

Large arterial stiffness and associated cardiovascular risk factors in black South Africans

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Psalm 139:14:

“I praise you because I am fearfully and wonderfully made; your works are wonderful, I know that full well.”

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PREFACE

The article format that was used to complete this thesis is an approved format and recommended by the North-West University. The thesis was written in English. It consists of three manuscripts that have already been published or submitted to a peer-reviewed journal, as well as an in-depth literature review and an interpretation of the results.

The layout of the thesis is as follows:

Chapter 1 includes a detailed literature study that offers background to the focused literature studies presented in the introduction of each manuscript, as well as the motivation, aim, objectives and hypotheses for each manuscript.

Chapter 2 offers a detailed overview of the protocol of both the African-PREDICT study and PURE-SA-NWP study. Statistical analyses performed for this thesis are also discussed.

Chapter 3 is the first manuscript of the thesis and it describes the relationship between large artery stiffness and markers of health behaviour in young black and white adults in South Africa. This manuscript was published in the *Journal of the American Society of Hypertension* in 2016.

Chapter 4 is the second manuscript of the thesis and it shows that a health profile associated with excessive alcohol use is predictive of large artery stiffness over a ten-year period in black South Africans. The *Journal of Hypertension* published this manuscript in 2017.

Chapter 5 contains the third manuscript of the thesis and explores the cross-sectional and longitudinal relationship between large artery stiffness and several biomarkers known to modulate arterial function in a younger and older black South African population. This manuscript was submitted to *Diabetes Research and Clinical Practice* in September 2017.

Chapter 6 is the final chapter and includes a critical discussion of the main findings of each manuscript, whereafter recommendations for future research are made and final conclusions are drawn.

For each manuscript, the first author listed is the PhD candidate and the rest of the authors included are the promoter and co-promoters, as well as collaborators who provided intellectual input on certain aspects and who participated in the design and the execution of the PURE-SA-NWP study. A reference list is included at the end of each chapter. In the interest of uniformity of the thesis, the Vancouver reference style was used throughout. However, each manuscript was prepared according to the author instructions of the individual journals and a summary of these instructions can be found at the beginning of the chapters that contain the manuscripts. Manuscripts one and two can be viewed in their journal format at the end of this thesis in annexures C and D.

AFFIRMATION BY AUTHORS

The following researchers contributed to this thesis:

Mrs M Maritz

Responsible for proposal of this study, extensive literature research, evaluation of study protocol and methodology, data collection, part of the biochemical analyses conducted in the laboratory, dataset cleaning and analyses, statistical analyses, design and planning of the research articles, interpretation of the results and writing of all sections of this thesis.

Prof. CMT Fourie (promoter), Prof. JM van Rooyen and Prof. AE Schutte (co-promoters)

The promoter and co-promoters supervised of the design, planning and writing of this thesis. In addition, they provided guidance, intellectual input and a critical evaluation of the statistical analyses and the final versions of the manuscripts and the thesis.

Prof. SJ Moss

Provided intellectual input in the manuscript presented in Chapter 3.

Dr. IM Kruger

In her capacity as project leader of the South African leg of the PURE study in the North West Province, provided intellectual input in the manuscript presented in Chapter 4.

The following is a statement of the co-authors verifying their individual contribution and involvement in this study and granting their permission that the relevant research articles may form part of this thesis:

Hereby, I declare that I approved the aforementioned manuscript and that my role in this thesis, as stated above, is representative of my actual contribution. I also give my consent that the manuscript may be published as the PhD thesis of Melissa Maritz.

Prof CMT Fourie

Prof JM van Rooyen

Prof AE Schutte

Prof SJ Moss

Dr IM Kruger

SUMMARY

Motivation

Sub-Saharan African countries face a double burden of disease due to a high prevalence of infectious diseases such as Human Immunodeficiency Virus infection (HIV) and tuberculosis, as well as non-communicable diseases such as cardiovascular disease. The high prevalence of cardiovascular disease in South Africa, especially in the black population, places significant strain on the overburdened public health system. Literature indicates that large artery stiffness is an early predictor of cardiovascular disease and mortality in various populations. Increased large artery stiffness places significant strain on the heart by increasing the afterload and by decreasing coronary blood flow during diastole. Furthermore, large artery stiffness is a risk factor for organ damage as it increases the transmission of pulsatile systolic pressure into the microcirculation of organs such as the brain and kidney.

Numerous reports indicate that blood pressure and age are the strongest predictors of large artery stiffness. However, other factors, including cardiometabolic risk factors and health behaviours, may also affect large artery stiffness. Obesity, lipids, inflammation, endothelial activation, renal function, liver function, oxidative stress and health behaviours such as alcohol use, tobacco use and physical inactivity have all been associated with large artery stiffness in previous reports.

The scantiness of longitudinal data concerning large artery stiffness measured with the gold standard method (carotid-femoral pulse wave velocity) has thus far inhibited an investigation into factors affecting large artery stiffness in black populations. The identification of such factors in black South Africans may enable policy makers to plan and implement prevention strategies that will successfully reduce the prevalence of morbidity and mortality due to cardiovascular disease in South Africa.

Aim

The central aim of this study was to investigate large artery stiffness (as measured by carotid-femoral PWV) and its associations with non-modifiable and modifiable risk factors in the understudied black South African population. Therefore, a young and older black population were included in this thesis and large artery stiffness and associated risk factors were investigated cross-sectionally in both populations, as well as longitudinally in the older population.

Methodology

This sub-study included data from both the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and HyperTension (African-PREDICT) and the South African leg of the international Prospective Urban and Rural Epidemiology (PURE) study, conducted in the North West Province (PURE-SA-NWP). For the purposes of this thesis, all HIV-infected participants were excluded from both study populations. The PURE-SA-NWP study is a longitudinal study with a baseline (conducted in the year 2005) and two follow-up data collections (conducted in 2010 and again in 2015). For manuscript two, baseline and 10-year follow-up data were used, and for manuscript three, five-year follow up data (representing baseline data for this manuscript) and 10-year follow-up data were used.

For both studies, data was collected according to standardised methods. Participants completed general and health questionnaires, in which socio-economic factors, alcohol, tobacco and medication use were reported. Anthropometric and cardiovascular measurements were performed. Carotid-femoral pulse wave velocity was measured with the Sphygmocor XCEL device according to the most recent recommendations. Biological sampling took place, which enabled the biochemical analyses of relevant metabolic, inflammatory, endothelial activation, oxidative stress, renal function and liver function markers.

As part of the statistical analyses, variables without a normal distribution were logarithmically transformed. I compared variable means and proportions with independent t-test and Chi-square tests, dependent t-tests and Wilcoxon tests, analyses of variance and analyses of covariance when adjustments were needed. Relationships between variables were established with Pearson's correlation coefficients and partial correlation coefficients (when adjustments were needed). Independent associations with and predictors of large artery stiffness were determined with multi-variate linear regression analyses. In all instances, a p-value of <0.05 were regarded as significant.

Results and conclusions of each manuscript

The central aim of this thesis was achieved by the results of three manuscripts. In the first manuscript, large artery stiffness was compared in young black and white adults and the associations of health behaviours with arterial stiffness were determined. Mean arterial pressure (MAP) was higher in the black participants ($p < 0.001$), but carotid-femoral pulse wave velocity (cfPWV) was similar in young black and white adults (6.37 ± 0.73 vs. 6.36 ± 0.73 m/s; $p = 0.89$) after adjustment for MAP. Higher levels of gamma-glutamyltransferase (GGT) ($p < 0.001$), cotinine, reactive oxygen species, interleukin-6 and monocyte-chemoattractant protein-1 (all $p < 0.02$) were found in the black group. GGT associated independently and positively with cfPWV in both black and white adults after multiple-adjustment in multiple regression analyses ($\beta = 0.15$; $p \leq 0.049$ in both groups). No association was found with smoking or physical activity, but cfPWV inversely associated with body mass index in the whites. These results indicated that, already at a young age, black populations may be more vulnerable to early vascular ageing and subsequent CVD development, due to higher GGT levels and an elevated cardiovascular risk profile.

Manuscript 2 investigated whether traditional cardiovascular risk factors and health behaviours predicted large artery stiffness in a black South African population 10 years later. At follow-up, 25.3 % of the population (age 65 ± 9.57 years) had a cfPWV greater than 10

m/s. In multivariate-adjusted regression analyses, the strongest predictors of cfPWV were MAP, age and heart rate (all $p < 0.024$). Urban locality (adjusted $R^2 = 0.31$, $\beta = 0.12$, $p = 0.001$), self-reported alcohol use ($\beta = 0.11$, $p = 0.018$) and plasma glucose ($\beta = 0.08$, $p = 0.023$) associated positively with follow-up cfPWV. Body mass index (BMI) associated negatively with cfPWV ($\beta = -0.15$, $p = 0.001$), but no associations with sex, smoking, inflammatory markers, lipids or antihypertensive medication were found. When self-reported alcohol use was replaced with GGT, the latter also associated independently with cfPWV ($\beta = 0.09$, $p = 0.028$). These results suggest that a health profile associated with excessive alcohol use, such as residing in an urban location, elevated plasma glucose levels and a low BMI may predispose black South Africans to stiffer arteries. This observation encourages the development of public health strategies that target excessive alcohol use in South Africa.

In the final manuscript, biomarkers known to modulate arterial function in other populations (metabolic, inflammatory, endothelial activation and oxidative stress) were investigated with regard to large artery stiffness in young and older black South Africans who self-reported no alcohol-use. Cross-sectional data from young (aged 24.7 ± 3.24 years) black adults and five-year follow-up data from older (aged 61.6 ± 9.77 years) black adults were included. Of the variety of biomarkers investigated in multivariable-adjusted regression analyses, only plasma glucose (adjusted $R^2 = 0.24$, $\beta = 0.21$, $p < 0.001$) and glycated haemoglobin (adjusted $R^2 = 0.22$, $\beta = 0.17$, $p = 0.002$) independently predicted cfPWV five years later in the older black adults. In the younger group, no associations were found. These results highlight the possible role of dysglycaemia in the development of CVD in Africa. Furthermore, it prompts public health education about the importance of managing sugar intake and body weight throughout the life course.

General conclusion

This study shows for the first time that health behaviour, especially alcohol use, is predictive of large artery stiffness over 10 years in black South Africans. Young black adults already

seem to be at a higher risk for cardiovascular disease due to a health profile which exhibits higher inflammation, oxidative stress, tobacco use and an independent positive association between arterial stiffness and GGT. In an older black population not consuming alcohol, arterial health is compromised by dysglycaemia. These results emphasise the importance of maintaining a healthy lifestyle throughout the life-course, in order to avoid early vascular ageing.

Keywords: large artery stiffness, carotid-femoral pulse wave velocity, black South Africans, health behaviour, gamma-glutamyltransferase, alcohol use, plasma glucose, predictors, prognostic, longitudinal

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- Figure 1 - Flow diagram depicting the main predictors of large artery stiffness in black South African populations.

LIST OF ABBREVIATIONS

AEE	-	active energy expenditure
African-PREDICT	-	the African Prospective study on the Early Detection and Identification of Cardiovascular disease and HyperTension
AGEs	-	advanced glycation end products
ALT	-	alanine aminotransferase
ANCOVA	-	analysis of covariance
ANOVA	-	analysis of variance
AST	-	aspartate aminotransferase
BMI	-	body mass index
BP	-	blood pressure
cfPWV	-	carotid-femoral pulse wave velocity
CI	-	confidence interval
CKD-EPI		chronic kidney disease epidemiology collaboration
CrCl	-	creatinine clearance
CRP	-	c-reactive protein
CVD	-	cardiovascular disease
DBP	-	diastolic blood pressure
eGFR	-	estimated glomerular filtration rate
ESH/ESC	-	European Society of Hypertension and European Society of Cardiology
Et al.	-	<i>et alia</i> 'and others'
EVA	-	early vascular ageing
GGT	-	gamma-glutamyltransferase
HbA1c	-	glycated haemoglobin type a1c
HDL-C	-	high density lipoprotein cholesterol
HIV	-	human immunodeficiency virus
ICAM-1	-	intercellular adhesion molecule-1
IL-6	-	interleukin-6
kg	-	kilogram

L	-	litre
LDL-C	-	low-density lipoprotein cholesterol
m/s	-	metres per second
MAP	-	mean arterial pressure
MCP-1	-	monocyte chemoattractant protein-1
mmHg	-	millimetres of mercury
mmol/l	-	millimole per litre
N	-	number of
NAFLD	-	non-alcoholic fatty liver disease
NCDs	-	non-communicable diseases
Ox-LDL-C	-	oxidized low-density lipoprotein cholesterol
p	-	probability
PP	-	pulse pressure
PURE	-	prospective urban and rural epidemiology
PURE-SA-NWP	-	South African leg of the Prospective Urban and Rural Epidemiology study in the North West Province
R ²	-	relative predictive power of a model
RAGE	-	receptor for advanced glycation end products
ROS	-	reactive oxygen species
SBP	-	systolic blood pressure
SD	-	standard deviation
SE	-	standard error
SES	-	socioeconomic status
SV/PP	-	stroke volume/pulse pressure ratio
TC	-	total cholesterol
TG	-	triglycerides
TNF- α	-	tumour necrosis factor-alpha
UACR	-	urinary albumin to creatinine ratio
μ mol/l	-	micromole per litre
WC	-	waist circumference

CHAPTER 1

Introduction and Literature Study

1. GENERAL INTRODUCTION

Globally, cardiovascular disease (CVD) causes more deaths than any other non-communicable or infectious disease [1]. The leading risk factor for CVD is high blood pressure, or hypertension [2]. Although global BP has decreased during the past four decades, low-income countries within sub-Saharan Africa are still at risk and currently exhibit the highest average blood pressures [3]. In addition, results obtained from 156 424 participants from 17 different countries as part of the Prospective Urban and Rural Epidemiology (PURE) study indicated that the prevalence of major CVD and death are significantly higher in low- than in high-income countries [4].

Large artery stiffness is an important cardiovascular risk estimator [5] and it may predict cardiovascular mortality better than BP [6, 7]. In addition, large artery stiffness predicts the occurrence of coronary events, stroke, type 2 diabetes and end-stage renal disease [8]. Both hypertension and increased large artery stiffness are more prevalent in black compared to white populations [9, 10]. Arterial stiffness is defined as a loss of elasticity, or increased rigidity of the large, central arteries [11] and it impacts the function of the artery by affecting BP and blood flow, as well as the changes in arterial diameter with each cardiac contraction [12]. Arterial stiffening occurs in the large arteries, as well as in the smaller peripheral arteries, although to a lesser extent [13]. This may be attributable to structural differences between large, elastic arteries and peripheral arteries [14].

Higher large artery stiffness in black populations may be partially explained by increased exposure to cardiovascular risk factors [15]. Seventy-five percent of urban black South Africans present with multiple risk factors for CVD and suffer from high rates of hypertension, resulting in hypertensive heart disease and stroke [16]. Arterial stiffness may be present before the development of hypertension [17], thus presenting a possible pathophysiological mechanism leading to the development and progression of CVD in black population. The measurement of carotid-femoral pulse wave velocity (cfPWV) is considered the gold

standard measurement for large artery stiffness [18]. Due to its ability to predict cardiovascular outcomes, large artery stiffness, measured with cfPWV, has received much attention in recent decades in various populations [5, 19-23], with a report regarding cfPWV in Chinese participants published in 1985 already [24]. The few studies that have focussed on arterial stiffness in sub-Saharan African populations mostly made use of PWV measurements that reflect stiffness in more peripheral arteries instead of central arteries. Thus, knowledge about large artery stiffness and its associations with cardiovascular risk factors in black populations residing in sub-Saharan Africa are severely limited. Consequently, early predictors of large artery stiffness in these populations are lacking.

This chapter consists of a broad overview of the literature, specifically focussing on large artery stiffness. The function of the arterial system, arterial stiffness and methods of measuring arterial stiffness are discussed. The relation between modifiable and non-modifiable cardiometabolic risk factors and arterial stiffness, as well as the role of arterial stiffness in CVD and mortality risk are discussed with specific reference to African populations.

2. LITERATURE OVERVIEW

2.1 Disease burden of the South African population

South Africa is a diverse and unique country with an estimated population of 55,91 million people, 51% of which are female and 80.1% of which are of African ancestry [25]. The population is multicultural and there are a variety of ethnic, urban-rural, class, age and gender differences amongst the population [26]. The average life expectancy is 59.7 years for men and 65.1 years for women [25]. A quarter of the population is unemployed [27].

Eastern and Southern Africa are most severely affected by Human Immunodeficiency Virus (HIV) infection in the world [28], with an estimated 12.7% of the South African population being HIV-infected in 2015 [25]. Since November 2003, free antiretroviral treatment has

been available to HIV-infected people [29]. The health of the South African population is impaired by perinatal and maternal disorders, injury, violence and a double burden of disease characterised by infectious diseases like tuberculosis and HIV-infection and non-communicable diseases (NCDs) like diabetes and CVD [30]. Health challenges in Africa are attributed to the so-called “Paradoxes of Africa” [31]: various natural resources and international financial aid are lost through corruption and impoverishment of African populations, while the health burden of infectious disease and NCDs continue to take its toll [31]. In South Africa, issues of inequality, poverty and human rights are being addressed, accompanied by changes in economic, societal and family structures [32], followed by rapid urbanisation and socio-demographic changes [33-35]. Consequently, while infectious diseases such as HIV-infection remain a health obstacle to millions of South Africans, the epidemiological transition has added NCDs like CVD to the health burden [32, 36].

Comparing hypertension to HIV-infection captures the size of the health threat posed by CVD to vulnerable sub-Saharan populations [37]. Both HIV-infection and hypertension are largely asymptomatic, easily diagnosed and fatal without lifelong management and treatment [37]. Over the next two decades, the mortality rate from hypertension may exceed that resulting from HIV-infection and associated diseases [37]. Indeed, cerebrovascular and other forms of heart disease are ranked as the 3rd and 4th leading causes of death in the South African population, only after tuberculosis and diabetes [38]. At 78%, South Africa has one of the highest hypertension rates in the world for people age 50 years and older [39]. Low rates of hypertension awareness and adequate control not only in South Africa (38% and 7.8%) [39], but in the whole of sub-Saharan Africa (27% and 7%) [40] contribute to the high CVD prevalence in this region.

With only 17.4% of South Africans currently belonging to a medical aid [41], the majority of the population is served by a public health system that faces challenges in terms of human and financial resources, management and implementation of policies [42]. Effective primary prevention strategies are urgently needed to curb the growing CVD epidemic and relieve the

strain on the public health system. The identification of early predictors of arterial stiffness may aid in the development of programmes that will adequately address risk factors for CVD.

2.2 The arterial system: structure and function

The arterial wall consists out of three concentric layers: the tunicas intima, media and adventitia [43]. The intima and media is separated by the internal elastic lamina, a layer of elastic fibres [44], while the media and adventitia is separated by the outer elastic lamina [45]. The intima consists mainly of vascular endothelium, while the media is made up of vascular smooth muscle cells, elastin fibres and collagen fibres [45]. The adventitia contains some elastin but primarily collagen fibres that merges with the surrounding connective tissue made up of fibroblasts, nerves and small blood vessels [45]. Central arteries, such as the aorta and its major branches, contain more elastin, while the more distal arteries such as the brachial artery is composed of more smooth muscle cells and collagen, giving smaller, distal arteries a greater intrinsic stiffness than the central arteries [46]. The collagen and elastin extracellular matrix is the load-bearing component of the arterial wall [44] and ensures that the artery is able to withstand deformations brought about by blood pressure changes [44]. While the collagen fibres prevent artery damage or rupture upon subjection to high pressure [47], the elastin fibres help 'spread' the stress load that the artery is subjected to over the whole arterial wall [48]. In the large arteries, elastin absorbs most of the energy created by the pulsatile ejection of blood [49].

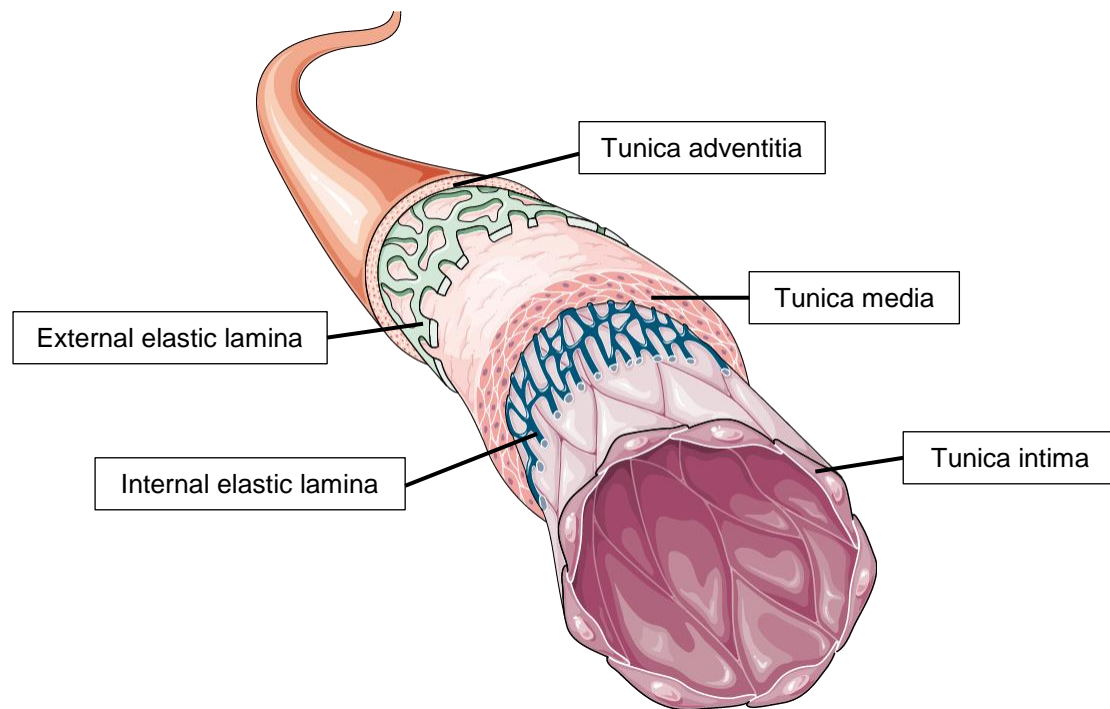


Figure 1. *The structure of the arterial wall. Image obtained from Sevier Medical Art.*

The heart acts as a pump to circulate oxygen and nutrients contained in the blood through an extensive network of arteries and veins [11]. While an important function of the arteries is to serve as pipes which deliver nutrients and oxygen to body tissues, another essential function involves the large arteries [50]. Blood is not compressible and the ejection of the stroke volume into the aorta means that space must be created to accommodate the stroke volume in a system that is already completely filled [50]. When the left ventricle ejects the stroke volume into the aorta, part of the energy created by the contraction of cardiac muscle is transmitted into the wall of the aorta, thereby distending the aorta and making room for the blood which has been newly ejected [50]. A pressure-gradient is needed to cause blood flow in the vascular system [12]. The increased pressure in the aorta just after ventricular ejection creates the needed pressure gradient in the arterial tree [50]. This pressure difference travels through the arterial wall down to the more distal arterial segments in the form of a pulse wave, thereby pushing the blood forward as it travels [50]. Therefore, an important function of the large, elastic arteries are to dampen the pressure pulse created when the heart contracts by expanding in volume in response to the increase in pressure [46]. When

the forward pressure wave generated by ventricular ejection reaches branching points in the arterial tree, it is reflected back towards the heart [51]. Pulse wave velocity (PWV) is the velocity by which the forward pressure pulse travels along the arterial tree until it reaches branching points [52].

During the diastolic phase of the cardiac cycle, the elastic large arteries recoil (gradually becomes smaller in diameter) [43], thereby ensuring constant blood flow during the 'relaxation' phase of the cardiac cycle [50]. This system delivers adequate coronary blood supply, nearly smooth, continuous capillary blood flow and constant organ perfusion [45, 46]. This specialised dampening function of the arteries decreases the further the artery is located from the heart [8, 45, 46], due to changes in arterial wall composition [11].

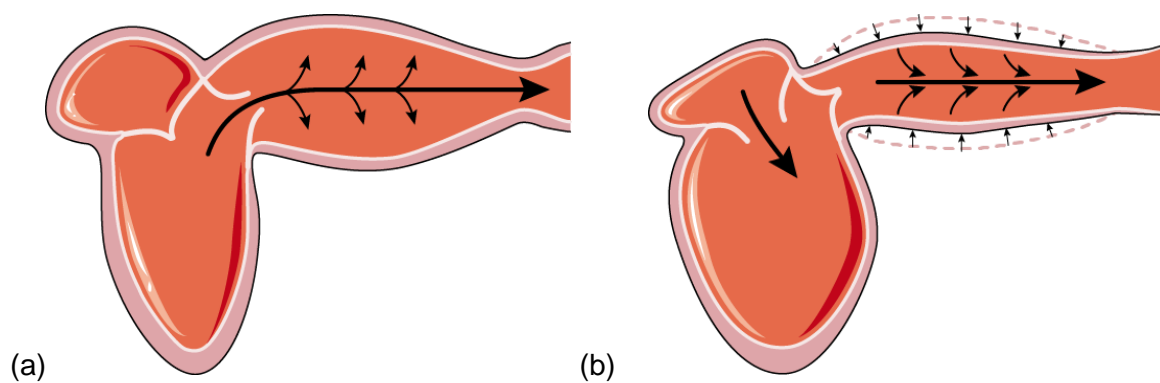


Figure 2. The dampening function of the large arteries. (a) Expansion of the aorta during systole and (b) elastic recoil of the aorta during diastole. This figure illustrates the theoretical concepts as explained in the literature [43, 50]. Images obtained from Servier Medical art.

2.3 Large arterial stiffness

Disease processes may hamper the two functions of the arterial system. Atherosclerosis affects the conduit function of the arteries by forming plaques that impede the flow of blood through the arteries [50]. Arteriosclerosis, or arterial stiffness, denotes the stiffening of the arteries [53], which affects the dampening function of the large arteries [50].

Arterial stiffness collectively refers to the distensibility, compliance and elastic modulus of the arterial system [54]. A loss of elasticity of the artery, or an increase in the rigidity of the arterial wall, increases arterial stiffness, which affects the buffering function of the arterial system [54]. Changes in arterial stiffness can be detected before the clinical manifestation of vascular disease, thus making it a useful marker for the future development of disease [55]. Indeed, several large studies have shown that arterial stiffness is an independent risk factor for mortality and morbidity relating to the cardiovascular system in the general population [56], hypertensive individuals [57] [19, 58, 59], patients with end-stage renal disease [60, 61] and in patients with impaired glucose tolerance [62].

The main structural elements of the arterial wall, elastin and collagen, are important determinants of wall stiffness [63]. Arterial stiffness is affected especially by structural changes in the medial layer of the arterial wall [64], however, it remains important to study all the layers of the arterial wall with regard to arterial stiffness [65]. For the same increase in distending pressure, larger arteries are able to expand more than smaller arteries due to differences in compliance and the composition of the arterial wall [46]. The pressure exerted on the artery wall by the blood flowing through is also an important determinant of arterial stiffness, with stiffness being higher at higher blood pressures, probably due to the recruitment of more of the stiffer collagen fibres when the arterial wall is stretched to a greater extent [63]. The tone of smooth muscle cells in the arterial wall, as well as the factors influencing the muscle tone, such as the endothelium, also affects arterial stiffness [66], although much less in the central arteries than in the peripheral arteries [64].

Pathophysiological processes such as fibrosis, increased thickness of the arterial intima and media, changes in collagen and elastin content, endothelial dysfunction and arterial calcification are characteristic responses to injury, disease or ageing in the arterial wall, a process also known as arterial remodelling [49, 67]. In healthy arteries, remodelling is a physiological response to alterations in blood flow and circumferential stress, aiming to

restore normal shear stress and wall tension [68]. Shear stress, which mainly affects endothelial cells, is the force exerted due to the friction of the blood on the vessel wall [69].

In normal, healthy arteries, a stiffness gradient exists between the elastic large arteries and the stiffer peripheral arteries [70]. Thus, the pulse wave created by ventricular ejection is reflected at branching points where the arteries become smaller and stiffer. This reflected pulse wave essentially reserves some energy for coronary perfusion, while it also decreases the amount of pulsatile stress transmitted into the smaller arteries and microcirculation [70]. However, with increases in large artery stiffness, the arterial stiffness gradient may eventually be reversed, with the large arteries becoming stiffer than the peripheral arteries [63, 70]. Upon the reversal of the stiffness gradient, a higher, potentially damaging increased pulsatile pressure is transmitted into the microcirculation [71]. The subsequent myogenic response may result in decreased organ perfusion, endothelial dysfunction and organ damage, especially in organs with high blood flow, such as the kidneys [72] and the brain [73]. In a Framingham Heart study cohort with minimal cardiovascular risk factors, cfPWV was lower than carotid-brachial PWV in participants younger than 50, however, at ages ≥ 50 years, cfPWV increased to values higher than carotid-brachial PWV, clearly showing the reversal of the stiffness gradient in older age [23].

Pharmacological therapy such as anti-hypertensive drugs (except diuretics and non-vasodilating beta-blockers) [74, 75], lipid-lowering drugs [76, 77] and anti-diabetic drugs [78] may effectively decrease arterial stiffness [79]. Of the anti-hypertensive drugs, the renin-angiotensin-aldosterone system inhibitors seem to be most effective in decreasing arterial stiffness so far [74]. Angiotensin-converting enzyme inhibitors effectively decreased arterial stiffness, beyond its effect on BP in patients with untreated hypertension [80]. Atorvastatin, a lipid-lowering drug, lowered arterial stiffness in elderly hypertensive patients possibly via a reduction in oxidative stress and improving endothelial function [77]. In addition, inhibitors of the formation of advanced glycation end products (AGEs), or breakers of the cross-links in collagen formed by AGEs show promise as effective destiffening-therapy [74, 81]. Physical

exercise may also lower arterial stiffness [79]. Aerobic exercise, but not resistance exercise, improves arterial stiffness, although to a greater extent in peripheral arteries (as measured by brachial-ankle PWV) than in central large arteries [82]. A randomised control study currently being conducted in France, named the SPARTE study, is comparing the ability of therapeutic interventions to reduce arterial stiffness. In the future, results from the SPARTE study may thus be able to shed light on whether the therapeutic effect of drugs, or the effect of controlling risk factors (such as BP) are more beneficial in reducing arterial stiffness [83].

2.3.1 Arterial stiffness measurement

Arterial stiffness can be measured with multiple invasive and non-invasive methods [84]. Four of the most used methods are devices that record the arterial pulse wave using a tonometer and transducer, devices that record the pulse wave oscillometrically, ultrasound devices and magnetic-resonance imaging (MRI) [12].

Carotid-femoral pulse wave velocity

Representing a direct measurement of large artery stiffness, cfPWV is a strong predictor of the occurrence of cardiovascular events and mortality [18]. This is reflected by the fact that the 2013 European Society of Cardiology-European Society of Hypertension (ESC/ESH) guidelines on hypertension management consider high cfPWV itself as target organ damage [85]. The measurement of cfPWV has the gold standard status due to it being the most simple, non-invasive, reproducible and relatively affordable measure of large artery stiffness currently available [8, 18]. Furthermore, cfPWV has established reference values and has been validated in large studies [84]. The validity of measuring pulse wave travel in an arterial segment is supported by the Moens-Korteweg and Bramwell Hill equations [86]. However, one of the disadvantages of PWV measurement is that it depends on the blood pressure at the time of measurement [87]. Carotid-femoral pulse wave velocity predicts cardiovascular events, cardiovascular- and all-cause mortality better than brachial systolic and diastolic blood pressure, as well as brachial and 24h pulse pressure [6, 7]. It is measured as the time the foot of the pulse wave take to travel between the carotid and femoral arteries [6] and

increases from approximately five metres per second (m/s) in childhood to about 15 m/s in old age [6].

During the measurement of cfPWV in healthy arteries, the reflection of the forward pulse wave created by ventricular ejection arrives back at the heart during diastole, which ensures that it does not affect the central blood pressure or increase the afterload of the heart [51]. However, in stiffened arteries, the pulse wave travels faster to the sites of reflection and the reflected wave arrives back at the aorta still during the systolic phase of contraction, thereby augmenting the central blood pressure [51]. The detrimental effects resulting from this earlier arrival of the reflected pulse wave includes increased cardiac afterload, [88] increased risk for left ventricular hypertrophy and heart failure [89] and decreased coronary blood flow [90].

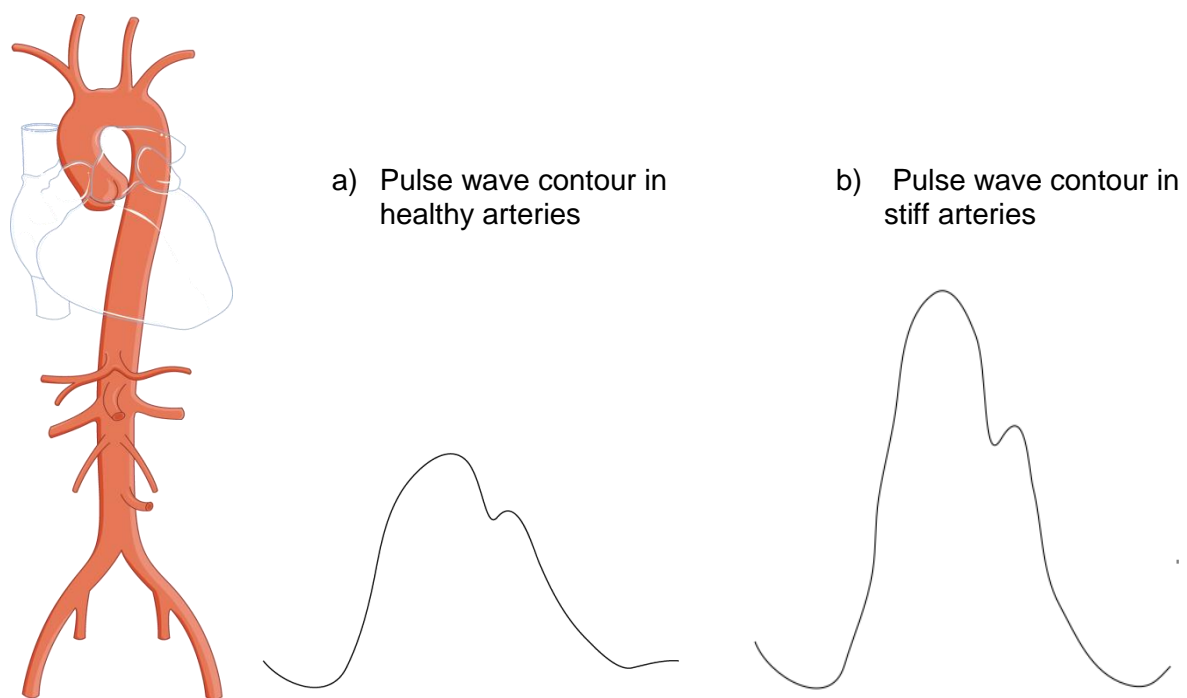


Figure 3. *The arterial pulse waveform in healthy, elastic large arteries and in stiff large arteries. In (a) the wave reflected from the branching points in the arterial tree arrives back at the heart during diastole, thus not augmenting the central pressure. In the stiffer arteries (b), the reflected wave travels faster from the branching points in the arterial tree back to the heart, arriving in systole and augmenting central pressure. This figure illustrates the theoretical concepts as explained in the*

literature [45, 50, 86]. Images obtained from Servier Medical Art (arterial tree) and created with Inkscape Illustrator software (arterial pulse wave forms).

Pulse wave velocity can be measured in several arterial segments. Large, elastic arteries are affected more by ageing and other cardiovascular risk factors than the more muscular, peripheral arteries [84]. PWV measures other than cfPWV, such as carotid-dorsalis-pedis PWV, carotid-radial PWV and brachial-radial PWV, are more representative of the stiffness of the peripheral arteries [91]. These measures of PWV provide information on the arterial stiffness of the local arterial segment and not of the whole arterial tree [55]. Brachial-ankle PWV measures the pulse wave over a longer distance and takes into account the stiffness of large and peripheral (brachial and tibial) arteries [92]. As an alternative to office measurements, the ambulatory arterial stiffness index can be derived from ambulatory blood pressure measurements, although the usefulness of this index is still debated [93]. Relatively recently, advances in oscillometric technology made possible the ambulatory measurement of PWV itself, thus enabling the study of 24h-variability in arterial stiffness [93].

Van Bortel and colleagues advises the use of 10 m/s as the cut-off value for cfPWV, above which the risk for CVD is increased [18]. However, this reference value was based mostly on populations from European descent. A threshold of 8.0 m/s has been proposed to diagnose increased arterial stiffness in young black adults, however, this needs validation in prospective outcome-based studies in young and older black populations [94].

Other methods of measuring arterial stiffness

The use of ultrasound is limited to large and easily accessible arteries. Several images of the vessel wall is taken and the maximum and minimum areas of the vessel wall are calculated by wall-tracking and edge-finding software, while blood pressure is measured simultaneously [95]. Some concerns exist about the reproducibility of this technique, but this may be improved by an experienced operator, or a robotic arm that fixes the ultrasound transducer in place [95]. The Atherosclerosis Risk in Communities study used ultrasound to determine decreased distensibility in the carotid artery and found that lower carotid distensibility

increased the future risk of developing hypertension [96]. Magnetic resonance imaging (MRI) allows for an accurate path length to be assessed non-invasively and for measurements to be made from arteries that are not easily accessed. However, this method is expensive, time-consuming and scanning facilities are limited [95].

Other markers of arterial stiffness

Other surrogate measures of arterial stiffness include arterial compliance, arterial distensibility and characteristic impedance, a measure that relates pressure changes to blood flow changes in the artery [12]. The intrinsic stiffness of the arterial wall can be calculated as the elastic modulus when the size and diameter measurements of the artery are available [12]. Pulse pressure (PP), calculated as the difference between the systolic and diastolic BP [97], is influenced by the stiffness of the large arteries, reflection of the pulse wave and the cardiac output [55]. An increased PP, attributable to large artery stiffness, is thought to be the leading cause of the age-related increase in hypertension [52]. However, PP alone cannot be used to measure arterial stiffness accurately [55], as it does not reflect the actual central pulse pressure when measured in the periphery, for instance the upper arm [55]. Systolic pressure augmentation, also called the augmentation index (AIx), compares the first and second systolic peaks in the central aortic waveform and expressed as a percentage of the PP [8]. The AIx is not a measurement of arterial stiffness alone [12] as it is influenced by the velocity of the pulse wave, the amplitude of the reflected wave, the point where the wave is reflected and the nature of ventricular ejection [98]. A relatively new measure of regional arterial stiffness, the cardio-ankle vascular index (CAVI) is theoretically independent of changes in blood pressure [99].

The aortic-brachial stiffness gradient, or the PWV ratio (carotid-radial PWV divided by cfPWV), predicted all-cause mortality better than cfPWV in a cohort of patients receiving renal dialysis [100]. The aortic-brachial PWV ratio has the potential advantage of being a blood-pressure independent measure of arterial stiffness [87]. However, cfPWV was still a better predictor of all-cause mortality than aortic-brachial PWV ratio in a community-based

sample of the Framingham Heart study, suggesting that the predictive value of the PWV ratio is related to the baseline cardiovascular risk and that cfPWV should remain the gold standard method of large artery stiffness evaluation in the general population [101]. The beta stiffness index, which takes into account blood pressure and arterial diameter changes, is another indicator of arterial stiffness, is thought to be more representative of the intrinsic stiffness of the arterial wall [55].

2.4 Large arterial stiffness and cardiovascular risk factors

2.4.1 Age

Already in the 17th century, Thomas Sydenham, an English physician remarked that “a man is as old as his arteries”. Various physiological processes in the human body deteriorate along with ageing [43]. Also in the vasculature, a gradual change in the structural and functional properties of blood vessels are seen with increasing age [51]. Cellular, enzymatic and biochemical changes of the arterial structure and the signals that affect these changes form part of this process [102]. Structural changes in the arterial wall associated with ageing include a decrease in the elastin content of the media, an increase in the collagen content, as well as increased cross-links between the collagen structures [103]. Differentiation of vascular smooth muscle cells from a contractile to a secretory or osteogenic phenotype may lead to increased vascular tone and increased arterial wall calcification [67]. While the large arteries are vulnerable to stiffening along with age, the radial, brachial and femoral arteries, which are more muscular, are resistant to stiffening induced by ageing [104].

In the presence of cardiovascular risk factors, ageing of the arteries may take place at an accelerated rate [43]. Changes in the vasculature of elderly people seem to be more substantial in the presence of hypertension or atherosclerosis at an earlier age [105]. Furthermore, in the presence of hypertension, increased arterial stiffness may be observed at an earlier age than in a normotensive person [106, 107]. This finding led to the development of a pathophysiological concept called early vascular ageing (EVA) [51].

Increased arterial stiffness, dilation of the large elastic arteries, endothelial dysfunction and impaired vasodilation of the peripheral arteries are important components of this early ageing process [51]. The EVA concept may help to identify individuals who are experiencing early insults to their cardiovascular health, thus enabling early intervention and prevention therapies [108].

Although mostly viewed as inevitable [109], early increases in arterial stiffness with advancing age may be partly due to pathophysiological processes [110] and may thus be partially preventable [111]. Recently, Niiranen *et al.* showed that 17.7% of a sample of 3196 Framingham Heart study participants aged ≥ 50 years exhibited healthy vascular ageing, which was defined as the absence of hypertension and a cfPWV of less than 7.6 m/s [111]. A younger age, female sex, low body mass index (BMI) the use of lipid-lowering drugs and the absence of diabetes mellitus were cross-sectionally associated with healthy vascular ageing [111]. In this study, the biggest threats to health vascular ageing were modifiable risk factors such as obesity and metabolic diseases such as diabetes [111]. However, after age 70, the maintenance of healthy vascular ageing is difficult [111].

2.4.2 Blood pressure

The elastic properties of the arterial wall are pressure-dependent [112] and physiologically, arterial stiffness is affected most by the mean arterial pressure (MAP) [12]. Therefore, the confounding effect of MAP should be accounted for when investigating arterial stiffness [12].

Blood pressure is the force that blood exerts against a unit area of the vessel wall and it is measured in millimetres mercury (mmHg) [113]. The distending force that blood pressure exerts on the arterial wall is known as circumferential stress [69]. The regulation of blood pressure is a complex, active process that matches tissue perfusion with metabolic demands under normal physiological circumstances [114]. Hypertension, the syndrome denoting high blood pressure, is associated with damage to the vasculature, kidneys, heart and brain, which may lead to premature morbidity and mortality [114, 115]. The 2011 South African

Hypertension Guidelines and the 2013 European Society of Hypertension and Cardiology (ESH/ESC) guidelines define hypertension as a systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure of ≥ 90 mmHg [85, 116]. Approximately 90% of hypertension cases are classified as essential hypertension, meaning that the cause of the hypertension is not clear [113]. Hypertension has been described as a “silent, invisible killer” as it rarely causes symptoms in the early stages [115, 117]. Low- and middle income countries are currently most affected by hypertension-related morbidity and mortality due to limited health resources [115].

Hypertension is a haemodynamic disorder that exposes the arterial system to increased pressure [118]. The arterial changes associated with hypertension include an increase in the total peripheral resistance, as well as a decrease in arterial compliance [8]. The structural damage caused by hypertension to large and small arteries may lead to endothelial dysfunction, reduced vascular compliance, increased vascular stiffness, reduced lumen diameter and formation of atherosclerotic plaques [63]. Arterial stiffness in turn affects blood pressure by increasing systolic and pulse pressure, endocardial ischemia and the pulsatile load on the microvasculature [119, 120], all while the diastolic BP remains relatively unchanged [8]. Increased pulsatile pressure in the microvasculature of the brain and kidney may increase the risk for stroke and renal disease [120]. The stiffening of large arteries may be a causative factor in essential hypertension, since evidence show that cfPWV is increased even at the early stages of hypertension, when BP is at the borderline level [121]. In Framingham Heart Study participants with a mean age of 60 years, increased large artery stiffness associated with the development of hypertension after seven years, but higher BP at baseline was not related to the risk of progressive large artery stiffening [122]. This result supports the theory that arterial stiffness contributes to the development of hypertension rather than the other way around [122].

When comparing arterial stiffness between normotensives and unsuccessfully treated hypertensives, the intravenous infusion of angiotensin II in the normotensive group raised

MAP, as well as cfPWV and carotid stiffness, confirming the role of blood pressure as a strong determinant of functional arterial stiffness [123]. However, upon infusion with nitroglycerine, cfPWV was still lower in the normotensives although the MAP of the two groups was now comparable. This suggests that structural changes are affecting the elasticity of the large arteries in the hypertensive individuals [123].

2.4.3 Sex

Sex differences exist in CVD and cardiovascular function [124]. Due to factors such as body size, sex hormones and the mechanical properties of arteries, the progression of large artery stiffness may differ for men and women [125]. Furthermore, sex differences in the manifestation of obesity, low-grade inflammation, fibrosis and endothelial dysfunction could contribute to sex disparities in arterial stiffness beyond the differences accounted for by body size and age [126]. While CVD risk in men develops linearly across the lifespan, women experience a sharp increase in CVD risk at the onset of menopause [127].

Despite the absence of a difference in cfPWV, healthy, middle aged women had higher reflected pulse waves than men, independent of body height [23]. Increases in large artery stiffness occur in both men and women with advancing age, but the rate of increase may be higher in women. This higher rate of increase provides a potential explanation for the increase in adverse cardiovascular events in menopausal women [128]. The arteries of women may be intrinsically less elastic than those of men, as studies at ages where sex hormones do not play a prominent role (pre-pubertal and postmenopausal) indicate higher arterial stiffness in women [129-131]. The finding of higher arterial stiffness in a small sample of healthy pre-pubertal girls was independent of body size, heart rate and cardiac output [129]. In post-menopausal women, arterial stiffness increased from pre-menopausal levels even without a concurrent increase in blood pressure [132]. This suggests that arterial stiffness is influenced by the menopausal transition in women [133]. Indeed, treatment with hormone-replacement therapy in menopausal women has shown some success in the reduction of large artery stiffness [134, 135]. In contrast, a large study in European

populations indicated a slightly higher arterial stiffness in men than in women, but the authors regarded this finding as negligible due to the large sample size [5]. Nevertheless, whether arterial stiffness is higher in either sex is still debated due to conflicting results and the use of different methodologies for measuring arterial stiffness [125].

2.4.4 Ethnicity

It is well-known that hypertension is more prevalent [10, 116, 136] and arterial stiffness more pronounced [9, 137-140] in black compared to white populations. Blacks are more prone to stroke, heart failure and renal failure than the other population groups of South Africa [10, 116] and arterial stiffness may be a pathophysiological mechanism contributing to the high prevalence of these adverse events.

In a population of black and white Americans, black adults presented with higher arterial stiffness and more impaired vasodilation in microvasculature independent of the prevalence of CVD risk factors [9]. Individual differences in arterial stiffness have been shown to be heritable and genetic factors may account for the higher arterial stiffness seen in black populations [141]. Sherva *et al.* found a 20% heritability of arterial stiffness in black Americans [142]. Comparable large artery stiffness data in black and white South Africans is limited. However, a previous report in young black and white South Africans found that black participants exhibited higher muscular artery stiffness, both in hypertensives and in those with normal BP [140].

2.4.5 Inflammation

The immune system is composed of a variety of cells and proteins that function to protect the body from foreign antigens [143]. Inflammation is a component of the immune system that can be triggered in any tissue of the body in response to traumatic, infectious, post-ischaemic, toxic or autoimmune injury [144]. The acute inflammatory process is self-limiting and usually results in a return of tissue homeostasis [145]. However, continuous inflammatory stimuli or a dysregulation of mechanisms that limit inflammation may result in a

chronic inflammatory response [146]. Chronic low-grade inflammation is associated with hypertension and cardiovascular disease [147-150]. Furthermore, acute and chronic inflammation is linked to the stiffness of the large arteries [151-157].

In a sample of 78 middle-aged white hypertensive participants, C-reactive protein (CRP), interleukin-6, (IL-6) and tumour necrosis factor-alpha (TNF- α) correlated positively with large artery stiffness [158]. CRP is an acute phase protein produced by the liver in response to IL-6 and interleukin-1 β [159] and it is known as a non-specific marker of low-grade systemic inflammation in the body [160]. IL-6, a pleiotropic cytokine, is synthesised by a wide range of cells, including immune, endothelial, smooth muscle and ischemic heart cells [161]. Its physiologic activity includes the mediation of a pro-inflammatory reactions and cytoprotection [161]. At some levels, IL-6 acts in a defensive manner, but in chronic inflammation it becomes pro-inflammatory [162]. Since the discovery of TNF- α as a factor that causes haemorrhage and death of tumours in mice, its role in the inflammatory process as a mechanism of defence against infection has become increasingly clearer [163]. Treatment with anti-TNF- α therapy improves aortic stiffness in patients with rheumatoid arthritis, possibly by decreasing inflammation [164].

Monocyte-chemoattractant protein-1 (MCP-1) is a chemokine that attract monocytes to sites of inflammation in the body [165]. Although the underlying mechanisms are unclear, a large body of evidence supports a role for MCP-1 in the development of cardiovascular disease, especially in the form of atherosclerosis [157, 166-168]. MCP-1 may exert effects on the arterial wall via increasing the activity of cytokines and promotion of inflammation [169] and by increasing the expression of adhesion molecules [170] and matrix metalloproteinases [170]. In young black South African women, MCP-1 associated positively with the thickness of the carotid wall, but not with large artery stiffness [171].

Whether vascular inflammation initiates arterial stiffening and hypertension or whether higher BP stiffens the arteries and initiates an inflammatory process is still unclear [158]. An

association between inflammation and large artery stiffness may only reflect the inflammatory burden of arterial stiffness or its determinants, but an experimental study showed that individuals who were vaccinated with *Salmonella typhi* exhibited increased cfPWV [172]. This finding and the results from two longitudinal studies supports the theory that an inflammatory response may induce large artery stiffening [21]. In addition, CRP predicted cfPWV 20 years later in older white men in the Caerphilly study [155], while CRP, IL-6, interleukin-1 and fibrinogen predicted cfPWV after 16 years of follow-up in British men and women [21]. Other evidence indicates that CRP is increased before the onset of hypertension [173, 174]. It may thus be likely that inflammation contributes to arterial stiffness [158]. Persistent low-grade inflammation and immune activation are also trademarks of infectious diseases, such as HIV-infection [175, 176]. Both treated and untreated HIV-infected individuals seem to be prone to large artery stiffness [177, 178], lending more support to the theory that chronic low-grade inflammation is linked to arterial stiffness.

Inflammation as measured by the soluble urokinase plasminogen activator receptor (suPAR) predicted all-cause and cardiovascular mortality in black South African adults [179]. However, whether arterial stiffness is involved in the mechanism by which inflammation influences the development of CVD in black populations is not clear.

2.4.6 Endothelial dysfunction

The endothelium is continuously exposed to mechanical stresses and biochemical factors [180]. A variety of endothelial response mechanisms exist to counteract these factors, with the purpose of maintaining vascular homeostasis [180, 181]. However, a change from the endothelium's normal vasorelaxant, anti-coagulant and anti-platelet characteristics to a vasoconstrictive, procoagulant and platelet-activating profile indicates endothelial dysfunction [181]. Low grade inflammation and endothelial dysfunction may be mediators of arterial stiffness by affecting the structure and composition of the arterial sub-endothelial

matrix [182] and by influencing the release of vasoactive substances in the large arteries [183].

The endothelium expresses several cell adhesion molecules, two of which are intercellular-adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) [180]. The endothelium becomes activated by the expression of cell surface adhesion molecules, such as ICAM-1 and VCAM-1, on its luminal surface [184]. ICAM-1 and VCAM-1 are structurally similar to immunoglobulins [181]. These molecules enhance the binding of leukocytes and platelets to the endothelial surface by acting as endothelial ligands for integrins that are expressed on leukocytes and platelets [181]. Furthermore, ICAM-1 and VCAM-1 are not exclusively expressed on endothelial cells; but are also observed on cell types such as vascular smooth muscle cells and monocytes [181]. Endothelial activation may be induced by proinflammatory cytokines (such as IL-6), hypercholesterolemia or smoking [181, 184]. Oxidised low-density lipoprotein-cholesterol (ox-LDL-C) may also lead to endothelial activation [181]. A small study (N=63) conducted in middle-aged Turkish adults failed to show an association between circulating adhesion molecules (ICAM-1 and VCAM-1) and aortic stiffness (aortic distensibility measured by echocardiography) [185].

2.4.7 Renal function

The reversal of the normal arterial stiffness gradient and the ensuing elevated pulsatile mechanical load characteristic of arterial stiffness could lead to endothelial and glomerular damage, resulting in microalbuminuria [186, 187]. Reporting on arterial stiffness in chronic kidney disease, Wang *et al.* indicated that arterial stiffness increased along with the stages of chronic kidney disease [188]. In end-stage kidney disease, large artery stiffness effectively predicts all-cause and cardiovascular mortality [60, 61].

The estimated glomerular filtration rate (eGFR) is the best indication of kidney health and function, but it is not easy to measure in practice [189]. Instead, the eGFR is calculated by equations. The Modification of Diet in Renal Disease (MDRD) equation is used widely, but it

may underestimate the eGFR at higher levels, possibly because it was originally developed in chronic kidney disease patients [190]. Recently, a new equation, named the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) was developed, which is more accurate than the MDRD equation at eGFR of $>60\text{ml/min}/1.73^2$ [189]. The CKD-EPI equation takes into account an individual's age, gender, ethnicity and serum creatinine level [189].

Albumin is a protein which is produced in the liver [191]. Its main physiological functions are the transport of substances in the blood to its main target organs, as well as the maintenance of the pH and osmotic pressure of blood plasma [191]. Albumin is the most abundant protein in the plasma, with a concentration of 5g/100ml [191]. Healthy kidneys excrete less than 150 mg of protein per day, 20mg of which is albumin [192]. Microalbuminuria is defined as the excretion of 30-300 mg of albumin per day [85]. A simple way to measure the urinary albumin excretion rate is measured by calculating the albumin to creatinine ratio (uACR) in spot urine samples [193].

Low glomerular filtration is characterised by the accumulation of creatinine in the plasma. The creatinine clearance (CrCl) rate, calculated by the Cockcroft-Gault formula, is thus another way of quantifying the eGFR [194]. A study of participants with mild to moderate renal damage, lower CrCl was associated with increased large artery stiffness independent of the effect of diabetes, dyslipidaemia, smoking and obesity [195]. An Icelandic study including 629 older participants (mean age 74 years) indicated that a relationship between aortic stiffness and lower eGFR may be mediated by MAP, thus lending support to the theory that the reversal of the arterial stiffness gradient impairs renal function via an increased pulsatile pressure effect [196]. However, this study found no association between arterial stiffness and the uACR [196]. In contrast, urinary albumin excretion independently associated with peripheral arterial stiffness in black South African men [197] and it also predicted all-cause and stroke mortality in black South Africans [198].

2.4.8 Dysglycaemia

Diabetes mellitus (hereafter 'diabetes') is a major risk factor for cardiovascular disease [85, 116, 199]. Type 1 diabetes is a chronic disease in which the pancreas do not produce enough of the hormone insulin, the regulator of blood glucose [200]. In type 2 diabetes, the pancreas produces insulin, but the body has become resistant and does not respond to insulin in a normal, physiological manner [201]. Glycated haemoglobin (HbA1c) is a type of haemoglobin that gives information on the average plasma glucose concentration over an extended time period [202]. A fasting plasma glucose level of ≥ 7.0 mmol/l and/or HbA1c of $\geq 6.5\%$ are used to diagnose diabetes [200].

Both type 1 [203] and type 2 diabetes [204] are associated with large artery stiffness. Aortic stiffness predicted mortality in multi-ethnic (white, Indian and African-Caribbean) diabetic and glucose-tolerance tested populations [62]. However, even before the manifestation of diabetes, a pre-diabetic state of insulin resistance affects elastic arteries [182, 205]. The association of deteriorating glucose tolerance with generalised increases in arterial stiffness may explain why diabetes and impaired glucose tolerance are associated with an increased risk for stroke, heart failure and myocardial infarction [206]. This is supported by the finding that individuals with undiagnosed diabetes have stiffer arteries than those with normal glucose levels [20] and that hyperglycaemia, dyslipidaemia and abdominal obesity predict arterial stiffness in a middle-aged population [20]. In a population of older (mean age of 60 years at baseline) British participants who were non-diabetic, HbA1c and an insulin resistance index associated with the progression of aortic stiffness over a follow-up period of four years [207]. However, no significant association with fasting glucose or 2- hour glucose levels were found [207].

A systematic review of cross-sectional studies found that apart from age and blood pressure, traditional cardiovascular risk factors such as lipids, smoking, body mass index and sex were not independently related to cfPWV [208]. Despite diabetes mellitus associating with cfPWV in 52% of the studies, the association was weak [208]. In contrast to the weak association

found in the aforementioned review, diabetes was a major obstacle to healthy vascular ageing in participants of the Framingham Heart Study [111].

Chronically increased plasma glucose and hyperinsulinemia may play a role in the increased production of reactive oxygen species, endothelial dysfunction, cardiovascular tissue fibrosis and vascular remodelling via increases in the activity of the renin-angiotensin-aldosterone system and the expression of angiotensin type I receptor in vascular tissue [209]. Part of the changes in arterial stiffness associated with dysglycaemia may be due to mechanisms such as glycation of proteins and formation of AGEs, which is also known as the Maillard reaction [210-212]. Arterial strength and elasticity are determined by cross-links formed between elastin and collagen fibres [213]. However, the non-enzymatic glycation of collagen and the formation of AGEs may lead to abnormal cross-link formation of collagen, thereby increasing arterial stiffness [213, 214]. Furthermore, AGEs may promote vasoconstriction by decreasing the bioavailability of the vasodilator nitric oxide [211], an effect that may be more pronounced in smaller, muscular arteries. AGEs bind to a plasma membrane receptor named RAGE (receptor for AGEs), thereby initiating a cascade of signalling which disrupts normal cell functioning [211].

Sub-Saharan Africa was relatively free of diabetes prior to the 1990s, but its prevalence is on the rise [215] due to factors such as changes from traditional lifestyles and diets, urbanisation and an ageing population [216]. A systemic review of reports (from the year 2000 to 2015) in older people in Africa revealed a type 2 diabetes prevalence of 13.7% across the region [217]. In 2012, a diabetes prevalence of 13.1% was found among black South Africans residing in the Western Cape Province[218]. The South African government is planning a taxation on sugar-sweetened beverages in South Africa, in an effort to curb the growing prevalence of obesity and diabetes in the country [219].

2.4.9 Dyslipidaemia

Dyslipidaemia refers to abnormal lipid (hydrophobic fat molecules such as cholesterol and fatty acids) and lipoprotein (molecules consisting of lipids and apolipoproteins) concentrations in the blood [220]. High total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) are considered “bad” cholesterol and was identified as a cardiovascular risk factor for the first time in 1959 in the Framingham Heart Study [221]. High-density lipoprotein cholesterol (HDL-C) is the “good” cholesterol and regarded as protective against CVD [222]. The assessment of an individual’s serum lipid levels may aid in the prediction of future cardiovascular events [223]. According to the ESH/ESC guidelines, the following values are indicators of dyslipidaemia: TC >4.9 mmol/L, LDL-C >3.0 mmol/L, HDL-C <1 mmol/L for men and <1.2 mmol/L for women and TG >1.7 mmol/L [85].

Cardiovascular events are preceded by progressive, subclinical alterations of the arterial wall [224]. Atherosclerosis is a disease of the intima which is characterised by the accumulation of lipids and inflammatory cells, vascular smooth muscle cell migration, foam cell development and the deposition of connective tissue and calcium [52]. The result is progressive occlusion of the arteries [225]. Atherosclerotic plaques frequently occur in arterial regions where turbulent flow and low shear stress are common [226]. Arterial stiffness and atherosclerosis are separate physiological processes [12], but these two disease states may influence one another [227]. Whereas atherosclerotic lesions may lead to arterial stiffening, increased arterial stiffness may also result in damage to the artery wall, thereby creating an initiation point for an atherosclerotic lesion [227].

Lipid abnormalities may lead to structural damage of the arterial wall, including the thickening of the intima-media [228] and atherogenesis [229]. In Chinese men and women aged 40 years and older, LDL-C associated with increased aortic stiffness, while HDL-C inversely associated with aortic and peripheral stiffness [230]. The proposed mechanisms for this association include the formation of atherosclerotic lesions, but also atherosclerosis-independent effects of lipids on the arterial wall [230]. Dyslipidaemia is associated with

inflammation and oxidative stress [231]. The oxidation of LDL-C is viewed as one of the first events in atherosclerotic plaque formation [232], but ox-LDL-C may also be involved in the pathogenesis of arterial stiffness [233]. Oxidised LDL-C may be involved in increased collagen production in the arterial wall [234], increased expression of matrix metalloproteinases in arterial endothelial cells which lead to degradation of extracellular matrix components [235] and increased vascular calcification [236]. HDL-C may compete with oxidised LDL-C for binding spots on the surface of platelets, leading to decreased platelet activation and a decrease in oxidative stress [237]. Furthermore, HDL-C is considered to be 'good cholesterol' since it plays a role in extracting excess cholesterol from the arterial wall and transporting it back to the liver for elimination, a process named "reverse cholesterol transport" [238].

Atherosclerosis and coronary artery disease are not highly prevalent in black South Africans, or at least to a lesser extent than arteriosclerosis, hypertension and stroke [10, 16]. This is reflected in the clinical expression of CVD, with congestive heart failure and stroke being more common in black South Africans than coronary artery disease [30]. However, coronary artery disease, even though historically rare in black South Africans, seems to be increasing in black adults older than 50 years [239]. This may be a consequence of urbanisation and a deviation from traditional lifestyles, which could be causing the favourable lipid profile generally attributed to black South Africans [240] to change into one more associated with disease risk [241].

2.4.10 Oxidative stress

Under normal physiological conditions, reactive oxygen species (ROS) play a role in cell signalling cascades, the expression of genes and apoptosis of cells [242]. The balance between oxidants and antioxidants is an important determinant of cellular homeostasis [242]. However, when the antioxidant system of the body is overwhelmed by oxidants, redox signalling may be disrupted and tissue damage may ensue [243]. ROS are generated via different pathways in the body, including the mitochondrial respiratory chain, nicotinamide

adenine dinucleotide phosphate oxidases, xanthine oxidases, lipoxygenase, uncoupled nitric oxide synthase and myeloperoxidase [242]. Harman proposed the 'free radical theory of ageing' in 1956, suggesting that the increased production of ROS partially causes many features of ageing [244]. Since ageing is an important determinant of cardiovascular health [245], the link between oxidative stress and CVD, as well as arterial stiffness, has received increasingly more attention.

Oxidative stress is associated with dysfunction of the large arteries, as elevated ox-LDL-C was associated with higher arterial stiffness in an elderly population (mean age 74 years) of black and white Americans [233]. An experimental study conducted in mice indicated that increased mitochondrial oxidative stress may increase the production of collagen, the intrinsic stiffness of smooth muscle cells, elastin rupture and a decrease in the elastin content of the aorta [246].

Oxidative stress may play a role in the development of early changes in vascular structure and function in black populations [35]. Along with higher BP, black South African adults had higher levels of ROS (as measured by serum peroxides) than white adults [247]. In another South African study, ROS was positively related to SBP and PP in black men, suggesting that oxidative stress may contribute to the development of arterial stiffness and hypertension [248]. In addition, decreased arterial compliance and increased peripheral vascular resistance was associated with oxidative damage independent of the presence of hypertension in a population of black South African teachers [249].

2.5 Large arterial stiffness, social risk factors and health behaviours

Health behaviours are important determinants of cardiovascular health [250]. Adopting a more healthy lifestyle may bring about an improvement in CVD without the aid of pharmacological therapy [251]. The establishment of certain health behaviours as part of an individual's lifestyle may already occur during early life phases, such as childhood and adolescence [252-256]. Health behaviours such as adequate physical activity, maintaining a

healthy weight, a healthy diet, moderate alcohol use and the avoidance of tobacco and drug use may prevent 80% of CVD [257, 258].

Health behaviours which may affect arterial stiffness include factors such as nutrition, salt intake, obesity and weight loss, physical activity, smoking, alcohol use and psychological stress [251]. In this section, the health behaviour and social risk factors relevant to this study is discussed with specific regards to large artery stiffness.

2.5.1 Socioeconomic status and locality

A low socioeconomic status (SES), generally determined by levels of income, education and employment, is related to poor health [259] and a higher CVD risk [260]. In American adolescents, arterial stiffness associated with lower SES [261]. Similarly, a five-year increase in cfPWV was related to lower household income, employment- and education level in 3484 black and white participants of the Whitehall II study [262]. These studies indicate that large artery stiffness may be a physiological link between low SES and increased CVD risk [262]. Indeed, SES greatly influences health behaviour and the maintenance of a healthy lifestyle are challenging in under-privileged communities [117, 263].

Ongoing urbanisation and socio-demographic changes are evident in sub-Saharan Africa [33-35]. Urbanisation can be viewed as “a cause of the causes of CVD” [250]. In developing countries, rapid, unplanned urbanisation overwhelms the local health care system and the existing infrastructure with regard to housing, sanitation, water and electrical services, while employment opportunities are limited [264]. Urban areas also provide readily accessible processed food, sugary drinks, tobacco products and alcohol [264]. These factors drive a deviation from traditional lifestyles [32, 36], the adoption of an unhealthy, high sugar and fat diet, chronic alcohol abuse [10, 265, 266] and psychosocial stressors due to high rates of poverty and unemployment [267]. Unhealthy coping mechanisms used to deal with these stressors, such as increased smoking and excessive alcohol use, impacts cardiovascular health negatively [263, 267]. Rural populations who adhere to traditional lifestyles exhibit

lower indices of CVD, evident in a report from 1972 which shows no increase in pulse pressure along with ageing in a sample of rural bushmen from Botswana [268]. In a small study conducted in a Cameroonian population, a hunter-gatherer tribe living in a rural setting had lower aortic stiffness than members of the tribe that migrated to a semi-urban area, or Bantu farmers that also reside in the semi-urban area [269]. Also in a Chinese population, rural participants exhibited lower age-related aortic stiffening than those living in an urban setting [24]. These populations had the following in common: good aerobic fitness, low obesity prevalence, a low salt diet, high intake of fruit and vegetables, less stress and almost no increased blood pressure as a result of ageing [24, 268, 269]. However, already more than a decade ago, a South African study reported that a drift of cardiovascular risk factors and an unhealthy lifestyle began to affect those living in a rural setting as well [36]. Indeed, recent evidence on increased sugar intake in a rural community indicates that the nutrition transition has reached rural South Africa as well [270].

Urbanisation has been linked to the manifestation of hypertension in a black South African population [271]. Thus, rapid urbanisation, together with the underlying physiological and metabolic processes, increases the risk for CVD development even further in this high-risk population [10, 32]. In the PURE-SA-NWP population, psychological distress and a high 'nervousness' score predicted hypertension development independently of the effect of alcohol use [267]. These results warn that strategies aimed at improving health behaviour in South Africa may not be effective when factors contributing to psychological stress in low SES urban or rural communities are not addressed [267].

2.5.2 Obesity

In 2015, excess body weight accounted for approximately four million deaths and 120 million disability-adjusted life-years [272]. In addition, nearly 70% of deaths associated with a high BMI were caused by CVD [272]. The WHO defines obesity as a BMI of $\geq 30\text{kg/m}^2$ [273] and worldwide, 5% of children and 12% of adults (603.7 million) are obese [272]. In South Africa, 68% of women and 31% of men are overweight or obese [274]. More specifically, 20% of

black women, but only 2% of black men in South African are severely obese [274]. As part of the global nutrition transition, a diet pattern often called the “Western diet” is becoming more common in middle and low-income countries, in both urban and rural areas [34, 270]. This diet is characterised by the increased intake of added sugars, carbohydrates, fats and animal-source food, while diets based on vegetable, legumes and other coarse grain intake are disappearing around the world [34].

Body weight is positively associated with the consumption of free sugar and sugar-sweetened beverages [275]. The increased intake of processed and energy-dense food may be driven by the increased availability, affordability and marketing of such foods [276]. Fifteen years ago, added sugar intake was lower in rural than in urban South Africans [277]. However, data from the PURE-SA-NWP study indicated a rapid increase in added sugar and sugar-sweetened beverage intake over five years in rural as well as urban areas (2005-2010) [270]. With over 50% of men and women consuming sugar-sweetened beverages in both the urban and rural settings, it is evident that the remote rural areas of South Africa are no longer protected against a nutrition transition [270].

Evidence from the Framingham Heart Study found obesity to be one of the most significant threats to healthy vascular ageing [111]. Furthermore, among both young and older white and black Americans, measures of body fat were among the strongest predictors of aortic stiffness [278]. This effect was found in adults as young as 20 years of age, indicating that being overweight begins to affect the arteries at a very early stage of vascular ageing [278]. Two recent studies, conducted in European men and women, show that abdominal obesity, as measured by waist circumference, predict aortic stiffness after 16 [21] and 17 years of follow-up [20].

Due to the possibility of overestimating PWV in obese individuals, it is recommended that the transit distance is not measured on the body surface with a tape measure, but rather with a sliding caliper or infantometer [18]. Different methods of measuring transit distance may

provide one explanation as to why reports on the association of arterial stiffness with obesity is not consistent [279]. While some studies show increased large artery stiffness in obese individuals independently of blood pressure, age and race [278-281], other studies found either no association [282], or an inverse association [283]. In an attempt to clarify this confusion, a study in 711 older obese Americans made use of computerised tomography (CT) scout images to accurately measure the pulse wave transit distance and found that only visceral fat associated with cfPWV in obesity [279]. In support of this, the association of aortic stiffness with waist circumference is stronger than the association with BMI [20, 280], pointing to the role of abdominal obesity in large artery stiffness [284]. Obesity is often accompanied by insulin resistance [285], inflammation [284] and changes in the concentrations of the hormone leptin [286, 287] and may contribute to arterial stiffness by way of these associations [278].

Authors that found negative associations between PWV and BMI suggested that the adaptation of the arterial wall to blood pressure changes may differ in some obese individuals [288]. The increased blood volume and the encapsulation of small conduit arteries by adipose tissue may buffer or blunt the reflecting wave in the pulse wave contour [289]. A positive correlation exists between the cross-sectional wall area of the ascending thoracic aorta and the abdominal aorta and BMI [290]. Therefore, the negative association between PWV and BMI may also be explained by the larger vessel size in obese individuals when compared to lean individuals, since vessel size is a determinant of pulse wave velocity [46]. A negative association between BMI and carotid-radial PWV was found in black South African men, indicating that low BMI may contribute to CVD in this population [291].

2.5.3 Physical inactivity

Men and women who are physically active are less prone to develop cardiovascular disease than their sedentary peers [292, 293]. Regular physical exercise is recommended as a strategy for lowering cardiovascular risk [85, 116, 294].

Physical activity may lower cardiovascular risk by improving arterial compliance [295, 296]. A 25% increase in arterial compliance and a 20% decrease in the Beta stiffness index were observed in previously sedentary middle-aged and older men after three months of regular aerobic exercise [296]. These changes were not associated with factors like changes in weight, blood pressure, plasma cholesterol or aerobic capacity, possibly indicating a direct effect of exercise on arterial compliance [296]. In older adults with type 2 diabetes, hypertension and hypercholesterolemia, aerobic training reversed arterial stiffness, as shown by significant decreases in both radial and femoral PWV, within three months [297]. The effects of exercise on the vasculature may be independent of other well-established benefits of exercise, as this improvement occurred without any significant improvements in aerobic fitness, weight, BMI, waist-to-hip ratio, or blood pressure [297].

A variety of possible mechanisms, including modulation of the sympathetic-adrenergic tone of smooth muscle cells [298], increases in parasympathetic activity [299], greater shear stress [300] and improvements in insulin sensitivity [301] may be responsible for the improvement of arterial compliance with physical activity. One study reported that exercise session immediately reduced arterial stiffness [302]. Because changes in the elastin-collagen composition of the arterial wall are believed to occur over several years, it is not likely that short-term physical activity exerts its effect on arterial compliance by altering the structure of the arterial wall [296]. Instead, the increased blood pressure generated by exercise probably causes greater mechanical distension of the artery, thus stretching collagen fibres and improving the pattern of their cross-links, thereby decreasing arterial stiffness [303].

In South Africa, the high rates of obesity and sedentary behaviour are causes for concern [274]. More than a decade ago, a study conducted in black South African men and women found a relationship between physical inactivity and cardiovascular risk factors, especially in overweight women [304]. Following these findings, the authors recommended that an effort should be made to identify factors causing a sedentary lifestyle in black South Africans and

that affordable physical activity programmes should be made available to low-resource communities [304].

2.5.4 Tobacco use

Smoking is an important preventable risk factor for CVD and fatal cardiovascular outcomes [85, 305]. Smoking may mediate cardiovascular disease through a variety of mechanisms [305], including effects on serum lipid quantities [306, 307] and the oxidation of lipids, especially LDL-C [308], through effects on platelets [309, 310], the endothelium [311-313], insulin resistance [314], inflammation [315, 316], haemostasis [317], oxidative stress [305, 318] and the autonomic nervous system [319]. In addition, smoking is detrimental to vascular health [320].

Young (aged 22 years), healthy university students who were current smokers had higher arterial stiffness than non-smokers [321]. The same result was found in a population of Hungarian university students (aged 19-33 years) [322] and an Irish population of never-treated hypertensive participants [320]. After the cessation of smoking, arterial stiffness does improve, but it may take more than 10 years to decrease to levels similar to non-smokers [320]. However, studies investigating the relationship between arterial stiffness and smoking has not yielded contradicting results, with some finding an association [21, 22] while other did not [20, 323, 324]. In a cross-sectional study, large artery stiffness measured in an older population did not associate with smoking status [323]. Similarly, no association between arterial stiffness and current or ever-smoking was found after 17 years of follow-up in a Swedish population [20]. In contrast, heavy smoking was one of the predictors of arterial stiffness after 20-years of follow-up in white Welsh men [155].

Smoking may increase blood pressure and heart rate [325-327], which in turn may increase large artery stiffness. The effect of smoking on arterial stiffness may also be mediated through endothelial dysfunction [328] and the generation of oxidative stress [329, 330]. Cigarette smoke contains more than 4000 different chemicals, most of which have not been

studied with regard to CVD [310]. Cotinine, as the major proximate metabolite of nicotine, is used as a biomarker for tobacco exposure [331, 332]. At similar daily levels of smoking, blacks have a higher serum cotinine concentration when compared to whites [333]. The possible reasons for this may be the slower metabolism of cotinine in blacks, or the higher nicotine intake per cigarette for blacks than whites [333, 334]. Indeed, after acute smoking, a larger increase in cfPWV and augmentation index was observed for black than for white adults [335].

In a South African population, carotid-radial and carotid-dorsalis-pedis PWV were significantly higher in black smokers than in white smokers and smoking associated more with cardiovascular dysfunction in black than in white adults [336]. Tax increases for tobacco were highly effective in South Africa, with a decrease of 33% in tobacco use in the 1990s [337]. However, according to the WHO, approximately 18.2% of South Africans older than 15 years still use tobacco [338]. Furthermore, the 2013 South African National Health and Nutrition Examination Survey estimated the prevalence of ever smoking tobacco in the South African population at 20.8% [255].

2.5.5 Alcohol use

The association between alcohol use and arterial stiffness is still being debated. Light to moderate alcohol use seems to have a protective effect on the cardiovascular system in older Dutch adults [339] and in middle-aged Japanese men and women, it is associated with a lower PWV [340]. Some studies found that alcohol use increase large artery stiffness when compared to non-drinkers [341], while others reported the opposite [342, 343]. One study indicated that acute alcohol use lowers arterial stiffness, while chronic and excess alcohol use increases arterial stiffness [344]. Moderate alcohol consumption increases the concentration of HDL-C [345, 346]. This may represent a mechanism by which moderate alcohol consumption is associated with lower large artery stiffness, since HDL-C plays a role in increased cellular cholesterol efflux and reverse cholesterol transport as already mentioned [225, 347]. This association between alcohol use and HDL-C was also found in

black South Africans, but alcohol use also associated with higher blood pressure, thereby possibly counteracting any benefit from the association with HDL-C [348]. Other evidence also indicates that alcohol use is associated with worse cardiovascular outcomes in black South Africans [348-351], as self-reported alcohol intake predicted a five-year increase in blood pressure [350].

Detrimental effects of alcohol use includes decreased magnesium levels in the body, which may lead to an increase in vascular tone [352]. In addition, the function of the sodium-potassium-ATPase pump is altered by acute and chronic alcohol use, thereby possibly altering vascular tone and the handling of sodium by the kidneys [353]. Ethanol, the main ingredient of alcohol, may regulate collagen and elastin content in myocytes via a pathway involving matrix metalloproteinases [354]. Furthermore, a metabolic product of ethanol, acetaldehyde, is oxidised to acetate which can lead to the generation of reactive oxidative species [355]. Oxidative stress may contribute to elastin rupture and collagen overproduction in the arterial wall, leading to arterial stiffness [246].

Alcohol abuse is a major social, economic and health problem in Southern Africa [356]. The average alcohol consumption per drinker in sub-Saharan Africa is 19.5 L a year, among the highest in the world [357]. The overuse of alcohol in sub-Saharan Africa may be the ease of access, but it may also be a sign of psychological distress in communities [267]. In South Africa, efforts to improve health behaviours may be undermined by social factors that cause psychological distress, especially in low SES, poverty-stricken areas [267]. Intervention strategies to curb excessive alcohol use may be less effective, due to the popularity of home-brewed alcohol in poor communities [358].

Liver enzymes

Serum hepatic enzymes like gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and carbohydrate-deficient transferrin [359] are elevated in alcohol abuse [360, 361]. Gamma-glutamyltransferase is a membrane-bound

glycoprotein enzyme that catalyses the transfer of the gamma-glutamyl portion of glutathione to peptides [362].

Gamma-glutamyltransferase has been associated with aortic stiffness in newly diagnosed hypertensive patients [363] and young (aged 33-34 years) Turkish patients with prehypertension [364]. In black and white Americans, GGT was higher in the blacks, even among lifetime abstainers [365]. Besides being a marker of alcohol use [361], GGT predicted cardiovascular events and mortality in a large Finnish population [366]. In addition, GGT independently predicted of all-cause and cardiovascular mortality in black South Africans [349].

The mechanistic link between GGT and arterial stiffness is not completely understood. Normally, GGT counteracts oxidative stress by breaking down extracellular glutathione, thus, GGT concentrations may be elevated when the body is subjected to high levels of oxidative stress in the body, for instance, in the case of excessive alcohol intake [367]. However, GGT may also induce oxidative stress, especially in the presence of iron [367]. As a result, GGT might be a useful marker of oxidative stress in the body [368, 369]. Furthermore, GGT levels are elevated in disease processes independent of alcohol use, such as non-alcoholic fatty liver disease (NAFLD) [370], which has also been associated with arterial stiffness [371].

2.6 Summary

The importance of arterial stiffness in the development of CVD is underscored by numerous reports [7, 8, 58]. While various methods exist for the measurement of arterial stiffness, cfPWV remains the gold standard measurement due to its relative ease, reliability and predictive value for adverse cardiovascular outcomes [18, 101].

Literature indicates that age and blood pressure are the strongest determinants of arterial stiffness [208] and that arterial stiffness increases with age in most populations [13]. However, emerging evidence indicates that healthy vascular ageing is potentially achievable when risk factors such as obesity and diabetes are avoided [111]. The figure below provides

a summary of the cardiovascular risk factors associated with large artery stiffness as reported in international literature.

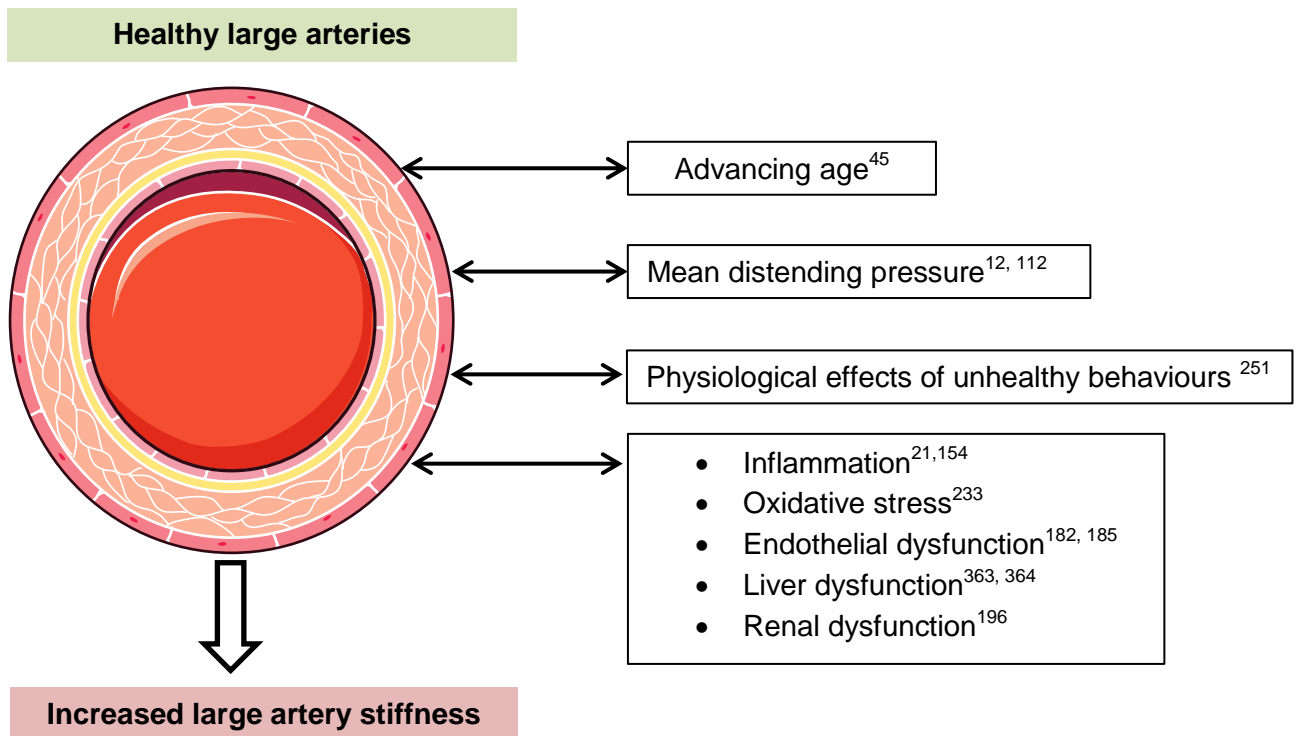


Figure 4. Multiple cardiovascular risk factors associated with large artery stiffness. Image obtained from Servier Medical Art.

However, whether these factors also associate or predict large artery stiffness in sub-Saharan black populations have remained largely undetermined. The measurement of large artery stiffness is potentially valuable marker of early changes in the arterial system that precedes the development of CVD. Therefore, the identification of early predictors of large artery stiffness may improve the ability of clinicians to detect individuals at a higher risk for CVD development and to select the most appropriate therapeutic approach. Furthermore, the identification of these early predictive factors may prevent arterial stiffening and subsequent CVD via multiple risk factor control strategies [65].

3. PROBLEM STATEMENT AND MOTIVATION

The central aim of this study was to investigate large artery stiffness (as measured by cfPWV) and its associations with cardiovascular risk factors in young (participating in the African-PREDICT study) and older (participating in the PURE-SA-NWP study) black South African populations. In addition, the aim was to identify independent predictors of arterial stiffness in older black adults.

3.1 Motivation, aim, objectives and hypotheses for each manuscript

This thesis contains three research articles that were submitted to peer reviewed journals for publication. A short motivation and problem statement, aim, objectives and hypothesis are stated in this section.

3.1.1 CHAPTER 3: Manuscript 1

Large artery stiffness and its association with health behaviours in healthy, young black and white South Africans

Motivation and problem statement

Health behaviours are important modifiable risk factors for CVD [250] and may also affect the stiffness of large arteries [20, 328, 344, 349, 372, 373]. Most health behaviours are likely to be established early in life, such as in adolescence or early adulthood [252-254, 256]. The prevention of increases in large artery stiffness is desirable, since increased large artery stiffness is a predictor of adverse cardiovascular events and mortality [7]. Literature indicates a higher cardiovascular risk for black populations due to higher hypertension rates and higher arterial stiffness compared to white populations [9, 35, 137, 138, 140]. However, whether large artery stiffness is already higher in young, healthy black compared to white adults is not known. In addition, the associations between arterial stiffness and health behaviours in young, healthy adults remain unexplored.

Aim

To compare arterial stiffness and explore the relationship between health behaviours and large artery stiffness in young (aged 20-30 years), apparently healthy black and white South Africans participating in the African-PREDICT Study.

Objectives

1. To compare arterial stiffness between young black and white South Africans.
2. To evaluate the roles of health behaviours (alcohol, smoking, obesity, physical activity) in large artery stiffness in young South Africans.

Hypotheses

1. Large artery stiffness is more pronounced in young black compared to young white South Africans.
2. Obesity (BMI), smoking (cotinine) and alcohol use (self-reported and GGT) are positively associated with large artery stiffness, while low physical activity is inversely associated with large artery stiffness.

3.1.2 CHAPTER 4: Manuscript 2

The identification of potential predictors of large artery stiffness over 10 years in older black South Africans

Motivation and problem statement

The high prevalence and poor prognosis of CVD faced by Sub-Saharan black populations [3, 16, 38, 40], in addition to the challenges being faced by African public health systems [42], creates a need for the identification early indicators of the development of CVD. The proven ability of large artery stiffness to predict adverse cardiovascular outcomes [7, 8] necessitates research on this topic in this understudied population. Although age and blood pressure are the strongest determinants of arterial stiffness, other factors such as health behaviour and cardiometabolic risk factors may accelerate arterial ageing beyond that of chronological age

[106]. The lack of longitudinal studies in sub-Saharan Africa has prevented the identification of potential early predictors of large artery stiffness. Consequently, there is also a lack of public health policies specifically aiming prevent large artery stiffness in Sub-Saharan Africa.

Aim

To identify potential risk factors for the prediction of large artery stiffness in Africans participating in the South African leg of the PURE study in the North West Province (PURE-SA-NWP) over 10 years.

Objectives

1. To investigate whether traditional cardiovascular risk factors, such as blood pressure, age, sex, obesity, lipids and glycaemic markers predict large artery stiffness in Africans.
2. To determine whether known cardiovascular risk factors in the black population, including inflammation, liver function and renal function predict large artery stiffness over 10 years (2005-2010).
3. To determine whether health behaviours such as alcohol and/or tobacco use predict arterial stiffness 10 years later.

Hypotheses

1. Blood pressure, age, female sex, obesity, lipids and glycaemic markers are predictors of large artery stiffness in black adults.
2. Inflammation, liver function and renal function predict large artery stiffness over 10 years.
3. Alcohol use and tobacco use predict large artery stiffness 10 years later.

3.1.3 CHAPTER 5: Manuscript 3

Associations of large artery stiffness with cardiovascular risk factors in young and older black South Africans who self-reported to not consume alcohol

Motivation

CVD shows a steady progression to epidemic proportions in the low- and middle income countries of Sub-Saharan Africa [33, 37, 39]. With overburdened health care systems and inadequate financial, human and drug resources, prevention of adverse cardiovascular outcomes is becoming an increasingly important goal [33]. Alcohol use is a known threat to cardiovascular health in black South Africans [349, 350]. However, a health profile associated with alcohol abuse may be masking other early predictors of arterial stiffness in this vulnerable population. The associations of biomarkers with and possible early predictors of arterial stiffness have not been investigated in young or older African populations who reported to not consume alcohol.

Aim

To investigate how biomarkers known to modulate arterial function [endothelial activation (ICAM-1, VCAM-1), inflammation (CRP, IL-6), oxidative stress (ROS, GGT) and metabolic markers (glucose, HbA1c, lipids)] relate to arterial stiffness in young and older black South Africans who self-report no alcohol use.

Objectives

1. To determine whether inflammatory markers (IL-6, CRP), endothelial activation markers (ICAM-1 and VCAM-1), oxidative stress (ROS, GGT) and metabolic markers (glucose, HbA1c, lipids) associate cross-sectionally with large artery stiffness in young, apparently healthy black adults (African-PREDICT study) who self-reported no alcohol use.

2. To determine whether the above mentioned biomarkers are predictive of large artery stiffness over five years (2010-2015) in an older black population (PURE-SA-NWP) who self-reported no alcohol use.

Hypotheses

1. Inflammation, endothelial activation, oxidative stress and metabolic markers associate cross-sectionally with large artery stiffness in young black adults who self-reported no alcohol use.
2. Inflammation, endothelial activation, oxidative stress, dysglycaemia and dyslipidaemia predict large artery stiffness five years later in an older black population who self-reported no alcohol consumption.

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CHAPTER 2

Study protocol and methodology

1. Introduction

To address the objectives of this thesis, data from both the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and HyperTension (African-PREDICT) and the South African leg of the international Prospective Urban and Rural Epidemiology study conducted in the North-West Province (PURE-SA-NWP) were included. This chapter contains the research methodology for both studies. The methodology appropriate to this sub-study is discussed in this chapter.



Figure 1. Communities in the North West Province of South Africa where data collection took place for the African-PREDICT study and the PURE-SA-NWP study. The 'A' marker shows the location of Potchefstroom, the research setting for the African-PREDICT study and the urban setting for the PURE-SA-NWP study. Areas 'B' and 'C' show the locations of the rural communities of Ganyesa and

Tlakgameng that were included in the PURE-SA-NWP study. Images obtained from Wikimedia Commons and Google Maps.

2. Ethical considerations

2.1 Legal and goodwill permission

Both the National and Provincial Departments of Health gave approval for the African-PREDICT Study and the PURE-SA-NWP Study. Furthermore, the Health Research Ethics Committee (HREC) of the North-West University gave approval for the African-PREDICT Study (NWU-00001-12-A1), the PURE-SA-NWP baseline, five-year and 10-year follow-up studies (ethics number: 04M10 and NWU-00016-10-A1), as well as this sub-study. The study protocol of both studies complies with the Declaration of Helsinki.

Prior to the commencement of the study, the principal investigator of the PURE-SA-NWP study fully informed the mayors of both the urban and rural communities included about the aims and possible outcomes and benefits of the study and obtained permission to proceed with the study. The rural communities included in the PURE-SA-NWP study are under tribal law. Therefore, verbal permission was obtained from the *Inkosi* (tribal chief) of the rural communities and from the community leaders of the urban areas (who acted as gatekeepers for each community) to perform the study in their communities. This was done prior to baseline, five- and 10-year follow-up data collection as an act of respect.

2.2 Appropriate skill level of researchers and field workers

Researchers and students who are or were involved in the data collection of these two studies were appropriately trained and evaluated before being allowed to conduct research procedures. The field workers of the PURE-SA-NWP study received extensive training in all aspects of the study in order to provide the participants with an overview of what could be expected on the research day.

2.3 Participant privacy and confidentiality

The privacy of participants was protected by conducting each research assessment, including questionnaires, in a private rooms or closed off areas. Only the researchers were present in the private areas during the assessment.

All information was kept confidential and no personal information was published. In both studies, participant numbers were allocated to each participant and this number was used on laboratory specimens, forms, questionnaires and all other records, eliminating the possibility of a participant being identified. For the PURE-SA-NWP study, the numbers allocated at baseline were used for both follow-up collections and in the master dataset. Data was captured and stored electronically and online (on the North-West University data servers) as well as on several external hard drives to ensure sufficient backup and storage. All data is password-protected and researchers only have access to data as approved by the HREC of the North-West University.

2.4 Benefits to the individual and to the South African community

In both studies, participants received feedback on results that were immediately available and were referred to local healthcare providers when necessary. An indirect benefit is derived from taking part in these studies, as the data obtained could lead to the development of more effective prevention strategies which may improve the health of all South Africans.

2.5 Incentives and reimbursement

For both the African-PREDICT and PURE-SA-NWP studies, the participants received the advanced clinical tests for free. Participants were provided with transport to the research facilities if needed and received a meal during the research day. Small reimbursements (cash or vouchers for supermarkets) were provided to the participants.

3. Measurement of carotid-femoral pulse wave velocity

Carotid-femoral PWV was measured according to the method recommended by the most recent expert consensus document on the measurement of aortic stiffness in daily practice

[1]. For both the African-PREDICT study and the 10-year follow-up PURE-SA-NWP study, cfPWV was measured with the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) with the participant in the supine position.



Figure 2. (a) Measurement of cfPWV as part of the PURE-SA-NWP study and (b) an illustration of tonometer placement on the carotid artery. Image (b) obtained from Servier medical Art.

The participant did not eat, drink, sleep or talk during the measurement. Two measurements were taken for each participant and the second reading was used for analysis. If the two cfPWV measurements differed by more than 0.5 m/s, a third measurement was performed and the median of the three measurements were used.

The transit-distance method was used to measure cfPWV along the descending thoraco-abdominal aorta. The transit distance between the carotid and femoral arteries was measured with an infantometer. The 80% rule was applied for the calculation of the distance used (common carotid artery- common femoral artery x 0.8). Of all currently used distances, 80% of the direct carotid to femoral distance appears to be the most accurate, only overestimating the real distance from the carotid to the femoral artery as measured by magnetic resonance imaging by 0.4% [2]. Pulse wave analyses were performed to obtain the central systolic and pulse pressures.

4. Study design and participant recruitment

4.1 *The African Prospective study on the Early Detection and Identification of Cardiovascular Disease and HyperTension (African-PREDICT) study*

The central aim of the African-PREDICT study is to understand the early pathophysiology accompanying cardiovascular disease development and to identify novel early markers or predictors for the development of cardiovascular disease in black South Africans. This will enable researchers to instigate more successful prevention programmes in Africans at younger ages. This study is one of very few longitudinal hypertension studies in sub-Saharan Africa which aims to take measurements of the highest sensitivity in young individuals where relatively minor cardiovascular changes are expected to occur in the initial phases of the development of hypertension. The knowledge to be gained from this study includes structural and functional vascular and cardiac changes, biopsychosocial changes, biomarkers (possibly proteomics, metabolomics and genomics) and the associated health behaviours. In order to address the central aim of the African-PREDICT study, a *Hypertension Research and Training Clinic* was established on the Potchefstroom Campus of the North-West University. This clinic enables internationally competitive cardiovascular research and the generation of high-quality data on a long-term basis.

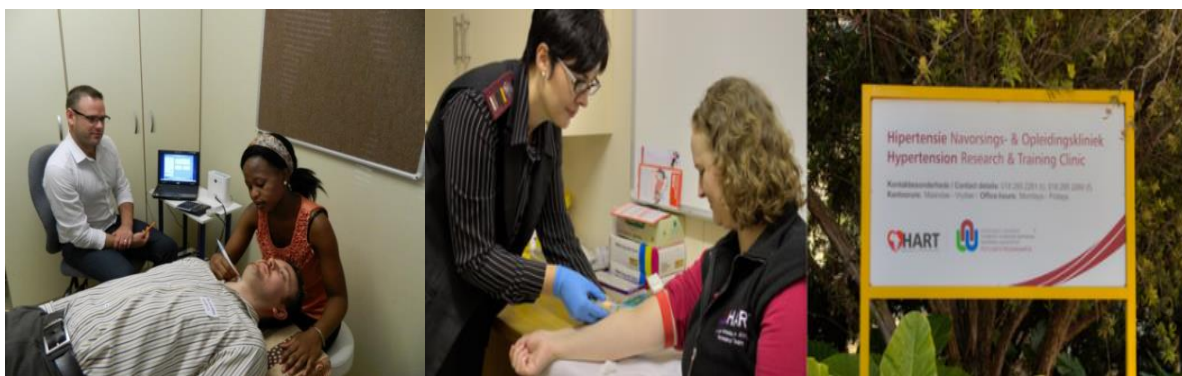


Figure 3. *Research procedures illustrated in the Hypertension in Africa Research and Training Clinic in Potchefstroom.*

To ensure maximum retention of participants during the follow-up period, industry-dependent strategies were used to recruit participants in different employment categories. Black and

white men and women were recruited by random sampling that took place at the level of invitations to the health screening step. In order to ensure a random sample and to stratify for socioeconomic status, local employers were contacted to identify and invite employees across different levels to participate in the study. To limit loss to follow-up, only employees who indicated that they intend to stay in the Potchefstroom region for five or more years were included.

4.2 The South African leg of the international Prospective Urban and Rural Epidemiology (PURE) study, conducted in the North West Province: PURE-SA-NWP

This sub-study is part of the South-African leg of the international PURE study which was conducted in the North West Province of South Africa (PURE-SA-NWP). The international PURE study was designed to collect information on the social, behavioural, environmental, biological and genetic factors that contribute to the development of CVD in 17 countries [3]. The two main objectives of the PURE study were to investigate the association between societal influence, cardiovascular risk factors and NCDs and to examine the relationship between societal determinants and the incidence of chronic NCDs [3].

For the PURE-SA-NWP study, the Potchefstroom and Ikageng districts were chosen as the urban areas, whereas rural participants were recruited from the Ganyesa district (450km west of Potchefstroom on the highway to Botswana) and a deep rural community named Tlakgameng, 35km east of Ganyesa (Please refer to Figure 1 on page 78). The urban research settings included the Metabolic Clinic and the HealthCare Centre on the North-West University's Potchefstroom campus. The baseline rural research setting was tents and gazebos on the grounds of the Setlhare Guest Lodge and the five-year and 10-year follow-up data collections took place in the buildings of the Setlhare Guest Lodge.

In the recruiting phase of the PURE-SA-NWP study, sampling at either the urban or rural settings focused on the issues of both "representativeness" and feasibility of long-term follow-up. In the urban area, it was attempted to enrol all eligible households from three

sources: a) households of industrial workers from specified factories, b) geographically defined middle class housing areas and c) defined slum or shanty town dwellers. A household census documenting the number of people, their ages and health profile was done in 6 000 houses. The head of every household gave written informed consent to fill out the census questionnaire. The questionnaire inquired about physical and psychological health, socio-economic background, lifestyle practices and available support systems. The household census was done by 16 intensively trained fieldworkers from the different communities. Eligible individuals from the 6 000 households were invited to participate, of which a total of 2010 individuals participated in the baseline phase of the PURE-SA-NWP study. Each fieldworker was responsible for 125 subjects for the next 10 years. They contacted the participants every three months to keep track of any changes in contact information and to capture event information.

5. Inclusion/exclusion criteria and total study population

5.1 The African-PREDICT study

Data collection for African-PREDICT started in the North West Province of South Africa in 2013 and is still continuing. A total of 1200 participants are to be included in the African-PREDICT study. At the time of starting statistical analyses for manuscript 1, the first 373 black and white participants who had complete data sets were included. For manuscript 3, the African-PREDICT study was further along in terms of data collection, resulting in an additional 218 (total N=591) black participants being included. To test the hypotheses of manuscript 3, those with missing PWV data or alcohol use data and those who self-reported to use alcohol were excluded.

Apparently healthy black and white men and women between the ages of 20-30 years who completed the health screening step were invited to take part in the African-PREDICT study. Those who gave informed consent were enrolled in the study. Detailed inclusion and exclusion criteria are shown in Table 1.

Table 1. *Inclusion and exclusion criteria for the African-PREDICT study*

Inclusion criteria	Exclusion criteria
1. African or Caucasian race	1. Indian, Asian or mixed origin race
2. Aged 20-30 years	2. Aged <20 or >30 years
3. Either gender (equally distributed)	3. Not a permanent resident of Potchefstroom or the surrounding areas
4. Apparently healthy	4. Type 1 or 2 diabetes mellitus, or a glycated haemoglobin level of $\geq 6.5\%$
5. Normotensive and pre-hypertensive blood pressure measurement (SBP<140mmHg DBP<90mmHg, based on the average of four measurements)	5. Regular medication use i.e. antihypertensive, anti-diabetic, antiretroviral or anti-inflammatory medication
	6. HIV-infected
	7. Tuberculosis, or currently receiving treatment for tuberculosis
	8. Fever on research day (internal ear temperature of $>37.5^{\circ}\text{C}$)
	9. Known liver or renal disease, cancer
	10. Microalbuminuria > 30 mg/ml in spot morning urine or proteinuria
	11. Pregnant or lactating women
	12. Recent surgery (within the past three months)
	13. Previous history of angina pectoris, myocardial infarction or stroke

5.2 PURE-SA-NWP study

At baseline, black individuals from the above mentioned areas 35 years and older were invited to participate in the PURE-SA-NWP study. Those who provided written informed consent were enrolled, while those who refused completed a non-responder form. Black men and women were included, while white, coloured and Indian individuals were excluded. Pregnant and lactating women were excluded from the study.

Baseline data for the PURE-SA-NWP study was collected in the year 2005, a five-year follow-up was conducted in 2010 and the 10 year follow-up in 2015. The total study population for the PURE-SA-NWP baseline, five-year and 10-year follow-up is shown in Figure 4. For the purposes of this sub-study, only HIV-uninfected participants with complete cfPWV data were included.

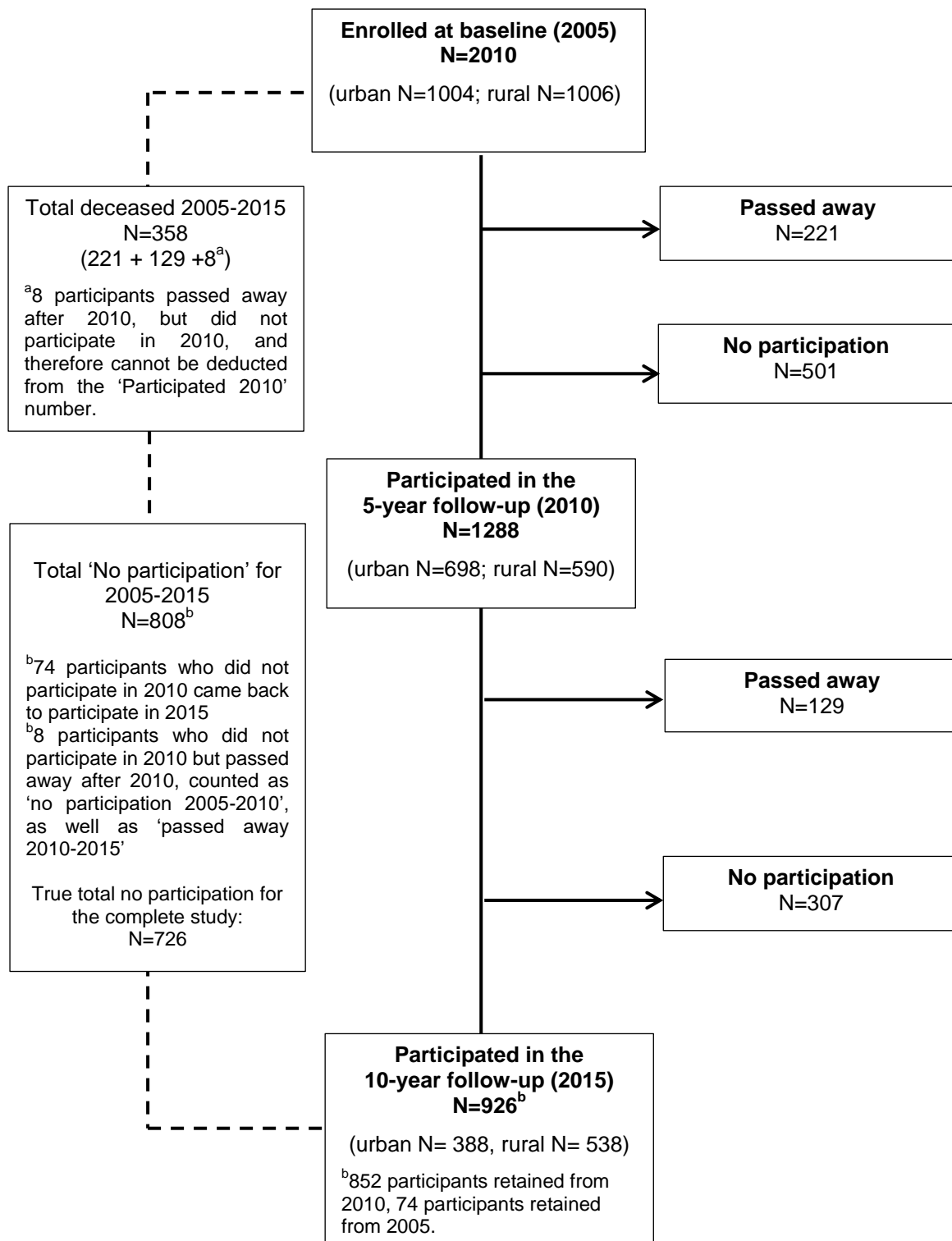


Figure 4. The total study population of the PURE-SA-NWP study at baseline, five-year and 10-year follow-up data collection.

6. Organisational procedures

**Please note: Only the research procedures applicable to this study are discussed in detail in the next sections.*

6.1 The African-PREDICT study

Eligible participants were identified by a health screening day, in building F11 on the Potchefstroom campus of the North-West University. Health screening was conducted in parallel with the research project which took place in building F12. Invitation to the screening took place through random selection. Screening was conducted on an appointment basis by the research nurse and trained post-graduate students. Participants in need of transportation were fetched at 07h00 by the bus. The participants arrived at 08h00 in the morning. The research coordinator was responsible for participant registration, explaining the research procedures to each participant and taking informed consent. The participants completed the General Health Questionnaire with the help of a post-graduate student. Next, anthropometric measurements were taken in a private room. After giving a urine sample, the participant's blood pressure and augmentation index were measured. Rapid tests (glucose, cholesterol, triglycerides, HbA1c (only if fasting glucose test > 5.6), a microalbuminuria test (only if urine dipstick indicates protein 2+) (15 min) and an ear temperature measurement were performed. HIV pre-counselling, testing and post counselling were done. After the completion of all health screening procedures, the research nurse provided the participant with feedback on immediately available results. If necessary, the participant was referred to further medical care. In the case of eligible participants, an appointment for participation in the study was made. Finally, the participants received a light lunch and were transported back. Reasons for refusing enrolment in the research project were recorded.

On the research day, participants were required to be fasted. Those in need of transportation were fetched at 07h00 and research procedures commenced at 08h00 in The Hypertension Research and Training Clinic in building F12 on the Potchefstroom Campus of the North-

West University. The research coordinator explained the procedures and informed consent was taken. A spot urine sample was collected, after which blood was drawn by the research nurse. The urine and blood samples were immediately taken to the laboratory on the first floor of building F12 to be prepared for storage. Anthropometric measurements were taken. Several questionnaires, including the General Health and World Health Organisation Global Physical Activity questionnaires were completed during the course of the day. Pulse wave velocity and analyses (SphygmoCor), were measured. The participants received a light meal. The ambulatory blood pressure device and the ActiHeart device were connected to the participant. After all the research procedures were completed, the research nurse provided feedback on the immediately available results. Thereafter the participants were transported back. The next day, the ambulatory BP device was collected or brought back to the clinic by the participant. After seven days, the ActiHeart device was collected.

6.2 PURE-SA-NWP study

During baseline, five-year and 10-year follow-up, an appointment with each person was made. At approximately 08h00 the day of their appointment, they were voluntarily picked up by the driver of the research team and brought to the research setting. Participants were introduced to the research set-up and all the research procedures were explained to them. Thereafter each participant gave informed consent. Each participant received information in their native language by field workers from the communities and were free to withdraw from the study at any time. Participants were asked to fast for approximately 10 hours, from 22h00 the evening before. All the participants underwent HIV-testing, but was given the choice whether they wanted to know their status or not. However, everyone received pre-test counselling in groups of 10 persons before the blood sample was taken. Post-test counselling was done individually when the participants received feedback on their results at the end of the research day. If deemed necessary by the research nurse, participants were referred to local healthcare providers.



Figure 5. Ten-year follow-up data collection in Ganyesa, North West Province, South Africa.

7. Questionnaires

African-PREDICT

For the African-PREDICT study, participants completed a general health questionnaire with the help of a trained researcher. The socio-economic status (SES) of a participant was derived from three categories included in the general health questionnaire, including skills level, education and household income. Points were awarded to each of these categories and the total number of points determined whether a participant had a low, middle or high SES. The classification of the SES was adapted from Patro *et al.* [4]. Alcohol and tobacco use were also reported in this questionnaire.

PURE-SA-NWP

Trained field workers assisted in the collection of the biographical data of participants. The use of any alcohol or tobacco product was recorded in the PURE Adult questionnaire in terms of the following codes: 0= not answered; 1= formerly used; 2= currently use; 3= never used. This format was recoded for the purpose of adjustment and Chi-square tests to the

following codes: not answered= missing data; 0= no history of alcohol consumption; 1= former and current use. Medication use and health history was also recorded by using the PURE Adult Questionnaire.

8. Anthropometric measurements

African-PREDICT

Height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany) weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany), waist circumference (WC) and hip circumference (Lufkin Steel Anthropometric Tape (W606PM), Lufkin, Apex, USA) were measured. Each participant was fitted with an ActiHeart physical activity monitor (CamNtech Ltd., England, UK) which recorded physical activity for seven days. Active energy expenditure was calculated with branched model equations.

PURE-SA-NWP

Baseline, five-year (Invicta Stadiometer IP 1465, Leicester, UK) and 10-year follow-up height (Leicester height measure, Seca, Birmingham, UK), weight (Precision Health Scale, A & D Company, Japan) and WC (Holtain unstretchable metal tape; Steel tape, Lufkin, Cooper Tools, Apex NC, USA for 10-year follow-up) were taken.

For both studies, standardised methods and calibrated instruments were used [5]. Measurements were taken to the nearest 0.1 cm or 0.01 kg. BMI (weight in kg/height² in m) and WC-to-height ratio were calculated for both studies.

9. Cardiovascular measurements

African-PREDICT

Office blood pressure measurements were made with the Dinamap® ProCare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA). After the correct cuff size was selected, duplicate measurements were made on both arms with five-minute intervals. During the measurements, the participant was sitting in a relaxed upright position with legs uncrossed. Each participant was fitted with a 24-hour ambulatory blood pressure and ECG

apparatus (CardioXplore®, CE0120, Meditech, Budapest, Hungary). The apparatus was fitted with the appropriate cuff size on the non-dominant arm. Instructions were given on how to ensure successful inflations. The participant also filled out an ambulatory diary card during the measurements. The mean arterial pressure (MAP) was calculated with the following equation: $(SBP-DBP)/3 + DBP$ where SBP, systolic BP; DBP, diastolic BP.

PURE-SA-NWP

During all three data collection periods, the validated OMRON HEM-757 and OMRON M6 (Omron Healthcare, Kyoto, Japan) devices were used to measure blood pressure. Each participant rested for 10 minutes prior to the blood pressure measurement. An appropriately sized cuff was fitted on the upper right arm, where after the brachial systolic (SBP) and diastolic blood pressure (DBP) were measured and recorded. After another five-minute resting period, the researcher performed a second blood pressure measurement. This second measurement was used for statistical analyses. During the measurements, the participant was seated in a relaxed upright position with legs uncrossed. The same protocol was followed during each data collection period. Hypertensive status was established with the following guidelines: $SBP \geq 140$ mmHg and/or $DBP \geq 90$ mmHg [6], as well as current use of anti-hypertensive medication.

10. Biological sampling

For both the African-PREDICT and PURE-SA-NWP studies, the participants were required to fast for 10 hours (from 22h00 the evening before). In the early morning, a research nurse took blood samples from each fasting participant with a sterile winged infusion set and syringes. The blood was drawn from the antebraial vein. Spot urine samples were collected. All blood and urine samples were immediately taken to the on-site laboratory and aliquoted into cryovials for storage in bio-freezers at -80°C .

The samples were prepared according to standardised methods and stored in the laboratory at -80°C . In the cases of blood collection in a rural area during the PURE-SA-NWP study,

samples were snap frozen and stored at -20°C for no more than five days. The samples were then transported to the laboratory and stored at -80°C.

All samples were stored in locked bio-freezers until analyses. Access to these bio-freezers was limited to key personnel. Sample integrity was protected by constant monitoring of the bio-freezers via a cellular-based monitoring system. Furthermore, the bio-freezers were connected to uninterrupted power supplies and centralised power generators to eliminate defrosting during power failures.

11. Biochemical analyses

African-PREDICT

An enzymatic colorimetric method (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) was used to determine serum total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), gamma-glutamyltransferase (GGT), creatinine, aspartate transferase (AST) and alanine transferase (ALT) as well as urinary creatinine and albumin levels.

High sensitivity C-reactive protein (CRP) was determined in serum and fasting glucose in fluoride plasma (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN). Glycosylated haemoglobin (HbA1c) was determined from ethylenediaminetetraacetic acid (EDTA) whole blood with an immunoturbidimetric method (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN). Interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor-alpha (TNF-alpha), intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1) were determined with high sensitivity Quantikine ELISA kits (R&D systems, Minneapolis, MN USA) and analysed on a Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA).

PURE-SA-NWP

The quantitative aspect of baseline TC, HDL-C, TG and GGT, creatinine and high-sensitivity CRP levels were analysed in serum samples (Konelab20i™ auto-analyzer, Thermo Fisher Scientific Oy, Vantoo, Finland), while for the five-year and 10-year follow-up, these analyses, as well as LDL-C, were done on the Cobas Intergra 400 Roche® Clinical System (Roche Diagnostics, Indianapolis, IN).

Baseline serum IL-6 (Cobas e411 Analyzer, Roche, Basel, Switzerland) and five-year follow-up IL-6 (Elecsys 2010, Roche, Basel, Switzerland) were determined with an electrochemiluminescence immunoassay. Baseline ferritin was measured from serum samples with an enzyme immunoassay procedure (Ramco Laboratories, Inc, Stafford Texas). Urinary creatinine was assessed with a kinetic colorimetric assay based on the Jaffe method, while urinary albumin levels were assessed with an immunoturbidimetric assay (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN).

At baseline, the fasting glucose (fluoride) levels were determined by an enzymatic reference method with hexokinase (Vitro DT6011 Chemistry Analyzer; Ortho Clinical Diagnostics, Rochester, New York, USA), while the Cobas Intergra 400 Roche® Clinical System was used for the five- and 10-year follow-up. The HbA1c levels were determined in with ion-exchange high-performance liquid chromatography from EDTA samples (D-10 Haemoglobin testing system, Bio-Rad #220-0101) for all three data collection periods.

Baseline and five-year follow-up ICAM-1 and VCAM-1 levels were determined in serum with sandwich ELISAs (Human sICAM-1 and human sVCAM-1 assay, IBL, Hamburg, Germany). Baseline LDL-C was calculated by the Friedewald Formula: $LDL-C = TC - HDL-C - VLDL-C$ where $VLDL-C = 0.456 * TG$ [7].

Both studies

Serum peroxides (representing reactive oxygen species, abbreviated as ROS), were determined with high-throughput spectrophotometric assay [Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA)] according to the method proposed by Hayashi *et al.* [8].

The creatinine clearance rate (CrCl) was calculated with the Cockcroft-Gault formula: CrCl in ml/min = $[(140 - \text{age}) \times (\text{weight in kg}) \times (0.85 \text{ if female})] / (72 \times \text{creatinine in mmol/l})$ [9]. The estimated glomerular filtration rate (eGFR) in ml/min/1.73m², with creatinine in umol/l, was calculated with the chronic kidney disease epidemiology collaboration equation (CKD-EPI): $141 \times \min(\text{Scr} / \kappa, 1)^\alpha \times \max(\text{Scr} / \kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018$ [if female] $\times 1.159$ [if black]; $\kappa = 61.88$ for female and 79.56 for male; $\alpha = -0.329$ for female and -0.411 for male [10]. The urinary albumin to creatinine ratio was calculated by the following formula: albumin (mg/l) / creatinine (mmol/l).

12. Statistical analysis

Statistical analyses were performed with Statistica 13 (StatSoft, Inc., Tulsa, OK, USA). and graphs were prepared using GraphPad Prism Version 5.03 (GraphPad Software Inc., California, USA). Power calculations were done for both the PURE-SA-NWP and African-PREDICT studies.

Interaction terms were used to determine the effect of ethnicity and sex in manuscript 1 and the effect of sex only in manuscript 2. Descriptive statistics, which include the mean and standard deviation, were performed on data with a normal distribution. Abnormally distributed variables were logarithmically transformed and the central tendency and spread were described as the geometric mean and the 5th and 95th percentiles.

Independent *t*-tests, analysis of variance (ANOVA) and for categorical data, Chi-square tests were used to compare means and proportions. Analysis of covariance (ANCOVA) was used to compare means while adjusting for covariates. Dependent *t*-tests and Wilcoxon tests were used to compare means and proportions between baseline and follow-up data within the PURE-SA-NWP population.

Dependent relationships between cfPWV and independent variables were determined with Pearson correlation and partial coefficients. Multi-variate linear regression analyses with cfPWV as the dependent variable were performed in each manuscript to test associations with various markers in young (African-PREDICT) and older (PURE-SA-NWP) black adults. The covariates entered into each regression model were based on literature, or identified with exploratory Pearson and/or partial correlations, as well as ANCOVA. All independent variables included in each model are listed in the footnotes and legends.

The measure in which a variable was expressed was specified next to the variable in all tables. In all instances, $p < 0.05$ was regarded as statistically significant.

13. Involvement of the candidate in data collection and analyses

During both the African-PREDICT and PURE-SA-NWP studies, I was involved in data-collection and analyses, as well as the compilation and cleaning of the master databases. Over the course of 5 years (2013-2017), I assisted study participants with the completion of the necessary questionnaires and conducted urinalysis for the screening day of the African-PREDICT study. As part of my duties on research day, I performed various cardiovascular measurements, including PWV measurement, and prepared urine and blood samples for storage in the laboratory.

For the PURE-SA-NWP study, I took part in the 2015 follow-up data collection where I specifically conducted PWV measurements in both the rural and urban locations. Furthermore, I was responsible for the analyses of blood and urine samples of the same data collection period in the laboratory, as well as the analysis of carotid distensibility from ultrasound clips. I was also involved in compiling and cleaning the master database of the study.

14. Acknowledgements

The figures and illustrations used in Chapters 1, 2 and 6 of this thesis were downloaded from the Servier Medical Art and Wikimedia Commons webpages. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License. Inkscape Illustration Software was used to create certain illustrations.

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CHAPTER 3

**Large artery stiffness is associated with
gamma-glutamyltransferase in young, healthy
adults: The African-PREDICT study**

Research Article

Large artery stiffness is associated with
gamma-glutamyltransferase in young, healthy adults:
The African-PREDICT study



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Summary of instructions to authors

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Impact factor	2.656		
Aims & Scope:	The <i>Journal of the American Society of Hypertension (JASH)</i> publishes peer-reviewed articles on the topics of basic, applied and translational research on blood pressure, hypertension and related cardiovascular disorders and factors ; as well as clinical research and clinical trials in hypertension		
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(Please note some of the format was changed to ensure uniformity throughout the thesis.)

ABSTRACT

Increased arterial stiffness is linked to cardiovascular disease (CVD) development, particularly in black populations. Since detrimental health behaviours in young adults may affect arterial stiffness, we determined whether arterial stiffness associates with specific health behaviours, and whether it is more pronounced in young healthy black compared to white adults. We included 373 participants, (49% black, 42% men) aged 20-30 years. Mean arterial pressure (MAP) was higher for blacks than whites ($p < 0.001$), but carotid-femoral pulse wave velocity (PWV) was similar (6.37 vs. 6.36 m/s; $p = 0.89$) after adjustment for MAP. The black group had higher gamma-glutamyltransferase (GGT) ($p < 0.001$), cotinine, reactive oxygen species, interleukin-6 and monocyte-chemoattractant protein-1 (all $p \leq 0.017$). PWV related positively and independently to GGT in both groups before and after multiple-adjustments (both $\beta = 0.15$; $p \leq 0.049$). Blacks had an unfavourable vascular profile and higher GGT, possibly indicating a higher vulnerability to CVD development, including changes in arterial stiffness. However, this observation needs confirmation.

Keywords: pulse wave velocity, gamma-glutamyltransferase, health behaviour, ethnicity, young adults, healthy

INTRODUCTION

The potential role of arterial stiffness in cardiovascular disease (CVD) development has become increasingly recognised in the past decade [1]. Pulse wave velocity (PWV) is considered to be the gold standard method to assess arterial stiffness and is predictive of cardiovascular events and mortality in the general population [2].

Detrimental health behaviours contribute to CVD development [3], and because of the early establishment of health behaviours during childhood and adolescence [4, 5] it should be a target for preventive strategies [6]. Arterial stiffness is associated primarily with blood pressure (BP), age and arterial wall properties [7]. In addition, it may be but may be influenced by poor health behaviours or unhealthy lifestyles such as smoking [8], excessive alcohol use [9], and physical inactivity [10], as well as the physiological effects thereof, such as obesity [11] and liver dysfunction [12, 13]. In South Africa, a change from traditional rural to modern, urban lifestyles includes changes in health behaviours, which affects the cardiovascular and metabolic health of the population [14].

Hypertension and increased arterial stiffness are more prevalent in black than white populations [15, 16]. Two thirds of urban black South Africans present with multiple risk factors for CVD and suffer from high rates of hypertension, resulting in alarming rates of hypertensive heart disease and stroke [17].

The need for effective and affordable markers of early cardiovascular deterioration as part of prevention programs is imperative, as poor health systems in Africa may hinder successful treatment programs. We therefore aimed to determine whether arterial stiffness is more pronounced in young healthy black compared to white South African adults, and whether large artery stiffness is associated with markers of health behaviours such as alcohol use, smoking, obesity, liver enzymes and physical activity in these individuals.

METHODS

Study population

This sub-study forms part of the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and HyperTension (African-PREDICT). The aim of the African-PREDICT study is to understand the early pathophysiology accompanying CVD development, and to identify novel early markers or predictors for the development of CVD by following young, healthy adults over a period of 10-20 years. The African-PREDICT study is currently being conducted at the *Hypertension Research and Training Clinic* on the Potchefstroom campus of the North-West University. Participants are recruited from the Potchefstroom and surrounding areas by field workers, via their workplace, or through advertisement by means of local newspapers or radio stations. Young (20-30 years of age), apparently healthy black and white men and women were included in the study after an initial screening day. Participants whose mean BP out of 4 measurements ≥ 140 mmHg and/or ≥ 90 mmHg, who were HIV infected, previously diagnosed with a chronic disease, pregnant or breastfeeding were excluded.

The African-PREDICT study complies with all applicable requirements of the Declaration of Helsinki. The Health Research Ethics Committee of the North-West University approved the protocol of the study. Before measurements commenced, all procedures were explained to the participants. The participants then gave written informed consent.

This sub-study includes cross-sectional data from the first 403 participants. Participants with incomplete PWV data (N=30) were excluded. The remaining 373 participants were divided into a black (N=183) and white (N=190) group.

Questionnaires

Participants completed a general health questionnaire with the help of a trained researcher. The socio-economic status (SES) of a participant was derived from three categories included in the general health questionnaire, including skills level, education and household income.

Points were awarded to each of these categories, and the total number of points determined whether a participant had a low, middle or high SES. The classification of the SES was adapted from Patro *et al* [18]. Alcohol and tobacco use were also reported in this questionnaire.

Body composition and physical activity measurements

Height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany), weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany) and waist circumference (Lufkin Steel Anthropometric Tape (W606PM), Lufkin, Apex, USA) were measured using standardised methods and calibrated instruments [19]. Body mass index (BMI) was calculated. Each participant was fitted with a combined heart rate and accelerometry device (ActiHeart, (CamNtech Ltd., Cambridge, UK) which was worn on the chest and recorded activity energy expenditure (AEE) continuously for 7 consecutive days.

Cardiovascular measurements

After at least a 10 minute rest period, duplicate office blood pressure measurements (Dinamap® ProCare 100 Vital Signs Monitor, GE Medical Systems, Milwaukee, USA) were made on both arms with 5 minute intervals. The participant was sitting in a relaxed upright position with legs uncrossed. An appropriate sized cuff was used for each individual.

The mean arterial pressure (MAP) was calculated with the following equation: $(SBP-DBP)1/3 + DBP$ where SBP, systolic BP; DBP, diastolic BP.

Each participant was fitted with a 24-hour ambulatory blood pressure and ECG apparatus (CardioXplore®, CE0120, Meditech, Budapest, Hungary). The participant was fitted with the appropriate cuff size on the non-dominant arm. Instructions were given on how to ensure successful inflations, and the participant filled out an ambulatory diary card during the measurements.

The carotid-femoral PWV was determined with the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia). The transit-distance method was used to measure

PWV along the descending thoraco-abdominal aorta. Pulse wave analyses (PWA) were also performed to obtain central SBP and pulse pressure.

Biological sampling

Participants were required to fast from 22h00 the evening before. Blood samples were taken with a sterile winged infusion set and syringes from the antebrachial vein. Spot urine samples were collected. All samples were immediately taken to the on-site laboratory and aliquoted into cryovials for storage in biofreezers at -80°C until analysis.

Biochemical analyses

An enzymatic colorimetric method (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) was used to determine serum total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), gamma-glutamyltransferase (GGT), creatinine, aspartate transferase (AST) and alanine transferase (ALT) as well as urinary creatinine and albumin levels.

High sensitivity C-reactive protein (hsCRP) was determined in serum, and fasting glucose in fluoride plasma (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN). Glycosylated haemoglobin (HbA1c) was determined from EDTA whole blood with an immunoturbidimetric method (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN). Interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-alpha), intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1) were determined with high sensitivity Quantikine ELISA kits (R&D systems, Minneapolis, MN USA) and analysed on a Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). Serum peroxides, representing reactive oxygen species (ROS), were determined with high-throughput spectrophotometric assay and analysed on Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). A chemiluminescence method was used to assess serum cotinine levels (Immulite, Siemens, Erlangen, Germany).

Creatinine clearance (CrCl) was calculated with the Cockcroft-Gault formula [20].

Statistical analysis

We used Statistica version 13 (StatSoft, Inc., Tulsa, OK, USA) and prepared graphs using GraphPad Prism Version 5.03 (GraphPad Software Inc., California, USA). Interactions of ethnicity and sex were tested for the relationship between PWV and health behaviours (WC, BMI, GGT, cotinine, AEE and SES). Descriptive statistics including the mean and standard deviation were performed on data with a normal distribution. Abnormally distributed variables were logarithmically transformed and the central tendency and spread described as the geometric mean, the 5th and 95th percentiles. Differences between the two ethnic groups were determined by independent t-tests and, for categorical data, by Chi-square tests. To determine the associations between PWV and measures of health behaviour we performed partial correlations, ANOVA and analysis of covariance (ANCOVA) with PWV as the dependent variable. PWV was plotted against age, SES, BMI and tertiles of GGT for both black and white groups, before and after adjustment for MAP. We investigated the independent associations of PWV with health behaviours by using linear multiple regression analyses with PWV as the dependent variable. Based on the literature, covariates considered for entry into the model included: ethnicity, SES (low, medium and high), age, MAP, sex, BMI, WC, height, TG, HDL-C, TG/HDL-C ratio, HbA1c, glucose, CRP, IL-6, MCP-1, TNF-alpha, ROS, urinary albumin/creatinine ratio (uACR), ICAM-1, VCAM-1, cotinine, self-reported smoking, GGT, self-reported alcohol use, CrCl, AEE. The final model is described in Table 3.

RESULTS

The characteristics of the study population are shown in Table 1. The blacks were younger, a larger percentage had a low SES and had lower WC and BMI than whites. After adjustment for MAP, PWV was similar between the groups (6.37 ± 0.73 vs. 6.36 ± 0.73 m/s; $p=0.89$). While ambulatory and central BP were similar between the groups, blacks had higher office systolic, diastolic and mean arterial pressure (all $p<0.001$). Although there were no difference in the self-reported alcohol intake, the black group exhibited higher GGT levels (24.5 vs. 17.2 U/l; $p<0.001$) compared to the whites. The blacks also had higher cotinine levels (63.7 ± 116 vs. 31.8 ± 84.0 ng/ml; $p=0.003$) and reported a higher rate of tobacco use (31.1% vs. 14.7% ; $p<0.001$). Objectively measured AEE were similar between the groups. The black group exhibited lower TC, LDL-C, TG and TG/HDL-C compared to the white group (all $p<0.02$). However, IL-6, MCP-1 and ROS were higher among the blacks (all $p<0.017$), whereas ICAM-1 was higher in whites. The uACR and the AST/ALT ratio were higher in the black group ($p\leq 0.001$).

TABLE 1. Characteristics of young black and white South Africans

	Black	White	p
	N= 183	N= 190	
Men, N (%)	86/183 (46.9)	72/190 (37.9)	0.08
Age, years	24.4 ± 3.22	25.5 ± 2.92	<0.001
<i>Socio-economic status</i>			
Low	111 (60.7)	19 (10.0)	<0.001
Middle	47 (25.7)	45 (23.7)	
High	25 (13.6)	126 (66.3)	
<i>Anthropometry</i>			
Waist circumference, cm	76.6 ± 10.6	80.8 ± 13.2	<0.001
Body mass index, kg/m ²	24.0 ± 5.07	25.5 ± 5.03	0.004
<i>Cardiovascular variables</i>			
Systolic BP, mmHg	120 ± 12.0	115 ± 12.0	<0.001
Diastolic BP, mmHg	80.4 ± 8.43	76.5 ± 7.35	<0.001
Pulse pressure, mmHg	40.2 ± 8.05	39.1 ± 8.15	0.18
Mean arterial pressure, mmHg	93.8 ± 9.01	89.5 ± 8.33	<0.001
Ambulatory SBP, mmHg	117 ± 9.60	117 ± 9.19	0.67
Ambulatory DBP, mmHg	69.6 ± 6.43	69.2 ± 5.95	0.48
Ambulatory heart rate, bpm	75.5 ± 13.5	74.1 ± 10.7	0.27
Central SBP, mmHg	111 ± 9.62	105 ± 8.69	<0.001
Central pulse pressure, mmHg	33.7 ± 5.82	34.5 ± 5.16	0.17
Carotid femoral PWV, m/s*	6.37 ± 0.73	6.36 ± 0.73	0.89
<i>Biochemical variables</i>			
Total cholesterol, mmol/l	3.78 [2.71; 5.54]	4.62 [3.30; 6.24]	<0.001
LDL-cholesterol, mmol/l	2.33 [1.31; 4.05]	2.92 [1.79; 4.49]	<0.001
Triglycerides, mmol/l	0.76 [0.41; 1.58]	0.96 [0.45; 2.18]	<0.001
Triglyceride/HDL-cholesterol ratio	0.60 [0.26; 1.55]	0.70 [0.26; 2.07]	0.016
Glucose, mmol/l	3.79 [2.79; 5.25]	4.76 [3.68; 5.47]	<0.001
HbA1c, %	5.48 [5.06; 6.01]	5.28 [4.88; 5.68]	<0.001
C-reactive protein, mg/l	1.17 [0.12; 7.47]	1.04 [0.14; 10.9]	0.39
Interleukin-6, pg/ml	0.95 [0.39; 3.21]	0.70 [0.27; 2.29]	<0.001
Monocyte chemotactic protein-1, pg/ml	175 [122; 270]	134 [91.5; 203]	<0.001
Tumour necrosis factor-alpha, pg/ml	1.64 [0.95; 4.70]	1.76 [1.06; 3.05]	0.26
Reactive oxygen species, mg/l H ₂ O ₂	180 [94.3; 337]	163 [83.5; 365]	0.017
Intercellular adhesion molecule-1, ng/ml	145 ± 78.2	179 ± 55.7	<0.001
Vascular adhesion molecule-1, ng/ml	526 [366; 809]	534 [356; 883]	0.09
<i>Renal function</i>			
Creatinine clearance, ml/min	128 [86.2; 199]	127 [86.6; 190]	0.82
Urinary albumin/creatinine ratio	0.71 [0.51; 1.00]	0.64 [0.48; 0.86]	<0.001
<i>Health behaviours</i>			
Self-reported alcohol intake, N./total (%)	112/183 (61.2)	126/190 (66.3)	0.30
GGT, U/l	24.5 [10.6; 64.3]	17.2 [7.50; 51.2]	<0.001
AST/ALT ratio	1.24 [0.66; 1.98]	1.10 [0.59; 1.94]	0.001
Self-reported tobacco use, N/ total (%)	57/183 (31.1)	28/190 (14.7)	<0.001
Cotinine, ng/ml	63.7 ± 116	31.8 ± 84.0	0.003
Activity energy expenditure, kCal/day	416 ± 168	435 ± 199	0.34

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables. N, number of participants; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; PWV, pulse wave velocity; HDL, high density lipoprotein; LDL, low density lipoprotein; HbA1c, glycated haemoglobin; GGT, gamma-glutamyltransferase; AST, aspartate transaminase; ALT, alanine transaminase.

* Adjusted for mean arterial pressure.

Interaction terms indicated an interaction of ethnicity for the relationship between PWV and BMI ($p=0.0012$) and PWV and WC ($p<0.001$), but we found no interactions with sex (Supplementary Table 1). Analyses were therefore performed separately in the black and white groups.

We performed partial correlations between PWV and health behaviours (Table 2) while adjusting for MAP. PWV correlated positively with age, male sex and GGT in both groups (all $p<0.05$). In blacks, we found a positive correlation between PWV and SES, AST, ALT and cotinine (all $p\leq 0.024$) and negative correlation between PWV and BMI ($p=0.001$), all of which are absent in the whites.

TABLE 2. Partial correlations of pulse wave velocity with measures of health behaviours, adjusted for MAP

	Black N=183	White N=190
Age, years	0.18 p=0.016	0.20 p=0.007
Sex, (0, women; 1, men)	0.34 p<0.001	0.18 p=0.012
Socioeconomic status	0.17 p=0.024	0.02 p=0.74
Waist circumference, cm	-0.14 p=0.06	0.04 p=0.59
Body mass index, kg/m ²	-0.24 p=0.001	-0.06 p=0.40
Self-reported alcohol intake, N, total (%)	0.03 p=0.65	0.08 p=0.25
GGT, U/l	0.15 p=0.049	0.19 p=0.012
AST/ALT ratio	-0.07 p=0.35	-0.11 p=0.12
Cotinine, ng/ml	0.17 p=0.022	0.12 p=0.11
Activity energy expenditure, kCal/day	-0.01 p=0.91	-0.03 p=0.71

Bold text indicates p -values <0.05 . GGT, Gamma-glutamyltransferase. AST, aspartate transaminase; ALT, alanine transaminase.

We plotted PWV against age, SES, BMI and tertiles of GGT, before and after adjustment for MAP (Figure 1). Before and after adjustments, PWV increased with age, only in blacks (p for trend=0.001 and $p=0.023$, respectively). PWV was higher in blacks than whites in age groups 23-25 and 26-28 years ($p<0.05$). For the black group, PWV was highest in the high SES group (p for trend=0.012). We found contrasting results for BMI, but after adjustment for MAP, no association remained in either ethnic group. Before adjustment for MAP, PWV increased with the tertiles of GGT for both the black and white groups, but after adjustment there was no significant trend in either group.

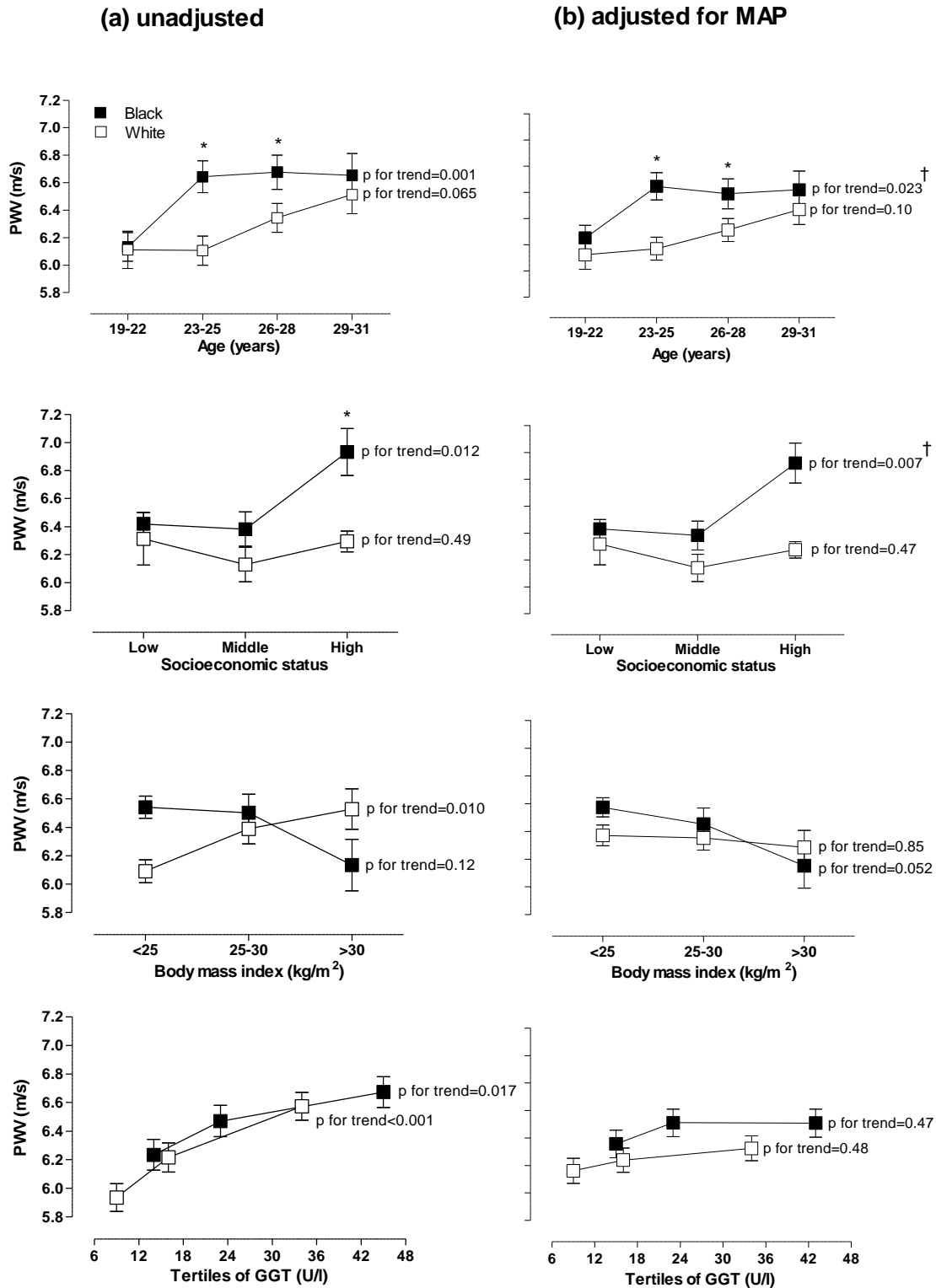


Figure 1. Pulse wave velocity plotted against age, socioeconomic status (SES), body mass index and tertiles of gamma-glutamyltransferase for young black and white groups. * indicates p -value (<0.05) between black and white group. † indicates p -value (<0.05) between the lowest and highest age group as well as low and high SES.

Multivariable-adjusted regression analyses with PWV as the dependent variable were performed (Table 3). The multivariate model accounted for 37%, 36% and 39% of the variability in PWV in the total, black and white groups, respectively. PWV associated with MAP and age in all three groups (all $p < 0.036$). Male sex associated with PWV in the total and black groups. We found a negative association between PWV and BMI in the total ($\beta = -0.15$, $p = 0.043$) and white ($\beta = -0.23$, $p = 0.049$) groups. PWV associated positively with higher SES ($\beta = 0.15$, $p = 0.044$) in blacks only. The relationship between PWV and GGT was confirmed in all groups (all $p < 0.05$).

TABLE 3. Linear multiple regression analyses with pulse wave velocity as dependent variable

	Total group (N=373)		Blacks (N=183)		Whites (N=190)	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Carotid-femoral pulse wave velocity, m/s						
Adjusted R²	0.37		0.36		0.39	
Ethnicity, white	0.03 (-0.10, 0.16)	0.66	-	-	-	-
Age, years	0.17 (0.07, 0.27)	<0.001	0.16 (0.01, 0.31)	0.036	0.25 (0.10, 0.39)	0.001
Sex, male	0.22 (0.11, 0.33)	<0.001	0.28 (0.10, 0.45)	0.003	0.10 (-0.06, 0.26)	0.20
BMI, kg/m ²	-0.15 (-0.30, -0.01)	0.043	-0.11 (-0.32, 0.10)	0.31	-0.23 (-0.45, -0.002)	0.049
SES	0.07 (-0.05, 0.19)	0.27	0.15 (0.01, 0.30)	0.044	-0.08 (-0.22, 0.07)	0.29
MAP, mmHg	0.40 (0.30, 0.51)	<0.001	0.34 (0.19, 0.50)	<0.001	0.45 (0.30, 0.60)	<0.001
GGT, U/l	0.13 (0.03, 0.24)	0.014	0.15 (0.003, 0.30)	0.048	0.15 (0.002, 0.30)	0.049
Cotinine, ng/ml	0.03 (-0.07, 0.13)	0.54	0.003 (-0.16, 0.16)	0.97	0.03 (-0.10, 0.16)	0.65
TG/HDL-C ratio	0.03 (-0.06, 0.13)	0.49	-0.09 (-0.23, 0.05)	0.19	0.14 (-0.01, 0.29)	0.07
CRP, mg/l	0.04 (-0.06, 0.14)	0.46	0.09 (-0.07, 0.24)	0.26	-0.04 (-0.18, 0.10)	0.59
Glucose, mmol/l	-0.09 (-0.21, 0.02)	0.12	-0.17 (-0.31, -0.02)	0.029	0.01 (-0.12, 0.14)	0.84
CrCl, ml/min	-0.03 (-0.16, 0.10)	0.68	-0.07 (-0.25, 0.10)	0.42	0.05 (-0.16, 0.26)	0.63
AEE, kCal	0.07 (-0.02, 0.16)	0.15	0.06 (-0.07, 0.20)	0.36	0.08 (-0.05, 0.21)	0.22

Data expressed as beta-values and 95% confidence intervals, p-values obtained with multiple regression analyses. BMI, body mass index; SES, socioeconomic status; MAP, mean arterial pressure; GGT, gamma-glutamyltransferase; TG, triglycerides; HDL-C, high density lipoprotein-cholesterol; CRP, C-reactive protein; CrCl, creatinine clearance; AEE, active energy expenditure

Sensitivity analysis

When excluding self-reported alcohol users, GGT was still higher in the blacks (22.2 vs. 14.4 U/l; $p < 0.001$). Regarding the regression models, neither the inclusion of the AST/ALT ratio, ROS, ICAM or VCAM, nor the replacement of CRP with IL-6 or MCP-1 in the model changed the results.

DISCUSSION

In young healthy adults, we found that large artery stiffness associated independently and positively with the liver enzyme, GGT, but not with health behaviours such as self-reported alcohol use, tobacco use or physical activity. Although we found no overall difference in arterial stiffness between young black and white adults (mean values in the normal range), blacks aged 23-28 years presented with significantly higher arterial stiffness, independent of MAP.

An association between PWV and GGT has been reported in other populations, including young (aged 33-34 years) Turkish patients with prehypertension [21]. However, studies in black populations are limited. We included GGT in this study initially in the conventional sense as a marker of alcohol use [22, 23]. However, our black and white group reported similar rates of alcohol use (61.2% vs. 66.3%, respectively), but GGT levels were higher in the blacks. This was an unexpected result, since alcohol use reported by black South Africans seems to be reliable, as our group found that it significantly predicted a 5-year change in BP [24]. In a sensitivity analysis, we found higher GGT in our black group who self-reported no alcohol use, supporting the theory of a racial difference in GGT levels. Similarly, in black and white Americans, GGT was higher in the blacks, even among lifetime abstainers [25].

GGT is a liver enzyme [22], but it is also present in other organs, including the nervous system, kidneys, pancreas and reproductive system [26]. GGT predicts CVD and cardiovascular (CV) events, incident diabetes and hypertension independently of alcohol consumption and other CVD risk factors [27, 28]. In older black South Africans, GGT also independently predicted CV and all-cause mortality, as well as hypertension development [13]. Therefore, GGT does not only reflect alcohol use, but also liver dysfunction, including non-alcoholic fatty liver disease [29].

The specific mechanism involved in the association between PWV and GGT is unclear. Physiologically, GGT counteracts oxidative stress by breaking down extracellular

glutathione, thus, GGT may be increased by conditions that elevate oxidative stress in the body, such as high alcohol consumption [30]. However, under certain conditions the products of the GGT reaction themselves may lead to free radical production [30]. GGT is thus regarded as a marker of oxidative stress [31]. Oxidative stress also associates with arterial stiffness [32] and could be the mechanism involved in the association between PWV and GGT, possibly via elastin rupture and increased collagen production in the arterial wall [33]. Future research should be aimed at elucidating the mechanism involved.

The association of PWV with GGT may point to an early underlying disease development process independent of alcohol use, such as the development of non-alcoholic fatty liver disease (NAFLD) or diabetes. GGT levels are elevated in patients with NAFLD [29]. Furthermore, NAFLD has been associated with arterial stiffness [34]. A mechanism involving inflammation, oxidative stress, and endothelial dysfunction [35] may explain the abnormal elastic properties of the large arteries found in patients with NAFLD [36]. Of three liver enzymes that associated with diabetes risk, namely AST, ALT and GGT, GGT associated most strongly with diabetes risk in older (over 60 years of age) black and white American adults, and even in those participants who were lifetime abstainers from alcohol [37]. The incidence of diabetes in the black South African population may also be on the rise [38], likely a consequence of urbanisation and the adoption of unhealthy lifestyles [14]. Compared to the whites, the black group may be at increased risk for future development of diabetes and NAFLD with increasing age due to their higher GGT levels. This, in turn, may also affect their vascular health and risk for future CVD [39, 40]. The higher GGT and AST/ALT ratio observed in our young black population may thus point to an early pathological metabolic process independent of alcohol use.

The association between PWV and GGT did not differ between the blacks and whites, and is weaker than with MAP and sex, but it is still independent of the main contributors to PWV. We found no association between PWV and inflammatory markers, cotinine or self-reported tobacco use. A possible reason for these observations could be the young, apparently

healthy status of this group. Nevertheless, the black group exhibited higher GGT, ROS, IL-6, MCP-1, cotinine and self-reported tobacco use (31.1% vs. 14.7%), all of which have been linked to arterial stiffness [8, 28, 32, 41, 42]. Furthermore, GGT predicts the development of diseases such as hypertension [13] and diabetes, even when the baseline GGT was in the lower to normal ranges [37]. Therefore, although our results showed a similar association between PWV and GGT in blacks and whites, young blacks may be at an increased risk for adverse health consequences as they age, including early changes in arterial stiffness, due to their elevated cardiovascular risk profile and higher GGT levels.

Our results confirm that BP and age are independently related to PWV [7]. Because of the well-known association between PWV and age [43], arterial stiffness research is more common in older groups than in young adults. A study in middle-aged black and white Americans indicated higher PWV in blacks independent of traditional CV risk factors [15]. This was not the case in our study, as we found no difference in PWV between the black and white groups. The latter may be explained by the relatively healthy cardiovascular profile and young age of our population, and specifically as we excluded individuals with a blood pressure exceeding 140/90 mmHg. Despite PWV and BP being in the normal range in our population, the 23-25 and 26-28 year old black groups had higher PWV compared to the white groups. Previously, we have shown that the stiffness of muscular and large arteries are already elevated in young blacks compared to whites, both in hypertensives and in those with normal BP [44]. Confirmed by the present study, it seems that young blacks may be more vulnerable to early vascular ageing than their white counterparts.

Apart from age and BP, low SES is also associated with arterial stiffness [45]. However, we found a positive association between PWV and high SES in our black group. This result suggests that the adaptation of an unhealthy, high sugar and fat diet, stressful situations and chronic alcohol abuse [16, 39, 46], characteristic of rapid urbanisation in South Africa [14], may be affecting the high SES black group. However, these results should be interpreted cautiously due to the uneven number of participants in the different SES categories.

We found an independent negative association between PWV and BMI in the white group. Authors who found similar results suggested that the adaptation of the arterial wall to blood pressure changes may differ in obese individuals [47], where the increased blood volume and the encapsulation of small conduit arteries by adipose tissue may buffer or blunt the reflecting wave in the pulse wave contour [48].

Strengths and limitations

Our study population consisted of individuals from specific urban areas in the North West Province of South Africa, and may not be representative of the whole population. However, this study included the understudied black South African population as well as information on arterial stiffness and variety of health behaviours, all of which may influence future CVD development in young populations. This study was conducted in highly controlled conditions in a well-equipped research facility. Due to the cross-sectional study design, causality cannot be inferred. Although the results were consistent after several adjustments, we cannot exclude residual confounding.

Conclusion

Large artery stiffness associates positively and independently with GGT in both black and white young, healthy individuals. Despite similar PWV values in black and white adults, blacks may be more vulnerable to future CVD development including changes in arterial stiffness, due to higher GGT levels, an elevated CV risk profile and a larger proportion of smokers. Whether the higher GGT levels in young blacks will translate to a higher risk for future cardiovascular disease should be confirmed in future studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY INFORMATION

Supplementary Table S1. Interaction Terms

Dependent variable: Carotid femoral pulse wave velocity	Ethnicity p	Sex p
Body mass index, kg/m ²	0.0012	0.87
Waist circumference, cm	<0.001	0.99
GGT, U/l	0.19	0.85
Cotinine, ng/ml	0.44	0.67
Activity energy expenditure, kCal/day	0.99	0.06
Socioeconomic status (low, middle, high)	0.24	0.83

Interaction terms p-values calculated with multiple regression analyses. GGT, gamma-glutamyltransferase.

CHAPTER 4

A health profile associated with excessive alcohol use independently predicts aortic stiffness over 10 years in black South Africans

Original Article

A health profile associated with excessive alcohol use independently predicts aortic stiffness over 10 years in black South Africans

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Ethical considerations	<p>The Editors reserve the right to judge the appropriateness of the use and treatment of humans or animals in experiments for publication in the journal.</p> <p><i>For human experiments:</i> All work must be conducted in accordance with the Declaration of Helsinki. Papers describing experimental work on human participants which carries a risk of harm must include (1) a statement that the experiments were conducted with the understanding and the consent of each participant, and (2) a statement that the responsible ethical committee has approved the experiments.</p>

Please note: Some format was changed to ensure uniformity throughout the thesis.

Abstract

Objective: Black populations exhibit higher arterial stiffness than whites and suffer a disproportionate burden of cardiovascular disease. It is therefore important to identify modifiable health behaviours predicting large artery stiffness in blacks. We examined whether traditional cardiovascular risk factors and health behaviours of black South Africans predict large artery stiffness 10 years later.

Methods: We included 650 HIV-free participants (32.8% men), and collected data in rural and urban areas of the North West Province in 2005 and 2015. We collected questionnaire data, anthropometry, blood pressure, and determined cardiometabolic and inflammatory markers from blood samples. We measured carotid-femoral pulse wave velocity (PWV) at follow-up.

Results: 25.3% of our population, aged 65 ± 9.57 years, had a PWV exceeding 10 m/s. In multivariable-adjusted regression analyses, the strongest predictors of PWV were mean arterial pressure (MAP), age and heart rate (all $p < 0.024$). Urban locality ($R^2 = 0.31$, $\beta = 0.12$, $p = 0.001$), self-reported alcohol use ($\beta = 0.11$, $p = 0.018$), and plasma glucose ($\beta = 0.08$, $p = 0.023$) associated positively with PWV at follow-up. We found a negative association between PWV and body mass index (BMI) ($\beta = -0.15$, $p = 0.001$), and no associations with sex, smoking, inflammatory markers, lipids, liver enzymes or antihypertensive medication. When replacing self-reported alcohol with gamma-glutamyltransferase, the latter associated positively with PWV ($\beta = 0.09$, $p = 0.023$).

Conclusion: A health profile associated with excessive alcohol use, including an urban setting, elevated plasma glucose and lower BMI predicts large artery stiffness independently of age and blood pressure in black South Africans over 10 years. This observation prompts urgent public health strategies to target alcohol overuse.

Keywords: arterial stiffness, pulse wave velocity; alcohol use, black South Africans, urban locality, longitudinal

Introduction

Black populations exhibit higher arterial stiffness than their white counterparts [1] and suffer a disproportionate burden of cardiovascular disease [2]. Large artery stiffness increases the risk of cardiovascular events including stroke and myocardial infarction, cardiovascular- and all-cause mortality [3]. Carotid-femoral pulse wave velocity (PWV) is the gold standard measurement of large artery stiffness [4] and is a better predictor of cardiovascular events, cardiovascular- and all-cause mortality than brachial systolic and diastolic blood pressure, as well as brachial and 24h pulse pressure [3, 5].

Large artery stiffness is largely dependent on age [6] and blood pressure [7]. However, other factors may also accelerate vascular ageing beyond the effect of chronological age by functional and structural alterations of the arterial wall of conduit vessels [8]. In older white men, circulating inflammatory markers and the level of repetitive cyclic stress in the artery were predictive of arterial stiffness over 20 years, while traditional cardiovascular risk factors had only a modest effect [9]. After 17 years, abdominal obesity, hyperglycaemia and dyslipidaemia, but not smoking, predicted arterial stiffness in middle-aged whites from Sweden [10]. In British middle-aged men and women, central obesity was a strong predictor of arterial stiffness after 16 years [11].

The pathophysiology of large artery stiffness is incompletely understood in black populations and limited longitudinal data in African populations has kept investigators from identifying traditional cardiovascular risk factors and health behaviours which may predict arterial stiffness. It is important to identify modifiable health behaviours leading to increases in arterial stiffness and cardiovascular disease in order to plan and implement effective preventive strategies. [12] Primary prevention, especially in Africa where health systems are weak and overburdened [13], is essential.

To contribute to a better understanding of cardiovascular disease development in black populations, we determined the prognostic value of traditional cardiovascular risk factors and

health behaviours, assessed over 10 years, in terms of large artery stiffness. The factors investigated as possible predictors include markers of obesity, glucose and lipid metabolism, renal function, liver function, inflammation and health behaviours.

Methods

Research design

This sub-study forms part of the South African leg of the international Prospective Urban and Rural Epidemiology (PURE) study [14]. This multi-country study was developed to examine the patterns of transition on health and the influence of changing communities on the prevalence and types of cardiovascular and other chronic diseases [14]. We collected data for this sub-study in the North West Province of South Africa in 2005 (baseline), with a 10 year follow-up conducted in 2015.

Recruitment

Baseline data collection included 2,010 (rural=1006 and urban=1004) black volunteers. Participants were fully informed about the objectives and procedures of the study before participation. Before measurements commenced, all procedures were explained to the participants in their home language, after which written informed consent was given. Black individuals older than 35 years were invited to participate in the study. Pregnant and lactating women were excluded. We collected longitudinal data for 926 participants, and excluded participants with incomplete PWV data (n=110) and those who were HIV-infected (n=166). The attrition rate of participants from baseline (n=2010) to follow-up (n=926) compares with other longitudinal studies due to frailty of elderly participants, refusal to participate, movement to other areas of the country and mortality. Included in the present study are 650 (rural=362 and urban=288) participants (Figure 1).

The Health Research Ethics Committee of the North-West University gave approval for the PURE-SA baseline (2005) and follow-up (2015) study, as well as this sub-study. All procedures comply with the Declaration of Helsinki.

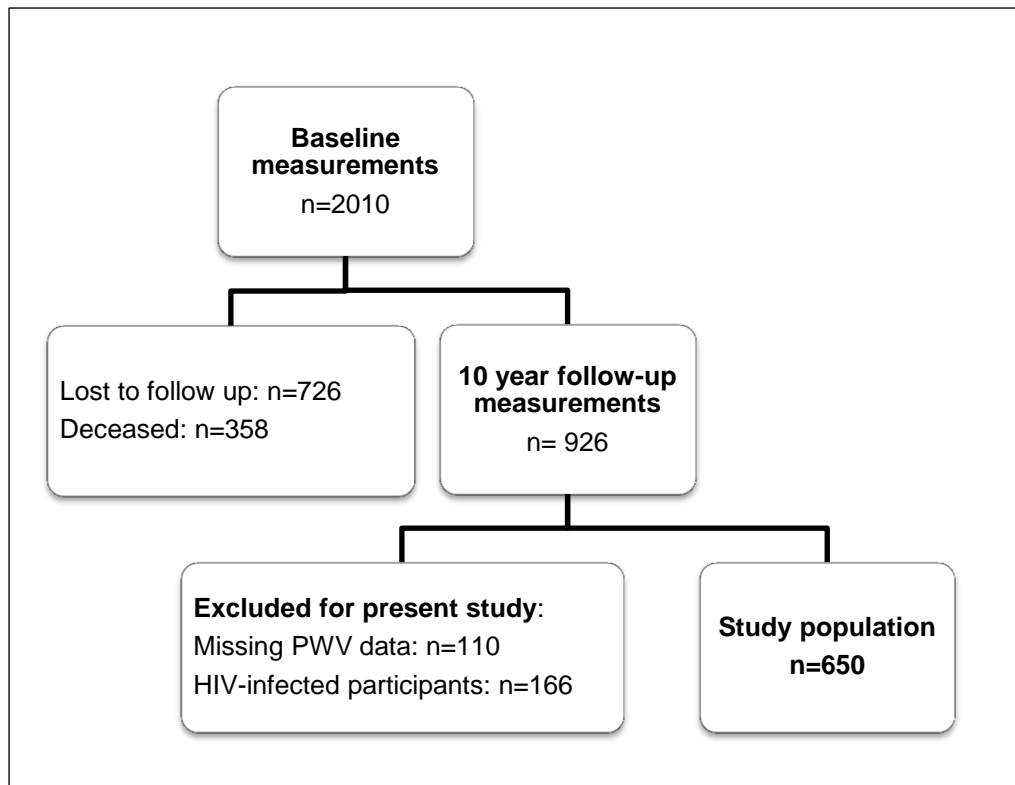


Figure 1. Flow diagram of study population.

Data collection

Questionnaires

Participants completed structured socio-demographic, lifestyle and physical activity questionnaires. Alcohol use and smoking were indicated with a yes/no answer, with yes for current or former use and no for never used. Trained African field workers assisted in the collection of the biographical data of participants.

Anthropometric measurements

Baseline and follow-up height (Invicta Stadiometer IP 1465, Leicester, UK; Leicester height measure, Seca, Birmingham, UK), weight (Precision Health Scale, A & D Company, Japan) and waist circumference (WC) (Holtain unstretchable metal tape) were taken using standardised methods and calibrated instruments. Body mass index (BMI) and WC-to-height ratio was calculated.

Cardiovascular measurements

The validated OMRON HEM-757 and OMRON M6 (Omron Healthcare, Kyoto, Japan) devices were used to measure blood pressure at baseline and follow-up, respectively. The correct cuff size was fitted on the right arm of each participant. The participant was seated in a relaxed upright position with legs uncrossed during the measurement. After a resting period of 10 minutes, the brachial systolic (SBP) and diastolic blood pressure (DBP) were measured and recorded twice with a 5 minute interval. The blood pressure of the second measurement was used for analysis.

At follow-up carotid-femoral PWV was determined in duplicate with the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) with the participant in the supine position, and the second reading used for analysis. The transit-distance method was used to measure PWV along the descending thoraco-abdominal aorta.

Biological sampling

Participants were asked to fast from 22h00 the evening before. In the early morning blood samples were taken from the antebrachial vein from each participant with a sterile winged infusion set and syringes. Morning spot urine samples were also collected. We used standardised methods to prepare serum and plasma, snap frozen on dry ice and stored in the laboratory at -80°C. In the cases of blood collection in a rural area, serum and plasma was snap frozen and stored at -20°C for not more than 5 days. The samples were then transported to the laboratory and stored at -80°C.

Biochemical analysis

We analysed serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), gamma-glutamyltransferase (GGT), creatinine, high-sensitivity C-reactive protein (CRP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Konelab20i™ auto-analyzer, Thermo Fisher Scientific Oy, Vantaa, Finland; Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) and urinary albumin and

creatinine (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) for baseline and follow-up.

The Friedewald formula was used to calculate the quantitative aspect of low-density lipoprotein cholesterol [15]. The creatinine clearance rate (CrCl) was calculated with the Cockcroft-Gault formula [16]. The estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration Equation (CKD-EPI) [17].

We determined plasma glucose at baseline (Vitro DT6011 Chemistry Analyzer; Ortho Clinical Diagnostics, Rochester, New York, USA) and follow-up (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN), as well as glycosylated haemoglobin (HbA1c) levels (D-10 Haemoglobin testing system, Bio-Rad #220-0101). Serum ferritin was determined with an enzyme immunoassay (Ramco Laboratories, Inc, Stafford Texas). Baseline interleukin-6 (IL-6) levels were determined with an electrochemiluminescence immunoassay (Elecsys 2010, Roche, Basel, Switzerland).

Statistical analyses

We performed statistical analyses using Statistica 13 (Statsoft Inc., Tulsa, OK, USA) and prepared graphs using GraphPad Prism Version 5.03 (GraphPad Software Inc., California, USA). Descriptive statistics including the mean and standard deviation were performed on data with a normal distribution. Abnormally distributed variables were logarithmically transformed and the central tendency and spread described as the geometric mean, the 5th and 95th percentiles. We tested for interactions of sex on the relationship between PWV and mean arterial pressure (MAP), and found a tendency for an interaction ($p=0.06$). Some analyses were therefore performed separately for men and women. We divided participants into tertiles based on PWV, and we determined differences between the tertiles using Chi-square tests, analysis of variance (ANOVA) and covariance (ANCOVA). We performed multiple regression analysis with PWV as the dependent variable in the total group, and in men and women separately. Covariates considered for entry into the model included all variables in Table 1 and those compared after adjustments in Table 2. The final model

included: baseline age, sex, locality, BMI, triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C), glucose, CRP, urinary albumin to creatinine ratio (uACR), GGT, self-reported alcohol use and tobacco use, and follow-up variables: heart rate, MAP and anti-hypertension medication use.

Results

The baseline characteristics of the total population (N=650), stratified by PWV tertiles are shown in Table 1. Those in the third PWV tertile were older, taller, a higher percentage were men (43%) and from an urban setting (52.2%) compared to those in the first two tertiles (P-trend ≤ 0.002). Hypertension prevalence was the highest in the third tertile (P<0.001). Sixty-seven percent of the study population had a PWV of at least 8 m/s while 25% had a PWV of ≥ 10 m/s. BMI decreased significantly with increased PWV (P-trend ≤ 0.023). All cardiovascular measures increased from the first to the third tertile (all P-trend ≤ 0.011). Biochemical measures including HDL-C, TG, TG/HDL-C, glucose, CRP, IL-6, GGT and uACR increased across the tertiles (all P-trend ≤ 0.042). Self-reported alcohol use was highest in the third PWV tertile (53% vs. 23.0% in the first tertile, P-trend <0.001), and although overall reported tobacco use was high (44.6% to 53.2%), no significant trend was seen according to PWV tertiles ($p=0.14$).

Table 1. Unadjusted baseline characteristics of a black South African population, stratified by tertiles of PWV

	1 st tertile (n = 214)	2 nd tertile (n = 215)	3 rd tertile (n = 221)	P-trend
Pulse wave velocity ranges, m/s	(< 8.0 m/s)	(8.0-9.4 m/s)	(≥ 9.5 m/s)	
Age	48.6 ± 8.70	50.0 ± 8.84	55.1 ± 9.63*	<0.001
Sex, men (%)	50/214 (23.4)	68/215 (31.6)	95/221 (43.0)	<0.001
Locality, urban (%)	75/214 (35.0)	97/215 (45.1)	116/221 (52.5)	0.002
Hypertension prevalence	83/214 (38.8)	99/215 (46.1)	140/221 (63.6)	<0.001
<i>Anthropometric measures</i>				
Body mass index (kg/m ²)	26.2 ± 6.53	25.4 ± 7.02	24.4 ± 6.66*	0.023
Waist circumference (cm)	80.5 ± 11.8	81.3 ± 13.4	80.2 ± 12.9	0.67
Height (m)	1.59 ± 0.07	1.60 ± 0.08	1.62 ± 0.08*	<0.001
WC/Height ratio	0.51 ± 0.08	0.51 ± 0.09	0.50 ± 0.08	0.21
<i>Cardiovascular measures</i>				
SBP (mmHg)	128 ± 19.4	131 ± 21.5	144 ± 25.7*	<0.001
DBP (mmHg)	85.4 ± 12.9	87.1 ± 13.29	92.2 ± 14.1*	<0.001
Pulse pressure (mmHg)	42.6 ± 11.9	44.8 ± 13.9	52.5 ± 16.5*	<0.001
Heart rate (bpm)	70.2 ± 14.6	72.6 ± 14.4	74.6 ± 16.6*	0.011
MAP (mmHg)	99.6 ± 14.3	102 ± 15.1	109 ± 17.1*	<0.001
<i>Biochemical measures</i>				
Total cholesterol, mmol/l	4.93 (4.76; 5.11)	5.11 (4.93; 5.30)	5.10 (4.92; 5.28)	0.31
LDL-C, mmol/l	2.88 (2.73; 3.04)	2.96 (2.81; 3.13)	2.78 (2.64; 2.94)	0.27
HDL-C, mmol/l	1.41 (1.34; 1.49)	1.40 (1.33; 1.48)	1.53 (1.45; 1.61)	0.033
Triglycerides, mmol/l	1.05 (0.98; 1.12)	1.23 (1.15; 1.31)*	1.21 (1.14; 1.29)*	0.001
TG/HDL-C ratio	0.74 (0.67; 0.82)	0.90 (0.81; 0.99)*	0.79 (0.72; 0.87)	0.029
Glucose, mmol/l	4.83 (4.69; 4.97)	4.85 (4.71; 4.99)	5.10 (4.96; 5.25)*	0.011
HbA1c, %	5.61 (5.51; 5.71)	5.66 (5.55; 5.76)	5.71 (5.61; 5.82)	0.37
Ferritin, µg/l	72.6 (60.1; 87.7)	88.4 (72.2; 108)	106 (85.3; 132)*	0.036
<i>Inflammatory markers</i>				
C-reactive protein, mg/l	2.56 (2.10; 3.13)	3.65 (2.98; 4.47)*	3.33 (2.73; 4.05)	0.042
Interleukin-6, pg/ml	2.21 (1.89; 2.58)	2.64 (2.25; 3.08)	3.12 (2.67; 3.64)*	0.009
<i>Liver enzymes</i>				
GGT, U/l	43.1 (38.6; 48.1)	53.3 (47.7; 59.6)	60.3 (54.1; 67.3)	<0.001
AST (U/L)	24.1 (22.2; 26.2)	26.6 (24.5; 28.9)	27.6 (25.4; 29.9)	0.06
ALT (U/L)	16.9 (15.6; 18.2)	17.9 (16.6; 19.4)	17.7 (16.4; 19.1)	0.50
AST/ALT ratio	1.43 (1.33; 1.53)	1.48 (1.38; 1.59)	1.55 (1.45; 1.67)	0.24
<i>Renal function</i>				
CrCl, ml/min	97.2 (92.7; 102)	97.4 (92.7; 102)	92.6 (88.3; 97.1)	0.25
uACR, mg/mmol	0.60 (0.51; 0.70)	0.58 (0.49; 0.68)	0.77 (0.66; 0.90)	0.025
<i>Health behaviour</i>				
Tobacco use, n (%)	95/213 (44.6)	112/213 (52.6)	117/220 (53.2)	0.14
Alcohol use, n (%)	50/214 (23.4)	78/213 (36.6)	116/219 (53.0)	<0.001
<i>Medication use</i>				
Anti-hypertensive, n (%)	33/214 (15.4)	36/215 (16.7)	49/221 (22.2)	0.15
Anti-diabetic, n (%)	2/214 (0.93)	0/215 (0.00)	7/221 (3.17)	0.014
Anti-inflammatory, n (%)	34/214 (15.9)	38/215 (17.7)	29/221 (13.1)	0.42
Statins, n (%)	0/214 (0.00)	0/215 (0.00)	1/221 (0.45)	0.38

Data are arithmetic mean ± standard deviation, geometric mean (5th; 95th percentiles), or % of n. P-values were obtained with analysis of variance and Chi-square tests. p≤0.05 were regarded as statistically significant. *Statistically different from the first PWV tertile. WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; LDL, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; TG: HDL-C, triglyceride: high density lipoprotein-cholesterol ratio; HbA1c, glycated haemoglobin; GGT, gamma-glutamyltransferase; CrCl, creatinine clearance; uACR, urinary albumin to creatinine ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; tobacco and alcohol use are self-reported.

Supplementary Table S1 shows the cross-sectional characteristics of the same population at follow-up, again stratified by tertiles of follow-up PWV. Similar findings were obtained as with baseline characteristics. In addition, those in the third PWV tertile had the lowest WC and CrCl (P-trend ≤ 0.014). Furthermore, AST, AST/ALT ratio, and tobacco use were highest in the third PWV tertile (all P-trend ≤ 0.039).

In Table 2 we determined which variables to enter into the multiple regression model. Therefore, we compared cardiometabolic baseline characteristics according to PWV tertiles, but adjusted for age, MAP, sex and rural/urban locality. We found results similar to those in Table 1, except for the loss of a significant trend for uACR (P=0.11). When additionally adjusting for HR (not shown), a significant trend remained only for TG/HDL-C ratio and glucose (P ≤ 0.015).

Table 2. Adjusted baseline characteristics of a black South African population, stratified by tertiles of PWV

	1 st tertile (n = 214)	2 nd tertile (n = 215)	3 rd tertile (n = 221)	P-trend
Pulse wave velocity ranges, m/s	(< 8.0 m/s)	(8.0 - 9.4 m/s)	(\geq 9.5 m/s)	
Body mass index (kg/m ²)	26.3 \pm 6.53	25.4 \pm 6.13	24.4 \pm 6.60*	0.020
Waist circumference, cm	81.4 \pm 13.2	81.4 \pm 12.4	79.3 \pm 13.4	0.20
TG/HDL-C ratio	0.72 (0.65; 0.80)	0.91 (0.83; 1.00)	0.80 (0.72; 0.89)	0.006
Glucose, mmol/l	4.82 (4.67; 4.97)	4.86 (4.72; 5.00)	5.11 (4.95; 5.27)*	0.027
HbA1c, %	5.60 (5.50; 5.71)	5.67 (5.57; 5.78)	5.70 (5.60; 5.81)	0.45
C-reactive protein, mg/l	2.58 (2.08; 3.19)	3.76 (3.07; 4.60)	3.21 (2.60; 3.96)	0.041
Interleukin-6, pg/ml	2.26 (1.91; 2.67)	2.67 (2.28; 3.13)	3.02 (2.55; 3.57)	0.08
GGT, U/l	45.8 (40.8; 51.3)	52.3 (46.9; 58.4)*	58.0 (51.8; 65.1)*	0.026
uACR, mg/mmol	0.63 (0.53; 0.74)	0.57 (0.49; 0.67)	0.73 (0.62; 0.87)	0.11
Creatinine clearance, ml/min	96.8 (92.3; 101)	95.7 (91.3; 100)	94.7 (90.2; 99.5)	0.84
eGFR, ml/min/1.73m ²	111 \pm 17.7	115 \pm 16.4	114 \pm 17.8	0.15

Data are arithmetic mean \pm standard deviation, geometric mean (5th and 95th percentile intervals), p-values obtained with ANCOVAs; TG: HDL-C, triglycerides: high density lipoprotein cholesterol ratio; HbA1c, glycated haemoglobin; GGT, gamma-glutamyltransferase; uACR, urinary albumin to creatinine ratio; eGFR, estimated glomerular filtration rate. Data adjusted for age, mean arterial pressure, sex and locality.

To identify predictors for PWV after 10 years, we performed multivariate-adjusted regression analyses with PWV as the dependent variable in the total group (Figure 2), and separately in men and women (Table 3). In the total group and in men and women separately, PWV associated positively with MAP, age and HR (all $P \leq 0.024$). In the total group and in women, urban locality ($\beta = 0.12$, $P = 0.001$; $\beta = 0.13$, $P = 0.005$) and self-reported alcohol use ($\beta = 0.11$, $P = 0.018$; $\beta = 0.11$, $P = 0.045$) associated positively with PWV. In the total group only, PWV associated with glucose ($\beta = 0.08$, $P = 0.023$). We found a negative association between PWV and BMI ($\beta = -0.15$, $P = 0.001$; $\beta = -0.13$, $P = 0.019$) for the total group and for women, respectively, and a trend for the same association in the men ($\beta = -0.16$, $P = 0.05$). In women, PWV also associated positively with the TG/HDL-C ratio ($\beta = 0.10$, $P = 0.047$).

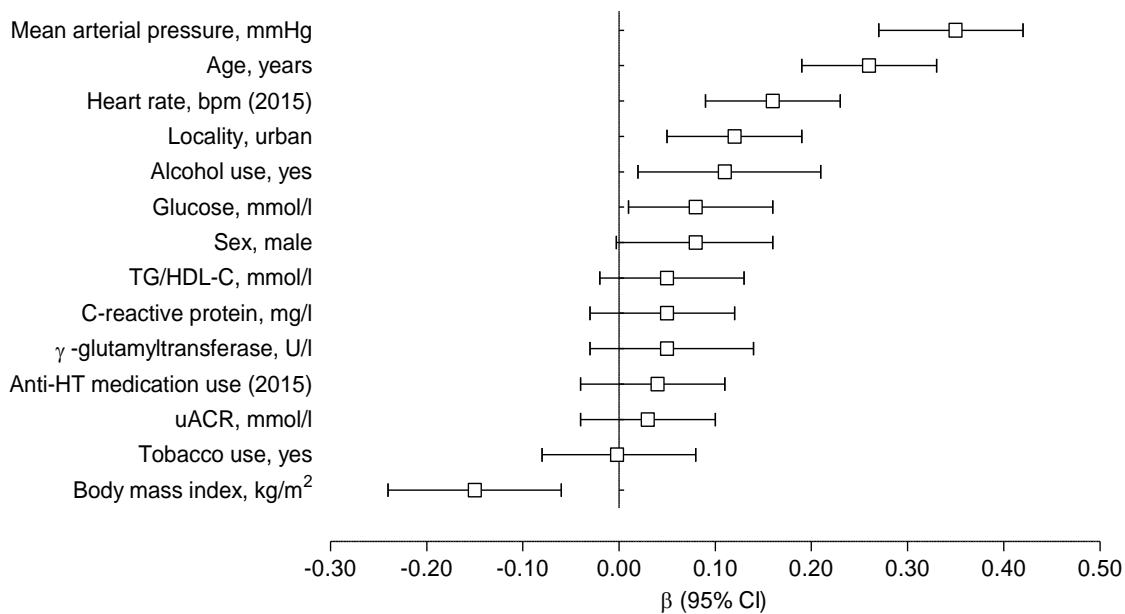


Figure 2. Multiple regression analyses in the total group with pulse wave velocity as dependent variable ($Adjusted R^2 = 0.31$). TG:HDL-C, triglyceride: high-density lipoprotein cholesterol ratio; Anti-HT, anti-hypertension; uACR, urinary albumin to creatinine ratio.

Table 3. Independent associations of 2015 PWV with baseline covariates in men and women

Dependent variable: Carotid-femoral pulse wave velocity, m/s				
	β (95% CI)	p	β (95% CI)	p
	Men (N= 213)		Women (N=437)	
Adjusted R²	0.32		0.28	
Age, years	0.23 (0.09; 0.36)	0.001	0.27 (0.18; 0.37)	<0.001
Locality, urban	0.11 (-0.02; 0.24)	0.088	0.13 (0.04; 0.23)	0.007
Body mass index, kg/m ²	-0.14 (-0.30; 0.02)	0.097	-0.12 (-0.23; -0.02)	0.021
Heart rate 2015, bpm	0.15 (0.02; 0.27)	0.024	0.19 (0.09; 0.28)	<0.001
MAP 2015, mmHg	0.44 (0.32; 0.57)	<0.001	0.30 (0.21; 0.40)	<0.001
TG:HDL-C, mmol/l	-0.02 (-0.17; 0.13)	0.79	0.10 (0.004; 0.20)	0.042
Glucose, mmol/l	0.12 (-0.01; 0.25)	0.074	0.07 (-0.03; 0.16)	0.18
Interleukin-6, pg/ml	0.11 (-0.03; 0.24)	0.14	0.03 (-0.07; 0.12)	0.56
uACR, mmol/ml	-0.05 (-0.19; 0.09)	0.48	0.07 (-0.02; 0.17)	0.13
γ -glutamyltransferase, U/l	0.11 (-0.04; 0.25)	0.15	0.02 (-0.09; 0.13)	0.71
Alcohol use, yes	0.11 (-0.07; 0.29)	0.24	0.11 (-0.005; 0.23)	0.061
Tobacco use, yes	0.002 (-0.18; 0.19)	0.98	-0.02 (-0.12; 0.08)	0.74
Anti-HT med use 2015	0.03 (-0.10; 0.16)	0.61	0.03 (-0.06; 0.13)	0.52

Data expressed as beta-values and 95% confidence intervals, p-values obtained with multiple regression analyses. PWV, pulse wave velocity; MAP, mean arterial pressure; TG:HDL-C, triglyceride/high-density lipoprotein cholesterol ratio; uACR, urinary albumin to creatinine ratio; Anti-HT med use 2015, anti-hypertension medication use 2015.

Sensitivity analyses

We repeated the multiple regression analyses but replaced self-reported alcohol with GGT to determine whether an independent association between PWV and GGT exists, as was previously found for SBP [18]. By doing so GGT associated positively with PWV in the total group (adjusted R²=0.31; β =0.09, P=0.023) but not in the separate analyses for men or women. Due to the known association between GGT and iron [19], we also included ferritin in the model, but no significant results were found for ferritin (N=357, adjusted R²=0.30, β =-0.02, P=0.65). Due to literature indicating an association between PWV and inflammation [20], we also tested the substitution of CRP with IL-6 in the model, but the results remained unchanged (adjusted R²=0.31, β =0.04, P=0.23). Similarly, the inclusion of baseline and/or follow-up anti-inflammatory medication in the model did not affect the original findings (adjusted R²=0.31, β =0.03, P=0.48).

Discussion

To the best of our knowledge, this study presents the first 10 year longitudinal findings on potential contributors towards large artery stiffness in a black South African population. We found that health behaviours related to excessive alcohol use including an urban setting, high plasma glucose and low BMI were predictive of PWV independently of age and blood pressure after 10 years.

Our results support the fact that alcohol abuse is a major health problem in Southern Africa [21]. Previously, we found that self-reported alcohol use predicted a 5-year change in blood pressure better than biochemical markers in the same population [22]. Therefore, we regarded self-reported alcohol use as a reliable measure of alcohol intake in this population. In our study we have also confirmed our findings when substituting self-report with GGT [23].

The relationship between arterial stiffness and alcohol use is, however, controversial. Moderate alcohol intake may decrease PWV, while alcohol abuse leads to increases in arterial stiffness [24]. The precise mechanism by which alcohol affects the arterial wall is not clear, but several possibilities exist. Alcohol decreases magnesium levels in the body, which may lead to an increase in vascular tone [25]. Both acute and chronic alcohol intake influence Na^+/K^+ -ATPase activity, which may alter both vascular tone and renal sodium handling [26]. Ethanol, the main ingredient of alcohol, may regulate collagen and elastin content in myocytes via a pathway involving matrix metalloproteinases [27]. Furthermore, a metabolic product of ethanol breakdown, acetaldehyde, is oxidised to acetate which can lead to the generation of reactive oxidative species [28]. Oxidative stress may contribute to elastin rupture and collagen overproduction in the arterial wall, leading to arterial stiffness [29]. GGT, a marker of alcohol intake and liver function [30], is also associated with oxidative stress [19] and may represent another mechanism by which alcohol damages the arterial wall. As mentioned, we found that GGT significantly predicts PWV when self-reported alcohol use was removed from the model. This is in line with previous results by our group,

which indicated a positive association between large artery stiffness and GGT in young, healthy black adults [31].

Awareness of alcohol-related problems and of the need for action in South Africa has improved, but the implementation of preventive strategies needs more attention [21]. New alcohol legislation has been proposed, which includes raising the legal drinking age to 21 and banning alcohol advertisements [32, 33]. Interventions such as tax increases for alcohol may be effective, however, home-brewed alcohol in poor communities is common and therefore a different strategy may be required to reduce alcohol abuse in South Africa [34, 35].

An urban setting was associated with future arterial stiffness in this black population. Nearly two decades ago, urbanisation already associated with changes in traditional lifestyles and health behaviour [36], as well as the manifestation of hypertension [37] in black South Africans. Furthermore, urbanisation gives rise to psychosocial stressors due to poverty and unemployment [35], which could manifest in changed health behaviours, such as anxiety, smoking and alcohol abuse [38]. A consequence of urbanisation may be increased sympathetic nerve activity [35, 37], translating into a higher heart rate [39]. Elastin fracture in the arterial wall may provide an explanation for the association between heart rate and arterial stiffness [40].

The association of glucose with future stiffness confirms previous reports [10, 41]. Chronically increased plasma glucose reduces the elastic properties of large arteries [41], confirmed by findings that diabetes mellitus and impaired glucose tolerance are associated with an increased risk for stroke, heart failure and myocardial infarction [41]. This may be due to mechanisms such as glycation of proteins and formation of advanced glycation end products (AGEs) [42], which are known to increase arterial wall stiffness by forming cross-links in collagen fibres and promoting vasoconstriction by decreasing the bioavailability of the vasodilator nitric oxide [43]. With obesity being a major cardiovascular risk factor and a

health burden in South Africa [44], especially in women [45], the negative association between BMI and PWV was unexpected. Our group found a similar negative association between BMI and PWV in two different population samples, including young [31] and older black South African adults [46]. Low BMI in blacks may be the result of unhealthy behaviours, such as excessive alcohol consumption and tobacco use [47], which in turn has a detrimental effect on vascular health. Other studies also indicated an inverse association between BMI and alcohol consumption [48, 49].

In our population, with a mean age of 61 years, 49% was hypertensive, which reflects the high cardiovascular risk in this general population sample. A quarter of the PWV measurements exceeded 10 m/s, which is the international cut-off value for PWV [50], indicating that at least 25% of our participants are at increased risk for cardiovascular and all-cause mortality [50, 51]. However, this reference value was based mostly on populations from European descent and needs validation in African populations.

Factors such as inflammation, endothelial dysfunction and smoking were expected to predict arterial stiffness after 10 years, but were not significant. More research is needed to elucidate the role of inflammation and endothelial dysfunction in arterial stiffening in this black population. Although we did not find any of the lipids to be predictors of PWV, we do see a concurrent rise in HDL-C, TG and the ratio of the two variables along with the increase in PWV, possibly indicating early atherosclerotic vascular injury. Heavy smoking was one of the predictors of arterial stiffness in European and US longitudinal studies [9, 52], but we and others [10, 53] found no association between PWV and tobacco use. Smoking remains a well-known harmful cardiovascular risk factor [54], but our findings suggest that other factors may have a greater effect on arterial stiffness in this population, thus potentially masking the effect of tobacco use.

Strengths and limitations

Our study included populations from specific urban and rural areas in the North West Province of South Africa, and may not be representative of the whole population. However, we focused on the understudied black population including longitudinal data on variety of health behaviours and arterial stiffness, using controlled conditions and well-equipped research facilities. Carotid-femoral PWV was measured at follow-up only, which prevents firm conclusions regarding change over time. Although the results were consistent after several adjustments, we cannot exclude residual confounding.

Conclusion

Health behaviours associated with alcohol abuse such as an urban setting, elevated plasma glucose and low BMI predict arterial stiffness independently of age and blood pressure in a black South African population over 10 years. These findings strongly support ongoing initiatives in South Africa to stem alcohol abuse.

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SUPPLEMENTARY INFORMATION

Supplementary Table S1. Ten year follow up characteristics of a black South African population, stratified by

	1 st tertile (n = 214)	2 nd tertile (n = 215)	3 rd tertile (n = 221)	P-trend
Pulse wave velocity ranges, m/s	(< 8.0 m/s)	(8.0-9.4 m/s)	(≥ 9.5 m/s)	
Age, years	58.6 ± 8.70	60.4 ± 8.84	65.1 ± 9.63	<0.001
Gender, men (%)	50/214 (23.4)	68/215 (31.6)	95/221 (43.0)	<0.001
Locality, rural (%)	139/214 (65.0)	118/215 (54.9)	105/221 (47.5)	0.001
<i>Anthropometric measures</i>				
Body mass index (kg/m ²)	27.6 ± 7.16	26.2 ± 6.94	24.4 ± 6.46	<0.001
Waist circumference (cm)	90.0 ± 14.2	89.7 ± 15.7	86.3 ± 13.9	0.014
Height (cm)	1.58 ± 0.07	1.59 ± 0.08	1.61 ± 0.08	<0.001
<i>Cardiovascular measures</i>				
SBP (mmHg)	124 ± 21.5	135 ± 20.9	147 ± 27.0	<0.001
DBP (mmHg)	80.7 ± 13.1	87.2 ± 12.6	89.7 ± 13.6	<0.001
Pulse pressure (mmHg)	43.6 ± 13.1	48.1 ± 13.0	57.6 ± 19.3	<0.001
Heart rate (bpm)	69.8 ± 12.9	72.7 ± 13.4	74.9 ± 15.5	<0.001
MAP (mmHg)	95.3 ± 15.2	103 ± 14.6	109 ± 16.8	<0.001
cfPWV (m/s)	7.02 ± 0.72	8.68 ± 0.42	11.2 ± 1.51	<0.001
<i>Biochemical measures</i>				
Total cholesterol, mmol/l	4.42 (4.27; 4.59)	4.48 (4.32; 4.65)	4.51 (4.36; 4.68)	0.74
LDL-C, mmol/l	2.83 ± 1.07	2.80 ± 1.13	2.72 ± 1.02	0.60
HDL-C, mmol/l	1.34 ± 0.58	1.36 ± 0.55	1.47 ± 0.63	0.06
Triglycerides, mmol/l	1.09 (1.02; 1.17)	1.21 (1.13; 1.30)	1.13 (1.06; 1.21)	0.10
TG/HDL-C ratio	0.88 (0.80; 0.98)	0.95 (0.86; 1.05)	0.83 (0.75; 0.92)	0.17
Glucose, mmol/l	5.11 (4.92; 5.30)	5.37 (5.17; 5.57)	5.58 (5.38; 5.80)*	0.004
HbA1c, %	5.75 (5.61; 5.89)	5.89 (5.75; 6.03)	5.87 (5.73; 6.01)	0.32
<i>Inflammatory markers</i>				
C-reactive protein, mg/l	3.24 (2.73; 3.85)	2.98 (2.51; 3.53)	3.75 (3.17; 4.44)	0.16
<i>Liver enzymes</i>				
GGT, U/l	29.3 (26.0; 33.1)	37.2 (32.9; 42.0)*	42.7 (37.9; 48.1)*	<0.001
AST (U/L)	20.8 (19.6; 22.1)	22.7 (21.3; 24.1)	23.1 (21.8; 24.6)*	0.039
ALT (U/L)	15.0 (14.1; 16.0)	16.0 (15.0; 17.0)	15.4 (14.5; 16.4)	0.41
AST/ALT ratio	1.38 (1.33; 1.45)	1.42 (1.36; 1.48)	1.50 (1.44; 1.57)	0.027
<i>Renal function</i>				
CrCl, ml/min	97.3 (92.2; 103)	93.0 (88.1; 98.2)	78.2 (74.1; 82.4)*	<0.001
uACR	1.25 (1.08; 1.46)	1.69 (1.45; 1.98)*	1.83 (1.57; 2.14)*	0.001
<i>Health behaviour</i>				
Tobacco use, n (%)	71/208 (34.1)	83/212 (39.2)	98/212 (46.2)	0.039
Alcohol use, n (%)	42/208 (20.2)	63/212 (29.7)	84/212 (39.6)	<0.001
<i>Medication use</i>				
Anti-hypertensive, n (%)	72/212 (34.0)	66/213 (31.0)	82/218 (37.6)	0.35
Anti-inflammatory, n (%)	10/212 (4.72)	16/213 (7.51)	13/218 (5.96)	0.48
Anti-diabetic, n (%)	7/212 (3.30)	10/213 (4.69)	21/218 (9.63)	0.014
Statins, n (%)	5/212 (2.36)	12/213 (5.63)	10/218 (4.59)	0.23

Data are arithmetic mean ± standard deviation, geometric mean (5th, 95th percentiles), or % of n. P-values for the comparison between groups were obtained with analysis of variance and Chi-square tests. p≤0.05 were regarded as statistically significant. *Statistically different from the first PWV tertile. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; LDL, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; TG: HDL-C, triglyceride: high density lipoprotein-cholesterol ratio; HbA1c, glycated haemoglobin; GGT, gamma-glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CrCl, creatinine clearance; uACR, urinary albumin to creatinine ratio; tobacco and alcohol use are self-reported.

CHAPTER 5

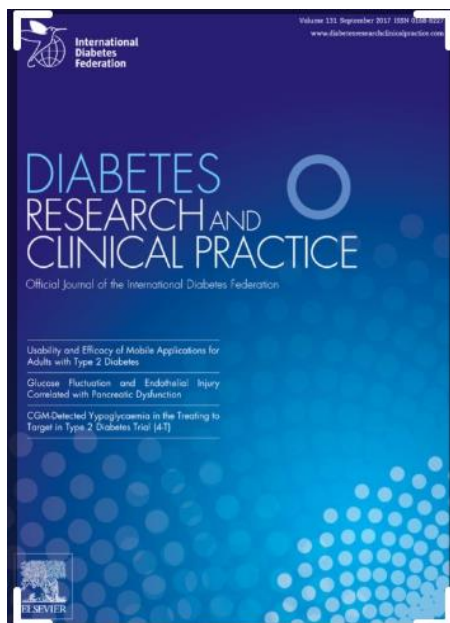
**Evaluating several biomarkers as predictors of
aortic stiffness in young and older Africans, not
consuming alcohol**

Evaluating several biomarkers as predictors of aortic stiffness in young and older Africans, not consuming alcohol

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(Please note some of the format was changed to ensure uniformity throughout the thesis.)

Abstract

Aims: Black populations from sub-Saharan Africa have a high prevalence of cardiovascular disease, which places significant strain on public health systems. Aortic stiffness is a prominent risk factor for cardiovascular disease development. We reported earlier that excessive alcohol use predicts aortic stiffness. However, we require a better understanding of other biomarkers involved in stiffness development, beyond alcohol use. Therefore, we determined which biomarkers (metabolic, inflammatory, endothelial activation and oxidative stress) relate to aortic stiffness in young and older black South Africans, self-reporting no alcohol-use.

Methods: We included cross-sectional data from young (aged 24.7 ± 3.24 years) black adults participating in the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and HyperTension (African-PREDICT) study (N=216), and five-year follow-up data from older (aged 61.6 ± 9.77 years) black adults (N=322) participating in the South African leg of the Prospective Urban and Rural Epidemiology study, conducted in the North West Province (PURE-SA-NWP). We excluded all participants self-reporting alcohol use. We determined biomarkers from blood samples, and measured carotid-femoral pulse wave velocity (PWV).

Results: Of all biomarkers investigated in multivariable-adjusted regression analyses, only plasma glucose ($R^2=0.24$, $\beta=0.21$, $p<0.001$) and glycated haemoglobin ($R^2=0.22$, $\beta=0.17$, $p=0.002$) independently predicted PWV five years later in older adults. We found no other associations in young or older black adults.

Conclusion: Dysglycaemia independently predicted aortic stiffness after five years in older black adults. Life-course management of body weight and sugar intake are important in preventing early vascular ageing and subsequent cardiovascular disease development in Africa.

Key words: aortic stiffness, glucose, black, Africans, longitudinal, predictors

1. Introduction

Stiffness of the large arteries, which is reflected by carotid-femoral pulse wave velocity (PWV) [1], predicts cardiovascular events and mortality better than blood pressure [2, 3]. The high incidence of cardiovascular disease in the black population of sub-Saharan Africa [4] places additional strain on the public health systems that also have to deal with the high prevalence of communicable diseases such as HIV and tuberculosis [5]. Therefore, the identification of early predictive risk factors for arterial stiffness is an important public health target, as it will help to direct the planning and implementation of prevention strategies that specifically address the development of cardiovascular disease in Africa.

Arterial stiffness is influenced by age, blood pressure and the properties of the arterial wall [6]. However, traditional cardiovascular risk factors such as dyslipidaemia, hyperglycaemia [7], inflammation [8], endothelial function and oxidative stress [9] are also known to modulate arterial structure and function by contributing to endothelial dysfunction and vascular remodelling [10], and by influencing the release of vasoactive substances in the large arteries [11].

Previously, we found alcohol use to be the main predictor of aortic stiffness over 10 years in black adults (mean age 65 years) [12] and that the liver enzyme, gamma-glutamyltransferase, independently associated with arterial stiffness in young black adults (mean age 24 years) [13]. However, in a country where excessive alcohol use is common and poses a significant threat to cardiovascular health [12, 14], the associations of arterial stiffness with other important vascular biomarkers may be masked by a cardiometabolic profile associated with alcohol use. We therefore excluded alcohol users from the present study and determined whether biomarkers known to modulate arterial structure and function, such as metabolic, inflammatory and endothelial activation markers, as well as oxidative stress, relate to aortic stiffness in young and older black South Africans who self-reported no alcohol use.

2. Methods

To address the aims of this sub-study, we included data from two studies conducted in South Africa, namely the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and HyperTension (African-PREDICT) study, and the South African leg of the international Prospective Urban and Rural Epidemiology study, conducted in the North West Province (PURE-SA-NWP). The detailed methods of the two studies are described elsewhere [12, 13], but herewith a succinct description of aspects relevant to this paper.

2.1 Ethical considerations

The Health Research Ethics Committee of the North-West University gave approval for the African-PREDICT Study, the PURE-SA baseline (2010) and follow-up (2015) study, as well as this sub-study. All procedures comply with the Declaration of Helsinki. Before measurements commenced, all procedures were explained to the participants, after which written informed consent was given.

2.3 The African-PREDICT Study

2.3.1 Research design

Young (20-30 years of age), apparently healthy black and white men and women were included in the study after an initial screening day. Participants whose mean blood pressure out of 4 measurements ≥ 140 mmHg and/or ≥ 90 mmHg, who were HIV infected, previously diagnosed with a chronic disease, pregnant or breastfeeding were excluded. This sub-study includes cross-sectional analysis of the first 591 black participants. Participants with incomplete PWV data (n=91), incomplete alcohol use data (n=6) and who reported using alcohol (n=278), were excluded. The study population is shown in Figure 1.

2.4 The PURE-SA-NWP study

2.4.1 Research design

Baseline data collection took place in the North West Province of South Africa in 2010, with a 5 year follow-up conducted in 2015. Baseline data collection included 1288 black men and

women, aged 38 to 98 years. Pregnant and lactating women were excluded. Longitudinal data were available for 852 participants. Participants with incomplete PWV data (n=119), those who were HIV-infected (n=130) and those who self-reported alcohol use (n=281) were excluded. Included for this study are 322 participants (Figure 1).

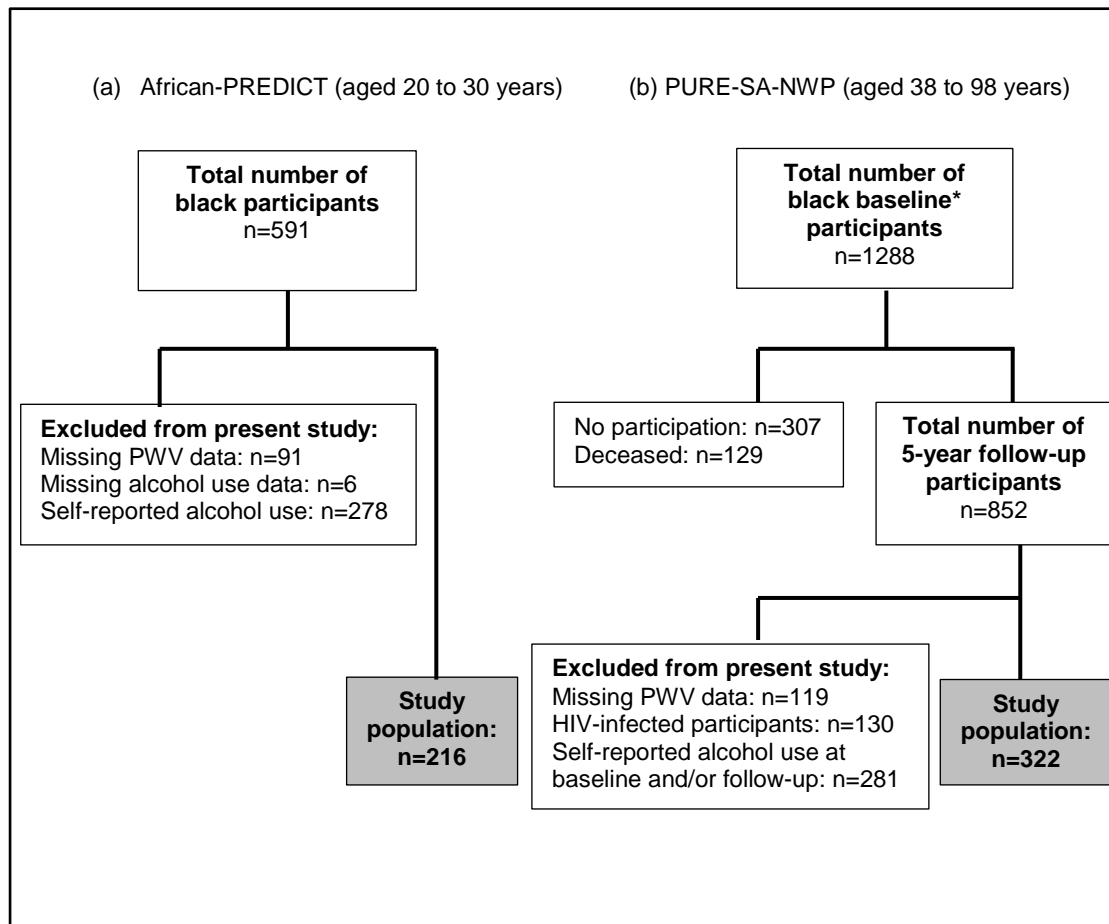


Figure 1. Study population: (a) Young black African-PREDICT participants; (b) Older black PURE-SA-NWP participants. *Baseline data collection for this sub-study refers to the 2010 data collection phase of the South African PURE-NWP study.

2.5 Questionnaires

The African-PREDICT participants completed a demographic and general health questionnaire, and the PURE-SA-NWP participants completed structured socio-demographic and lifestyle questionnaires. In both studies, alcohol and tobacco use were reported as a yes or no answer.

2.6 Anthropometric measurements

For African-PREDICT, height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany), weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany) and waist circumference (Lufkin Steel Anthropometric Tape (W606PM), Lufkin, Apex, USA) were measured. For PURE-SA-NWP, baseline and follow-up height (Invicta Stadiometer IP 1465, Leicester, UK; Leicester height measure, Seca, Birmingham, UK), weight (Precision Health Scale, A & D Company, Japan) and waist circumference (WC) (Holtain unstretchable metal tape for baseline and Steel tape, Lufkin, Cooper Tools, Apex NC, USA for follow-up) were measured. Standardised methods and calibrated instruments were used in both studies [15]. Body mass index (BMI) was calculated.

2.7 Cardiovascular measurements

After a 10 minute rest period, duplicate office blood pressure measurements (Dinamap® ProCare 100 Vital Signs Monitor, GE Medical Systems, Milwaukee, USA) were made on both arms of African-PREDICT participants with 5 minute intervals. The participant was in a seated position. For the PURE-SA-NWP participants, the validated OMRON HEM-757 and OMRON M6 (Omron Healthcare, Kyoto, Japan) devices were used to measure duplicate sitting blood pressure after a 10 minute rest at baseline and follow-up, respectively. Mean arterial pressure (MAP) was calculated for both studies.

For both studies, the carotid-femoral PWV was determined in duplicate with the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) with the participant in supine position. The transit-distance method was used to measure PWV along the descending thoraco-abdominal aorta. The 80% rule was applied for the calculation of the distance used.

2.8 Biological sampling

Blood samples were taken from the antebraial vein of each fasting participant. Using standardised methods to prepare serum and plasma in the laboratory, it was stored at -80°C until analysis. In the cases of blood collection in a rural area as part of the PURE-SA-NWP study, samples were snap frozen and stored at -20°C for not more than 5 days. The samples were then transported to the laboratory and stored at -80°C.

2.9 Biochemical analysis for African-PREDICT

An enzymatic colorimetric method (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) was used to determine serum total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), gamma-glutamyltransferase (GGT) and creatinine.

Serum high sensitivity C-reactive protein (CRP), fasting glucose in fluoride plasma, as well as glycosylated haemoglobin (HbA1c) from EDTA whole blood, were determined with the Cobas Integra 400 Roche® Clinical System (Roche Diagnostics, Indianapolis, IN). Interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1) were determined with high sensitivity Quantikine ELISA kits (R&D systems, Minneapolis, MN USA). Serum peroxides, representing reactive oxygen species (ROS), were determined with high-throughput spectrophotometric assay and analysed on a Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA).

2.10 Biochemical analysis for PURE-SA-NWP

We analysed baseline and follow-up serum TC, HDL-C, LDL-C, TG, creatinine, CRP, glucose, and GGT with the Cobas Integra 400 plus Roche Clinical System, and HbA1c levels with the D-10 Haemoglobin testing system (Bio-Rad #220-0101). Baseline IL-6 was determined with an electrochemiluminescence immunoassay (Elecsys 2010, Roche, Basel, Switzerland). Baseline ICAM-1 and VCAM-1 levels were determined in serum with sandwich ELISAs (Human sICAM-1 and human sVCAM-1 assay, IBL, Hamburg, Germany). Baseline

serum peroxides (ROS), were determined with high-throughput spectrophotometric assay [Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA)].

Creatinine clearance (CrCl) [16] and the estimated glomerular filtration rate [eGFR CKD-EPI(Cr)] in ml/min/1.73m² were calculated [17] for both studies.

2.11 Statistical analyses

We used Statistica version 13 (StatSoft, Inc., Tulsa, OK, USA) and prepared graphs using GraphPad Prism Version 5.03 (GraphPad Software Inc., California, USA). Descriptive statistics including the mean and standard deviation were performed on data with a normal distribution. Abnormally distributed variables were logarithmically transformed and the central tendency and spread described as the geometric mean and the 5th and 95th percentiles. For the PURE-SA-NWP study, the 5-year changes in continuous variables were determined with dependent t-tests, and for categorical variables, with the Wilcoxon test. We performed multi-variate linear regression analysis with PWV as the dependent variable to test associations with various markers in young and older black adults who self-reported not using alcohol. Each model included one main independent variable (an inflammatory, endothelial activation, oxidative stress or metabolic marker) and a set model of covariates listed in the footnote in Table 2. In addition, we used analysis of covariance (ANCOVA) to determine the differences in PWV between tertiles of glucose, while adjusting for age, sex, BMI and MAP.

3. Results

The characteristics of the young African-PREDICT and older PURE-SA-NWP populations (both for baseline and 5-year follow up) are shown in Table 1. The young adults (35.2% men) had a mean age of approximately 25 years. Hypertension prevalence based on clinic blood pressure on the day of data collection was 10.2%. When comparing the older PURE-SA-NWP participants at baseline and five years later, they had an increased waist circumference ($p<0.001$) and a higher heart rate ($p<0.001$). Diastolic blood pressure and mean arterial pressure were lower at follow-up ($p<0.001$). All lipids, except for triglycerides, decreased significantly over 5 years, but the TG/HDL-C ratio showed an increasing trend ($p=0.058$). Serum glucose increased significantly with 0.21 mmol/l ($p=0.008$), but HbA1c decreased by 0.27% (<0.001). Levels of GGT also decreased over 5 years ($p=0.005$). Fewer participants smoked at follow-up (39.9% vs. 20.8%, $p<0.001$), while more participants used anti-hypertensive medication (30.5% vs. 39.1%, $p=0.011$) and statins ($p=0.002$).

Table 1. Profile of young (African-PREDICT) and older (PURE-SA-NWP) black South Africans who self-reported no alcohol use

	Young black adults (n=216)	Older black adults (n=322)		p
	African-PREDICT	PURE-SA-NWP Baseline	PURE-SA-NWP 5-year Follow up	
Age, years	24.7 ± 3.24	56.7 ± 9.77	61.6 ± 9.77	<0.001
Gender, men (%)	76/216 (35.2)	56/322 (17.4)	56/322 (17.4)	-
Locality, urban (%)	216/216 (100)	125/322 (38.8)	125/322 (38.8)	-
Hypertension, n(%) ^a	22/216 (10.2)	202/322 (62.7)	217/320 (67.8)	0.13
Diabetes, n(%) ^b	0/63 (0.00)	71/315 (22.5)	55/293 (18.7)	0.048
<i>Anthropometric measures</i>				
Body mass index, kg/m ²	25.0 ± 5.74	28.1 ± 6.87	28.2 ± 6.85	0.17
Waist circumference, cm	77.9 ± 11.4	84.2 ± 13.22	91.9 ± 14.69	<0.001
Weight, kg	66.6 ± 15.0	70.3 ± 17.2	70.4 ± 17.1	0.76
Height, m	1.63 ± 0.09	1.59 ± 0.07	1.58 ± 0.08	<0.001
<i>Cardiovascular measures</i>				
SBP, mmHg	116 ± 10.6	136 ± 23.5	134 ± 24.0	0.15
DBP, mmHg	78.0 ± 8.28	87.8 ± 12.8	84.9 ± 13.7	<0.001
Pulse pressure, mmHg	37.1 ± 7.77	48.3 ± 16.3	49.4 ± 15.4	0.19
Heart rate, bpm	65.2 ± 10.6	62.1 ± 15.0	70.3 ± 12.6	<0.001
MAP, mmHg	93.3 ± 7.78	103 ± 15.4	101 ± 16.2	0.002
PWV, m/s	6.26 ± 0.82	-	8.58 ± 1.87	-
<i>Biochemical measures</i>				
Total cholesterol, mmol/l	3.67 (2.65; 4.90)	5.03 (3.44; 6.98)	4.56 (3.01; 6.63)	<0.001
LDL-cholesterol, mmol/l	2.42 (1.42; 3.84)	3.28 (1.82; 5.16)	2.87 (1.51; 4.80)	<0.001
HDL-cholesterol, mmol/l	1.17 (0.81; 1.72)	1.27 (0.76; 2.04)	1.18 (0.74; 1.96)	<0.001
Triglycerides, mmol/l	0.65 (0.35; 1.21)	1.17 (0.59; 2.33)	1.16 (0.61; 2.66)	0.65
TG/HDL-C ratio	0.55 (0.28; 1.22)	0.92 (0.34; 2.66)	0.99 (0.35; 3.34)	0.058
Glucose, mmol/l	4.53 (3.31; 5.56)	5.23 (4.14; 8.11)	5.44 (4.17; 8.61)	0.008
HbA1c, % ^c	5.59 [38] (5.10; 6.09)	6.25 [45] (5.30; 9.60)	5.98 [42] (5.00; 8.80)	<0.001
<i>Inflammatory markers</i>				
C-reactive protein, mg/l	1.32 (0.14; 11.9)	3.57 (0.36; 28.4)	3.42 (0.51; 18.1)	0.31
Interleukin-6, pg/ml	1.32 (0.51; 5.25)	2.97 (0.75; 9.57)	-	-
<i>Oxidative stress</i>				
ROS, U ^d	198 (124; 337)	250 ± 74.4	-	-
GGT, U/l	21.5 (10.1; 54.8)	28.5 (11.7; 84.5)	25.9 (11.1; 80.8)	0.005
<i>Endothelial function</i>				
ICAM-1, ng/ml	125 (25.4; 316.4)	273 (148; 422)	-	-
VCAM-1, ng/ml	656 (401; 1002)	740 (440; 1547)	-	-
<i>Renal function</i>				
CrCl, ml/min	124 (86.7; 193)	94.8 (54.5; 180)	94.5 (48.9; 180)	0.60
eGFR, ml/min/1.73m ²	134 (104; 155)	106 ± 18.6	106 ± 20.1	0.33
<i>Health behaviour</i>				
Tobacco use, n (%)	20/216 (9.26)	126/316 (39.9)	67/322 (20.8)	<0.001
<i>Medication use</i>				
Anti-hypertensive, n (%)	0/216 (0.00)	98/321 (30.5)	125/320 (39.1)	0.011
Anti-diabetic, n (%)	0/216 (0.00)	16/321 (4.98)	23/320 (7.19)	0.14
Anti-inflammatory, n (%)	1/216 (0.46)	24/321 (7.48)	18/320 (5.63)	0.35
Statins, n (%)	0/216 (0.00)	2/321 (0.62)	18/320 (5.63)	0.002

Data are arithmetic mean ± standard deviation, geometric mean (5th; 95th percentiles), or % of n.

SBP, systolic blood pressure; DBP, diastolic BP; MAP, mean arterial pressure; PWV, pulse wave velocity; LDL, low density lipoprotein; HDL, high density lipoprotein; TG: HDL-C, triglyceride: high density lipoprotein-cholesterol ratio; HbA1c, glycated haemoglobin; ROS, reactive oxygen species; GGT, gamma-glutamyltransferase; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular adhesion molecule-1; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; tobacco use is self-reported. ROS for African-PREDICT: n= 61, Glucose for African-PREDICT: n=161.

^aHypertension prevalence: all baseline or follow-up anti-hypertension medication users and/or participants with a clinic blood pressure measurement of SBP ≥140 mmHg and/or DBP ≥90 mmHg.

^bDiabetes prevalence: all baseline or follow-up anti-diabetes medication users and/or participants with glucose ≥ 7.0 mmol/l and/or HbA1c ≥ 6.5 %.

^cHbA1c given as mmol/mol in square brackets (IFCC)

^dROS units: 1.0 mg H₂O₂/l.

In figure 2, we plotted PWV against age for the young and older black populations, while adjusting for MAP. In the African-PREDICT participants, all within a narrow age bracket of 20-30 years, no trend for PWV with age was found. For the PURE-SA-NWP participants, a significant trend for increased PWV was evident from age 40 to ≥ 85 years (p for trend < 0.001).

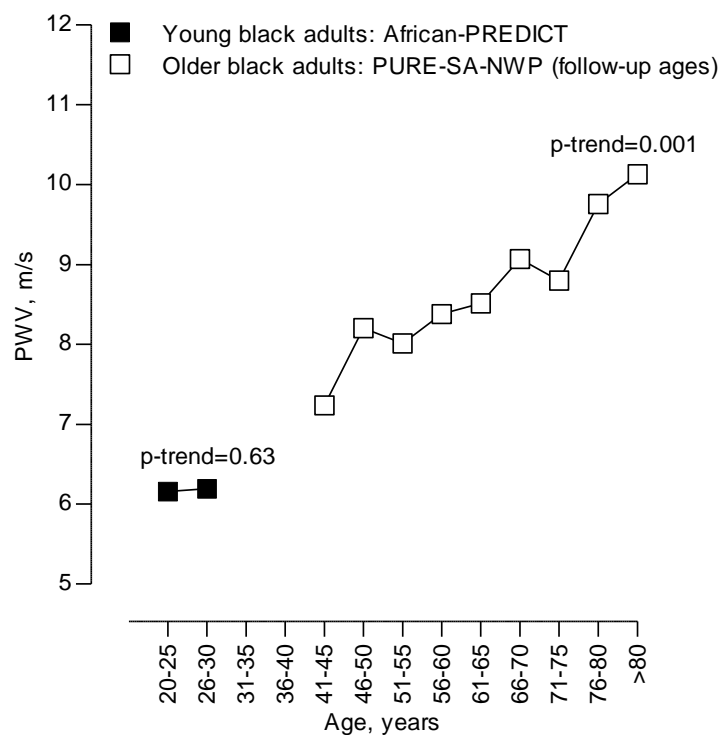


Figure 2. Pulse wave velocity plotted against age, adjusted for mean arterial pressure for African-PREDICT and PURE-SA-NWP participants who reported no alcohol use (cross-sectional). PWV, pulse wave velocity.

To identify associations with PWV for the young and older participants, as well as predictors of PWV after 5 years in the older group, we performed multivariate-adjusted regression analyses with PWV as the dependent variable in the young and older group (Table 2). For the young group, apart from significant independent associations with MAP (all $p < 0.001$), sex ($p < 0.002$) and BMI (negatively) ($p \leq 0.042$) (shown in Supplementary Table S1), we found no significant associations with metabolic, inflammatory, endothelial activation or oxidative

stress markers. In the older group, apart from significant associations with age, BMI (negatively), MAP and heart rate (all $p \leq 0.028$), PWV associated significantly with baseline glucose ($R^2=0.24$, $\beta=0.21$, $p<0.001$) and HbA1c ($R^2=0.22$, $\beta=0.17$, $p=0.002$). The full models with glucose and HbA1c, as main independent variable, are shown in Supplementary Table S1.

TABLE 2. Independent associations of PWV with vascular biomarkers in young (African-PREDICT) and older (PURE-SA) black South Africans who self-reported no alcohol use

Dependent variable: Carotid-femoral pulse wave velocity, m/s									
Main independent variable	African-PREDICT					PURE-SA (baseline covariates)			
	<i>n</i>	<i>R</i> ²	β (95% CI)	<i>p</i>		<i>n</i>	<i>R</i> ²	β (95% CI)	<i>p</i>
<i>Inflammatory markers</i>									
CRP, mg/l	182	0.34	-0.06 (-0.21; 0.10)	0.47		310	0.20	-0.05 (-0.16; 0.07)	0.43
IL-6, pg/ml	192	0.34	-0.07 (-0.20; 0.07)	0.34		310	0.20	-0.07 (-0.18; 0.04)	0.19
<i>Endothelial activation markers</i>									
ICAM-1, ng/ml	202	0.34	-0.03 (-0.15; 0.09)	0.62		311	0.20	-0.03 (-0.13; 0.08)	0.61
VCAM-1, ng/ml	202	0.35	0.09 (-0.03; 0.20)	0.14		310	0.20	0.02 (-0.09; 0.12)	0.77
<i>Oxidative stress markers</i>									
ROS, U ^a	61	0.29	-0.16 (-0.44; 0.12)	0.28		315	0.21	0.07 (-0.04; 0.18)	0.21
GGT, U/l	184	0.35	0.10 (-0.02; 0.22)	0.11		307	0.20	0.02 (-0.08; 0.13)	0.67
<i>Metabolic markers</i>									
Glucose, mmol/l	168	0.34	0.04 (-0.09; 0.17)	0.52		314	0.24	0.21 (0.10; 0.31)	<0.001
HbA1c, %	63	0.29	0.14 (-0.09; 0.37)	0.23		315	0.22	0.17 (0.06; 0.27)	0.003
TC, mmol/l	183	0.34	-0.06 (-0.18; 0.06)	0.34		311	0.20	-0.06 (-0.17; 0.04)	0.22
TG, mmol/l	184	0.34	-0.04 (-0.17; 0.09)	0.57		312	0.21	0.10 (-0.004; 0.20)	0.061
LDL-C, mmol/l	167	0.34	-0.06 (-0.18; 0.07)	0.40		311	0.21	-0.10 (-0.20; 0.004)	0.062
HDL-C, mmol/l	184	0.34	-0.03 (-0.16; 0.10)	0.67		311	0.20	-0.06 (-0.17; 0.05)	0.28
TG/HDL-C ratio	184	0.34	-0.02 (-0.15; 0.12)	0.81		311	0.21	0.10 (-0.01; 0.20)	0.072

Data expressed as beta-values and 95% confidence intervals, p-values obtained with multiple regression analyses. Included in each model for African-PREDICT participants is one main independent variable as listed in the table, plus the following covariates: Age, sex, socioeconomic status, body mass index, mean arterial pressure, heart rate, tobacco use. Included in each model for PURE-SA participants is one main independent variable as listed in the table, plus the following covariates: Age, sex, locality, body mass index, follow-up mean arterial pressure, follow-up heart rate, tobacco use, follow-up anti-hypertension medication use. CRP, C-reactive protein; IL-6, interleukin-6; ICAM-1, intercellular adhesion molecule-1, VCAM-1, vascular adhesion molecule-1; ROS, reactive oxygen species; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG/HDL-C, triglyceride/high-density lipoprotein cholesterol ratio.

^aROS unit: 1.0 mg H₂O₂/l

In Figure 3, we plotted PWV against tertiles of glucose in the younger and older groups after adjusting for MAP and other covariates. Baseline and follow-up tertiles of glucose were calculated for the older group. Only in the older group, PWV increased with the tertiles of baseline (p for trend=0.013) and follow-up glucose levels (p for trend=0.007).

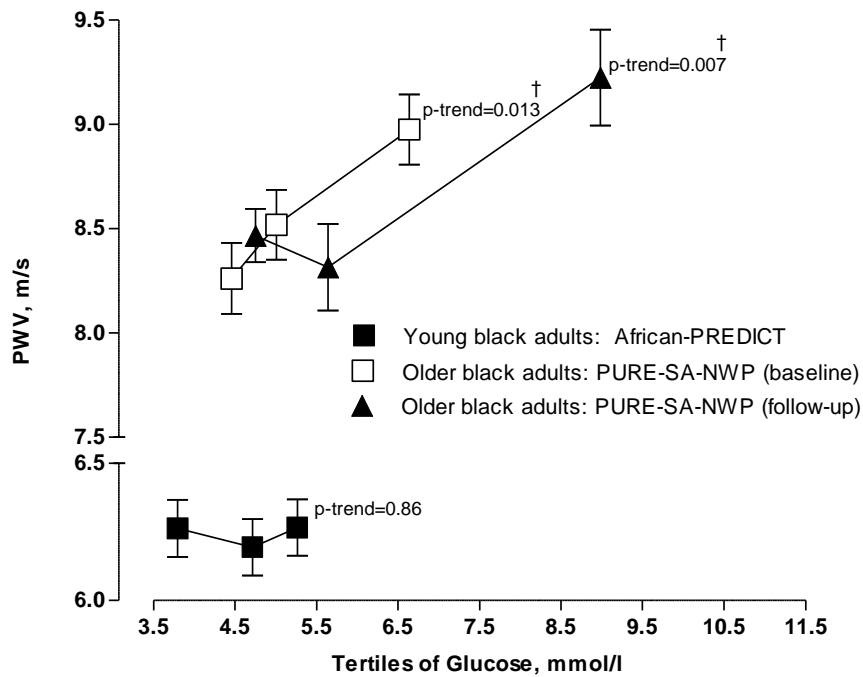


Figure 3. Pulse wave velocity plotted against tertiles of glucose, adjusted for age, sex, body mass index and mean arterial pressure for young (African-PREDICT) and older (PURE-SA-NWP) black non-alcohol users (cross-sectionally and longitudinal). PWV, pulse wave velocity. † indicates p-value (<0.05) between tertile 1 and tertile 3. *PWV for PURE-SA study obtained only at follow-up.

3.1 Sensitivity analyses

For the older group, we repeated the multiple regression analyses while excluding participants who were using diabetes medication and whose fasting glucose and HbA1c values were above the cut-off points (≥ 7.0 mmol/l for glucose and $\geq 6.5\%$ for HbA1c) [18]. Upon exclusion of participants with glucose (n=30) and HbA1c (n=74) above the cut-off points [first separately, and secondly as a combined variable with either glucose or HbA1c above cut-off, or diabetes medication use (n=77)] both glucose and HbA1c became non-significant in their separate models.

We calculated the 5-year percentage change for glucose, HbA1c, BMI, MAP and HR and included these variables in a multivariate regression model along with age, sex, locality, follow-up tobacco and anti-hypertension medication use. However, no significant result was found for 5-year percentage change in glucose ($p=0.55$) or HbA1c ($p=0.46$). We also performed cross-sectional multivariate-adjusted regression analyses with PWV as the dependent variable and 5-year follow-up covariates in the older group (Supplementary Table S2). PWV associated significantly with follow-up glucose ($R^2=0.22$, $\beta=0.15$, $p=0.005$), HbA1c ($R^2=0.22$, $\beta=0.13$, $p=0.017$) and TG/HDL-C ($R^2=0.21$, $\beta=0.11$, $p=0.048$).

4. Discussion

In the present study we aimed to identify possible predictors of aortic stiffness in the cardiovascular disease-prone black population of South Africa. From an array of well-known biomarkers, only dysglycaemia independently predicted aortic stiffness after five years in older black adults who do not consume alcohol. In young, healthy black adults who reported no alcohol consumption, aortic stiffness was within normal ranges (mean 6.26 ± 0.82 m/s) and did not associate with any biomarkers. The lack of any association in the younger black adults may be explained by the health status of this group, as individuals who participated in the African-PREDICT study were free of chronic diseases or hypertension.

To the best of our knowledge, this is the first evidence indicating the predictive value of dysglycaemia for aortic stiffness in black adults who do not consume alcohol. Our result is supported by similar findings in Dutch, Italian and South Korean populations [19-21], however, alcohol users were not excluded in these studies. Diabetes was uncommon in sub-Saharan Africa prior to the 1990s, but its prevalence is on the rise due to factors such as changing cultural practices and dietary habits, urbanisation and an aging population [22]. In addition, societal obstacles such as poor education, illiteracy, low socio-economic status and weak health systems hinder successful prevention and treatment strategies [23]. In South Africa, alcohol abuse is common and may be a coping-mechanism for the societal obstacles just mentioned [24, 25]. Self-reported alcohol use, which is thought to be a reliable indicator of alcohol intake in the PURE-SA-NWP population [26], was a significant independent predictor of arterial stiffness over 10 years in the PURE-SA-NWP population [12]. Building upon this knowledge, the results of the present study indicate that for non-alcohol users, plasma glucose poses the next biggest threat to cardiovascular health. In this population, a change from traditional lifestyles and the increased consumption of unhealthy foods may contribute to dysglycaemia. Indeed, the added sugar and sucrose-sweetened beverage intake of the PURE-SA participants doubled over five years, confirming that a nutritional

transition has reached rural South Africa [27]. Furthermore, increased sugar intake also associated with increased risk factors for non-communicable diseases [27].

Cardiovascular disease is a common cause of mortality in patients with diabetes [28]. The association of deteriorating glucose tolerance with generalised increases in aortic stiffness may explain the link between impaired glucose tolerance or diabetes and the increased risk for adverse cardiovascular events such as stroke, heart failure and myocardial infarction [21]. However, even before the clinical diagnosis of diabetes, increased fasting plasma glucose within the normal range associates with increased stiffness [7].

Elevated plasma glucose may affect arterial stiffness via several mechanisms. Chronically increased plasma glucose and hyperinsulinemia increase the activity of the renin-angiotensin-aldosterone system and the expression of angiotensin type I receptor in vascular tissue, which play a role in the increased production of reactive oxygen species, endothelial dysfunction, cardiovascular tissue fibrosis and vascular remodelling [29]. Furthermore, the non-enzymatic glycation of plasma proteins leads to the production of advanced glycation end products, which may accumulate in cells and disrupt their intra- and extracellular structure and function by cross-linking collagen fibres, proteins and possibly lipids and nucleic acids [30, 31].

Results from the Framingham Heart study indicated that diabetes is one of the biggest threats to healthy vascular ageing [32]. Type 2 diabetes prevalence among older people in Africa is estimated to be 13.7% [33]. Recent estimates on the prevalence of diabetes in black South Africans is sparse, but, half a decade ago, a diabetes prevalence of 13.1% was found among black South Africans residing in the Western Cape Province [34]. We found a baseline diabetes prevalence of 22% in the older black adults. These statistics and our results support indicate that diabetes is becoming increasingly prevalent in the black population of sub-Saharan Africa [22], which in turn may lead to increased cardiovascular morbidity and mortality in an already at-risk population. Legislation and taxation on sugar-

sweetened beverages in South Africa is likely to be implemented soon [35]. Whether this will curb the growing prevalence of obesity and diabetes remains to be seen.

4.1 Strengths and limitations

Our study population included specific urban and rural areas in the North West Province of South Africa and may not be applicable to the whole South African population. Insulin data were not available for this study, but it does provide novel longitudinal data on arterial stiffness and biomarkers of vascular function in the understudied black population. Data collection took place under controlled conditions in well-equipped research facilities. In the older group, PWV was measured at follow-up only, which prevents firm conclusions regarding change over time. Although the results were consistent after several adjustments, we cannot exclude residual confounding.

5. Conclusion

When comparing the predictive value of an array of cardiovascular biomarkers for aortic stiffness, only dysglycaemia independently predicted aortic stiffness 5 years later in black South Africans who do not consume alcohol. This prioritises the life-course management of a healthy body weight and dietary intake of refined carbohydrates and sugars for the prevention of early vascular ageing and cardiovascular disease in Africans.

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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY TABLE S1. Complete multiple regression models with glucose or HbA1c as independent variables in young (African-PREDICT) and older (PURE-SA-NWP) black South Africans who self-reported no alcohol use

Dependent variable: Carotid-femoral pulse wave velocity, m/s				
	African-PREDICT (n=213)		PURE-SA (baseline covariates) (n=322)	
Adjusted R²	0.34		0.24	
Complete model for:	<i>β</i> (95% CI)	<i>p</i>	<i>β</i> (95% CI)	<i>p</i>
<i>Independent variable: Glucose, mmol/l</i>	0.04 (-0.09; 0.17)	0.52	0.21 (0.10; 0.31)	<0.001
Age, years	0.11 (-0.02; 0.25)	0.11	0.25 (0.15; 0.35)	<0.001
Sex, male	0.26 (0.10; 0.41)	0.002	0.04 (-0.06; 0.15)	0.45
SES, high	-0.08 (-0.21; 0.05)	0.21	-	-
Locality, urban	-	-	0.09 (-0.01; 0.19)	0.089
Body mass index, kg/m ²	-0.22 (-0.37; -0.08)	0.002	-0.14 (-0.25; -0.03)	0.012
Mean arterial pressure, mmHg	0.35 (0.22; 0.48)	<0.001	0.31 (0.20; 0.41)	<0.001
Heart rate, bpm	-0.03 (-0.17; 0.10)	0.63	0.11 (0.01; 0.21)	0.028
Tobacco use, yes	0.10 (-0.03; 0.24)	0.13	-0.02 (-0.12; 0.08)	0.71
Anti-HT medication use, yes	-	-	0.05 (-0.05; 0.15)	0.32
Adjusted R²	0.29		0.22	
Complete model for:	<i>β</i> (95% CI)	<i>p</i>	<i>β</i> (95% CI)	<i>p</i>
<i>Independent variable: HbA1c, %</i>	0.14 (-0.09; 0.37)	0.23	0.17 (0.06; 0.27)	0.003
Age, years	0.12 (-0.11; 0.36)	0.30	0.25 (0.15; 0.36)	<0.001
Sex, male	0.26 (0.004; 0.52)	0.051	0.05 (-0.06; 0.16)	0.36
SES, high	-0.09 (-0.31; 0.13)	0.44	-	-
Locality, urban	-	-	0.10 (-0.01; 0.20)	0.064
Body mass index, kg/m ²	-0.27 (-0.51; -0.02)	0.042	-0.14 (-0.25; -0.03)	0.011
Mean arterial pressure, mmHg	0.34 (0.12; 0.56)	0.004	0.31 (0.21; 0.41)	<0.001
Heart rate, bpm	-0.04 (-0.27; 0.20)	0.75	0.13 (0.03; 0.23)	0.012
Tobacco use, yes	0.10 (-0.12; 0.33)	0.38	-0.04 (-0.14; 0.06)	0.46
Anti-HT medication use, yes	-	-	0.06 (-0.04; 0.16)	0.26

Data expressed as beta-values and 95% confidence intervals, p-values obtained with multiple regression analyses. SES, socio-economic status; Anti-HT, anti-hypertension.

Supplementary TABLE S2. Independent associations of PWV with follow-up vascular biomarkers in older (PURE-SA-NWP) black adults who self-reported no alcohol use

Dependent variable: Carotid-femoral pulse wave velocity, m/s					
Main independent variable	PURE-SA-NWP (5-year follow-up)				
	<i>n</i>	<i>R</i> ²	<i>β</i> (95% CI)	<i>p</i>	
<i>Inflammatory markers</i>					
CRP, mg/l	306	0.21	0.05 (-0.06; 0.16)	0.38	
GGT, U/l	302	0.20	0.05 (-0.06; 0.15)	0.39	
<i>Metabolic markers</i>					
Glucose, mmol/l	300	0.22	0.15 (0.05; 0.26)	0.005	
HbA1c, %	305	0.22	0.13 (0.02; 0.23)	0.017	
TC, mmol/l	306	0.20	-0.03 (-0.14; 0.07)	0.54	
TG, mmol/l	306	0.21	0.09 (-0.01; 0.19)	0.09	
LDL-C, mmol/l	305	0.20	-0.04 (-0.14; 0.06)	0.44	
HDL-C, mmol/l	306	0.21	-0.07 (-0.18; 0.03)	0.18	
TG/HDL-C ratio	307	0.21	0.11 (0.001; 0.21)	0.048	

Data expressed as beta-values and 95% confidence intervals, p-values obtained with multiple regression analyses. Included in each model is one main independent variable as listed in the table, plus the following covariates: Age, sex, locality, body mass index, follow-up mean arterial pressure, follow-up heart rate, tobacco use, follow-up anti-hypertension medication use.

CRP, C-reactive protein; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG/HDL-C, triglyceride/high-density lipoprotein cholesterol ratio.

CHAPTER 6

General findings and final conclusions

1. Introduction

The central aim of this study was to investigate large artery stiffness (as measured by carotid-femoral PWV) and its associations with cardiovascular risk factors in the understudied black South African population. The results of the manuscripts both support and contradict existing knowledge on the subject of arterial stiffness. These results are the first to shed light on possible risk factors for increased arterial stiffness in the black population of South Africa who are vulnerable specifically to cardiovascular morbidity and mortality. A young and older population, as well as 5- and 10-year follow-up data (PURE-SANWIP study) were used to discern between the possible effects of age and to evaluate whether the factors that associate most strongly with arterial stiffness differ over a shorter or longer time period. Since information on large artery stiffness in black adults from South Africa is limited, I will also compare these findings to those in other populations from around the world.

2. Summary of main findings and comparison to relevant literature

The main findings of the three manuscripts reported in this thesis are as follows:

2.1 Manuscript 1, published in the Journal of the American Society of Hypertension:

Large artery stiffness is associated with gamma-glutamyltransferase in young, healthy adults: The African-PREDICT study

In the first manuscript, we cross-sectionally investigated the relationship between health behaviours and large artery stiffness in young (aged 20-30 years), apparently healthy black and white adults participating in the African-PREDICT study. In the first instance, we compared arterial stiffness between young black and white adults. Secondly, we determined associations between health behaviours such as alcohol use, smoking, obesity, liver enzymes and physical inactivity and large artery stiffness.

Hypothesis 1: *Large artery stiffness is more pronounced in young black compared to young white South Africans.*

Similar overall PWV values were found in black and white adults after adjustment for MAP. Therefore, the first hypothesis is rejected, as no overall difference was found in arterial stiffness between the young black and white adults. In contrast, the largest study comparing aortic stiffness between middle-aged black and white Americans found higher arterial stiffness in black adults, even in a subgroup who were free from cardiovascular risk factors such as hypertension, diabetes, currently smoking and obesity [1]. The age difference of the participants in this study and in the American study may account in part for the contradicting results. Furthermore, the absence of a difference in aortic stiffness between the black and white adults in this study may be explained by the inclusion criteria of the African-PREDICT study, which allowed only normotensive young adults with no chronic diseases to participate.

Hypothesis 2: *Obesity (BMI), smoking (cotinine) and alcohol use (self-reported and GGT) are positively associated with large artery stiffness, while low physical activity is inversely associated with large artery stiffness.*

Large artery stiffness associated positively and independently with the liver enzyme GGT in both black and white young, healthy individuals. However, the majority of health behaviours included such as alcohol use, smoking or physical activity showed no association with arterial stiffness, while obesity did not associate with PWV in black participants and associated negatively with PWV in white adults. Therefore, hypothesis 2 is rejected, with the exception of an association between PWV and GGT. In agreement with the higher GGT levels in black compared to white Americans (in former, current and non-alcohol users) [2], the black participants of the African-PREDICT study also exhibited higher GGT levels, even in the sub-group of non-alcohol users. This is a major cause for concern, since GGT was shown to be predictive of not only hypertension, by also all-cause and cardiovascular mortality in older black South Africans [3]. GGT is a marker of high alcohol consumption [4], but it may also be indicative of oxidative stress [5] and pathological processes independent

of alcohol use, such as non-alcoholic fatty liver disease [6]. The higher levels of GGT, oxidative stress and inflammation as measured by IL-6 and MCP-1 in young black adults in this study confirm similar results obtained in other South African studies [7-9]. In addition, tobacco use was more prevalent amongst black than white participants. These results add to existing knowledge by showing that a health profile pertaining to higher CVD risk is already present in young, healthy black adults. In conclusion, young black adults may have an increased risk for early vascular ageing and subsequent cardiovascular disease.

2.2 Manuscript 2, published in the Journal of Hypertension:

A health profile associated with excessive alcohol use independently predicts aortic stiffness over 10 years in black South Africans

Due to the lack of longitudinal PWV data in the black adult population of South Africa, uncertainty exists as to which cardiovascular risk factors could be involved in early vascular ageing in this group. Thus, in manuscript 2, we determined the predictive value of traditional cardiovascular risk factors and health behaviours, assessed over 10 years, in terms of large artery stiffness. Factors investigated as possible predictors included blood pressure, age, sex, markers of obesity, glucose and lipid metabolism, renal function, liver function, inflammation and health behaviours such as alcohol and tobacco use.

For the sake of discussing the results found for this manuscript as a unit, the three hypotheses stated in chapter one is combined into a single hypothesis: *Blood pressure, age, female sex, obesity, lipids, glycaemic markers, inflammation, liver function, renal function, alcohol use and tobacco are predictors of large artery stiffness in blacks.*

The results of this manuscript indicated that alcohol use, residing in an urban location, plasma glucose and low BMI predicted arterial stiffness in black adults. Therefore, the hypothesis for this manuscript is partially accepted, as several of the factors investigated were able to predict higher PWV. On the other hand, low BMI instead of obesity was predictive of stiffness and sex, dyslipidaemia, renal and liver function, inflammation and

tobacco use did not associate with PWV, prompting a rejection of this part of the hypothesis.

It is well-established that age and blood pressure are the two most important determinants of large artery stiffness [10] and this was also observed in the two populations included in this thesis. However, the inevitability of stiffer arteries and higher blood pressure as a consequence of advancing age is increasingly being questioned [11, 12]. Lifestyle and health behaviour are the most important modifiable risk factors for CVD [13] and may also be important role-players in the progression of arterial stiffness beyond the effect of chronological ageing [12]. A recent study in the Framingham cohort used the absence of hypertension and a PWV of <7.6 m/s to define healthy vascular ageing and found that 17.7% of individuals older than 50 years had healthy vascular ageing. When using the same criteria in this study, only 9.5% of black adults aged >50 years had healthy vascular ageing. In addition, a quarter of this population had a PWV exceeding the international cut-off value of 10 m/s [14]. Taken together, these results confirm the high risk for CVD in this black South African population [15-19].

Health behaviours of African populations are influenced by urbanisation, which includes changes from traditional lifestyles and diets [3, 18-24]. The results of this study is novel in showing that apart from the strong influence of age and blood pressure, an urban setting and unhealthy behaviours such as excessive alcohol use also predict of arterial stiffness in a black population. Alcohol use has been implicated in the deterioration of cardiovascular health of black populations in other South African studies [3, 25-27]. For instance, despite self-reported alcohol use associating with higher HDL-C levels in the PURE-SA-NWP population, it also associated with higher blood pressure, thereby possibly counteracting any beneficial effect [25]. Also in this population, self-reported alcohol intake predicted a five-year increase in blood pressure [26]. The results of this thesis expand this knowledge by showing that alcohol use also predicts large artery stiffness in this population, thus representing another mechanism by which alcohol abuse threatens cardiovascular health. In

a country where alcohol is the most commonly abused substance [28], this finding supports initiatives aiming to decrease the prevalence of alcohol abuse in South Africa.

While some studies show increased large artery stiffness in obese individuals independently of blood pressure, age and race [29-32], other studies found either no association [33], or an inverse association [34]. The results of this study consistently showed an inverse association between PWV and BMI. Similar results were obtained in another black South African population with regard to peripheral arterial stiffness and BMI [35]. The mechanism responsible for this inverse association is unclear, but unhealthy behaviour such as excessive alcohol use may play a role, since alcohol may replace up to 60% of the daily calorie intake in alcohol abusers [15]. Other possible explanations for this association include a larger vessel size in obese participants that may result in decreased PWV [36, 37] and an increased blood volume and more adipose tissue surrounding the arteries, which effectively blunts the reflecting wave in the pulse wave contour [38]. Nevertheless, this observation warrants further research on the association between PWV and BMI in black populations, while supporting the notion of a J-shaped curve that indicates a pernicious effect of low BMI on cardiovascular health in black South Africans [35].

Literature further indicates an important role for inflammation in the process of arterial stiffening [39, 40]. In middle-aged white, healthy adults, CRP associated independently with PWV [41]. Furthermore, inflammatory markers predicted cfPWV in two longitudinal studies with long follow-up periods [42, 43]. In a South African study, inflammation as measured by the soluble urokinase plasminogen activator receptor (suPAR) predicted all-cause and cardiovascular mortality in black adults [44]. Rather surprisingly, the results of this study did not show any association between large artery stiffness and the inflammatory markers, endothelial activation markers, or oxidative stress. It is possible that stiffening of the large arteries is influenced by mechanisms independent of inflammation in this black population. However, a limited variety of inflammatory markers were available for this study and the

possibility exists that other inflammatory markers will better elucidate the potential role of inflammation in arterial stiffness in this population.

Another unexpected finding of this study was that tobacco use did not relate to arterial stiffness, even after ten years. These results directly contradict those found in a 20-year follow-up study conducted in white men, where heavy smoking indeed predicted arterial stiffness [43]. Smoking is a well-known cardiovascular risk factor [45], however, the aetiology of large artery stiffness may involve different pathophysiological mechanisms in black South Africans, with other factors exerting a more prominent influence. Our result does agree with a 17-year follow-up study conducted in a Swedish population [46], where smoking was not linked to arterial stiffness. It may be plausible that smoking is more related to an 'atherosclerotic' than an 'arteriosclerotic' disease process [47], or that the effect of smoking on arterial stiffness may be masked by other factors in black South Africans.

Renal function, as tested by several biomarkers, did not associate with or predict arterial stiffness in the populations included in this study. In contrast, urinary albumin excretion associated with a [48] and predicted all-cause and stroke mortality in black South Africans [49]. In this manuscript, urinary albumin excretion did increase along with the tertiles of PWV, but these results were not significant after adjustments. The possibility exists that in this population, renal dysfunction does not predict, but rather occurs concurrently along with arterial stiffening.

2.3 Manuscript 3: Submitted to Diabetes Research and Clinical Practice

Evaluating several biomarkers as predictors of aortic stiffness in young and older Africans, not consuming alcohol

In manuscript 2, we found that a health profile associated with excessive alcohol use predicted large artery stiffness over a 10-year period. However, this type of health profile may be masking the predictive value of other biomarkers. Therefore, we investigated which biomarkers previously shown to modulate arterial function such as endothelial activation

(ICAM-1, VCAM-1), inflammation (CRP, IL-6) and oxidative stress (ROS) [50, 51], as well as metabolic markers (glucose, HbA1c, lipids) [46, 52] relate to arterial stiffness in young (cross-sectional investigation) and older (longitudinal investigation) black South Africans who self-reported no alcohol use. We specifically included young and older groups in an attempt to discern whether different biomarkers associated with arterial stiffness in a young and older population. Self-reported alcohol use was regarded as a reliable marker in this population due to previous reports [26]. For the purposes of manuscript 3, five-year follow-up data from the PURE-SA-NWP study was used as baseline data to obtain a five-year follow-up period.

Hypothesis 1: *Inflammation, endothelial activation, oxidative stress and metabolic markers associate cross-sectionally with large artery stiffness in young black adults.*

In younger adults, no cross-sectional association was found between PWV and biomarkers in those who self-reported no alcohol consumption. Therefore, the first hypothesis is rejected. The lack of any association in this young, healthy group may be explained by their normal blood pressure and PWV ranges and also due to the fact that these participants reported no alcohol consumption.

Hypothesis 2: *Inflammation, endothelial activation, oxidative stress, dysglycaemia and dyslipidaemia predict large artery stiffness five years later in an older black population who do not consume alcohol.*

When excluding alcohol users, only glucose and glycated haemoglobin predicted aortic stiffness over five years in older black adults. Thus, the second hypothesis is accepted in part due to the independent positive association of markers of dysglycaemia with aortic stiffness in the older black adults. However, the rest of the hypothesis is rejected as none of the other biomarkers predicted aortic stiffness over five years.

To the best of our knowledge, this is the first study that examined large artery stiffness in a population of black South Africans who do not use alcohol. This result agrees with findings in other populations with and without diabetes [46, 53-56]. However, alcohol users were not

excluded in these studies. It is noteworthy that the β -value obtained with multiple regression analysis for glucose is only slightly lower than obtained for age (0.21 versus 0.25, both $p < 0.001$), potentially indicating that blood glucose level can be nearly as valuable as age in predicting PWV of older black adults who do not consume alcohol. This finding also confirms and expands upon the results of manuscript 2, where plasma glucose also independently predicted large artery stiffness. However, its predictive value was weaker ($\beta = 0.08$, $p = 0.023$) when alcohol users is included in the study population.

The diabetes prevalence found in this manuscript (22% at baseline) is higher than the 13.7% found in a review of diabetes prevalence across the African continent [57]. Although the prevalence found in this study is only representative of a black population in the North West Province of South Africa, it agrees with the growing trend of diabetes prevalence in sub-Saharan Africa [58]. Together, these results add to the existing body of knowledge regarding large artery stiffness in black populations by indicating that glycaemic status is linked to future large artery stiffness.

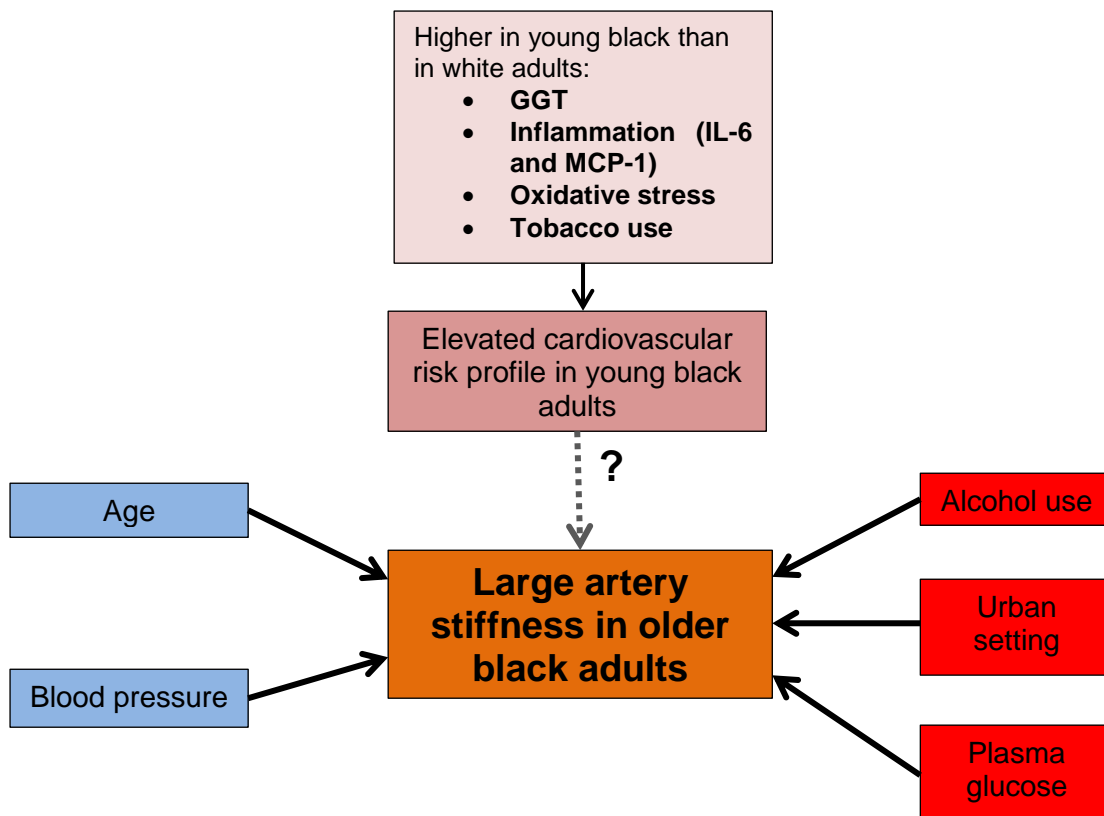


Figure 1. Flow diagram depicting the main findings from this PhD study. The factors predicting large artery stiffness over 10 years in an older black South African population are shown, as well as factors contributing to an elevated cardiovascular risk profile in younger black South Africans. GGT, gamma-glutamyltransferase.

3. Chance and confounding

A critical evaluation of some of the factors that may have confounded the results of this study is paramount. Relevant methodological issues are discussed here.

African-PREDICT study data was analysed cross-sectionally and causality cannot be inferred from the results obtained. Only follow-up cfPWV was available for the PURE-SANWNP study, thus preventing firm conclusions about change in aortic stiffness over the follow-up period and the factors contributing to it. However, this study does provide the first longitudinal data concerning cfPWV, the gold standard measurement of large artery stiffness, in a black South African population. Overall, both the African-PREDICT and PURE-

SA-NWP studies were conducted by well-trained researchers in highly controlled conditions and well-equipped research facilities.

The study populations of the African-PREDICT and PURE-SA-NWP studies were recruited from the North West Province of South Africa and are not representative of the cardiovascular health of the entire black South African population. A direct comparison between the African-PREDICT and PURE-SA-NWP study populations was not feasible due to differences in age, socioeconomic status and living environments. Most of the participants of the PURE-SA-NWP study were unemployed, with low education and income levels; results found for this group are thus not representative of populations with a higher socioeconomic status. Although self-reported alcohol use seems to be reliable in the PURE-SA-NWP population [26], complete abstinence from alcohol use in the five years between baseline data collection and follow-up data collection, as used for manuscript 2 (2010-2015), cannot be guaranteed. Regarding tobacco use, only self-reported data was available for the PURE-SA-NWP study, while a biomarker such as cotinine may have yielded more sensitive results regarding the relationship between large artery stiffness and tobacco use in this population. Even though the participants were asked to be in a fasted state on the morning of data collection, the possibility that some did not comply with this requirement cannot be excluded.

The possibility of chance findings should be taken into account. However, the results remained consistent upon repetition of the statistical analyses and for different multivariable regression analysis models. Adjustments for potential confounders such as age, MAP, sex, heart rate, BMI, medication use, creatinine clearance, tobacco use, active energy expenditure, fasting glucose or glycated haemoglobin, inflammatory (CRP or IL-6), endothelial activation (ICAM-1 or VCAM-1) and oxidative stress markers (ROS) could have resulted in an inaccurate estimation of the associations between large artery stiffness and the variables investigated in each manuscript. In addition, data on other potential confounders, such as diet, psychological stress, genetic characteristics and undiagnosed

infections or diseases such as non-alcoholic fatty liver disease were not available and the potential influence of these factors should be taken into account.

All statistical results were interpreted from a physiological perspective, but statistical significance does not automatically mean that a result is physiologically significant and vice versa. Each manuscript included a relatively large sample of the black South African population residing in the North West Province, thus lending adequate statistical power to the results found in each case.

4. Recommendations

In order to improve cardiovascular and overall health and quality of life, aortic stiffness remains an important research topic in this understudied black population. This is the first longitudinal study involving aortic stiffness in this population, but studies currently being conducted in South Africa will be able to shed more light on the pathological processes leading to increases in aortic stiffness. The following are recommended for future studies in sub-Saharan populations:

- Longitudinal studies that include baseline and follow-up PWV measurements are needed to confirm causality in the prediction of PWV.
- The ability of cfPWV to predict cardiovascular events or mortality needs to be established and compared to other cardiovascular markers (such as brachial and ambulatory blood pressure) in black South Africans. Such reports will also indicate the value and feasibility of measuring PWV in clinical practice as part of early screening for increased cardiovascular risk. The planned follow-up phases of the African-PREDICT study will probably enable these reports.
- Whether higher GGT levels and an elevated cardiovascular risk profile in young black adults manifests as a higher prevalence of CVD should be confirmed in longitudinal studies.

- Additional markers of inflammation such as soluble urokinase plasminogen activator receptor, other interleukins, TNF- α , MCP-1 should be determined to further explore the relationship between inflammation and large artery stiffness.
- Biomarkers of arterial stiffness, such as matrix-metalloproteinases and collagen markers should be investigated in black populations.
- Health behaviours not included in the study, such as dietary intake (especially salt intake), should be investigated with regard to large artery stiffness.
- The association between arterial stiffness and insulin levels should be investigated in black South African populations.
- The inverse relationship between arterial stiffness and BMI should be confirmed and further investigated in black populations.
- Future studies should attempt to identify factors relating to healthy vascular ageing in black populations. Such results may be able to increase the effectiveness of prevention strategies by recommending certain actions that has the potential to increase vascular health.
- Cotinine, a biomarker of tobacco use, should be included in future longitudinal investigations in order to shed more light on the potential relationship between large artery stiffness and tobacco use.
- A large percentage of the South African population is HIV-infected. Large artery stiffness should be investigated in this sub-group of the population in order to determine whether unique risk factors exist in HIV-infection.

Implications for public health policies

Future research should attempt to establish whether public health policies, such as the taxation of sugar and alcohol, are effectively preventing CVD. This is especially important in impoverished communities where unhealthy behaviour is used as a coping mechanism for violence, unemployment and a high level of psychological distress. Currently proposed

strategies for the improvement of health behaviours may not be effective unless these issues are addressed.

5. Final conclusions

The results of this study emphasise the importance of modifiable risk factors for the maintenance of large artery health in black populations. Young black adults have a general health profile already predisposing them to the development of cardiovascular disease. Apart from age and blood pressure, unhealthy behaviour over the life-course, such as excessive alcohol use and sugar intake, may accelerate arterial ageing in black South Africans. Considering that large artery stiffness is an important mechanism leading to the development of CVD, prevention strategies should focus on promoting healthy vascular ageing and education on the physiological effects of unhealthy behaviour, especially because these effects are not directly observed by an individual in his or her daily life. In order to address the high burden of CVD and alleviate the strain on the public health system in South Africa, the arterial health of the black population of South Africa should be addressed, starting with legislation and strategies to improve health behaviours.

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ANNEXURES

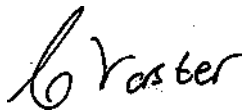
Annexure A

Declaration of language editing

DECLARATION

I, C Vorster (ID: 710924 0034 084), Language editor and Translator, and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of a thesis written by ms M Maritz (student number 22212337).

Title of the thesis: Large arterial stiffness and associated cardiovascular risk factors in black South Africans



11 September 2017

C Vorster

Date

Annexure B

Turnitin report



Turnitin Originality Report

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Annexure C

Published version of Manuscript 1

Research Article

Large artery stiffness is associated with gamma-glutamyltransferase in young, healthy adults: The African-PREDICT study



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Abstract

Increased arterial stiffness is linked to cardiovascular disease development, particularly in black populations. Since detrimental health behaviors in young adults may affect arterial stiffness, we determined whether arterial stiffness associates with specific health behaviors, and whether it is more pronounced in young healthy black compared to white adults. We included 373 participants (49% black, 42% men) aged 20–30 years. Mean arterial pressure was higher for blacks than whites ($P < .001$), but carotid-femoral pulse wave velocity was similar (6.37 vs. 6.36 m/s; $P = .89$) after adjustment for mean arterial pressure. The black group had higher gamma-glutamyltransferase (GGT) ($P < .001$), cotinine, reactive oxygen species, interleukin-6, and monocyte-chemoattractant protein-1 (all $P \leq .017$). Pulse wave velocity related positively and independently to GGT in both groups before and after multiple adjustments (both $\beta = 0.15$; $P \leq .049$). Blacks had an unfavorable vascular profile and higher GGT, possibly indicating a higher vulnerability to cardiovascular disease development, including changes in arterial stiffness. However, this observation needs confirmation. *J Am Soc Hypertens* 2016;10(10):772–781. © 2016 American Society of Hypertension. All rights reserved.

Keywords: Ethnicity; health behavior; pulse wave velocity; young adults.

Introduction

The potential role of arterial stiffness in cardiovascular disease (CVD) development has become increasingly recognized in the past decade.¹ Pulse wave velocity (PWV) is considered to be the gold standard method to assess arterial stiffness and is predictive of cardiovascular (CV) events and mortality in the general population.²

Detrimental health behaviors contribute to CVD development,³ and because of the early establishment of health behaviors during childhood and adolescence,^{4,5} it should be a target for preventive strategies.⁶ Arterial stiffness is associated primarily with blood pressure (BP), age, and arterial wall properties.⁷ In addition, it may be influenced by poor health behaviors or unhealthy lifestyles such as smoking,⁸ excessive alcohol use,⁹ and physical inactivity,¹⁰

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Conflict of interest: The project is financially supported by the corporate social investment grants from Pfizer, Boehringer Ingelheim, Novartis, the MediClinic Hospital group, and in-kind contributions of Roche Diagnostics (all of South Africa).

Opinions, findings, conclusions, and recommendations expressed in this article are those of the authors and not the NRF.

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as well as the physiological effects thereof, such as obesity¹¹ and liver dysfunction.^{12,13} In South Africa, a change from traditional rural to modern, urban lifestyles includes changes in health behaviors, which affects the CV and metabolic health of the population.¹⁴

Hypertension and increased arterial stiffness are more prevalent in black than white populations.^{15,16} Two-thirds of urban black South Africans present with multiple risk factors for CVD and suffer from high rates of hypertension, resulting in alarming rates of hypertensive heart disease and stroke.¹⁷

The need for effective and affordable markers of early CV deterioration as part of prevention programs is imperative, as poor health systems in Africa may hinder successful treatment programs. We therefore aimed to determine whether arterial stiffness is more pronounced in young healthy black compared to white South African adults, and whether large artery stiffness is associated with markers of health behaviors such as alcohol use, smoking, obesity, liver enzymes, and physical activity in these individuals.

Methods

Study Population

This substudy forms part of the African Prospective study on the Early Detection and Identification of Cardiovascular Disease and HyperTension (African-PREDICT). The aim of the African-PREDICT study is to understand the early pathophysiology accompanying CVD development and to identify novel early markers or predictors for the development of CVD by following young, healthy adults over a period of 10–20 years. The African-PREDICT study is currently being conducted at the *Hypertension Research and Training Clinic* on the Potchefstroom campus of the North-West University. Participants are recruited from the Potchefstroom and surrounding areas by field workers, via their workplace, or through advertisement by means of local newspapers or radio stations. Young (20–30 years of age), apparently healthy black and white men and women were included in the study after an initial screening day. Participants whose mean BP out of 4 measurements ≥ 140 mm Hg and/or ≥ 90 mm Hg, who were human immunodeficiency virus infected, previously diagnosed with a chronic disease, pregnant, or breastfeeding were excluded.

The African-PREDICT study complies with all applicable requirements of the Declaration of Helsinki. The Health Research Ethics Committee of the North-West University approved the protocol of the study. Before measurements commenced, all procedures were explained to the participants. The participants then gave written informed consent.

This substudy includes cross-sectional data from the first 403 participants. Participants with incomplete PWV data

(N = 30) were excluded. The remaining 373 participants were divided into a black (N = 183) and white (N = 190) group.

Questionnaires

Participants completed a general health questionnaire with the help of a trained researcher. The socioeconomic status (SES) of a participant was derived from three categories included in the general health questionnaire, including skills level, education, and household income. Points were awarded to each of these categories, and the total number of points determined whether a participant had a low, middle, or high SES. The classification of the SES was adapted from Patro et al.¹⁸ Alcohol and tobacco use were also reported in this questionnaire.

Body Composition and Physical Activity Measurements

Height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany), weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany), and waist circumference (Lufkin Steel Anthropometric Tape (W606PM), Lufkin, Apex, USA) were measured using standardized methods and calibrated instruments.¹⁹ Body mass index (BMI) was calculated. Each participant was fitted with a combined heart rate and accelerometry device (ActiHeart, CamNtech Ltd., Cambridge, UK) which was worn on the chest and recorded activity energy expenditure (AEE) continuously for 7 consecutive days.

CV Measurements

After at least a 10-minute rest period, duplicate office BP measurements (Dinamap ProCare 100 Vital Signs Monitor, GE Medical Systems, Milwaukee, USA) were made on both arms with 5-minute intervals. The participant was sitting in a relaxed upright position with legs uncrossed. An appropriate sized cuff was used for each individual.

The mean arterial pressure (MAP) was calculated with the following equation: $(SBP - DBP)1/3 + DBP$ where SBP, systolic BP; DBP, diastolic BP.

Each participant was fitted with a 24-hour ambulatory BP and ECG apparatus (CardioXplore, CE0120, Meditech, Budapest, Hungary). The participant was fitted with the appropriate cuff size on the nondominant arm. Instructions were given on how to ensure successful inflations, and the participant filled out an ambulatory diary card during the measurements.

The carotid-femoral PWV was determined with the SphygmoCor XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia). The transit-distance method was used to measure PWV along the descending thoraco-abdominal

aorta. Pulse wave analyses (PWAs) were also performed to obtain central SBP and pulse pressure.

Biological Sampling

Participants were required to fast from 10 PM the evening before. Blood samples were taken with a sterile winged infusion set and syringes from the antebraial vein. Spot urine samples were collected. All samples were immediately taken to the onsite laboratory and aliquoted into cryovials for storage in biofreezers at -80°C until analysis.

Biochemical Analyses

An enzymatic colorimetric method (Cobas Integra 400 Roche Clinical System, Roche Diagnostics, Indianapolis, IN, USA) was used to determine serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, triglycerides (TGs), gamma-glutamyltransferase (GGT), creatinine, aspartate transferase (AST), and alanine transferase (ALT) as well as urinary creatinine and albumin levels.

High sensitivity C-reactive protein was determined in serum and fasting glucose in fluoride plasma (Cobas Integra 400 Roche Clinical System, Roche Diagnostics, Indianapolis, IN). Glycosylated hemoglobin was determined from EDTA whole blood with an immunoturbidimetric method (Cobas Integra 400 Roche Clinical System, Roche Diagnostics, Indianapolis, IN). Interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule 1 (VCAM-1) were determined with high sensitivity Quantikine ELISA kits (R&D systems, Minneapolis, MN, USA) and analyzed on a Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). Serum peroxides, representing reactive oxygen species (ROS), were determined with high-throughput spectrophotometric assay and analyzed on Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). A chemiluminescence method was used to assess serum cotinine levels (Immulate, Siemens, Erlangen, Germany). Creatinine clearance was calculated with the Cockcroft-Gault formula.²⁰

Statistical Analysis

We used Statistica version 13 (StatSoft, Inc., Tulsa, OK, USA) and prepared graphs using GraphPad Prism version 5.03 (GraphPad Software Inc., CA, USA). Interactions of ethnicity and sex were tested for the relationship between PWV and health behaviors (waist circumference, BMI, GGT, cotinine, AEE, and SES). Descriptive statistics including the mean and standard deviation were performed on data with a normal distribution. Abnormally distributed

variables were logarithmically transformed and the central tendency and spread described as the geometric mean, the 5th and 95th percentiles. Differences between the two ethnic groups were determined by independent t-tests and, for categorical data, by chi-square tests. To determine the associations between PWV and measures of health behavior, we performed partial correlations, analysis of variance, and analysis of covariance with PWV as the dependent variable. PWV was plotted against age, SES, BMI, and tertiles of GGT for both black and white groups before and after adjustment for MAP. We investigated the independent associations of PWV with health behaviors by using linear multiple regression analyses with PWV as the dependent variable. Based on the literature, covariates considered for entry into the model included: ethnicity, SES (low, medium, and high), age, MAP, sex, BMI, WC, height, TG, HDL-C, TG/HDL-C ratio, glycated hemoglobin, glucose, C-reactive protein, IL-6, MCP-1, tumor necrosis factor alpha, ROS, urinary albumin/creatinine ratio (uACR), ICAM-1, VCAM-1, cotinine, self-reported smoking, GGT, self-reported alcohol use, creatinine clearance, and AEE.

Results

The characteristics of the study population are shown in [Table 1](#). The blacks were younger, and a larger percentage had a low SES and had lower WC and BMI than whites. After adjustment for MAP, PWV was similar between the groups (6.37 ± 0.73 vs. 6.36 ± 0.73 m/s; $P = .89$). While ambulatory and central BP were similar between the groups, blacks had higher office systolic, diastolic, and MAP (all $P < .001$). Although there were no difference in the self-reported alcohol intake, the black group exhibited higher GGT levels (24.5 vs. 17.2 U/l; $P < .001$) compared to the whites. The blacks also had higher cotinine levels (63.7 ± 116 vs. 31.8 ± 84.0 ng/mL; $P = .003$) and reported a higher rate of tobacco use (31.1% vs. 14.7% ; $P < .001$). Objectively measured AEE were similar between the groups. The black group exhibited lower total cholesterol, low-density lipoprotein cholesterol, TG, and TG/HDL-C compared to the white group (all $P < .02$). However, IL-6, MCP-1 and ROS were higher among the blacks (all $P < .017$), whereas ICAM-1 was higher in whites. The uACR and the AST/ALT ratio were higher in the black group ($P \leq .001$).

Interaction terms indicated an interaction of ethnicity for the relationship between PWV and BMI ($P = .001$) and PWV and WC ($P < .001$), but we found no interactions with sex ([Supplementary Table 1](#)). Analyses were therefore performed separately in the black and white groups.

We performed partial correlations between PWV and health behaviors ([Table 2](#)) while adjusting for MAP. PWV correlated positively with age, male sex, and GGT in both groups (all $P < .05$). In blacks, we found a positive

Table 1
Characteristics of young black and white South Africans

Variables	Black	White	P
	N = 183	N = 190	
Men, N (%)	86/183 (46.9)	72/190 (37.9)	.08
Age, y	24.4 ± 3.22	25.5 ± 2.92	<.001
Socioeconomic status			
Low	111 (60.7)	19 (10.0)	<.001
Middle	47 (25.7)	45 (23.7)	
High	25 (13.6)	126 (66.3)	
Anthropometry			
Waist circumference, cm	76.6 ± 10.6	80.8 ± 13.2	<.001
Body mass index, kg/m ²	24.0 ± 5.07	25.5 ± 5.03	.004
Cardiovascular variables			
Systolic BP, mm Hg	120 ± 12.0	115 ± 12.0	<.001
Diastolic BP, mm Hg	80.4 ± 8.43	76.5 ± 7.35	<.001
Pulse pressure, mm Hg	40.2 ± 8.05	39.1 ± 8.15	.18
Mean arterial pressure, mm Hg	93.8 ± 9.01	89.5 ± 8.33	<.001
Ambulatory SBP, mm Hg	117 ± 9.60	117 ± 9.19	.67
Ambulatory DBP, mm Hg	69.6 ± 6.43	69.2 ± 5.95	.48
Ambulatory heart rate, bpm	75.5 ± 13.5	74.1 ± 10.7	.27
Central SBP, mm Hg	111 ± 9.62	105 ± 8.69	<.001
Central pulse pressure, mm Hg	33.7 ± 5.82	34.5 ± 5.16	.17
Carotid femoral PWV, m/s*	6.37 ± 0.73	6.36 ± 0.73	.89
Biochemical variables			
Total cholesterol, mmol/l	3.78 (2.71, 5.54)	4.62 (3.30, 6.24)	<.001
LDL cholesterol, mmol/l	2.33 (1.31, 4.05)	2.92 (1.79, 4.49)	<.001
Triglycerides, mmol/l	0.76 (0.41, 1.58)	0.96 (0.45, 2.18)	<.001
Triglyceride/HDL cholesterol ratio	0.60 (0.26, 1.55)	0.70 (0.26, 2.07)	.016
Glucose, mmol/l	3.79 (2.79, 5.25)	4.76 (3.68, 5.47)	<.001
HbA1c, %	5.48 (5.06, 6.01)	5.28 (4.88, 5.68)	<.001
C-reactive protein, mg/L	1.17 (0.12, 7.47)	1.04 (0.14, 10.9)	.39
Interleukin-6, pg/mL	0.95 (0.39, 3.21)	0.70 (0.27, 2.29)	<.001
Monocyte chemotactic protein-1, pg/mL	175 (122, 270)	134 (91.5, 203)	<.001
Tumor necrosis factor-alpha, pg/mL	1.64 (0.95, 4.70)	1.76 (1.06, 3.05)	.26
Reactive oxygen species, mg/L H ₂ O ₂	180 (94.3, 337)	163 (83.5, 365)	.017
Intercellular adhesion molecule-1, ng/mL	145 ± 78.2	179 ± 55.7	<.001
Vascular adhesion molecule-1, ng/mL	526 (366, 809)	534 (356, 883)	.09
Renal function			
Creatinine clearance, mL/min	128 (86.2, 199)	127 (86.6, 190)	.82
Urinary albumin/creatinine ratio	0.71 (0.51, 1.00)	0.64 (0.48, 0.86)	<.001
Health behaviors			
Self-reported alcohol intake, N/total (%)	112/183 (61.2)	126/190 (66.3)	.30
GGT, U/l	24.5 (10.6, 64.3)	17.2 (7.50, 51.2)	<.001
AST/ALT ratio	1.24 (0.66, 1.98)	1.10 (0.59, 1.94)	.001
Self-reported tobacco use, N/total (%)	57/183 (31.1)	28/190 (14.7)	<.001
Cotinine, ng/mL	63.7 ± 116	31.8 ± 84.0	.003
Activity energy expenditure, kCal/d	416 ± 168	435 ± 199	.34

ALT, alanine transaminase; AST, aspartate transaminase; BP, blood pressure; DBP, diastolic blood pressure; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N, number of participants; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation.

Data are arithmetic mean ± SD or geometric mean (5th and 95th percentile intervals) for logarithmically transformed variables.

* Adjusted for mean arterial pressure.

correlation between PWV and SES and PWV and cotinine (all $P \leq .024$) and negative correlation between PWV and BMI ($P = .001$), all of which are absent in the whites.

We plotted PWV against age, SES, BMI, and tertiles of GGT before and after adjustment for MAP (Figure 1). Before and after adjustments, PWV increased with age, only in

Table 2

Partial correlations of pulse wave velocity with measures of health behaviors, adjusted for MAP

Variables	Black	White
	N = 183	N = 190
Age, y	0.18	0.20
	<i>P</i> = .016	<i>P</i> = .007
Sex (0, women; 1, men)	0.34	0.18
	<i>P</i> < .001	<i>P</i> = .012
Socioeconomic status	0.17	0.02
	<i>P</i> = .024	<i>P</i> = .74
Waist circumference, cm	-0.14	0.04
	<i>P</i> = .06	<i>P</i> = .59
Body mass index, kg/m ²	-0.24	-0.06
	<i>P</i> = .001	<i>P</i> = .40
Self-reported alcohol intake, N, total (%)	0.03	0.08
	<i>P</i> = .65	<i>P</i> = .25
GGT, U/l	0.15	0.19
	<i>P</i> = .049	<i>P</i> = .012
AST/ALT ratio	-0.07	-0.11
	<i>P</i> = .35	<i>P</i> = .12
Cotinine, ng/mL	0.17	0.12
	<i>P</i> = .022	<i>P</i> = .11
Activity energy expenditure, kCal/d	-0.01	-0.03
	<i>P</i> = .91	<i>P</i> = .71

ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyltransferase; MAP, mean arterial pressure.

Bold text indicates *P*-values < .05.

blacks (*P* for trend = .001 and *P* = .023, respectively). PWV was higher in blacks than whites in age groups 23–25 and 26–28 years (*P* < .05). For the black group, PWV was highest in the high SES group (*P* for trend = .012). We found contrasting results for BMI, but after adjustment for MAP, no association remained in either ethnic group. Before adjustment for MAP, PWV increased with the tertiles of GGT for both the black and white groups, but after adjustment, there was no significant trend in either group.

Multivariable-adjusted regression analyses with PWV as the dependent variable were performed (Table 3). The multivariate model accounted for 37%, 36%, and 39% of the variability in PWV in the total, black, and white groups, respectively. PWV associated with MAP and age in all three groups (all *P* < .036). Male sex associated with PWV in the total and black groups. We found a negative association between PWV and BMI in the total ($\beta = -0.15$, *P* = .043) and white ($\beta = -0.23$, *P* = .049) groups. PWV associated positively with higher SES ($\beta = 0.15$, *P* = .044) in blacks only. The relationship between PWV and GGT was confirmed in all groups (all *P* < .05).

Sensitivity Analysis

When excluding self-reported alcohol users, GGT was still higher in the blacks (22.2 vs. 14.4 U/l; *P* < .001).

Regarding the regression models, neither the inclusion of the AST/ALT ratio, ROS, ICAM, or VCAM, nor the replacement of C-reactive protein with IL-6 or MCP-1 in the model changed the results.

Discussion

In young healthy adults, we found that large artery stiffness associated independently and positively with the liver enzyme, GGT, but not with health behaviors such as self-reported alcohol use, tobacco use, or physical activity. Although we found no overall difference in arterial stiffness between young black and white adults (mean values in the normal range), blacks aged 23–28 years presented with significantly higher arterial stiffness, independent of MAP.

An association between PWV and GGT has been reported in other populations, including young (aged 33–34 years) Turkish patients with prehypertension.²¹ However, studies in black populations are limited. We included GGT in this study initially in the conventional sense as a marker of alcohol use.^{22,23} However, our black and white group reported similar rates of alcohol use (61.2% vs. 66.3%, respectively), but GGT levels were higher in the blacks. This was an unexpected result because alcohol use reported by black South Africans seems to be reliable, as our group found that it significantly predicted a 5-year change in BP.²⁴ In a sensitivity analysis, we found higher GGT in our black group who self-reported no alcohol use, supporting the theory of a racial difference in GGT levels. Similarly, in black and white Americans, GGT was higher in the blacks, even among lifetime abstainers.²⁵

GGT is a liver enzyme,²² but it is also present in other organs, including the nervous system, kidneys, pancreas, and reproductive system.²⁶ GGT predicts CVD and CV events, incident diabetes, and hypertension independently of alcohol consumption and other CVD risk factors.^{27,28} In older black South Africans, GGT also independently predicted CV and all-cause mortality, as well as hypertension development.¹³ Therefore, GGT does not only reflect alcohol use, but also liver dysfunction, including nonalcoholic fatty liver disease (NAFLD).²⁹

The specific mechanism involved in the association between PWV and GGT is unclear. Physiologically, GGT counteracts oxidative stress by breaking down extracellular glutathione; thus, GGT may be increased by conditions that elevate oxidative stress in the body, such as high alcohol consumption.³⁰ However, under certain conditions, the products of the GGT reaction themselves may lead to free radical production.³⁰ GGT is thus regarded as a marker of oxidative stress.³¹ Oxidative stress also associates with arterial stiffness³² and could be the mechanism involved in the association between PWV and GGT, possibly via elastin rupture and increased collagen production in the arterial wall.³³ Future research should be aimed at elucidating the mechanism involved.

Table 3

Linear multiple regression analyses with pulse wave velocity as dependent variable

Variables	Total Group (N = 373)		Blacks (N = 183)		Whites (N = 190)	
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
	Carotid-femoral pulse wave velocity, m/s					
Adjusted R²	0.37		0.36		0.39	
Ethnicity, white	0.03 (−0.10, 0.16)	.66				
Age, y	0.17 (0.07, 0.27)	<.001	0.16 (0.01, 0.31)	.036	0.25 (0.10, 0.39)	.001
Sex, male	0.22 (0.11, 0.33)	<.001	0.28 (0.10, 0.45)	.003	0.10 (−0.06, 0.26)	.20
Body mass index, kg/m ²	−0.15 (−0.30, −0.01)	.043	−0.11 (−0.32, 0.10)	.31	−0.23 (−0.45, −0.002)	.049
Socioeconomic status	0.07 (−0.05, 0.19)	.27	0.15 (0.01, 0.30)	.044	−0.08 (−0.22, 0.07)	.29
Mean arterial pressure, mm Hg	0.40 (0.30, 0.51)	<.001	0.34 (0.19, 0.50)	<.001	0.45 (0.30, 0.60)	<.001
GGT, U/l	0.13 (0.03, 0.24)	.014	0.15 (0.003, 0.30)	.048	0.15 (0.002, 0.30)	.049
Cotinine, ng/mL	0.03 (−0.07, 0.13)	.54	0.003 (−0.16, 0.16)	.97	0.03 (−0.10, 0.16)	.65
Triglyceride/HDL cholesterol ratio	0.03 (−0.06, 0.13)	.49	−0.09 (−0.23, 0.05)	.19	0.14 (−0.01, 0.29)	.07
C-reactive protein, mg/l	0.04 (−0.06, 0.14)	.46	0.09 (−0.07, 0.24)	.26	−0.04 (−0.18, 0.10)	.59
Glucose, mmol/l	−0.09 (−0.21, 0.02)	.12	−0.17 (−0.31, −0.02)	.029	0.01 (−0.12, 0.14)	.84
Creatinine clearance, mL/min	−0.03 (−0.16, 0.10)	.68	−0.07 (−0.25, 0.10)	.42	0.05 (−0.16, 0.26)	.63
Active energy expenditure, kCal	0.07 (−0.02, 0.16)	.15	0.06 (−0.07, 0.20)	.36	0.08 (−0.05, 0.21)	.22

CI, confidence interval; GGT, gamma-glutamyltransferase; HDL cholesterol, high-density lipoprotein cholesterol.

Data expressed as beta-values and 95% confidence intervals, *P*-values obtained with multiple regression analyses.Bold text indicates the independent variable as well as adjusted R² values.

The association of PWV with GGT may point to an early underlying disease development process independent of alcohol use, such as the development of NAFLD or diabetes. GGT levels are elevated in patients with NAFLD.²⁹ Furthermore, NAFLD has been associated with arterial stiffness.³⁴ A mechanism involving inflammation, oxidative stress, and endothelial dysfunction³⁵ may explain the abnormal elastic properties of the large arteries found in patients with NAFLD.³⁶ Of three liver enzymes that associated with diabetes risk, namely AST, ALT, and GGT, GGT associated most strongly with diabetes risk in older (over 60 years of age) black and white American adults, and even in those participants who were lifetime abstainers from alcohol.³⁷ The incidence of diabetes in the black South African population may also be on the rise,³⁸ likely a consequence of urbanization and the adoption of unhealthy lifestyles.¹⁴ Compared to the whites, the black group may be at increased risk for future development of diabetes and NAFLD with increasing age due to their higher GGT levels. This, in turn, may also affect their vascular health and risk for future CVD.^{39,40} The higher GGT and AST/ALT ratio observed in our young black population may thus point to an early pathologic metabolic process independent of alcohol use.

The association between PWV and GGT did not differ between the blacks and whites and is weaker than with MAP and sex, but it is still independent of the main contributors to PWV. We found no association between PWV and inflammatory markers, cotinine, or self-reported tobacco use. A possible reason for these observations could be the young, apparently healthy status of this group. Nevertheless, the

black group exhibited higher GGT, ROS, IL-6, MCP-1, cotinine, and self-reported tobacco use (31.1% vs. 14.7%), all of which have been linked to arterial stiffness.^{8,28,32,41,42} Furthermore, GGT predicts the development of diseases such as hypertension¹³ and diabetes, even when the baseline GGT was in the lower to normal ranges.³⁷ Therefore, although our results showed a similar association between PWV and GGT in blacks and whites, young blacks may be at an increased risk for adverse health consequences as they age, including early changes in arterial stiffness, due to their elevated CV risk profile and higher GGT levels.

Our results confirm that BP and age are independently related to PWV.⁷ Because of the well-known association between PWV and age,⁴³ arterial stiffness research is more common in older groups than in young adults. A study in middle-aged black and white Americans indicated higher PWV in blacks independent of traditional CV risk factors.¹⁵ This was not the case in our study, as we found no difference in PWV between the black and white groups. The latter may be explained by the relatively healthy CV profile and young age of our population and specifically as we excluded individuals with a BP exceeding 140/90 mm Hg. Despite PWV and BP being in the normal range in our population, the 23- to 25- and 26- to 28-year-old black groups had higher PWV compared to the white groups. Previously, we have shown that the stiffness of muscular and large arteries are already elevated in young blacks compared to whites, both in hypertensives and in those with normal BP.⁴⁴ Confirmed by the present study, it seems that young blacks may be more vulnerable to early vascular aging than their white counterparts.

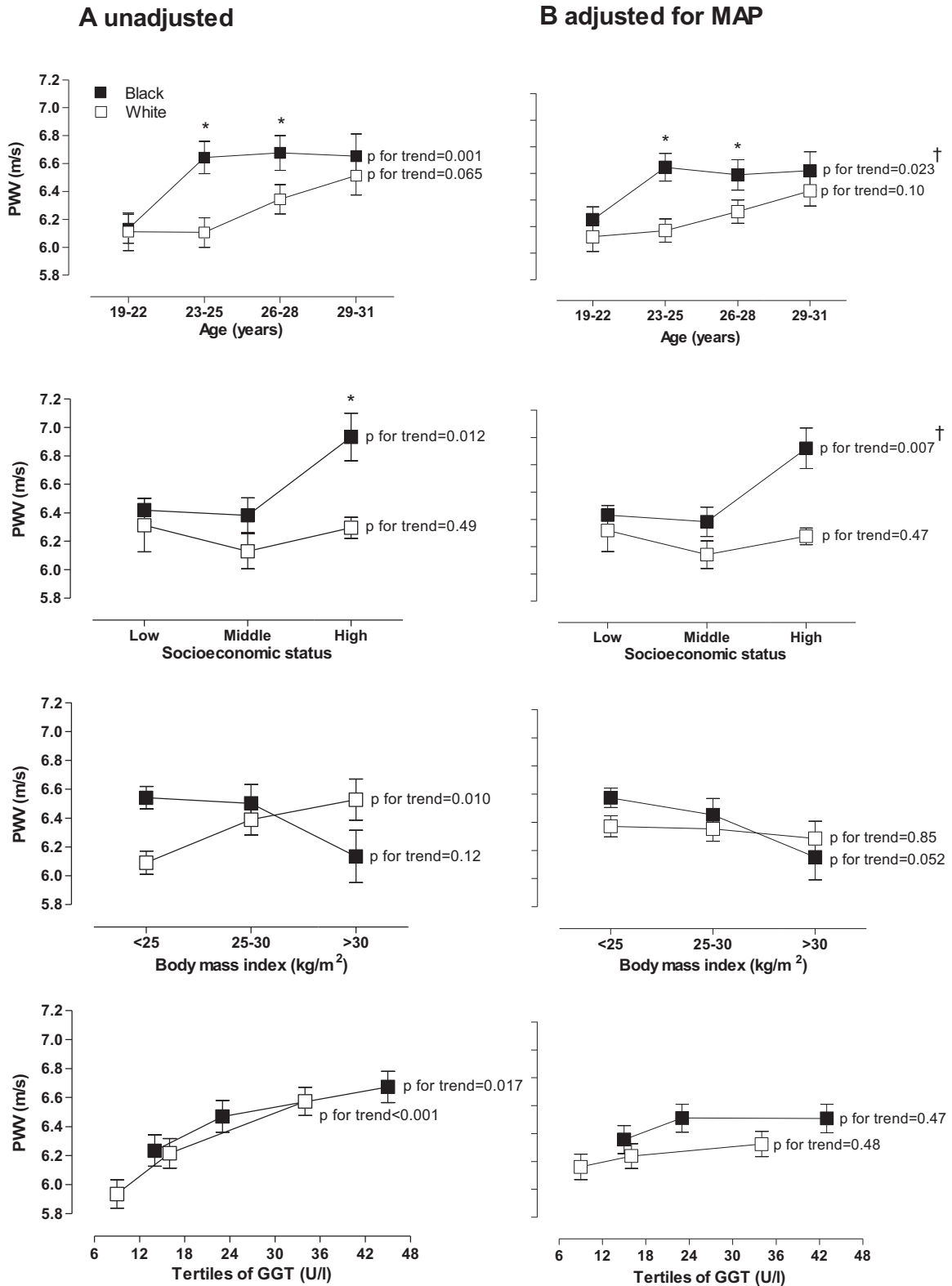


Figure 1. Pulse wave velocity plotted against age, socioeconomic status (SES), body mass index, and tertiles of gamma-glutamyltransferase for young black and white groups. * indicates P -value ($<.05$) between black and white group. † indicates P -value ($<.05$) between the lowest and highest age group as well as low and high SES. GGT, gamma-glutamyltransferase; MAP, mean arterial pressure; PWV, pulse wave velocity.

Apart from age and BP, low SES is also associated with arterial stiffness.⁴⁵ However, we found a positive association between PWV and high SES in our black group. This result suggests that the adaptation of an unhealthy, high sugar and fat diet, stressful situations and chronic alcohol abuse,^{16,39,46} characteristic of rapid urbanization in South Africa¹⁴ may be affecting the high SES black group. However, these results should be interpreted cautiously due to the uneven number of participants in the different SES categories.

We found an independent negative association between PWV and BMI in the white group. Authors who found similar results suggested that the adaptation of the arterial wall to BP changes may differ in obese individuals,⁴⁷ where the increased blood volume and the encapsulation of small conduit arteries by adipose tissue may buffer or blunt the reflecting wave in the pulse wave contour.⁴⁸

Strengths and Limitations

Our study population consisted of individuals from specific urban areas in the North West Province of South Africa and may not be representative of the whole population. However, this study included the understudied black South African population as well as information on arterial stiffness and variety of health behaviors, all of which may influence future CVD development in young populations. This study was conducted in highly controlled conditions in a well-equipped research facility. Due to the cross-sectional study design, causality cannot be inferred. Although the results were consistent after several adjustments, we cannot exclude residual confounding.

Conclusion

Large artery stiffness associates positively and independently with GGT in both black and white young, healthy individuals. Despite similar PWV values in black and white adults, blacks may be more vulnerable to future CVD development including changes in arterial stiffness, due to higher GGT levels, an elevated CV risk profile, and a larger proportion of smokers. Whether the higher GGT levels in young blacks will translate to a higher risk for future CV disease should be confirmed in future studies.

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Supplementary Table S1

Interaction terms

Dependent Variable:	Ethnicity, <i>P</i>	Sex, <i>P</i>
Carotid Femoral Pulse Wave Velocity		
Body mass index, kg/m ²	.0012	.87
Waist circumference, cm	< .001	.99
GGT, U/l	.19	.85
Cotinine, ng/mL	.44	.67
Activity energy expenditure, kCal/d	.99	.06
Socioeconomic status (low, middle, high)	.24	.83

GGT, gamma-glutamyltransferase.

Interaction terms *P*-values calculated with multiple regression analyses.

Annexure D

Published version of Manuscript 2

A health profile associated with excessive alcohol use independently predicts aortic stiffness over 10 years in black South Africans

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Objective: Black populations exhibit higher arterial stiffness than whites and suffer a disproportionate burden of cardiovascular disease. It is therefore important to identify modifiable health behaviours predicting large artery stiffness in blacks. We examined whether traditional cardiovascular risk factors and health behaviours of black South Africans predict large artery stiffness 10 years later.

Methods: We included 650 HIV-free participants (32.8% men) and collected data in rural and urban areas of the North West Province in 2005 and 2015. We collected questionnaire data, anthropometry, blood pressure and determined cardiometabolic and inflammatory markers from blood samples. We measured carotid–femoral pulse wave velocity (PWV) at follow-up.

Results: A total of 25.3% of our population, aged 65 ± 9.57 years, had a PWV exceeding 10 m/s. In multivariable-adjusted regression analyses, the strongest predictors of PWV were mean arterial pressure, age and heart rate (all $P < 0.024$). Urban locality ($R^2 = 0.31$, $\beta = 0.12$, $P = 0.001$), self-reported alcohol use ($\beta = 0.11$, $P = 0.018$) and plasma glucose ($\beta = 0.08$, $P = 0.023$) associated positively with PWV at follow-up. We found a negative association between PWV and BMI ($\beta = -0.15$, $P = 0.001$), and no associations with sex, smoking, inflammatory markers, lipids, liver enzymes or antihypertensive medication. When replacing self-reported alcohol with gamma-glutamyltransferase, the latter associated positively with PWV ($\beta = 0.09$, $P = 0.023$).

Conclusion: A health profile associated with excessive alcohol use, including an urban setting, elevated plasma glucose and lower BMI predicts large artery stiffness independently of age and blood pressure in black South Africans over 10 years. This observation prompts urgent public health strategies to target alcohol overuse.

Keywords: alcohol use, arterial stiffness, black South Africans, longitudinal, pulse wave velocity, urban locality

Abbreviations: AGE, advanced glycation endproducts; ALT, alanine aminotransferase; ANCOVA, analysis of covariance; ANOVA, analysis of variance; AST, aspartate aminotransferase; CKD-EPI, chronic kidney disease epidemiology collaboration equation; CrCl, creatinine clearance rate; CRP, C-reactive protein; eGFR, estimated

glomerular filtration rate; GGT, gamma-glutamyltransferase; HbA1c, glycosylated haemoglobin; HDL-C, HDL cholesterol; IL-6, interleukin-6; MAP, mean arterial pressure; Na⁺/K⁺ ATPase, sodium-potassium ATPase; PURE, prospective urban and rural epidemiology; PWV, pulse wave velocity; TC, total cholesterol; TG, triglycerides; TG/HDL-C, triglyceride to HDL cholesterol ratio; uACR, urinary albumin to creatinine ratio; WC, waist circumference

INTRODUCTION

Black populations exhibit higher arterial stiffness than their white counterparts [1] and suffer a disproportionate burden of cardiovascular disease [2]. Large artery stiffness increases the risk of cardiovascular events, including stroke and myocardial infarction, cardiovascular and all-cause mortality [3]. Carotid–femoral pulse wave velocity (PWV) is the gold standard measurement of large artery stiffness [4] and is a better predictor of cardiovascular events, cardiovascular and all-cause mortality than brachial SBP and DBP, as well as brachial and 24-h pulse pressure [3,5].

Large artery stiffness is largely dependent on age [6] and blood pressure (BP) [7]. However, other factors may also accelerate vascular ageing beyond the effect of chronological age by functional and structural alterations of the arterial wall of conduit vessels [8]. In older white men, circulating inflammatory markers and the level of repetitive cyclic stress in the artery were predictive of arterial stiffness over 20 years, whereas traditional cardiovascular risk factors had only a modest effect [9]. After 17 years, abdominal obesity,

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hyperglycaemia and dyslipidaemia, but not smoking, predicted arterial stiffness in middle-aged whites from Sweden [10]. In British middle-aged men and women, central obesity was a strong predictor of arterial stiffness after 16 years [11].

The pathophysiology of large artery stiffness is incompletely understood in black populations and limited longitudinal data in African populations has kept investigators from identifying traditional cardiovascular risk factors and health behaviours that may predict arterial stiffness. It is important to identify modifiable health behaviours leading to increases in arterial stiffness and cardiovascular disease to plan and implement effective preventive strategies [12]. Primary prevention, especially in Africa where health systems are weak and overburdened [13], is essential.

To contribute to a better understanding of cardiovascular disease development in black populations, we determined the prognostic value of traditional cardiovascular risk factors and health behaviours, assessed over 10 years, in terms of large artery stiffness. The factors investigated as possible predictors include markers of obesity, glucose and lipid metabolism, renal function, liver function, inflammation, and health behaviours.

METHODS

Research design

The current substudy forms part of the South African leg of the international Prospective Urban and Rural Epidemiology (PURE) study [14]. This multicountry study was developed to examine the patterns of transition on health and the influence of changing communities on the prevalence and types of cardiovascular and other chronic diseases [14]. We collected data for this substudy in the North West Province of South Africa in 2005 (baseline), with a 10-year follow-up conducted in 2015.

Recruitment

Baseline data collection included 2010 (rural = 1006 and urban = 1004) black volunteers. Participants were fully informed about the objectives and procedures of the study before participation. Before measurements commenced, all procedures were explained to the participants in their home language, after which written informed consent was given. Black individuals older than 35 years were invited to participate in the study. Pregnant and lactating women were excluded. We collected longitudinal data for 926 participants and excluded participants with incomplete PWV data ($n = 110$) and those who were HIV infected ($n = 166$). The attrition rate of participants from baseline ($n = 2010$) to follow-up ($n = 926$) compares with other longitudinal studies due to frailty of elderly participants, refusal to participate, movement to other areas of the country and mortality. Included in the present study are 650 (rural = 362 and urban = 288) participants (Fig. 1).

The Health Research Ethics Committee of the North-West University gave approval for the PURE-SA baseline (2005) and follow-up (2015) study, as well as this substudy. All procedures comply with the Declaration of Helsinki.

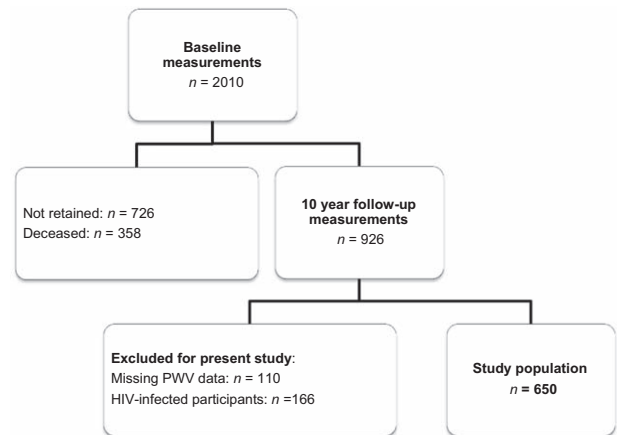


FIGURE 1 Flow diagram of study population.

Data collection

Questionnaires

Participants completed structured sociodemographic, life-style and physical activity questionnaires. Alcohol use and smoking were indicated with a yes/no answer, with yes for current or former use and no for never used. Trained African field workers assisted in the collection of the biographical data of participants.

Anthropometric measurements

Baseline and follow-up height (Invicta Stadiometer IP 1465, Leicester, UK; Leicester height measure, Seca, Birmingham, UK), weight (Precision Health Scale, A&D Company, Tokyo, Japan) and waist circumference (Holtain unstretchable metal tape) were taken using standardized methods and calibrated instruments. BMI and waist circumference-to-height ratio was calculated.

Cardiovascular measurements

The validated OMRON HEM-757 and OMRON M6 (Omron Healthcare, Kyoto, Japan) devices were used to measure BP at baseline and follow-up, respectively. The correct cuff size was fitted on the right arm of each participant. The participant was seated in a relaxed upright position with legs uncrossed during the measurement. After a resting period of 10 min, the brachial SBP and DBP were measured and recorded twice with a 5-min interval. The BP of the second measurement was used for analysis.

At follow-up, carotid-femoral PWV was determined in duplicate with the SphygmoCor XCEL device (AtCor Medical Pty. Ltd., Sydney, New South Wales, Australia) with the participant in the supine position, and the second reading used for analysis. The transit-distance method was used to measure PWV along the descending thoraco-abdominal aorta.

Biological sampling

Participants were asked to fast from 2200 h the evening before. In the early morning blood samples were taken from the antebrachial vein from each participant with a sterile winged infusion set and syringes. Morning spot urine samples were also collected. We used standardized

methods to prepare serum and plasma, snap frozen on dry ice and stored in the laboratory at -80°C . In the cases of blood collection in a rural area, serum and plasma was snap frozen and stored at -20°C for not more than 5 days. The samples were then transported to the laboratory and stored at -80°C .

Biochemical analysis

We analysed serum total cholesterol, HDL cholesterol (HDL-C), triglycerides, gamma-glutamyltransferase (GGT), creatinine, high-sensitivity C-reactive protein (CRP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Konelab20i auto-analyzer; Thermo Fisher Scientific Oy, Vantaa, Finland; Cobas Integra 400 Roche Clinical System; Roche Diagnostics, Indianapolis, Indiana, USA) and urinary albumin and creatinine (Cobas Integra 400 Roche Clinical System; Roche Diagnostics) for baseline and follow-up.

The Friedewald formula was used to calculate the quantitative aspect of LDL cholesterol [15]. The creatinine clearance rate (CrCl) was calculated with the Cockcroft–Gault formula [16]. The estimated glomerular filtration rate was calculated with the Chronic Kidney Disease Epidemiology Collaboration Equation [17].

We determined plasma glucose at baseline (Vitro DT6011 Chemistry Analyzer; Ortho Clinical Diagnostics, Rochester, New York, USA) and follow-up (Cobas Integra 400 Roche Clinical System; Roche Diagnostics), as well as glycosylated haemoglobin levels (D-10 Haemoglobin testing system, Bio-Rad #220-0101). Serum ferritin was determined with an enzyme immunoassay (Ramco Laboratories, Inc, Stafford, Texas, USA). Baseline IL-6 levels were determined with an electrochemiluminescence immunoassay (Elecsys 2010; Roche, Basel, Switzerland).

Statistical analyses

We performed statistical analyses using Statistica 13 (Statsoft Inc., Tulsa, Oklahoma, USA) and prepared graphs using GraphPad Prism Version 5.03 (GraphPad Software Inc., La Jolla, California, USA). Descriptive statistics, including the mean and SD, were performed on data with a normal distribution. Abnormally distributed variables were logarithmically transformed and the central tendency and spread described as the geometric mean, the 5th and 95th percentiles. We tested for interactions of sex on the relationship between PWV and mean arterial pressure (MAP) and found a tendency for an interaction ($P=0.06$). Some analyses were therefore performed separately for men and women. We divided participants into tertiles based on PWV, and we determined differences between the tertiles using chi-square tests, analysis of variance and covariance. We performed multiple regression analysis with PWV as the dependent variable in the total group, and in men and women separately. Covariates considered for entry into the model included all variables in Table 1 and those compared after adjustments in Table 2. The final model included: baseline age, sex, locality, BMI, triglyceride-to-HDL-C ratio (TG/HDL-C), glucose, CRP, urinary albumin-to-creatinine ratio (uACR), GGT, self-reported alcohol use and tobacco use and follow-up variables: heart rate (HR), MAP and antihypertension medication use.

RESULTS

The baseline characteristics of the total population ($n=650$), stratified by PWV tertiles are shown in Table 1. Those in the third PWV tertile were older, taller, a higher percentage were men (43%) and from an urban setting (52.2%) compared with those in the first two tertiles (P trend ≤ 0.002). Hypertension prevalence was the highest in the third tertile ($P < 0.001$). Sixty-seven percent of the study population had a PWV of at least 8 m/s, whereas 25% had a PWV of at least 10 m/s. BMI decreased significantly with increased PWV (P trend ≤ 0.023). All cardiovascular measures increased from the first to the third tertile (all P trend ≤ 0.011). Biochemical measures including HDL-C, triglycerides, TG/HDL-C, glucose, CRP, IL-6, GGT and uACR increased across the tertiles (all P trend ≤ 0.042). Self-reported alcohol use was highest in the third PWV tertile (53 vs. 23.0% in the first tertile, P trend < 0.001), and although overall reported tobacco use was high (44.6–53.2%), no significant trend was seen according to PWV tertiles ($P=0.14$).

Supplementary Table S1, <http://links.lww.com/HJH/A802> shows the cross-sectional characteristics of the same population at follow-up, again stratified by tertiles of follow-up PWV. Similar findings were obtained as with baseline characteristics. In addition, those in the third PWV tertile had the lowest waist circumference and CrCl (P trend ≤ 0.014). Furthermore, AST, AST/ALT ratio and tobacco use were highest in the third PWV tertile (all P trend ≤ 0.039).

In Table 2, we determined which variables to enter into the multiple regression model. Therefore, we compared cardiometabolic baseline characteristics according to PWV tertiles, but adjusted for age, MAP, sex and rural/urban locality. We found results similar to those in Table 1, except for the loss of a significant trend for uACR ($P=0.11$). When additionally adjusting for HR (not shown), a significant trend remained only for TG/HDL-C ratio and glucose ($P \leq 0.015$).

To identify predictors for PWV after 10 years, we performed multivariate-adjusted regression analyses with PWV as the dependent variable in the total group (Fig. 2), and separately in men and women (Table 3). In the total group and in men and women separately, PWV associated positively with MAP, age and HR (all $P \leq 0.024$). In the total group and in women, urban locality ($\beta = 0.12$, $P = 0.001$; $\beta = 0.13$, $P = 0.005$) and self-reported alcohol use ($\beta = 0.11$, $P = 0.018$; $\beta = 0.11$, $P = 0.045$) associated positively with PWV. In the total group only, PWV associated with glucose ($\beta = 0.08$, $P = 0.023$). We found a negative association between PWV and BMI ($\beta = -0.15$, $P = 0.001$; $\beta = -0.13$, $P = 0.019$) for the total group and for women, respectively, and a trend for the same association in the men ($\beta = -0.16$, $P = 0.05$). In women, PWV also associated positively with the TG/HDL-C ratio ($\beta = 0.10$, $P = 0.047$).

Sensitivity analyses

We repeated the multiple regression analyses but replaced self-reported alcohol with GGT to determine whether an independent association between PWV and GGT exists, as was previously found for SBP [18]. By doing so GGT

TABLE 1. Unadjusted baseline characteristics of a black South African population, stratified by tertiles of pulse wave velocity

Pulse wave velocity ranges (m/s)	1st tertile, n = 214	2nd tertile, n = 215	3rd tertile, n = 221	P trend
	<8.0	8.0–9.4	≥9.5	
Age	48.6 ± 8.70	50.0 ± 8.84	55.1 ± 9.63 ^a	<0.001
Sex [men (%)]	50/214 (23.4)	68/215 (31.6)	95/221 (43.0)	<0.001
Locality [urban (%)]	75/214 (35.0)	97/215 (45.1)	116/221 (52.5)	0.002
Hypertension prevalence	83/214 (38.8)	99/215 (46.1)	140/221 (63.6)	<0.001
Anthropometric measures				
BMI (kg/m ²)	26.2 ± 6.53	25.4 ± 7.02	24.4 ± 6.66 ^a	0.023
Waist circumference (cm)	80.5 ± 11.8	81.3 ± 13.4	80.2 ± 12.9	0.67
Height (m)	1.59 ± 0.07	1.60 ± 0.08	1.62 ± 0.08 ^a	<0.001
WC/height ratio	0.51 ± 0.08	0.51 ± 0.09	0.50 ± 0.08	0.21
Cardiovascular measures				
SBP (mmHg)	128 ± 19.4	131 ± 21.5	144 ± 25.7 ^a	<0.001
DBP (mmHg)	85.4 ± 12.9	87.1 ± 13.29	92.2 ± 14.1 ^a	<0.001
Pulse pressure (mmHg)	42.6 ± 11.9	44.8 ± 13.9	52.5 ± 16.5 ^a	<0.001
Heart rate (bpm)	70.2 ± 14.6	72.6 ± 14.4	74.6 ± 16.6 ^a	0.011
MAP (mmHg)	99.6 ± 14.3	102 ± 15.1	109 ± 17.1 ^a	<0.001
Biochemical measures				
Total cholesterol (mmol/l)	4.93 (4.76; 5.11)	5.11 (4.93; 5.30)	5.10 (4.92; 5.28)	0.31
LDL-C (mmol/l)	2.88 (2.73; 3.04)	2.96 (2.81; 3.13)	2.78 (2.64; 2.94)	0.27
HDL-C (mmol/l)	1.41 (1.34; 1.49)	1.40 (1.33; 1.48)	1.53 (1.45; 1.61)	0.033
Triglycerides (mmol/l)	1.05 (0.98; 1.12)	1.23 (1.15; 1.31) ^a	1.21 (1.14; 1.29) ^a	0.001
TG/HDL-C ratio	0.74 (0.67; 0.82)	0.90 (0.81; 0.99) ^a	0.79 (0.72; 0.87)	0.029
Glucose (mmol/l)	4.83 (4.69; 4.97)	4.85 (4.71; 4.99)	5.10 (4.96; 5.25) ^a	0.011
HbA1c (%)	5.61 (5.51; 5.71)	5.66 (5.55; 5.76)	5.71 (5.61; 5.82)	0.37
Ferritin (μg/l)	72.6 (60.1; 87.7)	88.4 (72.2; 108)	106 (85.3; 132) ^a	0.036
Inflammatory markers				
C-reactive protein (mg/l)	2.56 (2.10; 3.13)	3.65 (2.98; 4.47) ^a	3.33 (2.73; 4.05)	0.042
Interleukin-6 (pg/ml)	2.21 (1.89; 2.58)	2.64 (2.25; 3.08)	3.12 (2.67; 3.64) ^a	0.009
Liver enzymes				
GGT (U/l)	43.1 (38.6; 48.1)	53.3 (47.7; 59.6)	60.3 (54.1; 67.3)	<0.001
AST (U/l)	24.1 (22.2; 26.2)	26.6 (24.5; 28.9)	27.6 (25.4; 29.9)	0.06
ALT (U/l)	16.9 (15.6; 18.2)	17.9 (16.6; 19.4)	17.7 (16.4; 19.1)	0.50
AST/ALT ratio	1.43 (1.33; 1.53)	1.48 (1.38; 1.59)	1.55 (1.45; 1.67)	0.24
Renal function				
CrCl (ml/min)	97.2 (92.7; 102)	97.4 (92.7; 102)	92.6 (88.3; 97.1)	0.25
uACR (mg/mmol)	0.60 (0.51; 0.70)	0.58 (0.49; 0.68)	0.77 (0.66; 0.90)	0.025
eGFR (ml/min per 1.73 m ²)	112 ± 21.4	116 ± 19.5	112 ± 20.3	0.12
Health behaviour				
Tobacco use, n (%)	95/213 (44.6)	112/213 (52.6)	117/220 (53.2)	0.14
Alcohol use, n (%)	50/214 (23.4)	78/213 (36.6)	116/219 (53.0)	<0.001
Medication use				
Antihypertensive, n (%)	33/214 (15.4)	36/215 (16.7)	49/221 (22.2)	0.15
Antidiabetic, n (%)	2/214 (0.93)	0/215 (0.00)	7/221 (3.17)	0.014
Anti-inflammatory, n (%)	34/214 (15.9)	38/215 (17.7)	29/221 (13.1)	0.42
Statins, n (%)	0/214 (0.00)	0/215 (0.00)	1/221 (0.45)	0.38

Data are arithmetic mean ± SD, geometric mean (5th and 95th percentiles) or % of n. P values were obtained with analysis of variance and Chi-square tests. P value 0.05 or less were regarded as statistically significant. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; MAP, mean arterial pressure; TG: HDL-C, triglyceride: HDL-cholesterol ratio; tobacco and alcohol use are self-reported; uACR, urinary albumin-to-creatinine ratio; WC, waist circumference.

^aStatistically significant difference between 1st and 3rd pulse wave velocity tertile.

associated positively with PWV in the total group ($R^2 = 0.31$; $\beta = 0.09$, $P = 0.023$) but not in the separate analyses for men or women. Due to the known association between GGT and iron [19], we also included ferritin in the model, but no significant results were found for ferritin ($n = 357$, $R^2 = 0.30$, $\beta = -0.02$, $P = 0.65$). Due to literature indicating an association between PWV and inflammation [20], we also tested the substitution of CRP with IL-6 in the model, but the results remained unchanged (adjusted $R^2 = 0.31$, $\beta = 0.04$, $P = 0.23$). Similarly, the inclusion of baseline and/or follow-up anti-inflammatory medication in the model did not affect the original findings (adjusted $R^2 = 0.31$, $\beta = 0.03$, $P = 0.48$).

DISCUSSION

To the best of our knowledge, this study presents the first 10-year longitudinal findings on potential contributors towards large artery stiffness in a black South African population. We found that health behaviours related to excessive alcohol use, including an urban setting, high plasma glucose and low BMI, were predictive of PWV independently of age and BP after 10 years.

Our results support the fact that alcohol abuse is a major health problem in Southern Africa [21]. Previously, we found that self-reported alcohol use predicted a 5-year change in BP better than biochemical markers in the same

TABLE 2. Adjusted baseline characteristics of a black South African population, stratified by tertiles of pulse wave velocity

Pulse wave velocity ranges (m/s)	1st tertile, n = 214	2nd tertile, n = 215	3rd tertile, n = 221	P trend
	<8.0	8.0–9.4	≥9.5	
BMI (kg/m ²)	26.3 ± 6.53	25.4 ± 6.13	24.4 ± 6.60 ^a	0.020
Waist circumference (cm)	81.4 ± 13.2	81.4 ± 12.4	79.3 ± 13.4	0.20
TG/HDL-C ratio	0.72 (0.65; 0.80)	0.91 (0.83; 1.00)	0.80 (0.72; 0.89)	0.006
Glucose (mmol/l)	4.82 (4.67; 4.97)	4.86 (4.72; 5.00)	5.11 (4.95; 5.27) ^a	0.027
HbA1c (%)	5.60 (5.50; 5.71)	5.67 (5.57; 5.78)	5.70 (5.60; 5.81)	0.45
C-reactive protein (mg/l)	2.58 (2.08; 3.19)	3.76 (3.07; 4.60)	3.21 (2.60; 3.96)	0.041
IL-6 (pg/ml)	2.26 (1.91; 2.67)	2.67 (2.28; 3.13)	3.02 (2.55; 3.57)	0.08
GGT (U/l)	45.8 (40.8; 51.3)	52.3 (46.9; 58.4) ^a	58.0 (51.8; 65.1) ^a	0.026
uACR (mg/mmol)	0.63 (0.53; 0.74)	0.57 (0.49; 0.67)	0.73 (0.62; 0.87)	0.11
Creatinine clearance (ml/min)	96.8 (92.3; 101)	95.7 (91.3; 100)	94.7 (90.2; 99.5)	0.84
eGFR (ml/min per 1.73 m ²)	111 ± 17.7	115 ± 16.4	114 ± 17.8	0.15

Data are arithmetic mean ± SD, geometric mean (5th and 95th percentile intervals) and P values obtained with ANCOVAs. Data adjusted for age, mean arterial pressure, sex and locality. eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin; TG:HDL-C, triglycerides:HDL cholesterol ratio; uACR, urinary albumin-to-creatinine ratio.
^aStatistical significant difference from the 1st tertile.

population [22]. Therefore, we regarded self-reported alcohol use as a reliable measure of alcohol intake in this population. In our study, we have also confirmed our findings when substituting self-report with GGT [23].

The relationship between arterial stiffness and alcohol use is, however, controversial. Moderate alcohol intake may decrease PWV, whereas alcohol abuse leads to increases in arterial stiffness [24]. The precise mechanism by which alcohol affects the arterial wall is not clear, but several possibilities exist. Alcohol decreases magnesium levels in the body, which may lead to an increase in vascular tone [25]. Both acute and chronic alcohol intake influence Na⁺/K⁺-ATPase activity, which may alter both vascular tone and renal sodium handling [26]. Ethanol, the main ingredient of alcohol, may regulate collagen and elastin content in myocytes via a pathway involving matrix metalloproteinases [27]. Furthermore, a metabolic product of ethanol breakdown, acetaldehyde, is oxidized to acetate which can lead to the generation of reactive oxidative species [28]. Oxidative stress may contribute to elastin rupture and collagen overproduction in the arterial wall, leading to arterial stiffness [29]. GGT, a marker of alcohol intake and liver function [30], is also associated with

oxidative stress [19] and may represent another mechanism by which alcohol damages the arterial wall. As mentioned, we found that GGT significantly predicts PWV when self-reported alcohol use was removed from the model. This is in line with previous results by our group, which indicated a positive association between large artery stiffness and GGT in young, healthy black adults [31].

Awareness of alcohol-related problems and of the need for action in South Africa has improved, but the implementation of preventive strategies needs more attention [21]. New alcohol legislation has been proposed, which includes raising the legal drinking age to 21 and banning alcohol advertisements [32,33]. Interventions such as tax increases for alcohol may be effective; however, home-brewed alcohol in poor communities is common and therefore a different strategy may be required to reduce alcohol abuse in South Africa [34,35].

An urban setting was associated with future arterial stiffness in this black population. Nearly 2 decades ago, urbanization already associated with changes in traditional lifestyles and health behaviour [36], as well as the manifestation of hypertension [37] in black South Africans. Furthermore, urbanization gives rise to psychosocial stressors due

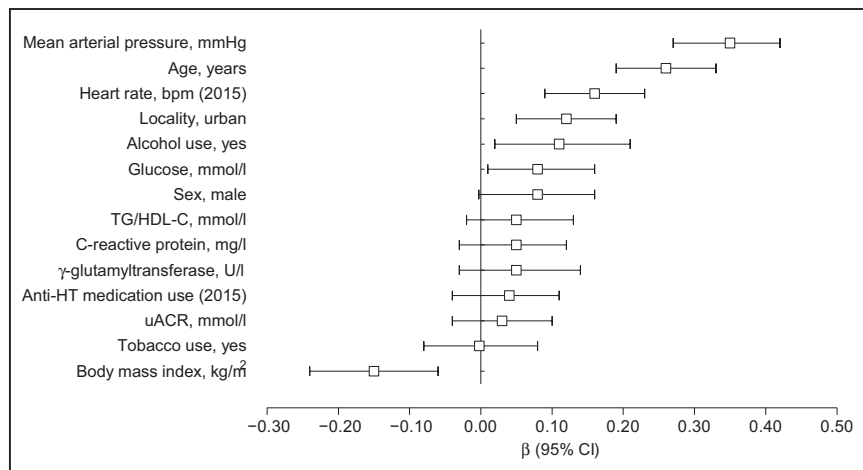


FIGURE 2 Multiple regression analyses in the total group with pulse wave velocity as dependent variable (adjusted R² = 0.31). TG:HDL-C, triglyceride:HDL cholesterol ratio; anti-HT, antihypertension; uACR, urinary albumin-to-creatinine ratio.

TABLE 3. Independent associations of follow-up pulse wave velocity with baseline covariates in men and women

Dependent variable: carotid–femoral pulse wave velocity (m/s)				
Adjusted R^2	Men, $n = 213$		Women, $n = 437$	
	β (95% CI)	P	β (95% CI)	P
	0.32		0.28	
Age (years)	0.25 (0.13; 0.37)	<0.001	0.27 (0.18; 0.36)	<0.001
Locality (urban)	0.10 (–0.02; 0.23)	0.10	0.13 (0.04; 0.23)	0.005
BMI (kg/m ²)	–0.16 (–0.31; –0.001)	0.050	–0.13 (–0.23; –0.02)	0.019
Heart rate 2015 (bpm)	0.14 (0.02; 0.27)	0.024	0.19 (0.09; 0.28)	<0.001
MAP 2015 (mmHg)	0.44 (0.32; 0.57)	<0.001	0.30 (0.21; 0.39)	<0.001
TG : HDL-C (mmol/l)	–0.01 (–0.16; 0.13)	0.85	0.10 (0.001; 0.19)	0.047
Glucose (mmol/l)	0.12 (–0.01; 0.25)	0.07	0.07 (–0.02; 0.16)	0.15
C-reactive protein (mg/l)	0.06 (–0.07; 0.19)	0.36	0.03 (–0.07; 0.13)	0.57
uACR (mmol/ml)	–0.03 (–0.17; 0.10)	0.60	0.07 (–0.02; 0.16)	0.12
γ -Glutamyltransferase (U/l)	0.12 (–0.02; 0.25)	0.10	0.02 (–0.08; 0.13)	0.67
Alcohol use (yes)	0.12 (–0.06; 0.29)	0.19	0.11 (0.002; 0.23)	0.045
Tobacco use (yes)	–0.01 (–0.18; 0.17)	0.95	–0.01 (–0.11; 0.08)	0.77
Anti-HT med use 2015	0.02 (–0.10; 0.15)	0.74	0.03 (–0.06; 0.13)	0.49

Data expressed as beta-values and 95% confidence intervals, P values obtained with multiple regression analyses. Anti-HT med use 2015, antihypertension medication use 2015; MAP, mean arterial pressure; PWV, pulse wave velocity; TG : HDL-C, triglyceride : HDL cholesterol ratio; uACR, urinary albumin-to-creatinine ratio.

to poverty and unemployment [35], which could manifest in changed health behaviours, such as anxiety, smoking and alcohol abuse [38]. A consequence of urbanization may be increased sympathetic nerve activity [35,37], translating into a higher HR [39]. Elastin fracture in the arterial wall may provide an explanation for the association between HR and arterial stiffness [40].

The association of glucose with future stiffness confirms previous reports [10,41]. Chronically increased plasma glucose reduces the elastic properties of large arteries [41], confirmed by findings that diabetes mellitus and impaired glucose tolerance are associated with an increased risk for stroke, heart failure and myocardial infarction [41]. This may be due to mechanisms such as glycation of proteins and formation of advanced glycation end-products [42], which are known to increase arterial wall stiffness by forming cross-links in collagen fibres and promoting vasoconstriction by decreasing the bioavailability of the vasodilator nitric oxide [43]. With obesity being a major cardiovascular risk factor and a health burden in South Africa [44], especially in women [45], the negative association between BMI and PWV was unexpected. Our group found a similar negative association between BMI and PWV in two different population samples, including young [31] and older black South African adults [46]. Low BMI in blacks may be the result of unhealthy behaviours, such as excessive alcohol consumption and tobacco use [47], which in turn has a detrimental effect on vascular health. Other studies also indicated an inverse association between BMI and alcohol consumption [48,49].

In our population, with a mean age of 61 years, 49% was hypertensive, which reflects the high cardiovascular risk in this general population sample. A quarter of the PWV measurements exceeded 10 m/s, which is the international cut-off value for PWV [50], indicating that at least 25% of our participants are at increased risk for cardiovascular and all-cause mortality [50,51]. However, this reference value was

based mostly on populations from European descent and needs validation in African populations.

Factors such as inflammation, endothelial dysfunction and smoking were expected to predict arterial stiffness after 10 years but were NS. More research is needed to elucidate the role of inflammation and endothelial dysfunction in arterial stiffening in this black population. Although we did not find any of the lipids to be predictors of PWV, we do see a concurrent rise in HDL-C, triglycerides and the ratio of the two variables along with the increase in PWV, possibly indicating early atherosclerotic vascular injury. Heavy smoking was one of the predictors of arterial stiffness in European and US longitudinal studies [9,52], but we and others [10,53] found no association between PWV and tobacco use. Smoking remains a well known harmful cardiovascular risk factor [54], but our findings suggest that other factors may have a greater effect on arterial stiffness in this population, thus potentially masking the effect of tobacco use.

Strengths and limitations

Our study included populations from specific urban and rural areas in the North West Province of South Africa and may not be representative of the whole population. However, we focused on the understudied black population, including longitudinal data on variety of health behaviours and arterial stiffness, using controlled conditions and well equipped research facilities. Carotid–femoral PWV was measured at follow-up only, which prevents firm conclusions regarding change over time. Although the results were consistent after several adjustments, we cannot exclude residual confounding.

In conclusion, health behaviours associated with alcohol abuse, such as an urban setting, elevated plasma glucose and low BMI predict arterial stiffness, independently of age and BP, in a black South African population over 10 years. These findings strongly support ongoing initiatives in South Africa to stem alcohol abuse.

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Conflicts of interest

There are no conflicts of interest.

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Reviewer's Summary Evaluation

Referee 2

This longitudinal study investigates predictors of elevated arterial stiffness in a black African population and finds that increased alcohol consumption is related to arterial stiffening independent of blood pressure changes. The findings provide potentially useful associative information for health

authorities. However, the study suffers from potential limitations of carotid-femoral pulse wave velocity being measured only once at the 10 year follow-up and not at baseline. In addition, some of the mechanisms proposed for the effect of alcohol on arterial stiffness may be related to changes in smooth muscle tone, preferentially affecting the measurement of pulse wave velocity in the peripheral muscular arteries.