse will make an appointment with the participant to collect the blood sample where it is most convenient for the participant e.g. at their workplace.

Once the research nurse has taken the samples, a research assistant, trained in the handling of biological samples (using latex gloves), collects the samples in the Hypertension Clinic and place the sample tubes in a closed container. The samples are taken immediately to the on-site temperature controlled laboratory. Samples are then aliquoted into cryovials for short- and long term (screw cap) storage in biofreezers at -80°C. This is done by trained postgraduate students under the supervision of the qualified laboratory manager (Prof. Carina Mels). All staff and students handling biological samples undergone extensive training by the laboratory manager to reduce any risks to the laboratory students. During all procedures protective laboratory coats as well as latex gloves are worn, and students were particulary trained on how to handle samples. When any abnormality or problem arises, the laboratory manager is immediately notified and the necessary steps are taken to ensure the safety of staff members. Staff and students working with biological samples are also part of a Hepatitis surveillance programme.

The biofreezer room in the F12 Physiology building is protected by a secure alarm system, sending SMS notifications to the full time academic staff member responsible for the security of the samples (Dr. Wayne Smith). Samples are further protected by a UPS system, and emergency electricity from the NWU, should it be required.

Samples will be stored in this facility for the duration of the study, i.e. at least until 2018. It is further possible that these samples may be stored for a longer term and until sufficient funding was obtained to ensure appropriate analyses in line with and as per the Informed Consent Form.

Biochemical measurements are wide-ranging, and include (amongst others) the following:

- immediate basic serum analyses (such as HDL-C, LDL-C, triglycerides, glucose, C-reactive protein, creatinine, liver enzymes, albumin, uric acid), glycated hemoglobin (HbA1c), vitamin D and cotinine
- full blood count
- 24-hour urinary sodium, potassium, creatinine, marinobufagenin
- markers of the renin angiotensin aldosterone system, such as renin, angiotensin II, aldosterone, angiotensin converting enzyme
- stress hormones, such as cortisol, testosterone, estradiol, FSH, SHBG
- N-terminal pro-brain natriuretic peptide, troponin T
- interleukin-6, tumor necrosis factor-α, P-selectin, E-selectin, soluble urokinase plasminogen activator receptor (suPAR)
- vascular endothelial growth factor-A, angiopoietin-2
- intracellular adhesion molecules (ICAM) and vascular cell adhesion molecule (VCAM)
- L-arginine, asymmetric dimethyl arginine, NO metabolites
- insulin, leptin, adiponectin, resistin, C-peptide, pro-insulin
- growth hormone, insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3
- extracellullar matrix: metalloproteinases 1 and 2, fibulin-1, elastin
- markers of oxidative stress, such as reactive oxygen species, superoxide dismutase, 3-nitrotyrosine
- markers of hemostasis, such as fibrinogen, prothrombin 1 and 2, von Willebrand factor antigen and activity, D-dimer, global fibrinolytic assay, plasminogen activator inhibitor-1
- in line with the aim of the study, *i.e.* to find new ways to identify early predictors for hypertension development, this study will also investigate other novel biomarkers that are not yet identified but are found to be associated with hypertension and CVD development, and may be measureable in the biological samples stored.

Omics:

Dedicated blood samples from cell preparation tubes and spot urine samples are stored for genomic, proteomic and metabolomic analyses in collaboration with local and international collaborators according to the appropriate procedures. The proteomic and metabolomic analyses are an extention of the abovementioned biomarker analyses, are will be analysed whilst particularly viewing the relationship to cardiovascular disease development. For proteomic and metabolic analyses spot urine samples (as described under Biological Sampling, Section 9.6) are used as follow:

Proteomic analyses

In collaboration with Professor Christian Delles at the Institute for Cardiovascular and Medical Sciences (ICAMS) at the University of Glasgow, spot urine samples are analysed for detailed proteomic profiles to establish biosignature predictors of CV deterioration. Different technologies are available for proteomic analyses. In this study we make use of capillary electrophoresis-mass spectrometry (CE-MS) technology. Proteins are separated by capillary electrophoresis based on their electrophoretic migration through a buffer-filled capillary. The separated proteins are subsequently

detected by mass spectrometry. This technique provides fast separation with high resolution, the capillaries used are less expensive than liquid chromatography columns, and it is compatible with various buffers and analytes. All analyses and data processing are done at ICAMS in Glasgow using only anonymous participant numbers. For each participants approximately 2000 urinary peptides are identified. Based on previous experience, the peptides are classified according to specific panels as observed previously in patients with certain conditions (e.g. coronary artery disease, chronic kidney disease, heart failure etc.) and healthy controls. Specific peptides are grouped as the most significant ones into classifiers. These are usually 50-300 peptides that can define a condition.

The classifiers are quite robust as they contain many features. Therefore, participants will be grouped and categories even if they do not show all e.g. 300 peptides differentially regulated but only e.g. 100. They also allow a degree of quantitation of disease severity, assuming that those with more differentially regulated peptides (higher classifier scores) are more affected than those with less (lower classifier scores). Participants with lower scores will usually exhibit asymptomatic disease, which falls within the direct scope of the African-PREDICT study, namely to early identify those at possible risk for future CVD development. Those individuals with low scores, and with the lowest scores are less likely to proceed to overt disease than people who are already at the higher end of the normal spectrum.

Therefore the classifiers, in theory, can be used as biomarkers for early detection, diagnosis, assessment of severity of disease, and prediction of disease.

The evidence for proteomics as clinically useful biomarker is not yet as robust as it is for other, traditional, biomarkers that are used in the African-PREDICT study. However, as the concept is convincing, and since there are already some evidence that it is effective, the participants from the study, and the larger South African population may benefit from the results of this study. Particularly if effective peptide panels can be identified as future prediction tool. Existing panels that will be evaluted in the study include:

1. Coronary artery disease. A diagnostic panel has been developed (PMID 20811296), it has been shown that it predicts events (PMID 25786980) and that it can be modified, e.g. by dietary measures (PMID 25527749).

2. Chronic kidney disease. Panel developed (PMID 20616184), further validated (PMID 24589724), predictive value shown (23086559), clinical trial underway (http://eu-priority.org/).

3. Heart failure panel (PMID 22789915).

Firstly the panels (classifiers) will be evaluated against other cardiovascular measurements in the study (arterial stiffnes, retinal microvasculature, echocardiography, ambulatory and central pressures), to determine if there are differences between appropriately defined cases and controls, between men and women, and between black and white groups. Once this was performed, further analyses will be done one the remaining 2000 peptides.

These baseline analyses will give insight into the differences in protein expression between the groups. Additional proteomic studies in the future will enable the detection of not only early changes (first follow-up after 5 years) within the urinary proteome but also long term changes over a period of 10+ years. As further indicated in a 2015 American Heart Association Position Statement on Proteomics, in the setting of CVD and health, proteomics is particularly useful for the early detection and identification of processes involved in several CVD states, including atherosclerosis, hypertension, and stroke.

Metabolomic analyses

The analysis of the urinary metabolome is the study of the metabolic phenotype of the particpants of the African-PREDICT study, and is also directly in line with the central aim of the study. Several platforms exist that can be applied to perform such analyses, and in collaboration with the Centre for Metabolomics at the North-West University (Prof. Carools Reyneke, Dr. Roan Louw, Prof. Francois van der Westhuizen), the following strategy will be used:

NMR spectroscopy – as it is quantitative in nature an doffers precise structural information that enables relatively easy metabolite identification. As the technique is not destructive, the samples will then also be used for further metabolomic analyses. As it has a low sensitivity, it will mainly allow the detection of high-abundance metabolites.

Mass spectrometry, coupled with separation techniques, namely liquid chromatography (LC), has a higher sensitivity than NMR spectroscopy, and will therefore also be employed to detect a significant number of very-low-abundance metabolites. Targetted LC-MS will be used for butylated compounds (amino acids and carnitines), whilst untargetted gas chromatography (GC-MS) will be used for organic acids, and untargetted LC-MS will be used for positive and negative ionisation.

The final analyses of the results will be done using participant characterisation based on sex, ethnicity and cardiovascular and metabolic profiles of participants.

Genetic analyses: Samples are presently being stored for genetic analyses at the Centre of Excellence for Nutrition, under the supervision of Dr. Wayne Towers. Future applications to the HREC and to funding bodies will be submitted to detail and specify these approaches.

9.7 Rationale/Justification for the Measurements

The decision to perform the specific measurements as listed previously are based on the existing knowledge, available literature, or lack thereof with reference to specific biomarkers, psychosocial measurements, cardiovascular assessments and markers of target organ damage.

Previous research performed by our group and others have found ethnic-specific differences with regards to the following measurements to be undertaken, and may potentially play a significant role in the development of cardiovascular disease:

Office and ambulatory blood pressure measurements are undertaken to confirm previous reports that some of the highest blood pressures have been recorded in African populations.[1,52] Our research has indicated significantly higher daytime and nighttime ambulatory BP in African school teachers compared to Caucasian school teachers.[53] Insufficient data is available on these values in healthy normotensive individuals. Additionally the dietary data and 24-hour urine sodium

concentrations should provide more clarity on salt-sensitive hypertension which is reported to be highly prevalent in black populations.[54]

Arterial stiffness is reported to be increased in African populations which has been confirmed in black populations across the globe.[55-57] Whether stiffness is already elevated at younger ages or during healthier periods is expected, but this requires confirmation. This is also relevant to the biomarkers related to the arteriosclerosis and atherosclerosis processes – such as extracellular matrix markers, e.g. fibulin-1,[58] markers of vascular calcification[59,60] and growth factors.[61,62]

Markers of NO bioavailability should be measured to establish during which stages of hypertension development these markers are dysregulated. We determined that Africans have lower L-arginine concentrations,[63] and that asymmetric dimethylarginine values are only significantly linked to pulse wave velocity in older African men.[64] Africans are also reported to present with a prothrombotic profile, with fibrinogen, plasminogen activator inhibitor-1 and von Willebrand factor being strongly linked to unfavourable cardiovascular and metabolic health.[65-67] The first results to be generated from this study on retinal microvascular reactivity in healthy individuals should provide greater insight into the early development of endothelial deterioration, whether it is ethnic specific and whether it may be a significant predictor of the development of hypertension later in life.

Renal function: Non-diabetic Africans with normal kidney function indicated significant associations between albumin-to-creatinine ratio and ambulatory blood pressures which was not present in Caucasian individuals.[68] Furthermore, due to the importance of low-renin hypertension and aldosterone levels, the occurrence of which is highly prevalent in African populations, these markers, as well as possible genetic polymorphisms affecting these levels, need to be tested as potential predictors for hypertension.[69-71]

Left ventricular hypertrophy: African populations are reported to have an increased risk of developing a left ventricular mass and a two- to three-fold higher prevalence of left ventricular hypertrophy than in the general population, with increased risk of developing a left ventricular mass already occurring from late childhood.[72,73] By using echocardiography we hope to obtain a clear profile on the occurrence of left ventricular mass in normotensive Africans and Caucasians, with additional biomarkers, such as Nt-proBNP and troponin T, supporting this data.[74]

Obesity and inflammatory markers: Globally, obesity is becoming an increasing trend, and even more so in black populations.[75,76] Its role in the expression of adipokines, such as leptin and adiponectin, is significant in the development of cardiovascular disease, especially in Africans.[77,78] The associated pro-atherogenic inflammatory profile of obese individuals may contribute to the elevated cardiovascular risk of Africans, and we have determined that in general Africans exhibit significantly higher levels of inflammatory markers, such as C-reactive protein and soluble urokinase plasminogen activator receptor (suPAR).[79,80]

Behaviour: smoking, alcohol and psychological distress: In Africans with lower socio-economic status, we have discovered the debilitating effects of the high prevalence of smoking, and alcohol abuse in these populations.[11,81] The inability to cope with the effects of rapid urbanisation and a westernised environment in older African populations is also strongly linked to cardiovascular deterioration.[82-84] By investigating these aspects inter-disciplinary in a bi-ethnic young population

with similar levels of socio-economic stress, we could obtain better insight into whether these behaviours may continue exerting their effects on cardiovascular disease development.

Omics: The significant potential that polyomics (e.g. genomics, metabolomics and proteomics) pose for future prediction of cardiovascular disease development cannot be ignored, especially due to the significant ongoing technological advances in these fields. We therefore aim to perform these measurement in collaboration with local and international expertise, and as funding allows. It forms a crucial part of the study and allows specifically to address the aim to identify novel biomarkers for hypertension and cardiovascular disease development.

10. Statistical Analyses Plan

The statistical analyses plan was developed in collaboration with the biostatistician and collaborator of the African-PREDICT study, namely Prof. Phil Gona, Boston, USA. The participants will be longitudinally monitored, with subjects returning to the Hypertension Centre every 5 years for 10+ years. At each post-baseline clinic visit, the same parameters assessed at the baseline visit will be measured. Prior to conducting formal statistical analysis, exploratory data analysis will be conducted to detect outliers and assess normality assumptions as well as to identify any notable features that could strongly affect results and conclusions. The Kolmogorov-Smirnov test will be used to test normality, and logarithmic transformation applied to variables with a skewed distribution. Data will be expressed as mean ± SD if normally distributed or median with interquartile range or 5^{th} and 95^{th} percentiles for non-Gaussian distributed variables. Categorical variables will be presented as frequency counts and proportions will be compared using Chi-squared tests. Chi-squared and Fisher's exact tests will be used to test univariate associations. Inference about the study hypotheses focusing on baseline ethnic comparisons will be made based on summarising all continuous variable outcome measures within strata to be compared across ethnicity using methods for continuous data. Independent T-tests, ANOVA, ANCOVA, as well as linear and partial regression models and multiple logistic and linear regression will be used to evaluate whether incidence rates or percentage change of each outcome variable vary across ethnicities and sub-groups. The analysis will include covariates to adjust for patient demographic characteristics. Stepwise regression will be used to select variables to include in the most parsimonious model. Weighted prevalence estimates accounting for the sampling design will be used if the data are collapsed across strata. Weights will be based on the population distribution of SA. Biomarker and –omics data will be natural log-transformed or standardised (to mean=0 and SD =1), if indicated.

To classify a protein signature sensitivity and specificity will be determined and ROC plots will be constructed to evaluate the area under the curve as measure of overall accuracy. Model fit will be assessed using R-squared, the overall F-test statistic, and Akaike information criterion (AIC), for continuous outcome linear regression models, and using the c-statistic, -2 log likelihood, the Bayesian information criterion (BIC), Pseudo R-squared for binary logistic regression models.

For repeatedly-measured continuous measures, mixed effect models for longitudinal data will be used. The correlation structures (e.g., compound symmetry or unstructured) will be determined by the data variance covariance matrices. To account for repeated measures we will use longitudinal multivariate analyses using random effects regression models, which allow for simultaneous estimation of within-subject (person-specific) intercepts and slopes and their association with other

predictors/ biomarkers for CV outcomes of interest. For repeatedly measured categorical outcomes, analysis will be implemented using generalized estimating equations (GEEs). Both linear mixed effects models and GEEs provide a practical method with reasonable statistical efficiency to analyse these kinds of longitudinal data and the methods help to provide deeper understanding in studies of change over time while appropriately accounting for the multi-level correlations arising from high dimensional repeated measurements. Missing data can be easily accommodated in these models. These models will be implemented for continuous, ordinal and dichotomous response data using SAS procedures PROC MIXED and PROC GLIMMIX. Every effort will be made during the study to minimise missing data.

It is recognised that analysis of such data is hindered by the presence of incomplete data. Prior to statistical analysis, all study variables will be reviewed for missing data by generating summary statistics by comparison groups (ethnicity, sex and age groups) to assess the degree of missing-ness. It's important to understand the mechanism generating the missing-ness because it affects the extent to which the missing data biases the results. That understanding will inform the choice of how we are to deal with the missing data. Missing-ness pattern could either be missing at random (MAR) or missing completely at random (MCAR). MAR refers to the propensity for a data point to be missing which is not related to the missing data but it is related to some of the observed data, i.e., missing-ness is conditional on another variable (ethnicity, sex and age groups, or the limits of detection of technology generating the data). On the other hand, MCAR means there's no relationship between missing-ness and any variable.

We will employ multiple imputation techniques to account for missing data in regression models and also perform sensitivity analyses to compare results with and without imputation. Multiple imputation is widely used to cope with missing data. The procedure fills in missing values to generate multiple complete datasets to preserve the main characteristics of the original data. The procedure entails that plausible values for missing data are drawn randomly from the underlying joint distribution of the variables, and each dataset analysed using standard methods. Across the datasets, parameters estimates and their variances are combined, accounting for between- and within-imputation variability, to generate overall estimates and standard errors. This process of estimation, reflecting our uncertainty in missing values, is called "Rubin's Rules". Multiple imputation, therefore, will serve to preserve the statistical power as presented in the proposal. To respond to the reviewer comments, we are not concerned about compromised power as a result of missing data because we will use standard analysis techniques that preserve the study statistical power. [References (1) Little, R. J. A., & Rubin, D. B. (2002). Statistical analysis with missing data. New York: John Wiley & Sons. To compare results with and without imputation. (2) Xiaoyan Yin, Daniel Levy, Christine Willinger, Aram Adourian,Martin G. Larson. Multiple imputation and analysis for high-dimensional incomplete proteomics data. Statistics in Medicine, Nov. 2015].

Regarding markers that are censored because their values are below or above the limit of detection or due to low concentrations, the first step is to generate distributions for each marker to determine if the distributions are truncated in the upper or lower tails. Our colleagues at the Framingham Heart Study, have confronted a similar problem. Pedigree-based linear mixed-effects models (see R package "lmekin" https://cran.r-project.org/web/packages/coxme/vignettes/lmekin.pdf) were used to analyse continuous values using logistic regression models to analyse binary values (i.e., below a cut-point vs above a cut-point). This was necessary because expression of the marker was not

universal, but varied from as low as 1.5% to as high as 99.9%. The observed marker values generally did not have a truncated normal distribution, which precluded Tobit regression modeling. [Reference: Tobin, J. (1958). Estimation of relationships for limited dependent variables. Econometrica 26: 24-36] Furthermore, imputation produced extreme bi-modal distributions and was not acceptable for response data in linear modeling. Therefore, they employed an adaptive approach as follows: for a given marker, if at least 90% of participants expressed it, they used the linear-model p value; if <10% of participants expressed it, they used the logistic-model p value; if between 10% and 90% of participants expressed the marker, they combined results from the two models. Specifically, they added their Chi-squared statistics and then calculated the p-value from the distribution of a Chi-squared variate with two degrees of freedom. [Reference: Professor Marty G. Larson, ScD, NHLBI Framingham Heart Study, personal communication to Phil Gona, 15 December, 2015] Other statistical methods that can incorporate approaches have been used with similar data [Reference: MinJae Lee, et al. Statist. Med. 2012, 31 1838–1848; Multiple imputation for left-censored biomarker data based on Gibbs sampling method. Statistics in Medicine, 2012, 31 1838–1848].

11. Data Handling and Management

The African-PREDICT study uses the REDCap (Research Electronic Data Capture, see http://project-redcap.org) system to capture all data elements. REDCap is a free, secure web-based, and user-friendly electronic database software which can be quickly developed and customized for studies for collecting and tracking information and data from research studies and scheduling patient visits.[85] A specific Data Manager, Dr. Lisa Uys, was appointed and trained to fulfil this function. When using this system all laboratory specimens, evaluation forms, reports, data and other records are identified only by the participant number to maintain subject confidentiality using the RedCap system. Apart from this system, data are also backed up on password protected hard drives – done by the Data Manager.

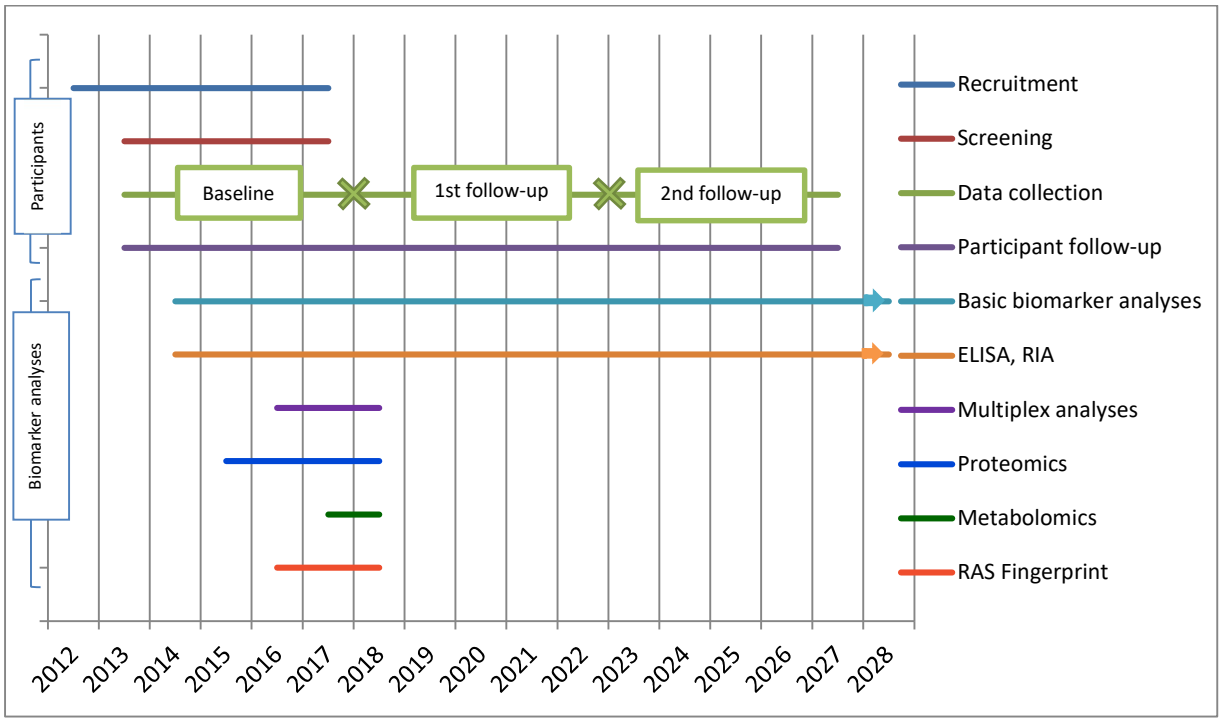
A six-digit ID number is used; the first 2 digits correspond with a particular examination where, for example "00" will refer to the baseline phase and "01" to the first follow-up; the next four digits denote the subject's unique four-digit number. Prior to participation in the study (also as part of screening) detailed information is conveyed individually to participants, with opportunities to ask questions. As explained orally and in the participant leaflet attached to the informed consent form, voluntary participation includes consent that all personally identifiable information (PII) as well as sensitive PII (SPII) will be captured as part of the study with the understanding that this information will be stored and handled very securely, and that anonymisation will take place promptly and equally securely. Furthermore, the minimum amount of PII is being captured within the study and only as far as the information contributes towards the aims of the study. As part of informed consent the implications of biological sample storage, international sample shipment and analyses are included, with samples being shipped only identifiable by the six-digit ID number, and thus blinded to international investigators. Data generated in other locations (e.g. Glasgow and Vienna) will either be uploaded directly onto REDCap or sent via password protected files to SA to be uploaded by the Data Manager.

All paper and computerised records are kept in a secured area (Hypertension Centre, Building F11 office with locked file drawers). All subject identification are done with the ID numbers only with all

identifying information kept in a separate file. Only three individuals (Data Manager, Head of the Hypertension Centre, and Head of Screening) have access to information linking ID numbers to participant names. Data entered on REDCap is saved online via secure and password protected fire-walls of the NWU computer systems. Clinical information will not be released without written permission of the subject, except as necessary for cleaning, monitoring, and statistical analysis by the authorised study team members or when the participant's well-being is at risk.

Although biological samples are shipped to international destinations for high-level biochemical analyses, the dataset is not accessed by international collaborators. Laboratories will receive anonymous data and send results linked to participant numbers to the Data Manager. Should it become necessary to share parts of the dataset with collaborators, appropriate material transfer agreements and confidentiality agreements will be signed beforehand.

12. Timeframe



- *Participant recruitment* started in 2012 and will continue until the baseline measurements are completed, and this is anticipated by end of 2017.
- *Screening* for eligibility commenced along with recruitment and will cease once 1200 participants have advanced through all data collection assessments of the study.
- *Participant follow-up* is a continuous process performed by clinic nurses, fieldworkers and research assistants. Participants are contacted twice annually via email, telephone or personal visits, and contact information updated. This will continue until completion of 2nd follow-up by 2027.
- *Basic biomarker analyses* including standard glucose, lipid, liver enzyme, full blood count analyse, done in-house at the Hypertension Research and Training Centre laboratory. It will be done for all three sampling sessions (baseline, 1st and 2nd follow-up).

- *ELISA and RIA analyses* for several novel but clear-cut cardiovascular biomarkers (such as renin and aldosterone) are performed, similarly to basic analyses in-house for all three sampling sessions.
- *Multiplex analyses* will be performed in Glasgow using Newton Funding and for the whole baseline sample (2016-2018). If funding permits, it will also be analysed for the two follow-up sessions.
- *Proteomics* will similarly be performed in Glasgow using Seed Funds (N=160) and Newton Funding.
- *Metabolomics* will be performed in South Africa once all baseline sampling are completed, i.e. 2017-2018, utilising funding from the Technology Innovation Agency (TIA) for the Metabolomics Platform.
- *RAS-Fingerprint* multiplex analyses will be performed in Vienna.
- Data management and cleaning will take place continuously.

13. Budget

Sufficient funding has been obtained from different sources to continue with the study for the next three years. The budget is reviewed at a continuous basis, with possible further funding applications planned. Security for continuation of the longer term part of the study (follow-up in the future) is evident from the SAREhI Chair and the MRC Unit Funding which are both available for a five year cycle (until 2018) and both being renewable for another 10 years. Funding from these grants would be sufficient to ensure continuing at least all data capturing (but not necessarily expensive biochemical analyses).

Item	Description of Item	TOTAL 2016-2018
Other Sources of Income:		
1	MRC Flagship Seed Fund (AE Schutte)	**R 500,000**
2	NRF/DST SA Research Chair, Running Costs (AE Schutte)	**R 1,063,327**
3	NRF/DST SA Research Chair, student bursaries	**R 2,250,430**
4	MRC Unit for Hypertension and CVD	**R 1,550,000**
5	MRC SIR Seed funds, Proteomics (CMC Mels)	**R 600,000**
6	Technology Innovation Agency (TIA) Metabolomics Platform	**R 420,000**
7	Newton Fund (MRC UK, SAMRC, GlaxoSmithKline)	**R11,000,000**
	TOTAL Income from other sources	**R17,383,757**

14. Remuneration

As participants arrive fasted for screening and research procedures, they receive healthy meals on these two occasions. After baseline phase measurements are completed they receive a R50 gift voucher from a supermarket as token of appreciation for their participation. For the first follow-up study the voucher is increased to R300.

15. Data Dissemination Plan

The REDCap system[85] used in the African-PREDICT study, allows direct sharing of all or selected data to any collaborator. **Publication plan:** Scientific research papers in high-impact journals on preliminary cross-sectional, full baseline cross-sectional, as well as findings from the 5 and 10-year follow-up will be of the highest priority to the PI, co-PI and scientific team members. Depending on the findings, journals such as the *EHJ, Hypertension, Circulation, JACC* and the *Lancet* will be targeted. It is expected that first authors will in all instances be from African origin, with expert input from other local and international collaborators and biostatisticians as co-authors and senior authors. With many postgraduate students, postdoctoral fellows, young and senior scientists already working in the study, several early publications are already expected in the next 3-5 years. These papers will mainly focus on cross-sectional analyses between ethnicities, to determine whether divergent cardiovascular phenotypes are evident in black and white normotensives, and whether these are accompanied by unique biomarkers or biosignatures. The most impactful publications, however, are expected to appear after 5-year follow-up and onwards, focusing on using precision health to predict and prevent hypertension in Africa. In addition, innovative strategies to translate this knowledge into cost-effective screening measures will be of high priority (e.g. rapid testing). **Meetings:** Accompanying publications, findings from the study will be presented at scientific meetings taking place in SA (e.g. SA Hypertension Society, SA Heart Society), Africa (e.g. Pan-African Society of Cardiology), and internationally (e.g. ISH, ESH, ESC, AHA Meetings, etc.) to engage in dialogue with other experts in the field. **Policymakers:** With existing open channels of communication with the Provincial and National Department of Health, as well as recent collaboration in publishing a new *Health Policymaker's Booklet for Africa*, there is no doubt that the findings of the study will be presented to these stakeholders on a continuous basis, with in-depth discussions. **Public:** Due to national and international leadership roles of the primary investigators, regular engagement with the media does take place especially to increase awareness of hypertension (e.g. radio, papers and television appearances, social media). Important findings from the study will therefore be easily conveyed to the public using these routes. The PI and others have also contributed to other successful online initiatives, such as The Conversation, where scientific findings are presented by scientists to the public, especially to increase impact and change in society. **Research participants:** After the screening procedures of the study the registered research nurse immediately makes the results available to the research participants, including results from blood pressure, HIV (with counselling prior/after HIV testing), lipids, glucose, body composition. If they have any health abnormalities they are referred for further testing or treatment and the referral letter is also included in the sealed envelope (see procedures below).

For the larger African-PREDICT study, participants get immediate feedback on site from the researchers as measurements are being made. During the follow-up phases, participants will receive a report on their health status as measured during the previous data collection phase. If

abnormalities are detected, they are appropriately and privately informed by the research nurse (Sr. Adele Burger) and referred by her using an official referral letter.

All referrals will depend on the abnormality:

- Psychological questionnaires: Once questionnaires have been completed, the psychologist can detect any abnormalities on the same day. If any abnormality is detected, the psychologist informs the research nurse, who will then privately debrief the participant (within her scope of practice as nurse with training in psychology) and if necessary, she will refer the participant for consultation at the Potchefstroom Campus Institute for Psychology and Wellbeing at no cost – or to the participant's preferred psychologist. An agreement was made with the Institute for the referral of participants.
- Eye abnormalities: As the researchers performing retinal vessel analyses are not trained ophthalmologists, and as the purpose of this measurement is not to detect eye abnormalities, the measurements made are not similar to those done during a normal visit to an ophthalmologist. However, as the researchers are trained on normal anatomy and physiology of the retinal vascular area, abnormalities are noted, and participants with a medical aid are referred to an ophthalmologist with whom the researchers have discussed this arrangement. Participants without a medical aid are referred to their clinic of choice, as once a month the clinics are visited by an ophthalmologist.
- Cardiac and blood pressure abnormalities: participants with a medical aid are referred to their general practitioner of choice, but depending on the abnormality they could also referred to an internist with whom the research team has had discussions. If necessary further referral are then made to a cardiologist. For participants without a medical aid, they are referred to a general practitioner at Potchefstroom Hospital, who will then refer participants to an internist or cardiologist at the Baragwanath hospital, should it be necessary.
- HIV infection: Should a participant be diagnosed with HIV during the screening phase, the research nurse will refer participants with a medical aid to their general practitioner of choice, and those without a medical aid to their clinic of choice.
- Abnormal full blood counts: Should any abnormalities be detected in the laboratory, these are referred to the research nurse and she will then refer the participant to their general practitioner or clinic of choice, as appropriate.

For all referrals, further contact with the participant is maintained to ensure that they have been to a healthcare facility and that treatment was initiated. The research team, particularly the Head of the Hypertension Clinic (Sr. Adele Burger), has communicated and has an agreement with all clinics from the Department of Health in the area that our research participants are referred to them. They also recognise our referral letter, and maintain communication with the research team.

16. Expected Outputs, Outcomes and Impact

16.1 Expected Outcomes

Although hypertension is generally considered to develop over a long period of time, we expect that of the original 600 young black and 600 young white participants, at least 10 % would have developed pre-hypertension or hypertension after four years (with possibly a higher percentage in the African sample).* Furthermore, a larger group will possibly develop smaller changes in vascular function, such as microvascular dysfunction and/or increased arterial stiffness, with possible changes in biomarkers.

- Based on the assumptions above, the first main outcome from this study will be the identification of markers (in any form such as behaviours, biomarkers, direct cardiovascular measurements or a biosignature based on e.g. proteomics) that may act as predictors for early vascular changes. As the project progresses over 10+ years in the same individuals, it would become even clearer which individuals are developing subclinical organ damage and which markers relate most strongly to these developments. Based on well-reported ethnic differences in hypertension development, this result will specifically address the development of hypertension in the black group (compared to white participants). Baseline data comparison should also shed light on the age-based differences in healthy normotensive black and white individuals. This advancement in knowledge on cardiovascular disease development will be imperative in understanding disease mechanisms and to develop strategies to curb the development of cardiovascular disease at young ages.
- Another outcome within this research programme, is the development of research capacity in all the disciplines participating in the project (BSc Honours, Masters, Doctoral students, as well as postdoctoral fellows and young emerging staff members). As with current research projects, it is impossible to perform such studies without the direct involvement of these students (for recruitment, cardiovascular measurements, laboratory analyses, feedback to the participants, etc.). Continuous mentoring needs to take place in order to develop these young individuals as scientists and to create the career pathways of especially those who are already appointed in permanent research positions.
- On a community level, an important outcome will be the greater awareness of hypertension as the “silent killer”.
- An indirect outcome of the study is that due to the study design and the advanced research methods used in this study, the results generated will be novel and highly competitive with international Hypertension Centers of Excellence.
- The final long-term outcome of this research will be to prevent the development of cardiovascular disease in individuals at increased cardiovascular risk based on identified predictors. The implementation of these specifically designed prevention programmes (based on the characteristics of the identified early predictor(s)) will take place in South Africa, and perhaps within other countries.

*This assumption is based on previous research by our group.[11,57]

16.2 Expected Outputs

During the first baseline phase of this prospective study, we expect that numerous publications in high-impact international journals will be generated. These manuscripts will typically focus on the baseline data generated, describing e.g. the methodology and cohorts collected in detail with respect to the various age, gender and ethnic reference groups included; and also the first data generated as part of the South African initiative to lower salt intake (based on the 24-h salt excretion data). The novel markers to be tested in this study have not been investigated in large bi-ethnic samples of young individuals, and the cross-sectional data collected will be highly publishable. Measures of cardiovascular structure and function and their association with different health exposures and biomarkers will be the focus of most papers, highlighting the different aspects with regard to ethnicity and gender. With respect to the first phase, we expect approximately 15-25 publications.

As the study progresses and the data from the first follow-up becomes available, the scientific impact will be significantly greater, and we expect to publish these results in journals with impact factors higher than 6.

Capacity development (as described above) will also allow several PhD and MSc students to complete their studies in this timeframe (2-4 postdoctoral fellows, 4 PhDs, 6 Masters, 10 Honours). Results from this study are also expected to be disseminated at numerous national and international scientific conferences.

16.3 Impact: Improvement in health outcomes

Should this project be able to identify novel markers for the early identification of cardiovascular disease in young black South Africans, then these results could impact significantly on the ultimate prevention or delay in the development of CVD by identifying young individuals at risk prior to disease development.

17. Reference List

1. Danaei G, Finucane MM, Lin JK, Singh GM, Paciorek CJ, Cowan MJ, Farzadfar F, Stevens GA, Lim SS, Riley LM, Ezzati M. National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants. *Lancet.* 2011;377:568-577.
2. Lloyd-Sherlock P, Beard J, Minicuci N, Ebrahim S, Chatterji S. Hypertension among older adults in low- and middle-income countries: prevalence, awareness and control. *Int J Epidemiol.* 2014;43:116-128.
3. Damasceno A, Azevedo A, Silva-Matos C, Prista A, Diogo D, Lunet N. Hypertension prevalence, awareness, treatment, and control in mozambique: urban/rural gap during epidemiological transition. *Hypertension.* 2009;54:77-83.
4. Weber MA, Schiffrin EL, White WB et al. Clinical practice guidelines for the management of hypertension in the community a statement by the American Society of Hypertension and the International Society of Hypertension. *J Hypertens.* 2014;32:3-15.
5. Department of Health. *South African Demographic and Health Survey, 2003. Preliminary Report.* Calverton, Maryland, USA: 2004.
6. Steyn K, Gaziano TA, Bradshaw D, Laubscher R, Fourie J. Hypertension in South African adults: results from the Demographic and Health Survey, 1998. *J Hypertens.* 2001;19:1717-1725.

7. Cappuccio FP, Micah FB, Emmett L, Kerry SM, Antwi S, Martin-Peprah R, Phillips RO, Plange-Rhule J, Eastwood JB. Prevalence, detection, management, and control of hypertension in Ashanti, West Africa. *Hypertension.* 2004;43:1017-1022.
8. Opie LH, Seedat YK. Hypertension in sub-Saharan African populations. *Circulation.* 2005;112:3562-3568.
9. Leeder S, Raymond S, Greenberg M, Lui H, Esson K. *A race against time: the challenge of cardiovascular disease in developing economies*. New York: Columbia University; 2004.
10. Donnison C. Blood pressure in the African natives: its bearing upon aetiology of hyperpiesa and arteriosclerosis. *Lancet.* 1929;1:6-7.
11. Schutte AE, Schutte R, Huisman HW et al. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in Black South Africans? A 5-year prospective study. *Int J Epidemiol.* 2012;41:1114-1123.
12. Flack JM, Sica DA, Bakris G et al. Management of high blood pressure in Blacks: an update of the International Society on Hypertension in Blacks consensus statement. *Hypertension.* 2010;56:780-800.
13. Jones DW, Hall JE. Racial and ethnic differences in blood pressure: biology and sociology. *Circulation.* 2006;114:2757-2759.
14. Dalal S, Beunza JJ, Volmink J, Adebamowo C, Bajunirwe F, Njelekela M, Mozaffarian D, Fawzi W, Willett W, Adami HO, Holmes MD. Non-communicable diseases in sub-Saharan Africa: what we know now. *Int J Epidemiol.* 2011;40:885-901.
15. Brown CE, McCarthy NS, Hughes AD, Sever P, Stalmach A, Mullen W, Dominiczak AF, Sattar N, Mischak H, Thom S, Mayet J, Stanton AV, Delles C. Urinary proteomic biomarkers to predict cardiovascular events. *Proteomics Clin Appl.* 2015;9:610-617.
16. Delles C, Schiffer E, von Zur MC, Peter K, Rossing P, Parving HH, Dymott JA, Neisius U, Zimmerli LU, Snell-Bergeon JK, Maahs DM, Schmieder RE, Mischak H, Dominiczak AF. Urinary proteomic diagnosis of coronary artery disease: identification and clinical validation in 623 individuals. *J Hypertens.* 2010;28:2316-2322.
17. Kuznetsova T, Mischak H, Mullen W, Staessen JA. Urinary proteome analysis in hypertensive patients with left ventricular diastolic dysfunction. *Eur Heart J.* 2012;33:2342-2350.
18. Zhang ZY, Thijs L, Petit T et al. Urinary Proteome and Systolic Blood Pressure as Predictors of 5-Year Cardiovascular and Cardiac Outcomes in a General Population. *Hypertension.* 2015;66:52-60.
19. Menni C, Graham D, Kastenmuller G et al. Metabolomic Identification of a Novel Pathway of Blood Pressure Regulation Involving Hexadecanedioate. *Hypertension.* 2015.
20. Shah SH, Sun JL, Stevens RD, Bain JR, Muehlbauer MJ, Pieper KS, Haynes C, Hauser ER, Kraus WE, Granger CB, Newgard CB, Califf RM, Newby LK. Baseline metabolomic profiles predict cardiovascular events in patients at risk for coronary artery disease. *Am Heart J.* 2012;163:844-850.
21. Soininen P, Kangas AJ, Wurtz P, Suna T, la-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet.* 2015;8:192-206.
22. van Deventer CA, Lindeque JZ, van Rensburg PJ, Malan L, Van der Westhuizen FH, Louw R. Use of metabolomics to elucidate the metabolic perturbation associated with hypertension in a black South African male cohort: the SABPA study. *J Am Soc Hypertens.* 2015;9:104-114.
23. Wurtz P, Havulinna AS, Soininen P et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation.* 2015;131:774-785.
24. Nkuipou-Kenfack E, Duranton F, Gayrard N, Argiles A, Lundin U, Weinberger KM, Dakna M, Delles C, Mullen W, Husi H, Klein J, Koeck T, Zurbig P, Mischak H. Assessment of metabolomic and proteomic biomarkers in detection and prognosis of progression of renal function in chronic kidney disease. *PLoS One.* 2014;9:e96955.

25. Imholz BPM, Wieling W, van Montfrans GA, Wesseling KH. Fifteen years experience with finger arterial pressure monitoring: assessment of the technology. *Cardiovascular Research.* 1998;38:605-616.
26. Guelen I, Westerhof BE, van der Sar GL, van Montfrans GA, Kiemeneij F, Wesseling KH, Bos WJW. Validation of brachial artery pressure reconstruction from finger arterial pressure. *Journal of Hypertension.* 2008;26:1321-1327.
27. Schutte AE, Huisman HW, van Rooyen JM, Malan NT, Schutte R. Validation of the Finometer device for measurement of blood pressure in black women. *Journal of Human Hypertension.* 2004;18:79-84.
28. Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol.* 1993;74:2566-2573.
29. Dahlof B, Devereux RB, Julius S et al. Characteristics of 9194 patients with left ventricular hypertrophy: the LIFE study. Losartan Intervention For Endpoint Reduction in Hypertension. *Hypertension.* 1998;32:989-997.
30. Julius S, Alderman MH, Beevers G, Dahlof B, Devereux RB, Douglas JG, Edelman JM, Harris KE, Kjeldsen SE, Nesbitt S, Randall OS, Wright JT, Jr. Cardiovascular risk reduction in hypertensive black patients with left ventricular hypertrophy: the LIFE study. *J Am Coll Cardiol.* 2004;43:1047-1055.
31. Okin PM, Roman MJ, Devereux RB, Kligfield P. Electrocardiographic identification of increased left ventricular mass by simple voltage-duration products. *J Am Coll Cardiol.* 1995;25:417-423.
32. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation.* 1978;58:1072-1083.
33. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise J, Solomon S, Spencer KT, St John SM, Stewart W. Recommendations for chamber quantification. *Eur J Echocardiogr.* 2006;7:79-108.
34. de SG, Daniels SR, Devereux RB, Meyer RA, Roman MJ, de DO, Alderman MH. Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. *J Am Coll Cardiol.* 1992;20:1251-1260.
35. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol.* 1986;57:450-458.
36. de SG, Verdecchia P, Pede S, Gorini M, Maggioni AP. Prognosis of inappropriate left ventricular mass in hypertension: the MAVI Study. *Hypertension.* 2002;40:470-476.
37. Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. *Am J Cardiol.* 1976;37:7-11.
38. de SG, Devereux RB, Ganau A, Hahn RT, Saba PS, Mureddu GF, Roman MJ, Howard BV. Estimation of left ventricular chamber and stroke volume by limited M-mode echocardiography and validation by two-dimensional and Doppler echocardiography. *Am J Cardiol.* 1996;78:801-807.
39. de SG, Devereux RB, Daniels SR, Mureddu G, Roman MJ, Kimball TR, Greco R, Witt S, Contaldo F. Stroke volume and cardiac output in normotensive children and adults. Assessment of relations with body size and impact of overweight. *Circulation.* 1997;95:1837-1843.
40. Casalnuovo G, Gerdts E, de SG, Izzo R, De MM, Giudice R, Trimarco B, De LN. Arterial stiffness is associated with carotid atherosclerosis in hypertensive patients (the Campania Salute Network). *Am J Hypertens.* 2012;25:739-745.
41. Lang RM, Borow KM, Neumann A, Janzen D. Systemic vascular resistance: an unreliable index of left ventricular afterload. *Circulation.* 1986;74:1114-1123.

42. de SG, Devereux RB, Roman MJ, Ganau A, Chien S, Alderman MH, Atlas S, Laragh JH. Gender differences in left ventricular anatomy, blood viscosity and volume regulatory hormones in normal adults. *Am J Cardiol.* 1991;68:1704-1708.
43. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation.* 1977;55:613-618.
44. de SG, Devereux RB, Roman MJ, Ganau A, Saba PS, Alderman MH, Laragh JH. Assessment of left ventricular function by the midwall fractional shortening/end-systolic stress relation in human hypertension. *J Am Coll Cardiol.* 1994;23:1444-1451.
45. Lang RM, Badano LP, Mor-Avi V et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2015;28:1-39.
46. Appleton CP, Jensen JL, Hatle LK, Oh JK. Doppler evaluation of left and right ventricular diastolic function: a technical guide for obtaining optimal flow velocity recordings. *J Am Soc Echocardiogr.* 1997;10:271-292.
47. Hamlin SK, Villars PS, Kanusky JT, Shaw AD. Role of diastole in left ventricular function, II: diagnosis and treatment. *Am J Crit Care.* 2004;13:453-466.
48. Touboul PJ, Hennerici MG, Meairs S et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis.* 2007;23:75-80.
49. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res.* 2003;27:143-149.
50. Kotliar KE, Lanzl IM, Schmidt-Trucksass A, Sitnikova D, Ali M, Blume K, Halle M, Hanssen H. Dynamic retinal vessel response to flicker in obesity: A methodological approach. *Microvasc Res.* 2011;81:123-128.
51. Kotliar K, Hanssen H, Eberhardt K, Vilser W, Schmaderer C, Halle M, Heemann U, Baumann M. Retinal pulse wave velocity in young male normotensive and mildly hypertensive subjects. *Microcirculation.* 2013;20:405-415.
52. Twagirumukiza M, De BD, Kips JG, de BG, Stichele RV, van Bortel LM. Current and projected prevalence of arterial hypertension in sub-Saharan Africa by sex, age and habitat: an estimate from population studies. *J Hypertens.* 2011;29:1243-1252.
53. Schutte AE, Schutte R, Huisman HW, van Rooyen JM, Fourie CM, Malan NT, Malan L. Blood pressure variability is significantly associated with ECG left ventricular mass in normotensive Africans: the SABPA Study. *Hypertens Res.* 2011;34:1127-1134.
54. Ergul A. Hypertension in black patients: an emerging role of the endothelin system in salt-sensitive hypertension. *Hypertension.* 2000;36:62-67.
55. Din-Dzietham R, Couper D, Evans G, Arnett DK, Jones DW. Arterial stiffness is greater in African Americans than in whites: evidence from the Forsyth County, North Carolina, ARIC cohort. *Am J Hypertens.* 2004;17:304-313.
56. Chaturvedi N, Bulpitt CJ, Leggetter S, Schiff R, Nihoyannopoulos P, Strain WD, Shore AC, Rajkumar C. Ethnic differences in vascular stiffness and relations to hypertensive target organ damage. *J Hypertens.* 2004;22:1731-1737.
57. Schutte AE, Huisman HW, Schutte R, van Rooyen JM, Malan L, Malan NT, Reimann M. Arterial stiffness profiles: investigating various sections of the arterial tree of African and Caucasian people. *Clin Exp Hypertens.* 2011;33:511-517.
58. Kruger R, Schutte R, Huisman HW, Argraves WS, Rasmussen LM, Olsen MH, Schutte AE. NT-proBNP is associated with fibulin-1 in Africans: the SAfrEIC study. *Atherosclerosis.* 2012;222:216-221.

59. Schutte R, Huisman HW, Malan L, van Rooyen JM, Smith W, Glyn MC, Mels CM, Fourie CM, Malan NT, Schutte AE. Alkaline phosphatase and arterial structure and function in hypertensive African men: The SABPA study. *Int J Cardiol.* 2012.
60. Gafane LF, Schutte R, Kruger IM, Schutte AE. Large artery stiffness and carotid intima-media thickness in relation to markers of calcium and bone mineral metabolism in African women older than 46 years. *J Hum Hypertens.* 2015;29:152-158.
61. Schutte AE, Huisman HW, van Rooyen JM, Malan L, Malan NT, Fourie CM, Louw R, Van der Westhuizen FH, Schutte R. A significant decline in IGF-I may predispose young Africans to subsequent cardiometabolic vulnerability. *J Clin Endocrinol Metab.* 2010;95:2503-2507.
62. Schutte AE, Volpe M, Tocci G, Conti E. Revisiting the relationship between blood pressure and insulin-like growth factor-1. *Hypertension.* 2014;63:1070-1077.
63. Glyn MC, Anderssohn M, Luneburg N, van Rooyen JM, Schutte R, Huisman HW, Fourie CM, Smith W, Malan L, Malan NT, Mels CM, Boger RH, Schutte AE. Ethnicity-specific differences in L-arginine status in South African men. *J Hum Hypertens.* 2012;26:737-743.
64. Schutte AE, Schutte R, Huisman HW, van Rooyen JM, Fourie CM, Malan L, Malan NT, Schwedhelm E, Strimbeanu S, Anderssohn M, Boger RH. Dimethylarginines: their vascular and metabolic roles in Africans and Caucasians. *Eur J Endocrinol.* 2010;162:525-533.
65. Vorster HH, Jerling JC, Steyn K, Badenhorst CJ, Slazus W, Venter CS, Jooste PL, Bourne LT. Plasma fibrinogen of black South Africans: the BRISK study. *Public Health Nutr.* 1998;1:169-176.
66. Schutte R, Schutte AE, van Rooyen JM, Huisman HW, Palmer IM, Fourie CM, Peter S, Malan L, Malan NT, Reimann M. Von Willebrand factor as marker of vascular function in South African women: the POWIRS Study. *Am J Hypertens.* 2008;21:1298-1303.
67. Greyling A, Pieters M, Hoekstra T, Oosthuizen W, Schutte AE. Differences in the association of PAI-1 activity with the metabolic syndrome between African and Caucasian women. *Nutr Metab Cardiovasc Dis.* 2006.
68. Schutte R, Schutte AE, Huisman HW, Glyn MC, van Rooyen JM, Malan NT, Fourie CM, Malan L. Arterial stiffness, ambulatory blood pressure and low-grade albuminuria in non-diabetic African and Caucasian men: the SABPA study. *Hypertens Res.* 2011;34:862-868.
69. Jones ES, Owen EP, Rayner BL. The association of the R563Q genotype of the ENaC with phenotypic variation in Southern Africa. *Am J Hypertens.* 2012;25:1286-1291.
70. Rayner BL, Owen EP, King JA, Soule SG, Vreede H, Opie LH, Marais D, Davidson JS. A new mutation, R563Q, of the beta subunit of the epithelial sodium channel associated with low-renin, low-aldosterone hypertension. *J Hypertens.* 2003;21:921-926.
71. Rayner BL, Myers JE, Opie LH, Trinder YA, Davidson JS. Screening for primary aldosteronism--normal ranges for aldosterone and renin in three South African population groups. *S Afr Med J.* 2001;91:594-599.
72. Drazner MH, Dries DL, Peshock RM, Cooper RS, Klassen C, Kazi F, Willett D, Victor RG. Left ventricular hypertrophy is more prevalent in blacks than whites in the general population: the Dallas Heart Study. *Hypertension.* 2005;46:124-129.
73. Dekkers C, Treiber FA, Kapuku G, Van Den Oord EJ, Snieder H. Growth of left ventricular mass in African American and European American youth. *Hypertension.* 2002;39:943-951.
74. Kruger R, Schutte R, Huisman HW, Hindersson P, Olsen MH, Schutte AE. N-terminal prohormone B-type natriuretic peptide and cardiovascular function in Africans and Caucasians: the SAfrEIC study. *Heart Lung Circ.* 2012;21:88-95.
75. Puoane T, Steyn K, Bradshaw D, Laubscher R, Fourie J, Lambert V, Mbananga N. Obesity in South Africa: The South African demographic and health survey. *Obesity Research.* 2002;10:1038-1048.
76. Schutte AE, Huisman HW, van Rooyen JM, Schutte R, Malan L, Reimann M, de Ridder JH, van der MA, Schwarz PE, Malan NT. Should obesity be blamed for the high prevalence rates of hypertension in black South African women? *J Hum Hypertens.* 2008;22:528-536.

77. Schutte R, Huisman HW, Schutte AE, Malan NT. Leptin is independently associated with systolic blood pressure, pulse pressure and arterial compliance in hypertensive African women with increased adiposity: the POWIRS study. *Journal of Human Hypertension.* 2005;19:535-541.
78. Schutte AE, Huisman HW, Schutte R, van Rooyen JM, Malan L, Fourie CM, Malan NT. Adipokines and cardiometabolic function: How are they interlinked? *Regul Pept.* 2010;164:133-138.
79. Schutte AE, van Vuuren D, van Rooyen JM, Huisman HW, Schutte R, Malan L, Malan NT. Inflammation, obesity and cardiovascular function in African and Caucasian women from South Africa: the POWIRS study. *J Hum Hypertens.* 2006;20:850-859.
80. Schutte AE, Myburgh A, Olsen MH, Eugen-Olsen J, Schutte R. Exploring soluble urokinase plasminogen activator receptor and its relationship with arterial stiffness in a bi-ethnic population: the SAfrEIC-study. *Thromb Res.* 2012;130:273-277.
81. Zatu MC, van Rooyen JM, Schutte AE. Smoking and vascular dysfunction in Africans and Caucasians from South Africa. *Cardiovasc J Afr.* 2011;22:18-24.
82. Malan L, Schutte AE, Malan NT, Wissing MP, Vorster HH, Steyn HS, van Rooyen JM, Huisman HW. Specific coping strategies of Africans during urbanization: comparing cardiovascular responses and perception of health data. *Biol Psychol.* 2006;72:305-310.
83. Malan L, Schutte AE, Malan NT, Wissing MP, Vorster HH, Steyn HS, van Rooyen JM, Huisman HW. Coping mechanisms, perception of health and cardiovascular dysfunction in Africans. *Int J Psychophysiol.* 2006;61:158-166.
84. Malan L, Hamer M, Schlaich MP et al. Facilitated defensive coping, silent ischaemia and ECG left-ventricular hypertrophy: the SABPA study. *J Hypertens.* 2012;30:543-550.
85. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009;42:377-381.