

The association between apolipoproteins and arterial stiffness in young adults: the African-PREDICT study

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PREFACE

This study, “The association between apolipoproteins and arterial stiffness in young adults: the African-PREDICT study” forms part of the dissertation for the degree Master of Health Sciences in Cardiovascular Physiology at the North-West University. The dissertation is compiled in the article format as described and recommended by the North-West University. Following this format, the chapter outline is as follows:

Chapter 1: Literature overview

Chapter 2: Methodology

Chapter 3: Manuscript

Chapter 4: Final Remarks and Recommendations for Future Studies

The manuscript was prepared for submission to the journal, *Atherosclerosis*. The referencing style for Chapters 1, 2 and 4 are also prepared according to the author instructions of this journal.

AUTHOR CONTRIBUTIONS

Ms. D Botha

Responsible for conducting literature search; writing of the initial research proposal and completion of the ethics application. Writing of the literature review; performing of statistical analyses; as well as the design, planning and writing of the manuscript and dissertation. Were responsible for aliquoting blood samples into cryovials and for data capturing of blood samples for the African-PREDICT study.

Dr. Y Breet

Study supervisor. Supervised the writing of the proposal, ethics application, literature review and manuscript, collecting and interpretation of data. Provided guidance regarding statistical analysis, initial planning and design of the manuscript.

Dr. LF Gafane-Matemané

Study co-supervisor. Co-supervised the writing of the proposal, ethics application, literature review and manuscript, collecting and interpretation of data. Provided guidance regarding statistical analysis, initial planning and design of the manuscript.

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



Ms. D Botha



Dr. Y Breet



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SUMMARY

Motivation: South Africa is facing a high burden of non-communicable diseases with cardiovascular disease (CVD) being the largest contributor. Changes in vascular structure and function, even at an early age, contributes to the future development of CVD. Dyslipidaemia is traditionally defined as increased low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol levels and is strongly related to vascular changes and CVD development. Ethnic differences in the traditional lipid profile are well documented, with white populations tending to present with a more atherogenic profile, characterised by increased low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol. In addition to the traditional lipid markers, the use of apolipoproteins as risk markers for CVD is showing increased credibility in recent studies. Evidence regarding associations between apolipoproteins and the development of changes within the vasculature are limited and whether any ethnic differences are at play also remains to be determined.

Aim: Our study aimed to investigate whether differences in apolipoproteins are evident in a young, apparently healthy bi-ethnic population and whether apolipoproteins are associated with arterial stiffness in each ethnic group.

Methods: This study included 1162 young, apparently healthy black and white, men and women from the African-PREDICT study. Measures of vascular structure and function were determined including: pulse wave velocity (PWV), augmentation index (AIx), central pulse pressure (cPP) and carotid intima-media thickness (cIMT). Apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) were determined from serum samples and a Apo B/ Apo A-1 ratio was calculated.

Results and conclusion: We found that Apo B, Apo B/Apo A-1 and Apo A-1 were all higher in the white group (all $P < 0.030$) as compared to the black group. Apolipoprotein B was independently associated with cPP ($\beta = 0.16$, $P < 0.027$) and cIMT ($\beta = 0.19$, $P < 0.022$) and Apo B inversely associated with PWV ($\beta = -0.15$, $P < 0.025$) in the black group. No associations between the vascular markers and any of the apolipoproteins were found in the white group. In conclusion, the results indicate that apolipoproteins may play an important role in the early changes of the vasculature in young, black South Africans.

Key words: Lipids; pulse wave velocity; augmentation index; intima-media thickness; ethnicity.

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LIST OF ABBREVIATIONS

African-PREDICT- African Prospective study on Early Detection and Identification of Cardiovascular Disease and Hypertension

Aix- Augmentation index

Apo A-1- Apolipoprotein A-1

Apo B- Apolipoprotein B

BMI- Body mass index

BP- Blood pressure

cIMT- Carotid intima-media thickness

cPP- Central pulse pressure

CRP- C-reactive protein

CVD- Cardiovascular disease

DBP- Diastolic blood pressure

GGT- Gamma-glutamyl transferase

HDL-c- High-density lipoprotein cholesterol

HIV- Human immunodeficiency virus

HREC- Health Research Ethics Committee

IDLs- Intermediate-density lipoproteins

LDL-c- Low-density lipoprotein cholesterol

MAP- Mean arterial pressure

NCDs- Non-communicable diseases

PWA- Pulse wave analysis

ROS- Reactive oxygen species

SBP- Systolic blood pressure

SES- Socioeconomic status

VLDLs- Very-low-density lipoproteins

VSMCs- Vascular smooth muscle cells

WC- Waist circumference

CHAPTER 1

LITERATURE OVERVIEW

1. GENERAL INTRODUCTION

Non-communicable diseases (NCDs) are the leading cause of death in many countries, accounting for 71% of deaths globally and representing 43% of the global burden of disease.^{1,2} Moreover, cardiovascular disease (CVD) continues to show significant detrimental health, social and economic consequences globally, particularly in resource-limited countries such as in the sub-Saharan Africa region.³ Various risk factors, including elevated blood pressure (BP), diabetes, obesity, alcohol usage, tobacco usage, unhealthy diet and physical inactivity, contribute to the development of CVD and increases the burden of NCDs.^{4,5} In particular, dyslipidaemia is associated with vascular alterations, including arterial stiffness and atherosclerosis, contributing to the development of future cardiovascular events.⁶⁻⁸ With data showing a global dyslipidaemia prevalence of over 50% in adult populations,⁶ dyslipidaemia remains a significant factor to target in order to reduce the burden of CVD.⁶⁻⁸

2. THE ROLE OF DYSLIPIDAEMIA AND THE RELATED ETHNIC DIFFERENCES IN CARDIOVASCULAR DISEASE

Dyslipidaemia – defined as an abnormal amount of free cholesterol within the circulation – is one of the major contributors to CVD development and progression.^{9,10} Dyslipidaemia does not tend to show any obvious symptoms but has the ability to potentiate CVD development.¹¹⁻¹³ Although CVD is more common among middle-aged and elderly populations, the prevalence of high cholesterol among young adults increases the risk of early CVD development.¹⁴⁻¹⁶

Risk factors related to dyslipidaemia include smoking, obesity, central obesity, hypertension and diabetes.^{17,18} Several ethnic disparities related to CVD-risk factors have been well documented,¹⁹⁻²¹ including differences in the lipid profile.²²⁻²⁴ Studies have shown that white populations are more prone to have an atherogenic lipid profile with an increase in low-density lipoprotein cholesterol (LDL-c) and a decrease in high-density lipoprotein cholesterol (HDL-c) than black populations.^{21,25,26} The tendency toward a more atherogenic lipid profile in white population groups may be due to increased amounts of visceral adipose tissue accumulation, as well as genetic and dietary factors²⁷⁻²⁹, when compared to black populations.^{21,25,26}

Elevated LDL-c and decreased HDL-c is known to be the current conventional indicators for lipid profiling used in evaluating cardiovascular risk.²²⁻²⁴ Alternative approaches for lipid profiling include the use of apolipoproteins, and evidence suggests that apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) may be better CVD-risk predictors than traditional lipoproteins.³⁰⁻³³

3. APOLIPOPROTEINS AND THEIR FUNCTION WITHIN THE CIRCULATION

Apolipoproteins are the main protein components of LDL-c and HDL-c.³⁴ Various studies suggest that Apo B may be a more valuable risk predictor for dyslipidaemia than traditional LDL-c, as Apo B has the ability to reflect the total number of atherogenic particles within the circulation.³⁰⁻³³ The measurement of Apo B is, therefore, a more representative method to reflect this atherogenic burden.³⁰⁻³³ Apolipoprotein B is the main protein constituent of lipoproteins such as very-low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs) and LDL-c.³⁵ This apolipoprotein has atherogenic properties as it facilitates the entrapment of lipoproteins within the arterial wall, peripheral tissue and the liver.³² It also transports cholesterol from the site of production to peripheral tissues, where it is used as an energy substrate.^{32,35} Apolipoprotein B is a major contributor to inflammation, atherosclerosis, calcification, oxidative stress and arteriosclerosis (Figure 1).^{32, 36}

In contrast, Apo A-1 is involved in reverse cholesterol transportation from peripheral tissue back to the liver, decreasing inflammation and plaque formation, thus serving an anti-atherogenic role (Figure 1).³⁷⁻⁴⁰ When Apo A-1 concentrations decrease within the circulation, the risk of CVD increases.^{37,38} In the presence of increased Apo B concentrations, these anti-atherogenic properties of Apo A-1 tend to disappear.⁴⁰ Apolipoprotein A-1 reduces oxidative stress, inhibits LDL-c oxidation and prevents platelet activation.⁴¹ Apolipoprotein A-1 plays a major anti-inflammatory role through the inhibition of endothelial cell activation, the promotion of M2 macrophages and by neutralising the pro-inflammatory effect of C-reactive protein (CRP).⁴¹⁻⁴³

The balance of cholesterol transport within the circulation is reflected through the Apo B/Apo A-1 ratio (Figure 1) – a major risk predictor of CVD.⁴⁰ The higher the Apo B/Apo A-1 ratio, the higher the amount of free circulating cholesterol.^{33,40,44} A higher Apo B/Apo A-1 ratio increases the risk of cholesterol deposition within the arterial wall and the development of atherosclerosis and arteriosclerosis,^{33,40,44} which will be discussed in the next section. However, when the Apo B/Apo A-

1 ratio decreases, reverse cholesterol transport increases with a subsequent decrease in cardiovascular risk.⁴⁰

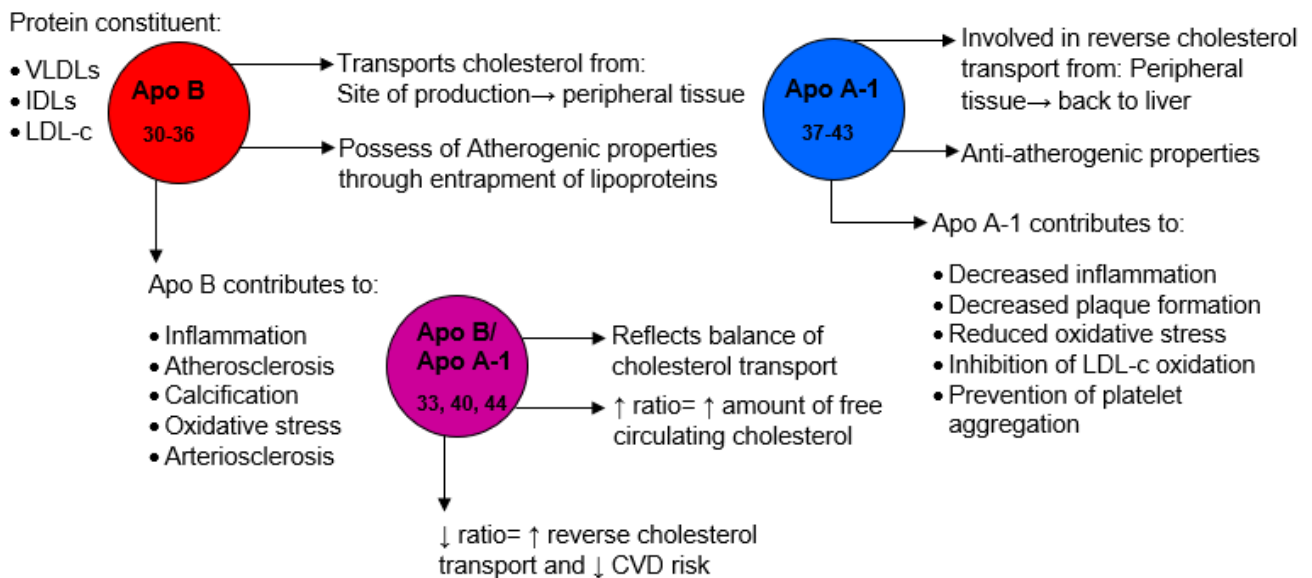


Figure 1: Summary of the function of the different apolipoproteins.³⁰⁻⁴⁴

4. PHYSIOLOGICAL STRUCTURE AND FUNCTION OF THE ARTERIAL SYSTEM

The function of the large arteries is to counteract the constant changes in BP through its buffer capacity, reducing pulse pressure and promoting optimal tissue perfusion.⁴⁵ The various arterial segments of the arterial system accomplishes this buffering function. This includes the large arteries (aorta and common carotid arteries) and the muscular arteries (femoral and brachial arteries).⁴⁶

The vascular wall consists of three layers: the intima, media and adventitia.⁴⁷ The intima is the innermost layer of the vascular wall, consisting of the endothelium and is responsible for regulating the vascular tone.⁴⁷ The layer bordering the intimal layer is the media, which consists mainly of vascular smooth muscle cells (VSMCs) and an elastin-rich extracellular matrix. The outermost layer of the vascular wall, the adventitia, is comprised mainly of collagen fibres and has very few elastin fibres.⁴⁷

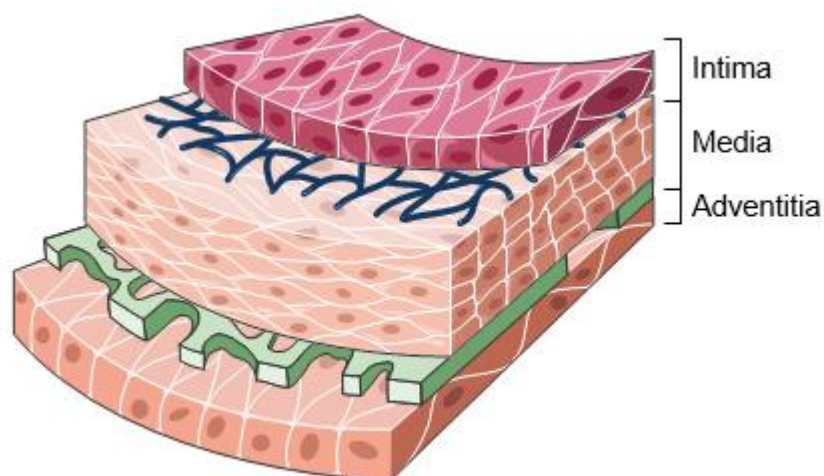


Figure 2: Three layers of the arterial wall. Image retrieved and adapted from Smart Servier Medical Art.

The various layers of the arterial wall work together to achieve the buffer capacity of the arterial system. When deterioration starts to occur within these vascular wall units, arterial stiffening and thickening occur, and the risk of arteriosclerotic plaque formation increases.⁴⁸ This deterioration increases the risk of cardiovascular disease and also increases with advancing age.⁴⁸

5. PATHOPHYSIOLOGICAL CHANGES IN VASCULAR STRUCTURE AND FUNCTION:

ARTERIOSCLEROSIS AND ATHEROSCLEROSIS

Arteriosclerosis is the consequence of lipid accumulation within the intimal layer of the vascular wall, resulting in stiffening, thickening and degeneration,^{49,50} whereas atherosclerosis is a consequence of atheroma formation.⁴⁹ Arteriosclerosis and atherosclerosis have the ability to potentiate one another through vascular remodelling – thus having a synergic process – as they share pathophysiological mechanisms.^{51,52} The accumulation of lipids within the intimal layer results in arterial alterations, including the loss of elasticity (loss of elastin and accumulation of collagen) and the thickening and stiffening of the vessel wall, thus leading to arterial stiffness.⁵³

5.1 PATHOPHYSIOLOGICAL MECHANISM OF ARTERIAL STIFFNESS

Arterial stiffness is a major predictor of cardiovascular risk and mortality.⁵¹ It is known that, throughout the course of life, aging^{45,54,55} and certain diseases (hypertension⁴⁵ and diabetes mellitus) can aggravate loss of elasticity, thickening and stiffening within the arterial wall.⁵³ Alterations can also be driven by haemodynamic forces within the circulation,⁵⁶ which results in a decreased buffer capacity of the arteries, increased central pulse pressure (cPP) and increased pulse wave velocity (PWV).⁵⁷ These changes within the vessel wall result in the early return of reflected waves, systolic BP

augmentation and decreased diastolic BP.^{57,58} As a result of this augmented pressure and velocity, the afterload and amount of sheer stress on the vessel walls increase.^{57, 58}

Arterial stiffness is the result of degeneration in the extracellular matrix of elastic arteries, which occurs over the course of life.⁵⁹ This degeneration is characterised by the fragmentation of elastin and the deposition of collagen within the arterial wall.^{56,59,60} When a dysregulation in the balance of these proteins occurs, it leads to the overproduction of collagen and diminished production of elastin.⁵³ Elastin has an inhibitory effect on the VSMCs, preventing migration and proliferation thereof.⁵¹ When extracellular matrix stiffening occurs, the integrity of the endothelial layer becomes compromised, and this results in increased permeability of the layer.^{51,61} Increased permeability results in increased cholesterol uptake from the circulation into the vessel wall and consequently plays a role in atherosclerotic plaque formation.^{51,62} This integral issue of the endothelial layer could explain the associations previously found between arterial stiffness and the presence of plaque found within the vessel wall.^{63,64}

Various ethnic disparities have been found regarding arterial stiffness: it tends to be more pronounced in black populations at an early age than in white populations.^{65,66} Changes in arterial stiffness in white populations tend to be more age-related, whereas in black populations, these changes are reported to be more pressure-related.^{65,67-69}

5.2 PATHOPHYSIOLOGICAL MECHANISM OF ATHEROSCLEROSIS

Atherosclerosis is characterised by the accumulation of lipids and inflammatory cells within the intimal layer of the artery, resulting in VSMC migration, foam cell development and plaque formation.^{51,70} Atherosclerosis is initiated mainly at sites of endothelial dysfunction, retention and accumulation of oxidised LDL-c within the arterial wall (Figure 3).⁷¹ This predominantly leads to the uptake of cholesterol-rich LDL, which results in the formation of cholesterol-rich monocyte-derived macrophage foam cells. This leads to the formation of fatty plaque streaks.^{71,72} Inflammation within this fatty-streak lesion occurs as pro-inflammatory cytokines are recruited from the circulation, further evolving the fatty-streak lesion.⁷³ Various cardiovascular risk factors are known to aggravate this inflammatory state, including hypertension, diabetes mellitus, hypercholesterolemia, smoking and genetics.^{71,73,74} The inflammatory state within the artery wall results in the migration of VSMCs to the

fatty-streak lesion.^{73,75} The VSMCs have equal ability to absorb modified lipoproteins and to transition further into foam cells.^{75,76} As the fatty-streak lesion progresses, apoptosis and necrosis occur within the inflammatory cells, resulting in the release of the intracellular contents.⁷¹ At this stage, advanced plaque is comprised mainly of a necrotic core and lipoprotein-derived lipids under a thin, fibrous cap.⁷¹ This thin, fibrous cap tends to rupture under high mechanical stress within the artery, resulting in the trigger of thrombosis that can lead to CVD complications (myocardial infarction or stroke).⁷¹

Previous studies have found differences in the prevalence of atherosclerosis among different ethnic groups.^{77,78} A higher burden of atherosclerosis has been reported among white populations in comparison to black populations. These differences may be due to the differences in cardiovascular risk factors among different ethnic groups, including central obesity and dyslipidaemia.⁷⁷⁻⁸⁰

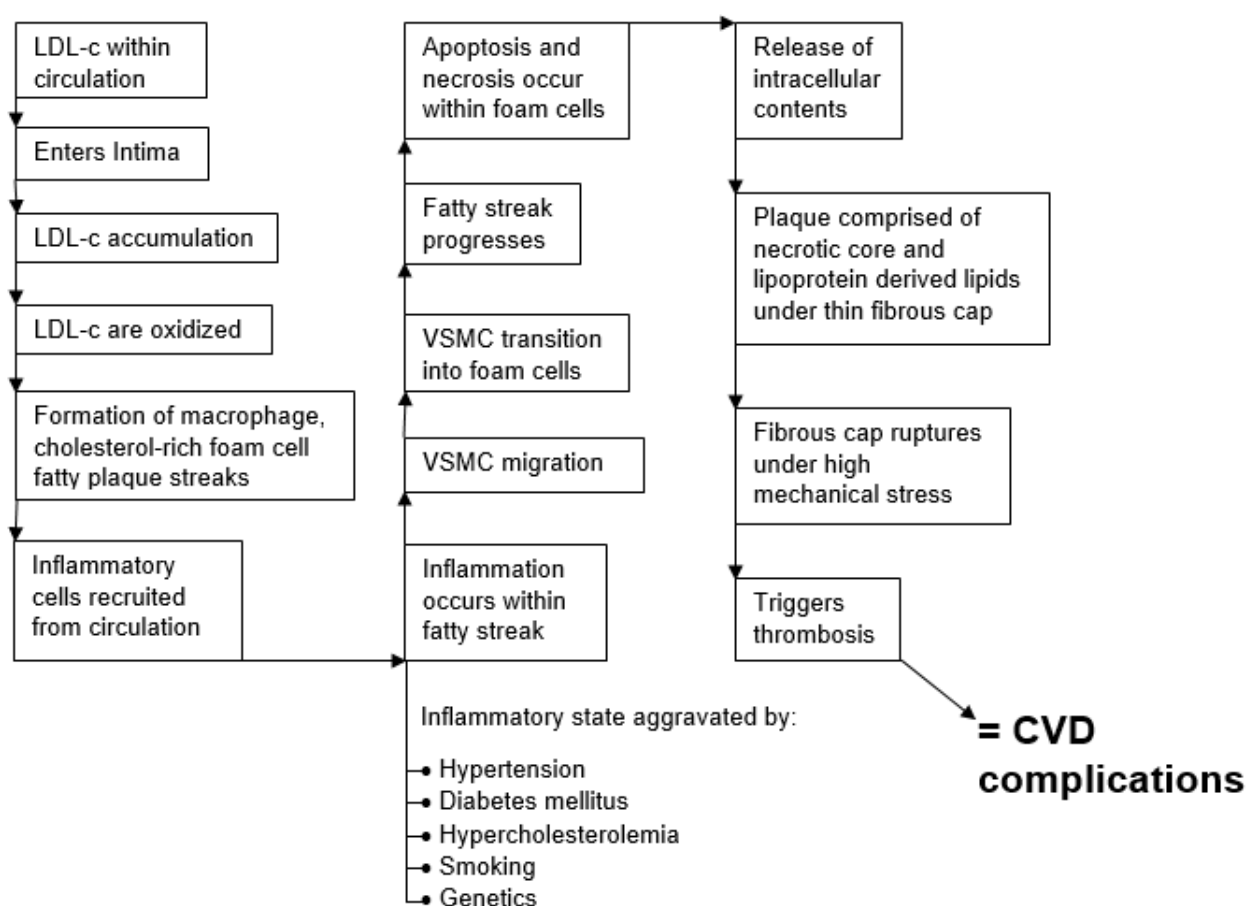


Figure 3: The pathway of atherosclerosis.

5.3 DETERMINANTS OF ARTERIAL STIFFNESS AND ATHEROSCLEROSIS

Various well-known markers exist to determine increased stiffness within the circulation, including PWV, augmentation index (Alx) and central pulse pressure. These markers have been established as major predictors of cardiovascular risk and mortality.^{58,81-84}

Pulse wave velocity measures the speed of pressure waves travelling along the aorta and large arteries. It is calculated by dividing the distance the pressure wave travelled between two points by the time it took to travel this distance within the arteries.^{85,86} Pulse wave velocity is known to be cost-effective, reliable and is determined non-invasively.⁸⁶⁻⁸⁸

Another useful marker to determine cardiovascular risk is Alx.⁸³ Augmentation index is a parameter that shows to which degree central arterial pressure is enhanced through the reflected pulse wave.⁸³ Strong associations have been found between Alx and PWV, with an increased PWV resulting in an early return of the reflected arterial waves, ultimately augmenting the central systolic pressure during systole.^{89,90}

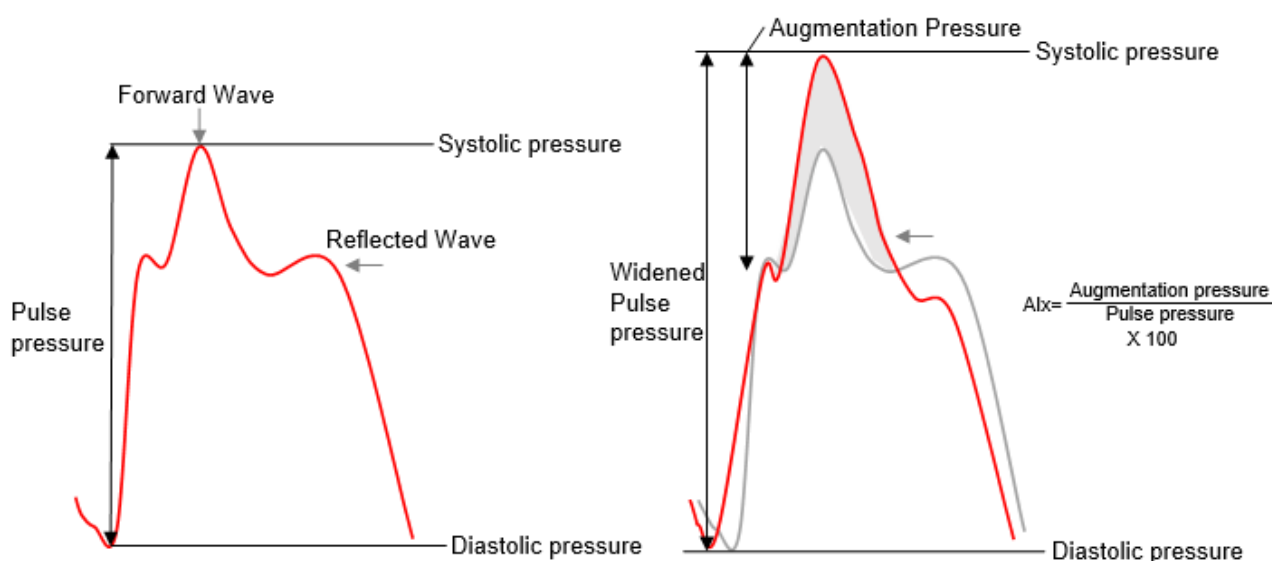


Figure 4: Arterial pressure wave. Image retrieved and adapted from Smart Servier Medical Art.

Pulse pressure is also a major determinant of cardiovascular risk.⁹¹⁻⁹³ It is a haemodynamic measurement affected by various cardiovascular risk factors, including hypercholesterolemia, hypertension, smoking and metabolic syndrome.⁸² However, pulse pressure is dependent on the degree of arterial stiffness, stroke volume and timing of the reflected pressure waves.⁹⁴

The structural properties of large arteries can be assessed by determining carotid intima-media thickness (cIMT), a subclinical marker of atherosclerotic burden. This non-invasive ultrasound technique measures the thickness of the intima and medial layers of the carotid artery and reflects the atherosclerotic burden at the site of measurement.^{84,95,96} When cIMT measures at less than 0.9 mm, it is seen as normal. With a cIMT greater than 1.5 mm, atherosclerotic plaque can be identified within the carotid artery.⁹⁷⁻⁹⁹

6. MOTIVATION

South Africa faces a high prevalence of NCDs, among which CVD is the largest contributor.^{100,101} The increase in the prevalence of NCDs, such as CVD, places a significant strain on the public health sector. Arterial stiffness has been identified as a risk for CVD, and previous studies have already shown ethnic differences to be evident here, with arterial stiffness being higher in six to eight-year-old black boys, showing differences in arterial stiffness already at an early age.^{65,102-104}

Numerous studies exist regarding the association between apolipoproteins and CVD risk,^{30,81,102,103} with only a few focusing on the direct associations between apolipoproteins and arterial stiffness.^{30,102} Existing studies, focusing on the direct association between apolipoproteins and arterial stiffness, were conducted in small population groups and populations already presenting with numerous other risk factors such as the metabolic syndrome.^{102,104} Limited data is available on the associations between apolipoproteins and arterial stiffness, and it remains to be determined whether any ethnic differences are at play. In addition, previous studies regarding the associations between apolipoproteins and vascular markers included older participants of European descent with existing cardiovascular related diseases.^{30,81,102,103} Therefore, this study investigated whether associations exist between apolipoproteins and arterial stiffness within a young bi-ethnic South African cohort. These findings could contribute to the understanding of the early development of arterial stiffness as a risk factor for CVD.

Aim

The aim of this study is to investigate apolipoproteins (Apolipoprotein A-1, Apolipoprotein B and the Apo B/Apo A-1 ratio) and their relationship with arterial stiffness in a young bi-ethnic cohort.

Objectives

In a bi-ethnic, young population, the objectives of this study are:

1. To determine if differences in apolipoproteins (Apolipoprotein A-1, Apolipoprotein B and the Apo B/Apo A-1 ratio) and markers of arterial stiffness (PWV, augmentation index, cIMT and central pulse pressure) are evident.
2. To determine whether associations exist between apolipoproteins and arterial stiffness (PWV, augmentation index, cIMT and central pulse pressure) within each ethnic group.

Hypothesis

1. Apo B and the Apo B/ApoA-1 ratio will be higher and Apo A-1 lower in the white population as compared to that of the black population.
2. Apo B and the Apo B/Apo A-1 ratio will positively associate with markers of vascular structure and function (PWV, AIX, cPP and cIMT), while a negative association between Apo A-1 and the markers of vascular structure and function (PWV, AIX, cPP and cIMT) will be evident. These associations are expected in both ethnic groups.

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CHAPTER 2

METHODOLOGY

1. STUDY DESIGN AND PARTICIPANTS

This MHSc study is a sub-study which includes the cross-sectional data from the full baseline cohort of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT).¹ The original, larger African-PREDICT study was designed to investigate the early pathophysiology accompanying cardiovascular disease development and to identify novel early markers or predictors by following young, apparently healthy adults over a period of 10 years. Participants were recruited from Potchefstroom and surrounding areas by trained field workers – via their workplace or through advertisement by means of local newspapers or radio stations. This sub-study included 1202 young, apparently healthy black and white men and women between the ages of 20 and 30 years who were first screened. Those who met the criteria were included in the study (Table 1). Forty participants with missing data for the main variables of interest were excluded, and the final study sample consisted of 1162 participants.

Table 1: Detailed eligibility criteria and concurrent justification for inclusion in the African-PREDICT study.

Inclusion criteria:	Justification:
1. Apparently healthy 2. Black or white ethnicity 3. Aged 20-30years 4. Normotensive or pre-hypertensive (systolic blood pressure (SBP) < 140 and diastolic blood pressure < 90mmHg) 5. Men and women (equally distributed)	1. The African-PREDICT study is a longitudinal study, aimed to include and track young, healthy individuals over a period of 10-20 years in order to monitor especially the early phases of cardiovascular disease (CVD) development. 2. In South Africa, black populations are disproportionately affected by CVD and early-onset hypertension compared to white populations.² 3. The study aims to follow the progression of vascular function in young normotensive individuals. 4. As hypertension is the primary endpoint, it was necessary to exclude those with hypertension (based on office blood pressure) and those on chronic medication or with CVD. International hypertension cut-off points were used to determine whether a participant was hypertensive or normotensive.³

	5. Both men and women were used to determine whether differences exist between genders.
Exclusion criteria: 1. Hypertensive 2. Indian, Asian or mixed-origin ethnicity 3. Not permanent resident of Potchefstroom surrounding area (i.e. intends to move to another area) 4. Self-reported diagnosis of Type 1 or 2 Diabetes Mellitus 5. Elevated glucose > 5.6 mmol/L and confirmed glycated haemoglobin (HbA1c) $\geq$ 6.5% 6. HIV-infected 7. Fever (internal ear temperature > 37.5°C on the research day) 8. Self-reported liver disease, cancer, tuberculosis or renal disease 9. Microalbuminuria > 30 mg/ml in spot morning urine or proteinuria 10. Medication use for chronic disease (antihypertensive, anti-diabetic, antiretroviral or anti-inflammatory) 11. Pregnant or lactating women 12. Phobia for needles 13. Recent surgery or trauma (past three months) 14. History of stroke, angina pectoris or myocardial infarction	Justification: 1. As hypertension development was an endpoint in this study, hypertensive participants were excluded from the start of the study. 2. From known populations, black individuals have elevated blood pressure and were therefore used in the study. The white population was used as a comparison group. 3. Due to the longitudinal nature of the study, researchers made sure that participants could be followed over the required time period. 4. Individuals with any known diseases or risk factors that may influence cardiovascular health were excluded (exclusion criteria 4-10, 13, 14). 11. Due to the known influences of hormones on cardiovascular health, pregnant and lactating women were excluded. 12. Since blood samples are important to meet the objectives of the African-PREDICT study, participants with a phobia for needles were excluded.

2. METHODOLOGY

2.1 ORGANISATIONAL PROCEDURES

The recruited participants underwent screening to determine eligibility based on the inclusion and exclusion criteria (Table 1) for participation in the African-PREDICT study. The African-PREDICT study (NWU-00001-12-A1) and this MHSc study (NWU-00368-20-A1) were evaluated and approved by the Health Research Ethics Committee of the North-West University (Appendix B). Additionally, both this study and the African-PREDICT study adhere to the principles set out in the Declaration of

Helsinki (2008). The African-PREDICT study was endorsed by both the National and Provincial Department of Health, and the study conformed to the Medical Research Council guidelines of good clinical practice.

Participants arrived at the North-West University in Potchefstroom at the Hypertension Research and Training Clinic at 08:00 in the morning. The measurements' procedures were explained to the participants by the research nurse, research assistant, and researchers. The informed consent form were discussed with the participants, and they had the opportunity to ask questions prior to giving written informed consent. Privacy of participants was always taken into consideration, and all measurements were taken in a private, temperature-controlled room. A maximum of four participants were accommodated per day to ensure detailed and quality measurements. During the study day, participants rotated around a number of measurement stations with the help of the research nurse and research assistant. In between stations, participants were requested to complete questionnaires with help from researchers. All procedures were performed according to good clinical practice.

2.2 GENERAL HEALTH AND DEMOGRAPHICS QUESTIONNAIRE

Data with regards to age, sex and ethnicity were collected using a general health and demographics questionnaire. This questionnaire was completed on iPads using a web-based program. It took approximately 15 minutes to complete. The questionnaire also captured data pertaining to alcohol consumption, smoking history and socioeconomic status (SES). The SES of a participant was determined using three categories included in the demographic questionnaire, namely skills level, education and household income. Points were scored for each of these categories, and the total number of points were used to determine whether a participant fell in low, middle or high SES.⁴

2.3 ANTHROPOMETRIC MEASUREMENTS

Anthropometric measurements were taken by trained anthropometrists. Standard procedures were used to determine height (m) by using the SECA 213 Portable Stadiometer (SECA, Hamburg, Germany), weight (kg) using the SECA 813 Electronic Scales with a weighing capacity of 200 kg (SECA, Hamburg, Germany) and waist circumference (cm) using a Lufkin Steel Anthropometric Tape W606PM (Lufkin, Apex, USA). These procedures were performed by a trained researcher. Body mass index (BMI) was subsequently calculated (kg/m^2). All of the anthropometric

measurements were performed according to the guidelines of ISAK (International Society for the Advancement of Kinanthropometry).⁵



Figure 1: Waist circumference being measured.

2.4 CLINIC BLOOD PRESSURE

With the use of the Dinamap Procare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA), the clinic blood pressure of participants was measured. Participants were fitted with an appropriately-sized GE Critikon latex-free Dura-Cuff. Before the measurement was conducted, participants were requested to refrain from eating, smoking and exercising for at least 30 minutes beforehand and to be seated in a resting state. After a 5 minute resting period, duplicate measurements were taken on the left and right arm (with 5 minute intervals), with the arm supported at heart level and feet rested flat on the floor. The systolic blood pressure, diastolic blood pressure and heart rate were captured after each measurement. An average of all four blood pressure measurements performed for each arm was used to calculate an average for both systolic and diastolic blood pressure.

2.5 AUGMENTATION INDEX, CENTRAL PULSE PRESSURE AND ARTERIAL STIFFNESS

The SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) was used to estimate central systolic blood pressure, central pulse pressure and the augmentation index by performing pulse wave analysis (PWA). The PWA was also used to measure the supine brachial systolic and diastolic blood pressure. From this, mean arterial pressure (MAP) was calculated. A brachial cuff was placed on the right upper arm. By using a general transfer function, the device estimated central

systolic blood pressure, and the measurement was performed in duplicate. Arterial stiffness was also assessed by determining carotid femoral pulse wave velocity (PWV) with the SphygmoCor® XCEL device. Approximately five minutes before the commencement of the measurement, participants were requested to be in a supine position and in a relaxed state. Through means of palpation, the participant's carotid artery was located to identify the strongest pulsating point. Pulse wave velocity was captured at the right carotid and femoral arterial pulse points. The femoral artery waveform was captured via an appropriately sized cuff placed around the thigh, and the carotid arterial waveform was captured simultaneously via applanation tonometry. The distances between the pulsated sites were measured using an infantometer, and 80% of these distances were used as the pulse wave travelled distance.⁶



Figure 2: SphygmoCor® XCEL device used to determine central systolic blood pressure, central pulse pressure, augmentation index and pulse wave velocity.

2.6 CAROTID INTIMA-MEDIA THICKNESS

Carotid intima-media thickness (cIMT) measurements were made from the far wall of the left and right common carotid artery and the internal carotid using the General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway). Carotid intima-media thickness measures the distance between the lumen-intima interface to the media-adventitia interface of the artery wall. Each side was measured and recorded for similar future assessments from various optimal angles. The images were digitised and imported in the Artery Measurement Systems software (Gustavsson, Sweden) for analyses.



Figure 3: General Electric Vivid E9 device.

2.7 BLOOD SAMPLING AND BIOCHEMICAL ANALYSES

Participants were requested to fast overnight for at least eight hours prior to attending the research measurement day. Early-morning blood samples were collected by a research nurse in a private, temperature-controlled room. Once the samples were taken, a research assistant trained in handling biological samples (using latex gloves) collected the samples in the clinic and placed the sample tubes in a closed container. Samples were immediately taken to the on-site temperature-controlled laboratory. Samples were then centrifuged and aliquoted into cryovials for storage at -80°C in biofreezers. Staff and students who handled the biological samples had received extensive training by the laboratory manager to reduce any risks. The staff and students were all part of the Hepatitis surveillance programme. During all procedures, protective laboratory coats and latex gloves were worn.



Figure 4: Samples aliquoted into cryovials for storage in biofreezers.

Serum samples were analysed using the Cobas Integra® 400 plus (Roche, Basel, Switzerland) to determine apolipoproteins (Apo B, Apo A-1), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-C), triglycerides, glucose, C-reactive protein and

gamma-glutamyl transferase. Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Reactive oxygen species (ROS) were determined in serum with high-throughput spectrophotometric assay by the Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA).



Figure 5: Cobas Integra® 400 plus.

3. DATA MANAGEMENT

The African-PREDICT study uses the REDCap (Research Electronic Data Capture, see <http://project-redcap.org>) system to capture all data elements.⁷ The Data Manager was appointed and trained to oversee REDCap. When using this system, all laboratory specimens, evaluation forms, reports, data and other records are identified only by the participant number to maintain participant confidentiality. Data entry is performed by research assistants and controlled and checked by the Data Manager. Only the Data Manager, Head of the Hypertension Clinic and Principal Investigator have access to information linking ID numbers to participant names. Apart from the REDCap system, data are also backed up on password-protected hard drives, and all paper and computerised records are kept in a secured area. Clinical information was not released without the participant's written permission except as necessary for cleaning, monitoring, and statistical analysis by the authorised study team members or when the participant's well-being was at risk. No new samples were collected since existing data were used for this sub-study. Password-protected computers were used to ensure confidentiality and, upon receiving HREC approval for the study, a password-protected dataset was shared with the student by the data manager. After completion of data analyses, the dataset and all copies thereof were given back to the principal investigator, who deleted the dataset.

4. STATISTICAL ANALYSES

Statistical analyses were performed using Statistica v13.3 (TIBCO Software Inc, Palo Alto, CA, USA). GraphPad Prism 5.03 (GraphPad Software, San Diego) was used for graphics. All variables were tested for normality using formal tests (Kolmogorov-Smirnov test and Shapiro-Wilks test) and graphical methods (histograms). In the case of non-Gaussian distribution, a logarithmic transformation was performed for each skewed variable and tested for normality again. The mean and spread of all descriptive variables (either standard deviation, 95% percentile interval or percentage) were presented to characterise the total group. Independent t-tests were performed to compare differences between black and white groups. Pearson, partial and multiple regression analyses were performed to determine the associations between measures of arterial stiffness and apolipoproteins. Covariates included in the models were age, sex, SES, WC, LDL-c, glucose, CRP, GGT and cotinine. Models with PWV and cIMT as dependent variables additionally included MAP.

5. REFERENCES

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CHAPTER 3

MANUSCRIPT

INSTRUCTIONS TO AUTHORS: ATHEROSCLEROSIS**Structure:****Title page:**

- Full manuscript title.
- Authors full names and affiliations (Institution where the actual work was done below the author names indicated with a lower-case superscript letter).
- Name of corresponding author.
- Permanent address of authors.

Abstract:

- Structured (Background and aims, Methods, Results, Conclusions).
- 250 words.
- 3-7 keywords.

Text:

- 4000 words.
- 5 figures and/or tables.
- Manuscript structure: Introduction, Methods, Results, Discussion, Conflict of interest, Financial support, Author contributions, Acknowledgements and References.
- Abbreviations defined when first used.

Figures and tables:

- Tables submitted as word files.
- Each table should have a brief title that describes the entire figure.

References:

- No strict requirements on reference formatting or style (used Vancouver).



The association between apolipoproteins and arterial stiffness in young adults: the African-PREDICT study

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Word count: 3556

ABSTRACT

Background and aims: Adverse lipid levels can potentiate changes within the vasculature, resulting in the development of cardiovascular disease. It is suggested that Apolipoproteins are better risk predictors for cardiovascular disease than traditional lipid profiling. However, data regarding the relationship between apolipoproteins and arterial stiffness are limited. Ethnic differences in apolipoproteins are not well documented and the role of ethnicity in the relationship between apolipoproteins and arterial stiffness also remains unclear. Therefore, the aim was to investigate whether differences in apolipoproteins are evident in a young, apparently healthy, bi-ethnic population and whether apolipoproteins are associated with markers of vascular structure and function in each ethnic group.

Methods: Young black and white men and women (aged 20–30, N=1162) from the African-PREDICT study were included. Carotid-femoral pulse wave velocity (PWV); central pulse pressure (cPP) and augmentation index (AIx) were determined, and carotid intima-media thickness (cIMT) was measured. Apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) were determined, and the ratio of Apo B/Apo A-1 was calculated.

Results: Apo B, Apo B/Apo A-1 and Apo A-1 were higher in the white group (All $P < 0.030$). Apolipoprotein B was independently associated with cPP ($\beta = 0.16$, $P < 0.027$) and cIMT ($\beta = 0.19$, $P < 0.022$) and inversely associated with PWV ($\beta = -0.15$, $P < 0.025$) in the black group. No associations between the vascular markers and any of the apolipoproteins were found in the white group.

Conclusions: Adverse associations between vascular measures and Apo B in young, apparently healthy black adults were found. No associations between vascular measures and apolipoproteins were found in the white adults.

Abstract word count: 254 words

Keywords: Lipids; pulse wave velocity; augmentation index; carotid intima-media thickness; ethnicity.

1. INTRODUCTION

The high prevalence of cardiovascular disease (CVD) continues to show detrimental health, social and economic consequences globally and in resource-limited settings such as sub-Saharan African countries.¹ Cardiovascular disease is recognised as one of the leading causes of morbidity and mortality globally.¹ The development of CVD is potentiated by various risk factors including elevated blood pressure, dyslipidaemia, smoking, diabetes and obesity.² These CVD risk factors potentiate vascular alterations including arterial stiffness. It is known that arterial stiffness manifests with increasing age. In the presence of CVD risk factors,² however, previous studies conducted in an African cohort, found that measures of arterial stiffness were already more pronounced in black South African children than when compared to their white counterparts.³

The current conventional approach for lipid profiling in cardiovascular risk evaluation are elevated low-density lipoprotein cholesterol (LDL-c) and decreased high-density lipoprotein cholesterol (HDL-c).^{4,5} An unfavourable lipid profile plays a major role in pathological processes leading to vascular abnormalities such as atherosclerosis, arterial stiffness and hypertension.⁶⁻⁸ Recent evidence suggests that apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) may be better CVD risk predictors in risk evaluation than the traditionally used lipoproteins.⁹⁻¹² Apolipoprotein B is the main protein constituent of lipoproteins, including very-low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs) and LDL-c.¹³ Apolipoprotein B is more of a representative marker of the atherogenic burden, as it facilitates the entrapment of lipoproteins within the arterial wall.⁹⁻¹² Therefore, it is a major contributor to inflammation, atherosclerosis, calcification, oxidative stress and arteriosclerosis.^{11,14} Apolipoprotein A-1 (Apo A-1), however, serves an anti-atherogenic role and, in the presence of increases in Apo B, these anti-atherogenic properties tend to disappear.¹⁵⁻¹⁸ Apolipoprotein A-1 is known to play a major anti-inflammatory role and also reduces oxidative stress, LDL-c oxidation and prevents platelet activation.¹⁹⁻²¹ The Apo B/Apo A-1 ratio reflects the balance of free cholesterol within the circulation.^{12,18,22} The higher the Apo B/Apo A-1 ratio, the higher the amount of free circulating cholesterol, which increases the risk of cholesterol deposition within the arterial wall and, therefore, increases the risk of atherosclerosis and arteriosclerosis development.^{12,18,22}

Data regarding apolipoproteins and arterial stiffness in young, healthy African populations are very limited. Existing studies were conducted in small European population groups and in populations already presenting with numerous other risk factors, such as the metabolic syndrome.²³ Therefore, the aim is to investigate whether any differences in apolipoproteins are evident in a young, apparently healthy, bi-ethnic population and whether apolipoproteins are associated with arterial stiffness in each ethnic group. These findings may aid in the early detection and prevention of CVD.

2. METHODS

2.1 Study design and population

This study is part of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT).²⁴ The main aim of African-PREDICT is to follow young, apparently healthy adults over a 10-year period to identify novel markers related to early cardiovascular disease development.

Participants were screened, and those who met the criteria were included in the study. The inclusion criteria were young (20–30 years), apparently healthy black and white men and women with a normal screening clinic BP (<140/90 mmHg), that are not infected with HIV, have no self-reported chronic disease (or treatment thereof) and are not pregnant or breastfeeding at the time of inclusion. This sub-study included the cross-sectional data from the full baseline cohort of 1202 participants. Forty of these participants were excluded because of missing data for the main variables of interest. The final study sample consisted of 1162 participants.

The study was approved (NWU-00368-20-A1) by the Health Research Ethics Committee (HREC) of the North-West University, and every participant gave written informed consent. The African-PREDICT study has also been registered at ClinicalTrials.gov (Identifier: NCT03292094).

2.2 Questionnaires

Participants completed the General Health and Demographic Questionnaire online on a web-based program that captured basic information such as sex, age, socioeconomic status, and alcohol and tobacco usage. The socioeconomic status (SES) of a participant was determined from three categories included in the demographic questionnaire: skills level, education and household income.

Points were scored for each of these categories, and the total number of points were used to determine whether a participant fell in low, middle or high SES.²⁵

2.3 Anthropometric measurements

Standard procedures were used to determine height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany), weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany) and waist circumference (WC) (Lufkin Steel Anthropometric Tape (W606PM) Lufkin, Apex, USA). Body mass index (BMI) was calculated.²⁶

2.4 Cardiovascular measurements

With the use of the DinamapProcure 100 Vital signs Monitor (GE Medical Systems, Milwaukee, USA), the clinical blood pressure of participants was measured. Participants were fitted with an appropriate-sized GE Critikon latex-free Dura-Cuff. Before the measurement was conducted, participants were requested to refrain from eating, smoking and exercise for at least 30 minutes prior to the measurement and to be seated in a resting state with the arms supported at heart level. After the participant was seated calmly for five minutes, the first measurement was taken on the left arm. The blood pressure was taken on the right arm in duplicate. A final measurement was then taken on the left upper-arm of the participant. The systolic blood pressure, diastolic blood pressure and heart rate were captured after each measurement. For statistical analysis, both the duplicate measures performed on each arm were used to calculate an average for both systolic and diastolic blood pressure separately.

The SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) was used to estimate central systolic blood pressure, central pulse pressure and the augmentation index by performing pulse wave analysis (PWA). A brachial cuff was placed on the right upper-arm and, by using a general transfer function, the device estimated central systolic pressure. The measurement was performed in duplicate. An average of the duplicate measures was used for statistical analysis. Carotid-femoral pulse wave velocity (PWV) was also measured with the SphygmoCor® XCEL device. The participant's carotid artery was located to identify the strongest pulse point, and the carotid pulse was measured using a tonometer, while the femoral pulse was measured by a femoral

cuff placed around the thigh of the participant. The distance between the carotid pulse point and upper femoral cuff was noted, and 80% of the distance was calculated and entered.²⁷

Carotid intima-media thickness (cIMT) measurements were taken on the left and right common carotid artery and the internal carotid using the General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway). Carotid intima media thickness measures the distance between the lumen-intima interface to the media-adventitia interface of the artery wall. Each side was measured and recorded for similar future assessments from various optimal angles. The images were digitised and analysed using the Artery Measurement Systems software (Gustavsson, Sweden). The left far wall measurements of the carotid artery were used in analysis.

2.5 Biological sampling and biochemical analysis

Fasting morning blood samples were taken by a registered nurse. All samples were immediately taken to the on-site laboratory. Blood samples were then centrifuged and aliquoted into cryovials for storage in biofreezers at -80°C until analysis. Serum samples were analysed using the Cobas Integra® 400 plus (Roche, Basel, Switzerland) to determine apolipoproteins (Apo B, Apo A-1), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerides, glucose, C-reactive protein (CRP) and gamma-glutamyl transferase (GGT). Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Reactive oxygen species (ROS) were determined in serum with high-throughput spectrophotometric assay by the Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA).

2.6 Statistical analyses

Statistical analyses were performed using Statistica v13.3 (TIBCO software Inc, Palo Alto, CA, USA), and GraphPad Prism 5.03 (GraphPad Software, San Diego) was used for graphics. All variables were tested for normality using formal tests (Kolmogorov-Smirnov test and Shapiro-Wilks test) and graphical methods (histograms). In the case of non-Gaussian distribution, a logarithmic transformation was performed for each skewed variable and tested again for normality. The mean and spread of all descriptive variables (either standard deviation, 95% percentile interval or %) were presented to characterise the total group. Independent t-tests were performed to compare differences between black and white groups. Pearson, partial and multiple regression analyses were

performed to determine the associations between measures of arterial stiffness and apolipoproteins. Covariates included in the models were age, sex, SES, WC, LDL-c, glucose, CRP, GGT and cotinine. Models with PWV and cIMT as dependent variables additionally included MAP.

3. RESULTS

The characteristics of the study population are presented in Table 1. The age and sex were comparable between the groups (all $P \geq 0.452$), while a larger percentage of the black group was from a low SES ($P < 0.001$). Overall, the black group presented with lower measures of body composition than the white group (all $P < 0.003$). The black group presented with a different cardiovascular profile, but values were still within normal ranges, with higher clinic and central BP measurements when compared to the white group (all $P < 0.005$). In addition, vascular measures, including PWV, Alx, and cIMT, were also higher in the black group (all $P < 0.034$). Apo B, Apo A-1 and Apo B/Apo A-1 were all higher in the white group (all $P < 0.030$).

Figure 1 and Supplementary Table 1 presents the unadjusted associations between vascular measures and apolipoproteins. In the black group, an inverse association between PWV and Apo B was evident ($P < 0.001$). An inverse association was found between PWV and Apo B/Apo A-1 ($p = 0.007$). A positive association was observed between Alx and Apo B ($P < 0.001$), as well as between Alx and Apo B/ Apo A-1 ($P < 0.001$). Furthermore, a positive association between cIMT and Apo A-1 ($P = 0.006$) was also encountered in the black group.

In the white group, positive associations between Alx and Apo B, and between Alx and Apo A-1 were found (all $P < 0.001$). No other associations were evident in this group.

In the partial correlations (Table 2), most of the associations remained robust. In the black group, an inverse association between PWV and Apo B ($r = -0.13$, $P = 0.001$), and between PWV and Apo B/Apo A-1 was found ($r = -0.10$, $P = 0.018$). A positive association between Alx and Apo B ($r = 0.17$, $P < 0.001$), and Apo B/Apo A-1 was also still evident ($r = 0.12$, $P = 0.004$). In addition, a positive association between central pulse pressure (cPP) and Apo B/Apo A-1 were found ($r = 0.10$, $P = 0.017$), as well as between cIMT and Apo B in the black group ($r = 0.09$, $P = 0.025$). Carotid intima-media thickness associated positively with Apo A-1 in the black group ($r = 0.13$, $P = 0.002$). In the white group, the positive association between Alx and all of the apolipoproteins (all $P \leq 0.001$) was also still evident.

The multivariate adjusted-regression analyses are shown in Figure 2 and Supplementary Table 2. In the black group, an independent inverse association between PWV and Apo B was evident ($\beta = -0.15$, $P = 0.025$) as well as a positive association between cPP and Apo B ($\beta = 0.16$, $P = 0.027$). In addition, a positive association was observed between cIMT and Apo B ($\beta = 0.19$, $P = 0.022$) in the black group. No associations between AIX and any of the apolipoproteins were found in either the black or white group.

Table 1: Characteristics of the study population

Variables	Black N=584	White N=578	P-values
Sex, men, n (%)	288 (49.3)	273 (47.2)	0.477
Age (years)	24.5 $\pm$ 3.18	24.6 $\pm$ 3.07	0.452
Socio-economic status			<0.001
Low, n (%)	345 (59.2)	114 (19.7)	
Middle, n (%)	159 (27.3)	182 (31.5)	
High, n (%)	79 (13.6)	282 (48.8)	
Anthropometric measurements			
Height (cm)	165 $\pm$ 8.35	173 $\pm$ 8.74	<0.001
Weight (kg)	65.6 $\pm$ 13.9	76.1 $\pm$ 17.6	<0.001
Body mass index (kg/m ²)	24.3 $\pm$ 5.36	25.4 $\pm$ 5.10	0.003
Waist circumference (cm)	77.4 $\pm$ 10.6	82.4 $\pm$ 13.1	<0.001
Cardiovascular measurements			
Brachial systolic blood pressure (mmHg)	119 $\pm$ 11.6	118 $\pm$ 11.8	0.055
Brachial diastolic blood pressure (mmHg)	80 $\pm$ 8.02	78 $\pm$ 7.18	<0.001
Mean arterial pressure (mmHg)	94 $\pm$ 8.64	93 $\pm$ 8.54	0.016
Central systolic blood pressure (mmHg)	110 $\pm$ 9.20	106 $\pm$ 9.11	<0.001
Central pulse pressure (mmHg)	38 $\pm$ 8.48	39 $\pm$ 8.59	0.070
Pulse wave velocity (m/sec) ^a	6.41 $\pm$ 0.04	6.28 $\pm$ 0.04	0.011

Carotid intima-media thickness (mm)	0.54 ± 0.07	0.44 ± 0.06	0.034
Augmentation index (%) ^b	5.81 ± 12.6	2.62 ± 12.2	<0.001
Biochemical markers			
Apolipoprotein B (g/L)	0.65 (0.33;1.13)	0.75 (0.40;1.44)	<0.001
Apolipoprotein A-1 (g/L)	1.25 (0.71;2.02)	1.31 (0.72;2.22)	0.030
Apo B/Apo A-1	0.52 (0.29;0.92)	0.57 (0.32;1.04)	<0.001
Total cholesterol (mmol/L)	3.32 (1.94;5.10)	3.82 (2.11;6.11)	<0.001
HDL-c (mmol/L)	1.09 (0.58;1.83)	1.09 (0.56;2.01)	0.976
LDL-c (mmol/L)	2.06 (0.99;3.70)	2.45 (1.20;4.42)	<0.001
Triglycerides (mmol/L)	0.64 (0.30;1.36)	0.80 (0.33;2.13)	<0.001
Glucose (mmol/L)	3.79 (2.33;5.44)	4.09 (2.60;5.57)	<0.001
CRP (mg/L)	0.96 (0.09;9.53)	0.77 (0.08;8.08)	0.012
Reactive oxygen species (Units)	42.9 (14.8;123)	34.7 (12.1;97.9)	<0.001
Lifestyle factors			
GGT (U/L)	22.1 (8.40;66.9)	14.9 (5.40;47.0)	<0.001
Self-reported alcohol use, n (%)	321 (55.5)	322 (55.8)	0.953
Self-reported tobacco use, n (%)	151 (25.9)	128 (22.1)	0.134
Cotinine for those >10 ng/mL	150 (18.6;492)	124 (15.3;462)	0.104

Data expressed as arithmetic mean ± standard deviation, geometric mean (fifth and 95th percentile boundaries) or % of n. Significance is set at $P \leq 0.05$. HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; CRP, C-reactive protein; GGT, γ -glutamyl transferase.

^a Adjusted for mean arterial pressure

^b Adjusted for a heart rate

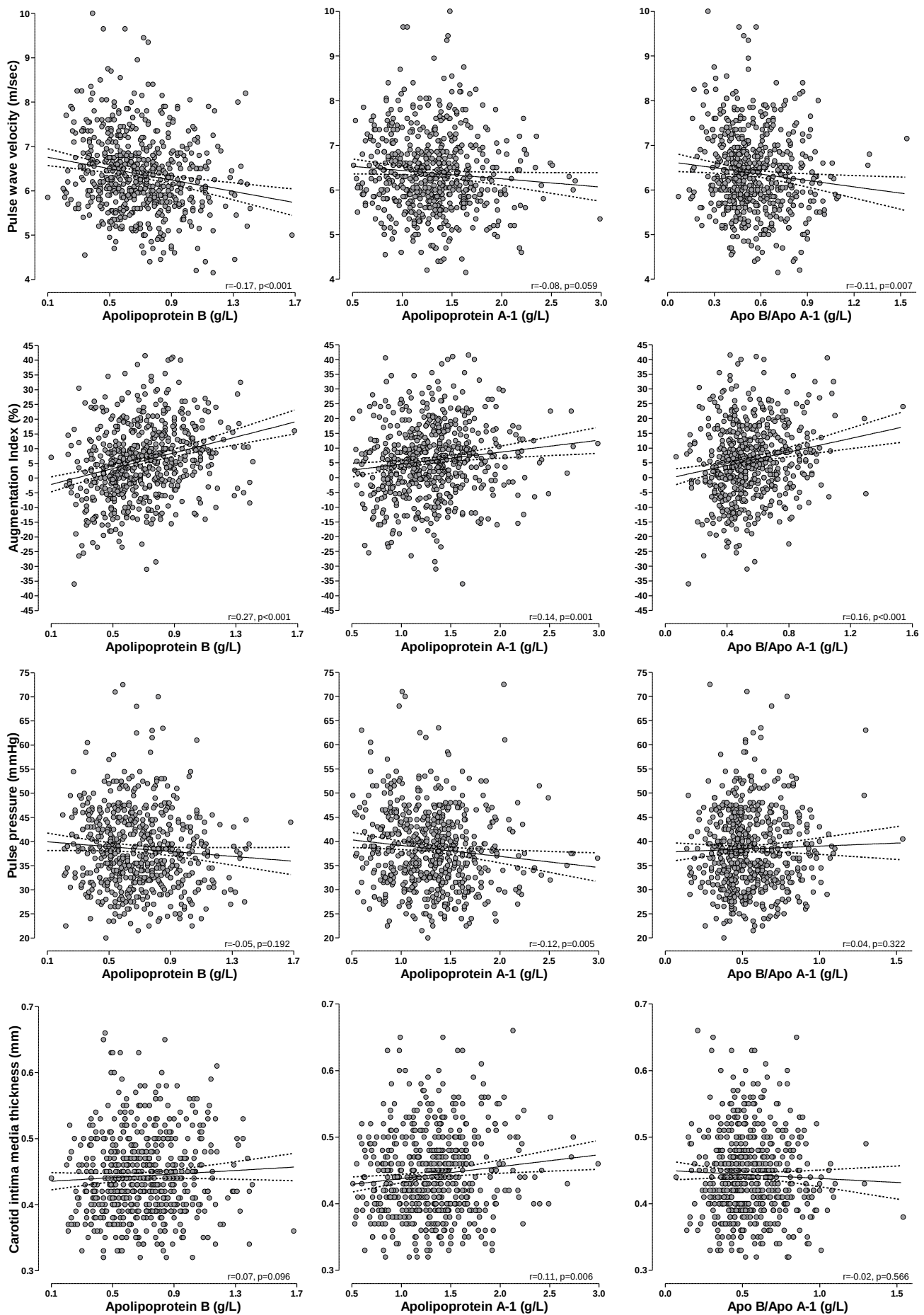


Figure 1a: Vascular markers plotted against apolipoproteins within the black group.

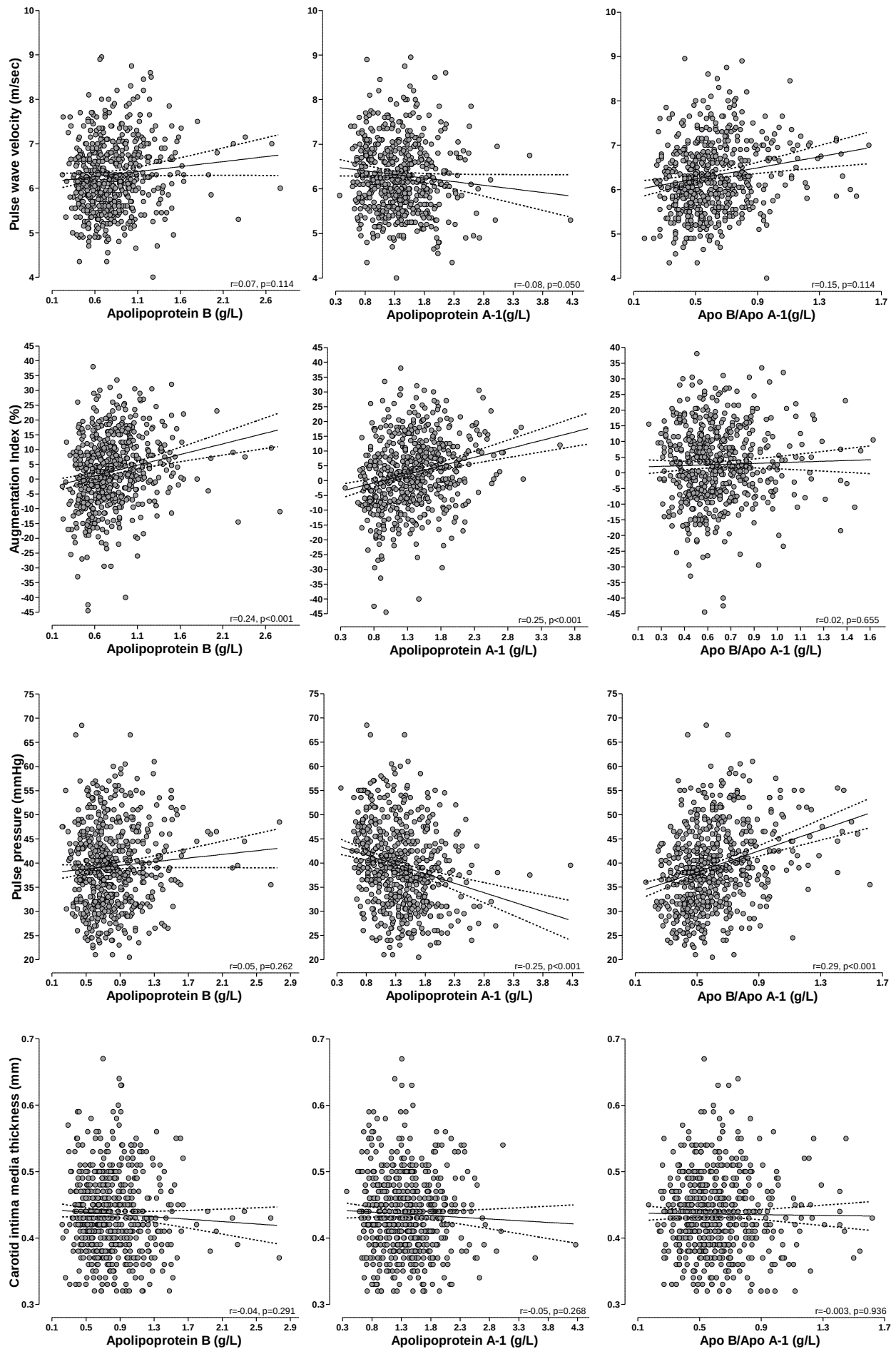


Figure 1b: Vascular markers plotted against apolipoproteins within the white group.

Table 2: Partial correlations of vascular markers with apolipoproteins

	PWV (m/sec)^a		Aix (%)^b		cPP (mmHg)^b		cIMT far wall (mm)^a	
	Black (n=584)	White (n=578)	Black (n=584)	White (n=578)	Black (n=584)	White (n=578)	Black (n=584)	White (n=578)
Apolipoprotein B (g/L)	r= -0.13 p= 0.001	r= -0.05 p= 0.277	r= 0.17 p< 0.001	r= 0.23 p< 0.001	r= 0.07 p= 0.107	r= -0.01 p= 0.892	r= 0.09 p= 0.025	r= -0.08 p= 0.064
Apolipoprotein A- 1 (g/L)	r= -0.04 p= 0.336	r= -0.02 p= 0.656	r= 0.06 p= 0.185	r= 0.14 p= 0.001	r= -0.04 p= 0.336	r= -0.06 p= 0.162	r= 0.13 p= 0.002	r= -0.06 p= 0.180
Apo B/Apo A-1	r= -0.10 p= 0.018	r= -0.04 p= 0.388	r= 0.12 p= 0.004	r= 0.14 p= 0.001	r= 0.10 p= 0.017	r= 0.05 p= 0.216	r= -0.01 p= 0.734	r= -0.04 p= 0.378

^a Adjusted for sex, age and MAP. ^b Adjusted for sex and age. Bold text indicates significant P-values ($P \leq 0.05$). Apo B/Apo A-1, apolipoprotein B to apolipoprotein A-1 ratio; MAP, mean arterial pressure; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; CRP, C-reactive protein; GGT, γ -glutamyl transferase.

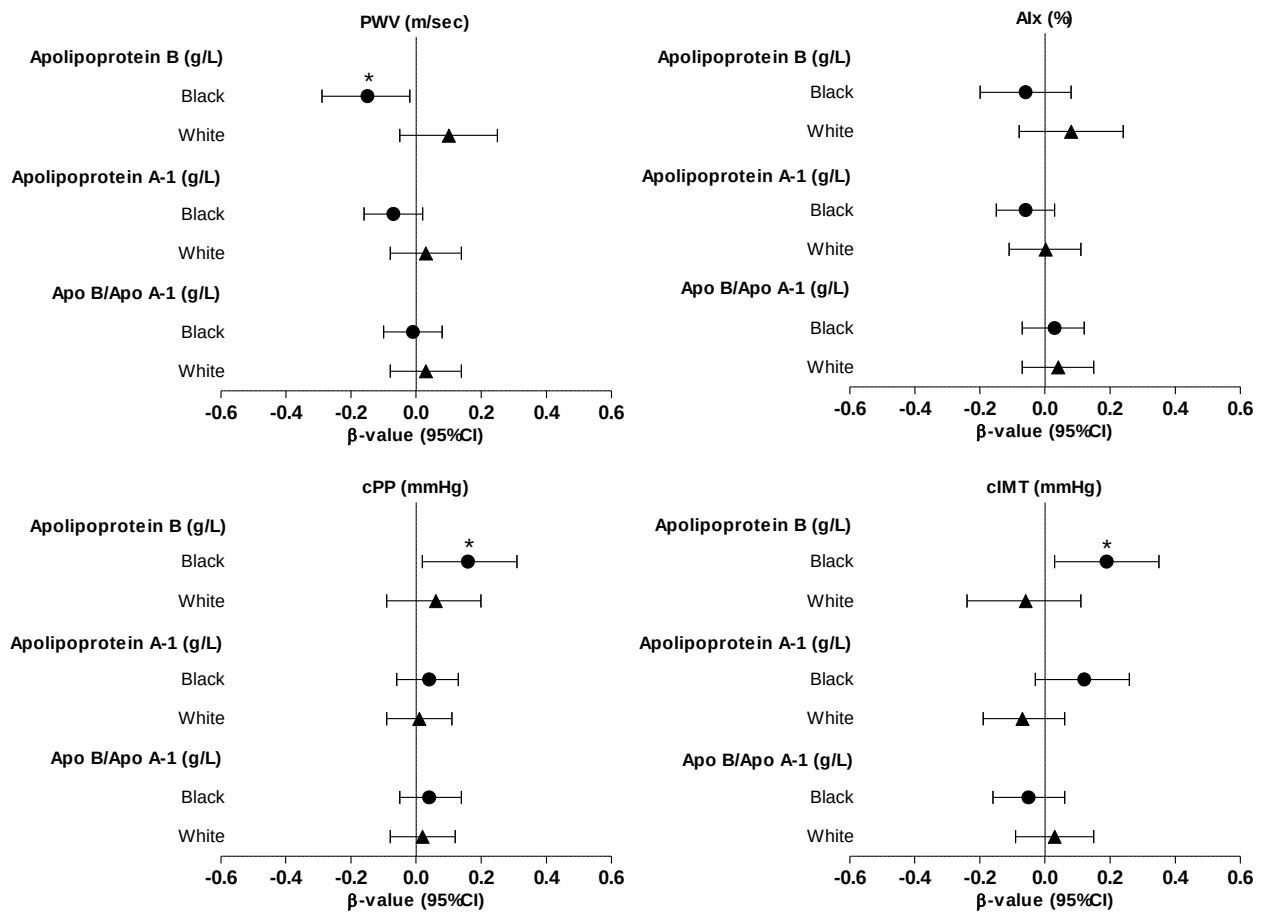


Figure 2: Multiple regression analysis * $P \leq 0.001$. All models included age, sex, SES, WC, LDL-c, glucose, CRP, GGT and cotinine. Models with PWV and cIMT as dependent variables additionally included MAP. PWV, pulse wave velocity; Alx, augmentation index; cPP, central pulse pressure; cIMT, carotid intima-media thickness; Apo B/Apo A-1, apolipoprotein B to apolipoprotein A-1 ratio; SES, socioeconomic status; MAP, mean arterial pressure; WC, waist circumference; LDL-c, low-density lipoprotein cholesterol; CRP, C-reactive protein; GGT, γ -glutamyl transferase

4. DISCUSSION

The aim was to investigate whether any differences in apolipoproteins are evident in a young, apparently healthy, bi-ethnic population and whether apolipoproteins are associated with markers of vascular structure and function (PWV, AIx, cPP and cIMT) in each ethnic group.

As expected, Apo B and the Apo B/Apo A-1 ratio were higher in the white group when compared to their black counterparts. It is known that traditional lipid markers, including total cholesterol, LDL-c and HDL-c, differ between African-Americans and Caucasian-Americans, and data shows that white populations are more prone to an atherogenic lipid profile than black populations.²⁸⁻³⁰ The findings of this study regarding the higher Apo B and Apo B/Apo A-1 levels in the white group are in line with a previous study that included 528 Caucasian and African-American participants. In that study, the authors found that the Caucasian-Americans had increased levels of Apo B and a higher Apo B/ Apo A-1 ratio compared to the African-American group.³¹ Apolipoprotein B can reflect the number of atherogenic particles within the circulation and contributes to the development of inflammation, atherosclerosis, calcification, oxidative stress and arteriosclerosis.⁹⁻¹² The balance of free cholesterol within the circulation is reflected through the Apo B/Apo A-1 ratio and, when increased, the risk of cholesterol deposition within the arterial wall also increases.¹⁸

An unexpected finding in our study was that Apo A-1 was also higher in the white group – a contrast to previous studies where African-American participants presented with higher levels of Apo A-1 compared to their Caucasian-American counterparts.³¹ Apolipoprotein A-1 is known to have a more anti-atherogenic role within the circulation. When ApoA-1 concentrations decrease within the circulation, it increases the risk of CVD development.^{15,16} Collectively, this study's findings show that, even though the white group had higher levels of Apo B and the Apo B/ Apo A-1 ratio (considered markers of an atherogenic state), they also had higher Apo-A1 levels compared to the black group. This may suggest a protective effect against the effects of Apo B.^{18,32-34}

Unexpectedly, an inverse association between Apo B and PWV was found in the black group. Only a limited number of previous studies investigated the association between Apo B and arterial stiffness. In contrast to this study's findings, the Cardiovascular Risk in Young Finns Study (aged 30-45 years) found that Apo B associated positively with pulse wave velocity, independent of risk

factors such as sex, age and heart rate in a Finnish population.⁹ In another study that included 247 adults with cardiovascular disease risk (hypertension, dyslipidaemia and diabetes), positive associations between Apo B and pulse wave velocity were encountered among treated participants.³⁵

Our study population included young black and white adults (20-30 years) with no history of chronic disease, which may explain the conflicting results. Other risk factors can affect this association later in life or within the presence of pathology. In addition, ethnic differences in traditional lipid markers show that white populations are more prone to an atherogenic lipid profile than black populations, and adverse associations are often found within white groups.^{28,30,36}

It was further found that Apo B associated positively with cPP and cIMT in the black group. These findings are in line with previous studies that found that Apo B directly associates with atherosclerosis and central pulse pressure.³⁷⁻⁴⁰ Apolipoprotein B initiates an inflammatory response through infiltration into the arterial wall, resulting in the development of atherosclerosis.^{37,38} While the white group did present with higher concentrations of Apo B, Apo B/Apo A-1 ratio and Apo A-1, no associations were encountered between any of the vascular markers and apolipoproteins in this group. These findings may be due to early changes within the vasculature of the black group suggesting that they may be more sensitive to changes in apolipoprotein levels.

The strengths and limitations should be taken into consideration when interpreting the results from this study. The study population consisted of young, apparently healthy individuals from specific urban areas of South Africa and may not be representative of the whole study population. Due to the cross-sectional study design of the study, causality cannot be inferred. The African-PREDICT study is longitudinal in design, with the future possibility of determining the contribution of apolipoproteins to changes in arterial stiffness over time. The African-PREDICT study was conducted in a highly-controlled research setting. Data regarding the association between apolipoproteins and markers of vasculature structure and function in African populations are very limited. The findings of this study may contribute to the understanding of how apolipoproteins effect the vasculature at a young age aiding in the early detection of CVD development.

In conclusion, adverse associations were found between vascular measures and Apo B in young, apparently healthy black adults. No associations were found between vascular measures and apolipoproteins in white adults. Overall, the findings highlight the possible role of apolipoproteins in the development of early detrimental vascular changes in young, healthy South African adults, which may contribute to the future development of cardiovascular disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Miss D Botha, responsible for performing of statistical analyses; as well as the design, planning and writing of the manuscript.

Dr Y Breet, supervised the writing of the manuscript, collecting and interpretation of data and provided guidance regarding statistical analysis, initial planning and design of the manuscript.

Dr LF Gafane-Mateman, co-supervised the writing of the manuscript, collection and interpretation of data and provided support in planning and design of the manuscript,

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SUPPLEMENTARY DATA

Supplementary table 1: Pearson correlations of vascular markers with apolipoproteins

	PWV (m/sec)		AIx (%)		cPP (mmHg)		cIMT (mm)	
	Black (n=584)	White (n=578)	Black (n=584)	White (n=578)	Black (n=584)	White (n=578)	Black (n=584)	White (n=578)
Sex (men/women)	r= 0.43 p< 0.001	r= 0.38 p< 0.001	r= -0.37 p< 0.001	r= -0.22 p< 0.001	r= 0.38 p< 0.001	r= 0.56 p< 0.001	r= 0.13 p= 0.001	r= 0.07 p= 0.107
Age (years)	r= 0.18 p< 0.001	r= 0.24 p< 0.001	r= 0.27 p< 0.001	r= 0.21 p< 0.001	r= -0.16 p< 0.001	r= -0.03 p= 0.498	r= 0.05 p= 0.195	r= 0.09 p= 0.025
SES	r= -0.01 p= 0.854	r= 0.14 p= 0.001	r= 0.20 p< 0.001	r= 0.10 p= 0.022	r= -0.11 p= 0.007	r= -0.66 p=0.113	r= -.03 p= 0.52	r= 0.06 p=0.143
Apolipoprotein B (g/L)	r= -0.17 p< 0.001	r= 0.07 p= 0.114	r= 0.27 p< 0.001	r= 0.24 p< 0.001	r= -0.05 p= 0.192	r= 0.05 p= 0.262	r= 0.07 p= 0.096	r= -0.04 p= 0.291
Apolipoprotein A-1 (g/L)	r= -0.08 p= 0.059	r= -0.081 p= 0.050	r= 0.14 p= 0.001	r= 0.25 p< 0.001	r= -0.12 p= 0.005	r= -0.25 p< 0.001	r= 0.11 p= 0.006	r= -0.05 p= 0.268
Apo B/Apo A-1	r= -0.11 p= 0.007	r= 0.15 p< 0.001	r= 0.16 p< 0.001	r= 0.02 p= 0.655	r= 0.04 p= 0.322	r= 0.29 p< 0.001	r= -0.02 p= 0.566	r= -0.003 p= 0.936
Total cholesterol (mmol/L)	r= -0.11 p= 0.007	r= -0.02 p= 0.614	r= 0.28 p< 0.001	r= 0.28 p< 0.001	r= -0.12 p= 0.004	r= -0.10 p= 0.013	r= 0.09 p= 0.023	r= -0.04 p= 0.295
HDL-c (mmol/L)	r=-0.04 p= 0.296	r= -0.16 p< 0.001	r= 0.07 p= 0.080	r= 0.16 p< 0.001	r= -0.15 p< 0.001	r= -0.35 p< 0.001	r= 0.14 p= 0.002	r= -0.03 p= 0.460
LDL-c (mmol/L)	r= -0.14 p= 0.001	r= -0.01 p= 0.743	r= 0.29 p< 0.001	r= 0.24 p< 0.001	r= -0.10 p= 0.02	r= -0.02 p= 0.621	r= 0.04 p= 0.383	r= -0.03 p= 0.472
Triglycerides (mmol/L)	r= 0.04 p= 0.396	r= 0.08 p= 0.048	r= 0.17 p< 0.001	r= 0.30 p< 0.001	r= 0.04 p= 0.395	r= 0.09 p= 0.037	r= 0.10 p= 0.012	r= -0.05 p= 0.267
Glucose (mmol/L)	r= -0.11 p= 0.008	r= 0.02 p= 0.703	r= 0.28 p< 0.001	r= 0.26 p< 0.001	r= -0.16 p< 0.001	r= -0.08 p= 0.044	r= -0.05 p= 0.269	r= -0.01 p= 0.765
CRP (mg/L)	r= -0.21 p< 0.001	r= -0.02 p= 0.640	r= 0.34 p< 0.001	r= 0.30 p< 0.001	r= -0.05 p= 0.217	r= -0.04 p= 0.321	r= -0.07 p= 0.111	r= -0.03 p= 0.477

Reactive oxygen species (units)	r= -0.15 p< 0.001	r= -0.04 p= 0.384	r= 0.19 p< 0.001	r= 0.19 p< 0.001	r= -0.12 p= 0.004	r= -0.13 p= 0.002	r= -0.02 p= 0.680	r= 0.02 p= 0.575
GGT (U/L)	r= 0.18 p< 0.001	r=0.19 p< 0.001	r= 0.11 p= 0.011	r= 0.24 p< 0.001	r= 0.04 p= 0.357	r= 0.20 p< 0.001	r= 0.09 p= 0.036	r= -0.12 p= 0.006
Self-reported alcohol use, n (%)	r= 0.07 p= 0.118	r= 0.19 p< 0.001	r= -0.08 p= 0.058	r= 0.06 p= 0.131	r= 0.02 p= 0.728	r= 0.05 p= 0.207	r= 0.05 p= 0.266	r= -0.05 p= 0.276
Self-reported tobacco use, n (%)	r= 0.16 p< 0.001	r= 0.11 p= 0.006	r= -0.19 p< 0.001	r= 0.07 p= 0.102	r= 0.06 p= 0.128	r= -0.012 p= 0.661	r= 0.08 p= 0.059	r= -0.09 p= 0.027
Cotinine (ng/mL)	r= 0.15 p< 0.001	r= 0.14 p= 0.001	r= -0.14 p= 0.001	r= 0.04 p= 0.338	r= 0.08 p= 0.050	r= -0.04 p= 0.350	r= 0.08 p= 0.060	r= -0.06 p= 0.131

Bold values indicate significant P-values (P< 0.001). PWV, pulse wave velocity; Alx, augmentation index; cPP, central pulse pressure; cIMT, carotid intima-media thickness; Apo B/Apo A-1, apolipoprotein B to apolipoprotein A-1 ratio; SES, socioeconomic status; WC, waist circumference; HDL-c, high-density lipoprotein; LDL-c; low-density lipoprotein cholesterol; CRP, C-reactive protein; GGT, γ -glutamyl transferase.

Supplementary table 2: Multiple regression analyses between various vascular measures and apolipoproteins

	Pulse Wave Velocity (m/sec) ^a					
	Model 1		Model 2		Model 3	
	Black (N=584)	White (N=578)	Black (N=584)	White (N=578)	Black (N=584)	White (N=578)
	β (5;95)	β (5;95)	β (5;95)	β (5;95)	β (5;95)	β (5;95)
Adjusted R ²	0.34	0.28	0.34	0.28	0.34	0.28
Apolipoprotein B (g/L)	-0.15 (-0.29; -0.02)*	0.10 (-0.05; 0.25)	-	-	-	-
Apolipoprotein A-1 (g/L)	-	-	-0.07 (-0.16; 0.02)	0.03 (-0.08; 0.14)	-	-
Apo B/Apo A-1 (g/L)	-	-	-	-	-0.01 (-0.10; 0.08)	0.03 (-0.08; 0.14)
Age (years)	0.18 (0.10; 0.25)**	0.19 (0.11; 0.28)**	0.17 (0.10; 0.25)**	0.19 (0.10; 0.28)**	0.17 (0.10; 0.25)**	0.20 (0.11; 0.28)**
Sex (women/men)	0.25 (0.33; 0.67)**	0.22 (0.25; 0.62)**	0.25 (0.33; 0.67)**	0.23 (0.27; 0.66)**	0.26 (0.35; 0.68)**	0.21 (0.23; 0.62)**
SES (score)	-0.002 (-0.10; 0.10)	0.04 (-0.06; 0.16)	0.01 (-0.10; 0.11)	0.04 (-0.06; 0.16)	0.01 (-0.10; 0.11)	0.04 (-0.06; 0.16)
MAP (mmHg)	0.34 (0.25; 0.40)**	0.36 (0.27; 0.45)**	0.33 (0.26; 0.41)**	0.36 (0.27; 0.45)**	0.33 (0.25; 0.40)**	0.36 (0.28; 0.45)**
WC (cm)	-0.13 (-0.21; 0.05)*	-0.09 (-0.19; 0.01)	-0.15 (-0.23; -0.06)**	-0.09 (-0.19; 0.02)	-0.13 (-0.22; 0.05)**	-0.01 (-0.20; 0.004)
LDL-c (mmol/L)	0.03 (-0.10; 0.16)	-0.17 (-0.32; -0.01)*	-0.08 (-0.16; 0.001)*	-0.09 (-0.19; 0.01)	-0.09 (-0.19; 0.01)	-0.10 (-0.22; 0.02)
Glucose (mmol/L)	-0.002 (-0.08; 0.07)	0.01 (-0.09; 0.10)	0.02 (-0.06; 0.11)	-0.004 (-0.11; 0.10)	-0.01 (-0.09; 0.07)	0.02 (-0.09; 0.12)
CRP (mg/L)	-0.06 (-0.14; 0.03)	0.002 (-0.08; 0.08)	-0.06 (-0.15; 0.02)	0.01 (-0.08; 0.09)	-0.06 (-0.14; 0.03)	0.01 (-0.07; 0.09)
GGT (U/L)	0.14 (0.06; 0.21)**	0.01 (-0.09; 0.11)*	0.14 (0.07; 0.22)**	0.02 (-0.09; 0.12)	0.13 (0.06; 0.21)**	0.02 (-0.08; 0.12)
Cotinine (ng/mL)	0.02 (-0.05; 0.09)	0.08 (0.01; 0.16)	-0.01 (-0.05; 0.10)	0.09 (0.01; 0.16)*	0.02 (-0.05; 0.09)	0.08 (0.01; 0.16)*
	Augmentation Index (%)					
	Model 1		Model 2		Model 3	
	Black (N=584)	White (N=578)	Black (N=584)	White (N=578)	Black (N=584)	White (N=578)
	β (5;95)	β (5;95)	β (5;95)	β (5;95)	β (5;95)	β (5;95)
Adjusted R ²	0.26	0.24	0.26	0.24	0.26	0.24
Apolipoprotein B (g/L)	-0.06 (-0.20; 0.08)	0.08 (-0.08; 0.24)	-	-	-	-
Apolipoprotein A-1 (g/L)	-	-	-0.06 (-0.15; 0.03)	0.002 (-0.11; 0.11)	-	-

Apo B/Apo A-1 (g/L)	-	-	-	-	0.03 (-0.07; 0.12)	0.04 (-0.07; 0.15)
Age (years)	0.18 (0.10; 0.26)**	0.11 (0.02; 0.20)*	0.18 (0.10; 0.25)**	0.11 (0.02; 0.20)*	0.17 (0.10; 0.25)**	0.11 (0.03; 0.20)*
Sex (women/men)	-0.26 (-0.69; -0.35)**	-0.39 (-0.96; -0.59)**	-0.26 (-0.69; -0.35)**	-0.38 (-0.95; -0.57)**	-0.26(-0.68; -0.34)**	-0.39(-0.99; -0.59)**
SES (score)	0.06 (-0.02; 0.19)	0.02 (-0.08; 0.14)	0.06 (-0.02; 0.20)	0.02 (-0.08; 0.14)	0.07 (-0.02; 0.20)	0.02 (-0.08; 0.14)
WC (cm)	0.06 (-0.02; 0.15)	0.25 (0.15; 0.35)**	0.05 (-0.04; 0.14)	0.25 (0.15; 0.35)**	0.05 (-0.03; 0.14)	0.24 (0.14; 0.34)**
LDL-c (mmol/L)	0.18 (0.04; 0.32)*	-0.04 (-0.20; 0.12)	0.14 (0.06; 0.23)*	0.03 (-0.07; 0.13)	0.11 (0.01; 0.22)*	0.00 (-0.12; 0.12)
Glucose (mmol/L)	0.08 (0.001; 0.16)	0.09 (-0.01; 0.19)	0.11 (0.02; 0.20)*	0.09 (-0.02; 0.20)	0.09 (0.001; 0.18)	0.11 (0.002; 0.22)
CRP (mg/L)	0.13 (0.04; 0.21)*	0.10 (0.02; 0.19)*	0.12 (0.03; 0.21)*	0.11 (0.03; 0.19)*	0.12 (0.03; 0.21)*	0.11 (0.03; 0.19)*
GGT (U/L)	0.02 (-0.07; 0.09)	0.10 (0.00; 0.20)	0.02 (-0.06; 0.10)	0.11 (0.004; 0.21)*	0.02 (-0.07; 0.10)	0.11 (0.01; 0.21)*
Cotinine (ng/mL)	0.003 (-0.07; 0.08)	0.08 (0.004; 0.16)*	0.007 (-0.07; 0.09)	0.08 (0.10; 0.16)*	0.01 (-0.07; 0.08)	0.08 (0.002; 0.16)

Central Pulse Pressure (mmHg)

	Model 1		Model 2		Model 3	
	Black (N=584)	White (N=578)	Black (N=584)	White (N=578)	Black (N=584)	White (N=578)
	β (5;95)	β (5;95)	β (5;95)	β (5;95)	β (5;95)	β (5;95)
Adjusted R ²	0.23	0.34	0.22	0.34	0.22	0.34
Apolipoprotein B (g/L)	0.16 (0.02; 0.31)*	0.06 (-0.09; 0.20)	-	-	-	-
Apolipoprotein A-1 (g/L)	-	-	0.04 (-0.06; 0.13)	0.01 (-0.09; 0.11)	-	-
Apo B/Apo A-1 (g/L)	-	-	-	-	0.04 (-0.05; 0.14)	0.02 (-0.08; 0.12)
Age (years)	-0.21(-0.29; -0.13)**	-0.05 (-0.13; 0.04)	-0.20 (-0.28; -0.12)**	-0.05 (-0.13; 0.04)	-0.20 (-0.28; -0.12)**	-0.04 (-0.13; 0.04)
Sex (women/men)	0.42 (0.66; 1.01)**	0.46 (0.75; 1.09)**	0.41 (0.65; 1.00)**	0.47 (0.75; 1.11)**	0.41 (0.65; 0.99)**	0.46 (0.73; 1.09)**
SES (score)	-0.05 (-0.17; 0.05)	-0.04 (-0.15; 0.05)	-0.05 (-0.18; 0.04)	-0.04 (-0.15; 0.06)	-0.05 (-0.18; 0.04)	-0.04 (-0.15; 0.05)
WC (cm)	0.25 (0.16; 0.34)**	0.20 (0.11; 0.29)**	0.26 (0.18; 0.35)**	0.21 (0.11; 0.30)**	0.25 (0.16; 0.34)**	0.20 (0.11; 0.29)**
LDL-c (mmol/L)	-0.13 (-0.28; 0.01)	-0.06 (-0.21; 0.09)	-0.01 (-0.09; 0.08)	-0.02 (-0.11; 0.07)	-0.03 (-0.13; 0.08)	-0.30 (-0.14; 0.08)
Glucose (mmol/L)	-0.06 (-0.15; 0.02)	-0.07 (-0.17; 0.02)	-0.07 (-0.17; 0.02)	-0.08 (-0.18; 0.02)	-0.04 (-0.13; 0.05)	-0.07 (-0.17; 0.04)
CRP (mg/L)	0.03 (-0.06; 0.12)	-0.04 (-0.11; 0.04)	0.03 (-0.06; 0.12)	-0.03 (-0.11; 0.05)	0.03 (-0.06; 0.12)	-0.03 (-0.11; 0.05)
GGT (U/L)	-0.03 (-0.11; 0.05)	0.02 (-0.07; 0.11)	-0.03 (-0.11; 0.05)	0.02 (-0.07; 0.12)	-0.02 (-0.10; 0.06)	0.03 (-0.07; 0.12)
Cotinine (ng/mL)	-0.08(-0.16;-0.003)*	-0.04 (-0.11; 0.03)	-0.08 (-0.16; -0.01)*	0.01 (-0.09; 0.11)	-0.08 (-0.16; 0.00)	-0.04 (-0.12; 0.30)

	Carotid Intima-Media Thickness (mm)					
	Model 1		Model 2		Model 3	
	0.05	0.04	0.06	0.04	0.04	0.04
Adjusted R ²						
Apolipoprotein B (g/L)	0.19 (0.03; 0.35)*	-0.06 (-0.24; 0.11)	-	-	-	-
Apolipoprotein A-1 (g/L)	-	-	0.12 (-0.03; 0.26)	-0.07 (-0.19; 0.06)	-	-
Apo B/Apo A-1 (g/L)	-	-	-	-	-0.05 (-0.16; 0.06)	0.03 (-0.09; 0.15)
Age (years)	0.06 (-0.03; 0.15)	0.13 (0.03; 0.23)*	0.07 (-0.02; 0.16)	0.13 (0.03; 0.23)*	0.07 (-0.02; 0.16)	0.13 (0.03; 0.23)*
Sex (women/men)	0.06 (-0.07; 0.33)	0.20 (0.18; 0.61)**	0.07 (-0.06; 0.34)	0.18 (0.13; 0.57)*	0.06 (-0.09; 0.32)*	0.18 (0.14; 0.60)**
SES (score)	-0.01 (-0.14; 0.11)	-0.01 (-0.13; 0.12)	-0.02 (-0.14; 0.10)	-0.01 (-0.13; 0.12)	-0.02 (-0.15; 0.09)	-0.01 (-0.13; 0.12)
MAP (mmHg)	0.15 (0.06; 0.24)**	0.05 (-0.05; 0.15)	0.14 (0.05; 0.23)*	0.05 (-0.05; 0.15)	0.15 (0.06; 0.24)**	0.05 (-0.05; 0.15)
WC (cm)	-0.15 (-0.24; -0.05)*	-0.17 (-0.28; -0.06)*	-0.11 (-0.21; -0.02)*	-0.18 (-0.30; -0.07)*	-0.13 (-0.23; -0.03)*	-0.18 (-0.29; -0.06)*
LDL-c (mmol/L)	-0.09 (-0.25; 0.07)	0.06 (-0.12; 0.24)	0.03 (-0.06; 0.13)	0.02 (-0.09; 0.13)	0.10 (-0.02; 0.21)	-0.01 (-0.15; 0.13)
Glucose (mmol/L)	-0.05 (-0.14; 0.04)	0.04 (-0.07; 0.16)	-0.11 (-0.22; -0.01)*	0.07 (-0.05; 0.20)	-0.06 (-0.16; 0.04)	0.06 (-0.07; 0.18)
CRP (mg/L)	-0.002 (-0.11; 0.10)	0.08 (-0.02; 0.17)	0.01 (-0.09; 0.11)	0.08 (-0.01; 0.17)	0.01 (-0.10; 0.11)	0.08 (-0.02; 0.17)
GGT (U/L)	0.06 (-0.04; 0.15)	-0.17 (-0.29; -0.06)*	0.04 (-0.05; 0.11)	-0.16 (-0.28; -0.05)*	0.06 (-0.03; 0.15)	-0.17 (-0.29; -0.06)*
Cotinine (ng/mL)	0.04 (-0.05; 0.13)	-0.04 (-0.12; 0.05)	0.03 (-0.06; 0.11)	-0.04 (-0.13; 0.04)	0.04 (-0.05; 0.12)	-0.04 (-0.13; 0.05)

*P<0.05, **P≤0.001. Apo B/Apo A-1, apolipoprotein B to apolipoprotein A-1 ratio; SES, socioeconomic status; MAP, mean arterial pressure; WC, waist circumference; LDL-c; low-density lipoprotein cholesterol; CRP, C-reactive protein; GGT, γ-glutamyl transferase.

CHAPTER 4

FINAL REMARKS AND RECOMMENDATIONS FOR FUTURE STUDIES

1. INTRODUCTION

In this conclusive chapter, the hypotheses made in the first chapter will be revisited and compared with the main findings. Comparisons with previous literature will be made as well as conclusions about the key findings of this study will be made. Recommendations will also be offered for future studies.

2. SUMMARY OF KEY FINDINGS

Hypothesis 1: Apo B and the Apo B/ApoA-1 ratio will be higher and Apo A-1 lower in the white population as compared to that of the black population. As expected, it was found that apolipoprotein B (Apo B) and the Apo B/Apo A-1 ratio was higher in the white group than in the black group. The results from previous studies of participants of European and African-American descent align with our results of this study.¹⁻³ The former studies found lipid markers including low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), Apo B, apolipoprotein A-1 (Apo A-1) and the Apo B/Apo A-1 ratio to differ between black and white groups. White groups presented with higher levels of LDL-c, Apo B and Apo B/Apo A-1 and lower levels of HDL-c and Apo A-1 than black groups.¹⁻³ Apolipoprotein B directly reflects the quantity of atherogenic particles present within the circulation, while the Apo B/Apo A-1 ratio reflects the balance of free circulating apolipoproteins. When the Apo B/Apo A-1 ratio is increased within the circulation, the risk of cholesterol deposition within vessel walls also increases.⁴⁻⁸ The results of this study therefore reflect findings from previous studies,¹⁻³ showing that white populations may be prone to a more atherogenic lipid profile and therefore an increased risk for future cardiovascular disease (CVD) development.

Against expectations, it was found that Apo A-1 was higher within the white group when compared to the black group. Previous studies including Caucasian and African-American participants found that the African-American group presented with higher levels of Apo A-1 than their European-American counterparts.^{2,9} It is known that Apo A-1 plays a more anti-atherogenic or protective role within the circulation, opposing the detrimental effects of Apo B. When the balance between Apo B and Apo A-1 shifts to favour Apo B, vascular damage may occur.^{2,10-14} The unexpected findings regarding Apo A-1 in the white individuals may be attributed to this study's population, which included

only healthy young individuals and, while the atherogenic apolipoproteins were higher in the white group, the mean levels were within normal ranges (Table 1).

Table 1: Normal reference ranges of apolipoproteins ^{15,16}			
	Normal ranges	Black	White
Apo B (mg/dL)	≤ 100	65	75
Apo A-1 (mg/dL)	≥ 120	125	131
Apo B/ Apo A-1	< 0.8	0.52	0.57
Apo B, Apolipoprotein B; Apo A-1, Apolipoprotein A-1			

Based on the higher levels of Apo B and the Apo B/Apo A-1 ratio and the unexpected higher levels of Apo-A1 in the white group, Hypothesis 1 is partially accepted.

Hypothesis 2: Apo B and the Apo B/Apo A-1 ratio will positively associate with markers of vascular structure and function (PWV, Alx, cPP and cIMT), while a negative association between Apo A-1 and the markers of vascular structure and function (PWV, Alx, cPP and cIMT) will be evident. These associations are expected in both ethnic groups. When examining the associations between apolipoproteins and markers of vascular structure and function (PWV, Alx, cPP and cIMT) it was found, against expectations, that Apo B inversely associated with pulse wave velocity (PWV) in the black group. This is a controversial finding as previous studies including participants of European descent showed Apo B to associate positively with markers of arterial stiffness. A previous study, including a Finnish population (aged 30-45 years), found that Apo B positively associated with PWV independent of risk factors including sex, age and heart rate⁴. Furthermore, in a separate study including older participants with a history of hypertension, dyslipidaemia and diabetes,¹⁷ Apo B positively associated with PWV. The participants included in this study were young adults with no history of chronic disease, and previous studies, where positive associations between Apo B and PWV were reported, included participants of European descent.

We found that Apo B associated positively with central pulse pressure (cPP) and carotid intima-media thickness (cIMT) within the black group. In previous studies that included participants without treatment for hypertension and participants with at least one atherosclerotic vascular event, similar results were reported.^{18,19} The development of atherosclerosis is known to be potentiated by Apo B.

Apolipoprotein B has the ability to initiate an inflammatory response within the vascular wall, resulting in the infiltration of Apo B and contributes to the development of atherosclerotic plaque.^{19,20} As Apo B has the ability to contribute to the development of atherosclerosis and consequently the stiffening of the arterial wall and increased early wave reflection, an increase in cPP is also expected.^{21,22} No associations were found between the apolipoproteins or any of the vascular markers within the white group. Against expectations no associations were found between Apo A-1 and any of the vascular markers within both groups. Based on these findings, hypothesis 2 is partially accepted.

3. STRENGTHS, LIMITATIONS AND RECOMMENDATION FOR FUTURE STUDIES

The baseline data from the African-PREDICT study was used for this dissertation. As a result of the cross-sectional design of this study, it is not possible to determine the pathophysiological effects of apolipoproteins over time. Our study did not include the data of the full apolipoprotein profile (Apo AII, Apo CII, Apo CIII, and Apo E), some of which may have additional effects on the vascular structure and function that could not be evaluated. Young, apparently healthy black and white individuals without pre-existing diseases were recruited for the African-PREDICT study and, thus, allowed investigation of the study population without the influence of underlying pathology. The African-PREDICT study included study participants from only one province in South Africa. As such, our results may not reflect the true effect of apolipoproteins on vascular structure and function of the South African population in its entirety. A large sample of black and white South African adults was included, which provided us with adequate statistical power. However, the possibility of chance should be considered. Further residual confounding is possible even though several covariates were included in the multiple regression analyses.

The following are recommendations for future research into the relationship between apolipoproteins and arterial stiffness:

- Longitudinal studies are required to identify changes in apolipoproteins of individuals as they age and how these changes contribute to pathophysiological changes within the vasculature over time.
- Research in larger study populations, including individuals from multiple regions of South Africa, is needed.

- Investigation of the effect of other apolipoproteins on the vasculature is needed.
- Further research showing the effects of sex hormones (estrogen) on the lipid profile of young South Africans.

4. CONCLUSION

Levels of Apo B, Apo A-1 and the Apo B/Apo A-1 ratio were higher in the white group when compared to the black group. Positive associations were found between markers of vascular structure and function and Apo B in the black group, while no associations were evident in the white group. Our findings show that apolipoproteins, specifically Apo B, may play an important role in the development of early changes within the vasculature in young, black South Africans.

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APPENDIX A

**APPROVAL FROM THE HEALTH RESEARCH
ETHICS COMMITTEE**

14 June 2020

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Health Research Ethics Committee (NWU-HREC) on 14/06/2020, the NWU-HREC hereby approves your study as indicated below. This implies that the NWU-HREC grants its permission that, provided the general conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: The association between apolipoproteins and arterial stiffness in young adults: The African-PREDICT study

Principal Investigator/Study Supervisor/Researcher: Dr Y Breet

Student: D Botha - 27188396

Ethics number:

N	W	U	-	0	0	3	6	8	-	2	0	-	A	1
Institution				Study Number						Year			Status	

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

Application Type: Single study

Commencement date: 14/06/2020

Expiry date: 30/06/2021

Risk:

Minimal

Approval of the study is provided for a year, after which continuation of the study is dependent on receipt and review of an annual monitoring report and the concomitant issuing of a letter of continuation. A monitoring report is due at the end of June annually until completion.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- The principal investigator/study supervisor/researcher must report in the prescribed format to the NWU-HREC:
 - annually on the monitoring of the study, whereby a letter of continuation will be provided annually, and upon completion of the study; and
 - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the principal investigator/study supervisor/researcher must apply for approval of these amendments at the NWU-HREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- Annually a number of studies may be randomly selected for active monitoring.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility, the NWU-HREC reserves the right to:

- request access to any information or data at any time during the course or after completion of the study;
- to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;
- withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected;
 - it becomes apparent that any relevant information was withheld from the NWU-HREC or that information has been false or misrepresented;
 - submission of the annual monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or
 - new institutional rules, national legislation or international conventions deem it necessary.
- NWU-HREC can be contacted for further information via Ethics-HRECAppl@nwu.ac.za or 018 299 1206

Special COVID-19 arrangements:

There may be certain changes that you may need to make to your study, after it has been approved, due to the impact of the COVID-19 pandemic or the lockdown e.g.:

- a. Changing the recruitment strategy e.g. from an in-person process to a telephonic process
- b. Changing the approved methodology to use data collection methods that ensure physical distancing
- c. The implementation of risk-mitigating strategies to prevent infection and not entering communities for data collection
- d. Changes in time schedules etc.

Please note that any 1) amendments, 2) request for extensions or 3) any other kind of modification to the proposal or other associated documentation must be submitted for review by the NWU-HREC, Faculty of Health Sciences, and must be approved, prior to these changes being implemented.

These amendment requests will be handled via the *expedited process*.

Process to be followed:

- a. Ensure all your amendments are indicated in **yellow highlight** in the amended documents.
- b. Submit your request to Ethics-HRECAppl@nwu.ac.za with a *cover letter* with a specific subject title indicating, "Amendment request (COVID-19): NWU-XXXXX-XX-XX". The letter should include the title of the approved study, the names of the researchers involved, the nature of the amendment/s being made (indicating what changes have been made as well as where they have been made), which documents have been attached and any further explanation to clarify the amendment request being submitted.
- c. Name the emails, to which you attach the documents that you send, with a *specific subject line* indicating that it is an amendment request e.g. "Amendment request (COVID-19): NWU-XXXXX-XX-XX". This e-mail should indicate the nature of the amendment.
- d. Send a short email to Prof Wayne Towers and Prof Petra Bester indicating to them that you have submitted an amendment request due to the COVID-19 impact.

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

Yours sincerely,



Digitally signed by Petra Bester
DN: cn=Petra Bester, o=AUTHeR,
ou=NWU, Faculty of Health Sciences,
email=petra.bester@nwu.ac.za, c=ZA
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APPENDIX B

TURN-IT-IN REPORT

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SUMMARY Motivation: South Africa is facing a high burden of non-communicable diseases with cardiovascular disease (CVD) being the largest contributor. Changes in vascular structure and function, even at an early age, contributes to the future development of cardiovascular disease. It is well known that lipids contribute to increased risk for CVD. Dyslipidaemia, is traditionally defined as increased <u>low-density lipoprotein cholesterol</u> and decreased <u>high-density lipoprotein cholesterol</u> levels and is strongly <u>related to</u> vascular <u>changes</u> and CVD development. Ethnic differences regarding differences in lipids is also well documented. The use of apolipoproteins as risk markers for cardiovascular disease is showing increased credibility in resent evidence. Evidence regarding associations between apolipoproteins and the development of changes within the vasculature are limited and whether any ethnic differences are at play also remains to be determined. Aim: Our study aimed to investigate whether any differences in apolipoproteins are evident in a young, apparently healthy bi-ethnic population and whether apolipoproteins are associated with arterial stiffness in each ethnic group. Methods: Our study included 1162 young, apparently healthy black and white, men and women from the African-PREDICT study. Measures of vascular structure and function were determined <u>including: pulse wave velocity (PWV), augmentation index (AIx), central pulse pressure (cPP) and carotid intima-media thickness (cIMT)</u>. Apolipoproteins including apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) were determined from serum samples and a ratio was also calculated (Apo B/ Apo A-1). Results and conclusion: We found that Apo B, Apo B/Apo A-1 and Apo A-1 were all higher in the white group (all $P < 0.030$) as compared to the black group. Apolipoprotein B was independently associated with cPP ($\beta = 0.16$, $P < 0.027$) and cIMT ($\beta = 0.19$, $P < 0.022$) and Apo B inversely associated with PWV ($\beta = -0.15$, $P < 0.025$) in the black group. No associations between the vascular markers and any of the apolipoproteins were found in the white group. In conclusion, our results indicate that apolipoproteins may <u>play an important role in the</u> early development <u>of</u> vascular changes <u>in</u> young, black South Africans. Key words: Lipids; <u>pulse wave velocity; augmentation index; intima-media thickness</u>; ethnicity. CHAPTER 1 1. GENERAL <u>INTRODUCTION</u> Non-communicable diseases (NCDs) are the <u>leading cause of death</u> in many countries, <u>accounting for 71% of deaths</u> globally and representing 43% of the global burden of disease.^{1,2} Moreover, cardiovascular disease (CVD) continues to show significant detrimental health, social and economic consequences globally, particularly in resource-limited countries such as in the sub-Saharan Africa region.³ Various risk factors, including elevated blood pressure (BP), diabetes, obesity, alcohol usage, tobacco usage, unhealthy diet and physical inactivity, contribute to the development of CVD and

increases the burden of NCDs.^{4,5} In particular, dyslipidaemia is associated with vascular alterations, including arterial stiffness and atherosclerosis, contributing to the development of future cardiovascular events.⁶⁻⁸ With data showing a global dyslipidaemia prevalence of over 50% in adult populations,⁶ dyslipidaemia remains a significant factor to target in order to reduce the burden of CVD.⁶⁻⁸

2. THE ROLE OF DYSLIPIDAEMIA AND THE RELATED ETHNIC DIFFERENCES IN CARDIOVASCULAR DISEASE

Dyslipidaemia – defined as an abnormal amount of free cholesterol within the circulation – is one of the major contributors to CVD development and progression.^{9,10} Dyslipidaemia does not tend to show any obvious symptoms but has the ability to potentiate CVD development.¹¹⁻¹³ Although CVD is more common among middle-aged and elderly populations, the prevalence of high cholesterol among young adults increases the risk of early CVD development.¹⁴⁻¹⁶ Risk factors related to dyslipidaemia include smoking, obesity, central obesity, hypertension and diabetes.^{17,18} Several ethnic disparities related to CVD-risk factors have been well documented,¹⁹⁻²¹ including differences in the lipid profile.²²⁻²⁴ Studies have shown that white populations are more prone to have an atherogenic lipid profile with an increase in low-density lipoprotein cholesterol (LDL-c) and a decrease in high-density lipoprotein cholesterol (HDL-c) than black populations.^{21,25,26} The tendency toward a more atherogenic lipid profile in white population groups may be due to increased amounts of visceral adipose tissue accumulation, as well as genetic and dietary factors²⁷⁻²⁹, when compared to black populations.^{21,25,26} Elevated LDL-c and decreased HDL-c is known to be the current conventional indicators for lipid profiling used in evaluating cardiovascular risk.²²⁻²⁴ Alternative approaches for lipid profiling include the use of apolipoproteins, and evidence suggests that apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) may be better CVD-risk predictors than traditional lipoproteins.³⁰⁻³³

3. APOLIPOPROTEINS AND THEIR FUNCTION WITHIN THE CIRCULATION

Apolipoproteins are the main protein components of LDL-c and HDL-c.³⁴ Various studies suggest that Apo B may be a more valuable risk predictor for dyslipidaemia than traditional LDL-c, as Apo B has the ability to reflect the total number of atherogenic particles within the circulation.³⁰⁻³³ The measurement of Apo B is, therefore, a more representative method to reflect this atherogenic burden.³⁰⁻³³ Apolipoprotein B is the main protein constituent of lipoproteins such as very-low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs) and LDL-c.³⁵ This apolipoprotein has atherogenic properties as it facilitates the entrapment of lipoproteins within the arterial wall, peripheral tissue and the liver.³² It also transports cholesterol from the site of production to peripheral tissues, where it is used as an energy substrate.^{32,35} Apolipoprotein B is a major contributor to inflammation, atherosclerosis, calcification, oxidative stress and arteriosclerosis (Figure 1).^{32, 36} In contrast, Apo A-1 is involved in reverse cholesterol transportation from peripheral tissue back to the liver, decreasing inflammation and plaque formation, thus serving an anti-atherogenic role (Figure 1).³⁷⁻⁴⁰ When Apo A-1 concentrations decrease within the circulation, the risk of CVD increases.^{37,38} In the presence of increased Apo B concentrations, these anti-atherogenic properties of Apo A-1 tend to disappear.⁴⁰ Apolipoprotein A-1 reduces oxidative stress, inhibits LDL-c oxidation and prevents platelet activation.⁴¹ Apolipoprotein A-1 plays a major anti-inflammatory role through the inhibition of endothelial cell activation, the promotion of M2 macrophages and by neutralising the pro-inflammatory effect of C-reactive protein (CRP).⁴¹⁻⁴³ The balance of cholesterol transport within the circulation is reflected through the Apo B/Apo A-1 ratio (Figure 1) – a major risk predictor of CVD.⁴⁰ The higher the Apo B/Apo A-1 ratio, the higher the amount of free circulating cholesterol.^{33,40,44} A higher Apo B/Apo A-1 ratio increases the risk of cholesterol deposition within the arterial wall and the development of atherosclerosis and arteriosclerosis,^{33,40,44} which will be discussed in the next section. However, when the Apo B/Apo A-1 ratio decreases, reverse cholesterol transport increases with a subsequent decrease in cardiovascular risk.⁴⁰

4. PHYSIOLOGICAL STRUCTURE AND FUNCTION OF THE ARTERIAL SYSTEM

The function of the large arteries is to counteract the constant changes in BP through its buffer capacity, reducing pulse pressure and promoting optimal tissue perfusion.⁴⁵ The various arterial segments of the arterial system accomplishes this buffering function. This includes the large arteries (aorta and common carotid arteries) and the muscular arteries (femoral and brachial arteries).⁴⁶ The vascular wall consists of three layers: the intima, media and adventitia.⁴⁷ The intima is the innermost layer of the vascular wall, consisting of the endothelium and is responsible for regulating the vascular tone.⁴⁷ The layer bordering the intimal layer is the media, which consists mainly of vascular smooth muscle cells (VSMCs) and an elastin-rich extracellular matrix. The outermost layer of the vascular wall, the adventitia, is comprised mainly of collagen fibres and has very few elastin fibres.⁴⁷ The various layers of the arterial wall work together to achieve the buffer capacity of the arterial system. When deterioration starts to occur within these vascular wall units, arterial stiffening and thickening occur, and the risk of arteriosclerotic plaque formation increases.⁴⁸ This deterioration increases the risk of cardiovascular disease and also increases with advancing age.⁴⁸

5. PATHOPHYSIOLOGICAL CHANGES IN VASCULAR STRUCTURE AND FUNCTION: ARTERIOSCLEROSIS AND ATHEROSCLEROSIS

Arteriosclerosis is the consequence of lipid accumulation within the intimal layer of the vascular wall, resulting in stiffening, thickening and degeneration,^{49,50} whereas atherosclerosis is a consequence of atheroma formation.⁴⁹ Arteriosclerosis and atherosclerosis have the ability to potentiate one another through vascular remodelling – thus having a synergic process – as they share pathophysiological mechanisms.^{51,52} The accumulation of lipids within the intimal layer results in arterial alterations, including the loss of elasticity (loss of elastin and accumulation of collagen) and the thickening and stiffening of the vessel wall, thus leading to arterial stiffness.⁵³

5.1 PATHOPHYSIOLOGICAL MECHANISM OF ARTERIAL STIFFNESS

Arterial stiffness is a major predictor of cardiovascular risk and mortality.⁵¹ It is known that, throughout the course of life, aging^{45,54,55} and certain diseases (hypertension⁴⁵ and diabetes mellitus) can aggravate loss of elasticity, thickening and stiffening within the arterial wall.⁵³ Alterations can also be driven by haemodynamic forces within the circulation,⁵⁶ which results in a decreased buffer capacity of the arteries, increased central pulse pressure (cPP) and increased pulse wave velocity (PWV).⁵⁷ These changes within the vessel wall result in the early return of reflected waves, systolic BP augmentation and decreased diastolic BP.^{57,58} As a result of this augmented pressure and velocity, the afterload and amount of shear stress on the vessel walls increase.^{57, 58} Arterial stiffness is the result of degeneration in the extracellular matrix of elastic arteries, which occurs over the course of life.⁵⁹ This degeneration is characterised by the fragmentation of elastin and the deposition of collagen within the arterial wall.^{56,59,60} When a dysregulation in the balance of these proteins occurs, it leads to the overproduction of collagen and diminished production of elastin.⁵³ Elastin has an inhibitory effect on the VSMCs, preventing migration and proliferation thereof.⁵¹ When extracellular matrix stiffening occurs, the integrity of the endothelial layer becomes compromised, and this results in increased permeability of the layer.^{51,61} Increased permeability results in increased cholesterol uptake from the circulation into the vessel wall and consequently plays a role in atherosclerotic plaque formation.^{51,62} This integral issue of the endothelial layer could explain the associations previously found between arterial stiffness and the presence of

plaque found within the vessel wall.^{63,64} Various ethnic disparities have been found regarding arterial stiffness: it tends to be more pronounced in black populations at an early age than in white populations.^{65,66} Changes in arterial stiffness in white populations tend to be more age-related, whereas in black populations, these changes are reported to be more pressure-related.^{65,67-69}

5.2 PATHOPHYSIOLOGICAL MECHANISM OF ATHEROSCLEROSIS

Atherosclerosis is characterised by the accumulation of lipids and inflammatory cells within the intimal layer of the artery, resulting in VSMC migration, foam cell development and plaque formation.^{51,70} Atherosclerosis is initiated mainly at sites of endothelial dysfunction, retention and accumulation of oxidised LDL-c within the arterial wall.⁷¹ This predominantly leads to the uptake of cholesterol-rich LDL, which results in the formation of cholesterol-rich monocyte-derived macrophage foam cells. This leads to the formation of fatty plaque streaks.^{71,72} Inflammation within this fatty-streak lesion occurs as inflammatory cells are recruited from the circulation, further evolving the fatty-streak lesion.⁷³ Various cardiovascular risk factors are known to aggravate this inflammatory state, including hypertension, diabetes mellitus, hypercholesterolemia, smoking and genetics.^{71,73,74} The inflammatory state within the artery wall results in the migration of VSMCs to the fatty-streak lesion.^{73,75} The VSMCs have equal ability to absorb modified lipoproteins and to transition further into foam cells.^{75,76} As the fatty-streak lesion progresses, apoptosis and necrosis occur within the inflammatory cells, resulting in the release of the intracellular contents.⁷¹ At this stage, advanced plaque is comprised mainly of a necrotic core and lipoprotein-derived lipids under a thin, fibrous cap.⁷¹ This thin, fibrous cap tends to rupture under high mechanical stress within the artery, resulting in the trigger of thrombosis that can lead to CVD complications.⁷¹ Previous studies have found differences in the prevalence of atherosclerosis among different ethnic groups.^{77,78} A higher burden of atherosclerosis has been reported among white populations in comparison to black populations. These differences may be due to the differences in cardiovascular risk factors among different ethnic groups, including central obesity and dyslipidaemia.⁷⁷⁻⁸⁰

5.3 DETERMINANTS OF ARTERIAL STIFFNESS AND ATHEROSCLEROSIS

Various well-known markers exist to determine increased stiffness within the circulation, including PWV, [augmentation index \(Aix\)](#) and [central pulse pressure](#). These markers [have been](#) established as major predictors of cardiovascular risk and mortality.^{58,81-84} Pulse wave velocity measures the speed of pressure waves travelling along the aorta and large arteries. It is calculated by dividing the distance the pressure wave travelled between two points by the time it took to travel this distance within the arteries.^{85,86} [Pulse wave velocity is considered to be the gold standard](#) measurement of [arterial](#) stiffness⁸⁷ and is known to be cost-effective, reliable and is determined non-invasively.^{86,88} Another useful marker to determine cardiovascular risk is Aix.⁸³ Augmentation index is a parameter that shows to which degree central arterial pressure is enhanced through the reflected pulse wave.⁸³ Strong associations have been found between Aix and PWV, with an increased PWV resulting in an early return of the reflected arterial waves, ultimately augmenting the central systolic pressure during systole.^{89,90} Pulse pressure is also a major determinant of cardiovascular risk.⁹¹⁻⁹³ It is a haemodynamic measurement affected by various cardiovascular risk factors, including hypercholesterolemia, hypertension, smoking and metabolic syndrome.⁸² However, pulse pressure is dependent on the degree of [arterial stiffness, stroke volume and timing of the reflected pressure waves](#).⁹⁴ The structural properties of large arteries can be assessed by determining [carotid intima-media thickness \(cIMT\)](#), [a subclinical marker of atherosclerotic burden](#). This [non-invasive ultrasound technique measures the thickness of the intima and medial layers of the carotid artery](#) and reflects the atherosclerotic burden at the site of measurement.^{84,95,96} When cIMT measures at less than 0.9 mm, it is seen as normal. With a cIMT greater than 1.5 mm, atherosclerotic plaque can be identified within the carotid artery.⁹⁷⁻⁹⁹

6. MOTIVATION

South Africa faces a high prevalence of NCDs, among which CVD is the largest contributor.^{100,101} The increase in the prevalence of NCDs, such as CVD, places a significant strain on the public health sector. Arterial stiffness has been identified as a risk for CVD, and previous studies have already shown ethnic differences to be evident here, with arterial stiffness being higher in six to eight-year-old black boys, showing differences in arterial stiffness already at an early age.^{65,102-104} Numerous studies exist regarding the association between apolipoproteins and CVD risk,^{30,81,102,103} with only a few focusing on the direct associations between apolipoproteins and arterial stiffness.^{30,102} Existing studies, focusing on the direct association between apolipoproteins and arterial stiffness, were conducted in small population groups and populations already presenting with numerous other risk factors such as the metabolic syndrome.^{102,104} Limited data is available on the associations between apolipoproteins and arterial stiffness, and it remains to be determined whether any ethnic differences are at play. Therefore, this study investigated whether associations exist between apolipoproteins and arterial stiffness within a young bi-ethnic South African cohort. These findings could contribute to the understanding of the early development of arterial stiffness [as a risk factor for CVD](#). [The aim of this study is to investigate](#) apolipoproteins (Apolipoprotein A-1, Apolipoprotein B and the Apo B/Apo A-1 ratio) and their relationship with arterial stiffness in a young bi-ethnic cohort. Objectives In a bi-ethnic, young population, the objectives of this study are: 1. To determine if differences in apolipoproteins (Apolipoprotein A-1, Apolipoprotein B and the Apo B/Apo A-1 ratio) and markers [of arterial stiffness \(PWV, augmentation index, cIMT and central pulse pressure\)](#) are evident. 2. To determine whether associations exist between apolipoproteins and [arterial stiffness \(PWV, augmentation index, cIMT and central pulse pressure\)](#) within each ethnic group. Hypothesis 1. Apo B and the Apo B/Apo A-1 ratio will be higher and Apo A-1 lower in the white population as compared to that of the black population. 2. Apo B and the Apo B/Apo A-1 ratio will positively associate with [markers of vascular structure and function \(PWV, Aix, cPP and cIMT\)](#), while a negative association between Apo A-1 [and the markers of vascular structure and function](#) (PWV, Aix, cPP and cIMT) will be evident. These associations are expected in both ethnic groups.

CHAPTER 2.1. STUDY DESIGN AND PARTICIPANTS

[This MHS study is a sub-study](#) which includes the cross-sectional data from the full baseline cohort of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT).¹ The original, larger African-PREDICT study was designed to investigate the early pathophysiology accompanying cardiovascular disease development and to identify novel early markers or predictors by following young, apparently healthy adults over a period of 10 years. Participants were recruited from Potchefstroom and surrounding areas by trained field workers – via their workplace or through advertisement by means of local newspapers or radio stations. This sub-study included 1202 young, apparently healthy black and white men and women between the ages of 20 and 30 years who were first screened. Those who met the criteria were included in the study (Table 1). Forty participants with missing data for the main variables of interest were excluded, and the final study sample consisted of 1162 participants. 2.

METHODOLOGY 2.1 ORGANISATIONAL PROCEDURES

Participants who were recruited first underwent screening to [determine eligibility based on the inclusion and exclusion criteria \(Table 1\)](#) for participation in the African-PREDICT study. The African- PREDICT study (NWU-00001-12-A1) and this MHS study (NWU-00368-20-A1)

were evaluated and [approved by the Health Research Ethics Committee of the North-West University](#) (Appendix B). Additionally, both this study and the African-PREDICT study adhere [to the principles](#) set out [in the Declaration of Helsinki](#) (2008). The African-PREDICT study was endorsed by both the National and Provincial Department of Health, and the study conformed to the Medical Research Council guidelines of good clinical practice. Participants arrived [at the North-West University in Potchefstroom](#) at the Hypertension Research and Training Clinic at 08:00 in the morning. The measurements' procedures were explained to the participants by the research nurse, research assistant, and researchers. The informed consent form was discussed with the participants, and they had the opportunity to ask questions prior to giving written informed consent. Privacy of participants was always taken into consideration, and all measurements were taken in a private, temperature-controlled room. A maximum of four participants was accommodated per day to ensure detailed and quality measurements. During the study day, participants rotated around a number of measurement stations with the help of the research nurse and research assistant. In between stations, participants were requested to complete questionnaires with help from researchers. All procedures were performed according to good clinical practice.

2.2 GENERAL HEALTH AND DEMOGRAPHICS QUESTIONNAIRE Data with regards to age, sex and ethnicity were collected using a general health and demographics questionnaire. This questionnaire was completed on iPads using a web-based program. It took approximately 15 minutes to complete. The questionnaire also captured data pertaining to alcohol consumption, smoking history and socioeconomic status (SES). The SES of a participant was determined using three categories included in the demographic questionnaire, namely skills level, education and household income. Points were scored for each of these categories, and the total number of points were used to determine whether a participant fell in low, middle or high SES.

2.3 ANTHROPOMETRIC MEASUREMENTS Anthropometric measurements were taken by trained anthropometrists. Standard procedures were used to determine height (m) by using the SECA 213 Portable Stadiometer (SECA, Hamburg, Germany), weight (kg) using the SECA 813 Electronic Scales with a weighing capacity of 200kg (SECA, Hamburg, Germany) and waist circumference (cm) using a Lufkin Steel Anthropometric Tape W606PM (Lufkin, Apex, USA). These procedures were performed by a trained researcher. [Body mass index \(BMI\) was subsequently calculated \(kg/m²\)](#). All of the anthropometric measurements were performed according to the [guidelines of ISAK \(International Society for the Advancement of Kinanthropometry\)](#).

2.4 CLINIC BLOOD PRESSURE With the use of the Dinamap Procare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, USA), the clinic blood pressure of participants was measured. Participants were fitted with an appropriately-sized GE Critikon latex-free Dura-Cuff. Before the measurement was conducted, participants were requested to refrain from eating, smoking and exercising for at least 30 minutes beforehand and to be seated in a resting state with the arms supported at heart level. After the participant was seated calmly for five [minutes, the first measurement was taken on the left arm](#). The blood pressure was taken on the right arm in duplicate. A final measurement was then made on the left upper-arm of the participant. The [systolic blood pressure, diastolic blood pressure and heart rate](#) were captured after each measurement. An average of all four blood pressure measurements performed for each arm was used to calculate an average for both systolic and diastolic blood pressure.

2.5 AUGMENTATION INDEX, CENTRAL PULSE PRESSURE AND ARTERIAL STIFFNESS The [SphygmoCor® XCEL device \(AtCor Medical Pty. Ltd., Sydney, Australia\)](#) was used to estimate [central systolic blood pressure, central pulse pressure and the augmentation index](#) by performing [pulse wave analysis \(PWA\)](#). The PWA was also used to measure the supine brachial systolic and [diastolic blood pressure](#). From this, [mean arterial pressure \(MAP\) was calculated](#). A brachial cuff was placed on the right upper arm. By using a general transfer function, the device estimated central systolic blood pressure, and the measurement was performed in duplicate. Arterial stiffness was also assessed by determining [pulse wave velocity \(PWV\) with the SphygmoCor® XCEL device](#). Approximately five minutes before the commencement of the measurement, participants were requested to be in a supine position and in a relaxed state. Through means of palpation, the participant's carotid artery was located to identify the strongest pulsating point. Pulse wave velocity was captured at the right carotid and femoral arterial pulse points. The femoral artery waveform was captured via an appropriately sized cuff placed around the thigh, and the carotid arterial waveform was captured simultaneously via applanation tonometry. The distances between the pulsated sites were measured using an infantometer, and 80% of these distances were used as the pulse wave travelled distance.

2.6 CAROTID INTIMA-MEDIA THICKNESS Carotid intima-media thickness (cIMT) measurements were made from the [far wall of the left and right common carotid artery](#) and the internal carotid using the [General Electric Vivid E9 device \(GE Vingmed Ultrasound A/S, Horten, Norway\)](#). Carotid [intima-media thickness](#) measures [the distance between the lumen-intima interface to the media-adventitia interface of the artery wall](#). Each side was [measured](#) and recorded for similar future assessments from various optimal angles. The images were digitised and imported in the Artery Measurement Systems software (Gustavsson, Sweden) for analyses.

2.7 BLOOD SAMPLING AND BIOCHEMICAL ANALYSES Participants were asked to fast overnight for at least [eight hours](#) prior to attending the research measurement day. Early-morning blood samples were collected by a research nurse in a private, temperature-controlled room. Once the samples were taken, a research assistant trained in handling biological samples (using latex gloves) collected the samples in the clinic and placed the sample tubes in a closed container. Samples were immediately taken to the on-site temperature-controlled laboratory. Tubes were then centrifuged and aliquoted into cryovials for storage at -80°C in biofreezers. Staff and students who handled the biological samples had received extensive training by the laboratory manager to reduce any risks. The staff and students were all part of the Hepatitis surveillance programme. During all procedures, protective laboratory coats and latex gloves were worn. Serum samples [were analysed using the Cobas Integra® 400 plus \(Roche, Basel, Switzerland\)](#) to determine apolipoproteins (Apo B, Apo A-1), [total cholesterol, high-density lipoprotein cholesterol \(HDL-c\), low-density lipoprotein cholesterol \(LDL-c\), triglycerides, glucose, C-reactive protein](#) and gamma-glutamyl transferase. Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Reactive oxygen species (ROS) were determined in serum with high-throughput spectrophotometric assay by the [Synergy H4 hybrid microplate reader \(BioTek, Winooski, VT, USA\)](#).

3. DATA MANAGEMENT The African-PREDICT study uses the [REDCap \(Research Electronic Data Capture, see <http://project-redcap.org>\)](#) system [to capture all data](#) elements.

7 The Data Manager was appointed and trained to oversee REDCap. When using this system, all laboratory specimens, evaluation forms, reports, data and other records are identified only by the participant number to maintain participant confidentiality. Data entry is performed by research assistants and controlled and checked by the Data Manager. Only the Data Manager, Head of the Hypertension Clinic and Principal Investigator have access to information linking ID numbers to participant names. Apart from the REDCap system, data are also backed up on password-protected hard drives, and all paper and computerised records are kept in a secured area. Clinical information was not released without the participant's written

permission except as necessary for cleaning, monitoring, and statistical analysis by the authorised study team members or when the participant's well-being was at risk. No new samples were collected since existing data were used for this sub-study. Password-protected computers were used to ensure confidentiality and, upon receiving HREC approval for the study, a password-protected dataset was shared with the student by the data manager. After completion of data analyses, the dataset and all copies thereof were given back to the principal investigator, who deleted the dataset.

4. STATISTICAL ANALYSES

Statistical analyses were performed using Statistica v13.3 (TIBCO Software Inc, Palo Alto, CA, USA), GraphPad Prism 5.03 (GraphPad Software, San Diego) was used for graphics. All variables were tested for normality using formal tests (Kolmogorov-Smirnov test and Shapiro-Wilks test) and graphical methods (histograms). In the case of non-Gaussian distribution, a logarithmic transformation was performed for each skewed variable and tested for normality again. The mean and spread of all descriptive variables (either standard deviation, 95% percentile interval or percentage) were presented to characterise the total group. Independent t-tests were performed to compare differences between black and white groups. Pearson, partial and multiple regression analyses were performed to determine the associations between measures of arterial stiffness and apolipoproteins. Covariates included in the models were age, sex, SES, WC, LDL-c, glucose, CRP, GGT and cotinine. Models with PWV and cIMT as dependent variables additionally included MAP.

CHAPTER 3 The association between apolipoproteins and arterial stiffness in young adults: the African-PREDICT study

ABSTRACT

Background and aims: Adverse lipid levels can potentiate changes within the vasculature, resulting in the development of cardiovascular disease. It is suggested that Apolipoproteins are better risk predictors for cardiovascular disease than traditional lipid profiling. However, data regarding the relationship between apolipoproteins and arterial stiffness are limited. Ethnic differences in apolipoproteins are not well documented and the role of ethnicity in the relationship between apolipoproteins and arterial stiffness also remains unclear. Therefore, the aim was to investigate whether differences in apolipoproteins are evident in a young, apparently healthy, bi-ethnic population and whether apolipoproteins are associated with markers of vascular structure and function in each ethnic group.

Methods: Young black and white men and women (aged 20–30, N=1162) from the African-PREDICT study were included. Carotid-femoral pulse wave velocity (PWV); central pulse pressure (cPP) and augmentation index (AIx) were determined, and carotid intima-media thickness (cIMT) was measured. Apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) were determined, and the ratio of Apo B/Apo A-1 was calculated. Results: Apo B, Apo B/Apo A-1 and Apo A-1 were higher in the white group (All $P < 0.030$). Apolipoprotein B was independently associated with cPP ($\beta = 0.16$, $P < 0.027$) and cIMT ($\beta = 0.19$, $P < 0.022$) and inversely associated with PWV ($\beta = -0.15$, $P < 0.025$) in the black group. No associations between the vascular markers and any of the apolipoproteins were found in the white group.

Conclusions: Adverse associations between vascular measures and Apo B in young, apparently healthy black adults were found. No associations between vascular measures and apolipoproteins were found in the white adults.

1. INTRODUCTION

The high prevalence of cardiovascular disease (CVD) continues to show detrimental health, social and economic consequences globally and in resource-limited settings such as sub-Saharan African countries.

1 Cardiovascular disease is recognised as one of the leading causes of morbidity and mortality globally.

The development of CVD is potentiated by various risk factors including elevated blood pressure, dyslipidaemia, smoking, diabetes and obesity.

2 These CVD risk factors potentiate vascular alterations including arterial stiffness.

It is known that arterial stiffness manifests with increasing age. In the presence of CVD risk factors, however, previous studies conducted in an African cohort, found that measures of arterial stiffness were already more pronounced in black South African children than when compared to their white counterparts.

3 The current conventional approach for lipid profiling in cardiovascular risk evaluation are elevated low-density lipoprotein cholesterol (LDL-c) and decreased high-density lipoprotein cholesterol (HDL-c).

4 An unfavourable lipid profile plays a major role in pathological processes leading to vascular abnormalities such as atherosclerosis, arterial stiffness and hypertension.

5-8 Recent evidence suggests that apolipoprotein B (Apo B) and apolipoprotein A-1 (Apo A-1) may be better CVD risk predictors in risk evaluation than the traditionally used lipoproteins.

9-12 Apolipoprotein B is the main protein constituent of lipoproteins, including very-low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs) and LDL-c.

13 Apolipoprotein B is more of a representative marker of the atherogenic burden, as it facilitates the entrapment of lipoproteins within the arterial wall.

9-12 Therefore, it is a major contributor to inflammation, atherosclerosis, calcification, oxidative stress and arteriosclerosis.

11,14 Apolipoprotein A-1 (Apo A-1), however, serves an anti-atherogenic role and, in the presence of increases in Apo B, these anti-atherogenic properties tend to disappear.

15-18 Apolipoprotein A-1 is known to play a major anti-inflammatory role and also reduces oxidative stress, LDL-c oxidation and prevents platelet activation.

19-21 The Apo B/Apo A-1 ratio reflects the balance of free cholesterol within the circulation.

12,18,22 The higher the Apo B/Apo A-1 ratio, the higher the amount of free circulating cholesterol, which increases the risk of cholesterol deposition within the arterial wall and, therefore, increases the risk of atherosclerosis and arteriosclerosis development.

12,18,22 Data regarding apolipoproteins and arterial stiffness in young, healthy African populations are very limited.

Existing studies were conducted in small population groups and in populations already presenting with numerous other risk factors, such as the metabolic syndrome.

23 Therefore, the aim is to investigate whether any differences in apolipoproteins are evident in a young, apparently healthy, bi-ethnic population and whether apolipoproteins are associated with arterial stiffness in each ethnic group.

2. METHODS

2.1 Study design and population

This study is part of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT).

24 The main aim of African-PREDICT is to follow young, apparently healthy adults over a 10-year period to identify novel markers related to early cardiovascular disease development.

Participants were screened, and those who met the criteria were included in the study. The inclusion criteria were young (20–30 years), apparently healthy black and white men and women with a normal screening clinic BP ($< 140/90$ mmHg), who are not infected with HIV, have no self-reported chronic disease (or treatment thereof) and are not pregnant or breastfeeding at the time of inclusion. This sub-study included the cross-sectional data from the full baseline cohort of 1202 participants. Forty of these participants were excluded because of missing data for the main variables of interest. The final study sample consisted of 1162 participants. The study was approved (NWU-00368-20-A1) by the Health Research Ethics Committee (HREC) of the North-West University, and every participant gave written informed consent. The African-PREDICT study has also been registered at ClinicalTrials.gov (Identifier: NCT03292094).

2.2 Questionnaires

Participants completed the General Health and Demographic Questionnaire online on a web-based program that captured basic information such as sex, age, socioeconomic status, and alcohol and tobacco usage. The socioeconomic status (SES) of a participant was determined from three categories included in the demographic questionnaire: skills level, education and household income. Points were scored for each of these categories, and the total

number of points were used to determine whether a participant fell in low, middle or high SES.²⁵ 2.3 Anthropometric measurements Standard procedures were used to determine height (SECA 213 Portable Stadiometer, SECA, Hamburg, Germany), weight (SECA 813 Electronic Scales, SECA, Hamburg, Germany) and waist circumference (WC) (Lufkin Steel Anthropometric Tape (W606PM) Lufkin, Apex, USA). Body mass index (BMI) was calculated.²⁶ 2.4 Cardiovascular measurements With the use of the DinamapProcare 100 Vital signs Monitor (GE Medical Systems, Milwaukee, USA), the clinical blood pressure of participants was measured. Participants were fitted with an appropriate-sized GE Critikon latex-free Dura-Cuff. Before the measurement was conducted, participants were requested to refrain from eating, smoking, and exercise for at least 30 minutes prior to the measurement and to be seated in a resting state with the arms supported at heart level. After the participant was seated calmly for five minutes, the first measurement was taken on the left arm. The blood pressure was taken on the right arm in duplicate. A final measurement was then taken on the left upper-arm of the participant. The systolic blood pressure, diastolic blood pressure and heart rate were captured after each measurement. For statistical analysis, both the duplicate measures performed on each arm were used to calculate an average for both systolic and diastolic blood pressure separately. The SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) was used to estimate central systolic blood pressure, central pulse pressure and the augmentation index by performing pulse wave analysis (PWA). A brachial cuff was placed on the right upper-arm and, by using a general transfer function, the device estimated central systolic pressure. The measurement was performed in duplicate. An average of the duplicate measures was used for statistical analysis. Carotid-femoral pulse wave velocity (PWV) was also measured with the SphygmoCor® XCEL device. The participant's carotid artery was located to identify the strongest pulse point, and the carotid pulse was measured using a tonometer, while the femoral pulse was measured by a femoral cuff placed around the thigh of the participant. The distance between the carotid pulse point and upper femoral cuff was noted, and 80% of the distance was calculated and entered.²⁷ Carotid intima-media thickness (cIMT) measurements were taken on the left and right common carotid artery and the internal carotid using the General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway). Carotid intima-media thickness measures the distance between the lumen-intima interface to the media-adventitia interface of the artery wall. Each side was measured and recorded for similar future assessments from various optimal angles. The images were digitised and analysed using the Artery Measurement Systems software (Gustavsson, Sweden). The left far wall measurements of the carotid artery were used in analysis. 2.5 Biological sampling and biochemical analysis Fasting morning blood samples were taken by a registered nurse. All samples were immediately taken to the on-site laboratory. Blood samples were then centrifuged and aliquoted into cryovials for storage in biofreezers at -80°C until analysis. Serum samples were analysed using the Cobas Integra® 400 plus (Roche, Basel, Switzerland) to determine apolipoproteins (Apo B, Apo A-1), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerides, glucose, C-reactive protein (CRP) and gamma-glutamyl transferase (GGT). Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Reactive oxygen species (ROS) were determined in serum with high-throughput spectrophotometric assay by the Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). 3. RESULTS The characteristics of the study population are presented in Table 1. The age and sex were comparable between the groups (all $P \geq 0.452$), while a larger percentage of the black group was from a low SES ($P < 0.001$). Overall, the black group presented with lower measures of body composition than the white group (all $P < 0.003$). The black group presented with a different cardiovascular profile, but values were still within normal ranges, with higher clinic and central BP measurements when compared to the white group (all $P < 0.005$). In addition, all vascular measures, including PWV, AIX, and cIMT, were also higher in the black group (all $P < 0.034$). Apo B, Apo A-1 and Apo B/Apo A-1 were all higher in the white group (all $P < 0.030$). Figure 1 and Supplementary Table 1 presents the unadjusted associations between vascular measures and apolipoproteins. In the black group, an inverse association between PWV and Apo B was evident ($P < 0.001$). An inverse association was found between PWV and Apo B/Apo A-1 ($p = 0.007$). A positive association was observed between AIX and Apo B ($P < 0.001$), as well as between AIX and Apo B/Apo A-1 ($P < 0.001$). Furthermore, a positive association between cIMT and Apo A-1 ($P = 0.006$) was also encountered in the black group. In the white group, positive associations between AIX and Apo B, and between AIX and Apo A-1 were found (all $P < 0.001$). No other associations were evident in this group. In the partial correlations (Table 2), most of the associations remained robust. In the black group, an inverse association between PWV and Apo B ($r = -0.13$, $P = 0.001$), and between PWV and Apo B/Apo A-1 was found ($r = -0.10$, $P = 0.018$). A positive association between AIX and Apo B ($r = 0.17$, $P < 0.001$), and Apo B/Apo A-1 was also still evident ($r = 0.12$, $P = 0.004$). In addition, a positive association between central pulse pressure (cPP) and Apo B (Apo A-1) were found ($r = 0.10$, $P = 0.017$), as well as between cIMT and Apo B in the black group ($r = 0.09$, $P = 0.025$). Carotid intima-media thickness associated positively with Apo A-1 in the black group ($r = 0.13$, $P = 0.002$). In the white group, the positive association between AIX and all of the apolipoproteins (all $P \leq 0.001$) was also still evident. The multivariate adjusted-regression analyses are shown in Figure 2 and Supplementary Table 2. In the black group, an independent inverse association between PWV and Apo B was evident ($\beta = -0.15$, $P = 0.025$) as well as a positive association between cPP and Apo B ($\beta = 0.16$, $P = 0.027$). In addition, a positive association was observed between cIMT and Apo B ($\beta = 0.19$, $P = 0.022$) in the black group. No associations between AIX and any of the apolipoproteins were found in either the black or white group. 4. DISCUSSION The aim was to investigate whether any differences in apolipoproteins are evident in a young, apparently healthy, bi-ethnic population and whether apolipoproteins are associated with markers of vascular structure and function (PWV, AIX, cPP and cIMT) in each ethnic group. As expected, Apo B and the Apo B/Apo A-1 ratio were higher in the white group when compared to their black counterparts. It is known that traditional lipid markers, including total cholesterol, LDL-c and HDL-c, differ between African-Americans and Caucasian-Americans, and data shows that white populations are more prone to an atherogenic lipid profile than black populations.²⁸⁻³⁰ The findings of this study regarding the higher Apo B and Apo B/Apo A-1 levels in the white group are in line with a previous study that included 528 Caucasian and African-American participants. In that study, the authors found that the Caucasian-Americans had increased levels of Apo B and a higher Apo B/Apo A-1 ratio compared to the African-American group.³¹ Apolipoprotein B can reflect the number of atherogenic particles within the circulation and contributes to the development of inflammation, atherosclerosis, calcification, oxidative stress and arteriosclerosis.⁹⁻¹² The balance of free cholesterol within the circulation is reflected through the Apo B/Apo A-1 ratio and, when increased, the risk of cholesterol deposition within the arterial wall also increases.¹⁸ An unexpected finding in our study was that Apo A-1 was also higher in the white group – a contrast to previous studies where African-American participants presented with higher levels of Apo A-1 compared to their Caucasian-American counterparts.³¹ Apolipoprotein A-1 is known to have a more anti-

atherogenic role within the circulation. When ApoA-1 concentrations decrease within the circulation, it increases the risk of CVD development.^{15,16} Collectively, this study's findings show that, even though the white group had higher levels of Apo B and the Apo B/ Apo A-1 ratio (considered markers of an atherogenic state), they also had higher ApoA-1 levels compared to the black group. This may suggest a protective effect.^{18,32-34} Unexpectedly, an inverse association between Apo B and PWV was found in the black group. Only a limited number of previous studies investigated the association between Apo B and arterial stiffness. In contrast to this study's findings, the Cardiovascular Risk in Young Finns Study (aged 30-45 years) found that Apo B associated positively with pulse wave velocity, independent of [risk factors such as sex, age and heart rate in a Finnish population](#).⁹ In another study that included 247 adults with cardiovascular disease risk (hypertension, dyslipidaemia and diabetes), positive associations between Apo B and pulse wave velocity were also encountered.³⁵ Our study population included young black and white adults (20-30 years) with no history of chronic disease, which may explain the conflicting results. Other risk factors can affect this association later in life or within the presence of pathology. In addition, ethnic differences in traditional lipid markers show that white populations are more prone to an atherogenic lipid profile than black populations, and adverse associations are often found within white groups.^{28,30,36} It was further found that Apo B associated positively with cPP and cIMT in the black group. [These findings are in line with previous studies that found](#) that Apo B directly associates with atherosclerosis and central pulse pressure.³⁷⁻⁴⁰ Apolipoprotein B initiates an inflammatory response through infiltration into the arterial wall, resulting in the development of atherosclerosis.^{37,38} While the white group did present with higher concentrations of Apo B, Apo B/Apo A-1 ratio and Apo A-1, no associations were encountered between any of the vascular markers and apolipoproteins in this group. These findings may be due to early changes within the vasculature of the black group suggesting that they may be more sensitive to changes in apolipoprotein levels. The strengths and limitations should be taken into consideration when interpreting the results from this study. The study population consisted of young, apparently healthy individuals from specific urban areas of South Africa and may not be representative of the whole study population. Due to the cross-sectional study design of the study, causality cannot be inferred. The African-PREDICT study is longitudinal in design, with the future possibility of determining the contribution of apolipoproteins to changes in arterial stiffness over time. The African-PREDICT study was conducted in a highly-controlled research setting. Data regarding the association between apolipoproteins and markers of vasculature structure and function in African populations are very limited. The findings of this study may contribute to the understanding of how apolipoproteins effect the vasculature at a young age aiding in the early detection of CVD development. In conclusion, adverse associations were found between vascular measures and Apo B in young, apparently healthy black adults. No associations were found between vascular measures and apolipoproteins in white adults. Overall, the findings highlight the possible role of apolipoproteins in the [development of early detrimental vascular changes in](#) young, healthy South African adults, which may [contribute to the future development of cardiovascular disease](#).

CHAPTER 4 1. INTRODUCTION

In this conclusive chapter, the hypotheses made in the first chapter will be revisited and compared with the main findings. Comparisons with previous literature will be made as well as conclusions about the key findings of this study will be made. Recommendations will also be offered for future studies.

2. SUMMARY OF KEY FINDINGS

Hypothesis 1: Apo B and the Apo B/ApoA-1 ratio will be higher and Apo A-1 lower in the white population as compared to that of the black population. As expected, it was found that [apolipoprotein B \(Apo B\)](#), and [the Apo B/Apo A-1 ratio](#) was [higher in the white group than in the black group](#). The results from previous studies of participants of European and African-American descent align with our results of this study. The former studies found lipid markers [including low-density lipoprotein cholesterol \(LDL-c\), high-density lipoprotein cholesterol \(HDL-c\)](#), Apo B, [apolipoprotein A-1 \(Apo A-1\)](#), and the Apo B/Apo A-1 ratio to differ between black and white groups. White groups presented with higher [levels of LDL-c, Apo B and](#) Apo B/Apo A-1 and lower [levels of HDL-c and Apo A-1](#) than black groups.¹⁻³ Apolipoprotein B directly reflects the quantity of atherogenic particles present within the circulation, while the Apo B/Apo A-1 ratio reflects the balance of free circulating apolipoproteins. When the Apo B/Apo A-1 ratio is increased within the circulation, the risk of cholesterol deposition within vessel walls also increases.⁴⁻⁸ Our results of this study therefore reflect findings from previous studies,¹⁻³ showing that white populations may be prone to a more atherogenic lipid profile and therefore an increased risk for future cardiovascular disease (CVD) development. Against expectations, it was found that Apo A-1 was higher within the white group when compared to the black group. Previous studies including Caucasian and African-American participants found that the African-American group presented with higher levels of Apo A-1 than their European-American counterparts.^{2,9} It is known that Apo A-1 plays a more anti-atherogenic or protective role within the circulation, opposing the detrimental effects of Apo B. When the balance between Apo B and Apo A-1 shifts to favour Apo B, vascular damage may occur.^{2,10-14} The unexpected findings regarding Apo A-1 in the white individuals may be attributed to our this study's population, which included only healthy young individuals and, while the atherogenic apolipoproteins were higher in the white group, the mean levels were within normal ranges (Table 1). Based on the higher levels of Apo B and the Apo B/Apo A-1 ratio and the unexpected higher levels of ApoA-1 in the white group, Hypothesis 1 is partially accepted.

Hypothesis 2: Apo B and the Apo B/Apo A-1 ratio will positively associate with [markers of vascular structure and function \(PWV, AIX, cPP and cIMT\)](#), while a negative association between Apo A-1 and the [markers of vascular structure and function](#) (PWV, AIX, cPP and cIMT) will be evident. These associations are expected in both ethnic groups. When examining the associations between apolipoproteins and [markers of vascular structure and function](#) (PWV, AIX, cPP and cIMT) it was found, against expectations, that Apo B inversely associated with pulse wave velocity (PWV) in the black group. This is a controversial finding as previous studies including participants of European descent showed Apo B to associate positively with markers of arterial stiffness. A previous study, including a Finnish population (aged 30-45 years), found that Apo B positively associated with PWV independent of risk factors including sex, age and heart rate.⁴ Furthermore, in a separate study including older participants with [a history of hypertension, dyslipidaemia and diabetes](#),¹⁷ Apo B positively associated with PWV. The participants included in this study were young adults with no history of chronic disease, and previous studies, where positive associations between Apo B and PWV were reported, included participants of European descent. We found that Apo B associated positively with [central pulse pressure \(cPP\) and carotid intima-media thickness \(cIMT\)](#), within the black group. In previous studies that included participants without treatment for hypertension and participants with at least one atherosclerotic vascular event, similar results were reported.^{18,19} The development of atherosclerosis is known to be potentiated by Apo B. Apolipoprotein B has the ability to initiate an inflammatory response within the vascular wall, resulting in the infiltration of Apo B and contributes to the development of atherosclerotic plaque.^{19,20} As Apo B has the ability to contribute to the development of atherosclerosis and consequently the

stiffening of the arterial wall and increased early wave reflection, an increase in cPP is also expected.^{21,22} No associations were found between the apolipoproteins or any of the vascular markers within the white group. Based on these findings, Hypothesis 2 is partially accepted.

3. STRENGTHS, LIMITATIONS AND RECOMMENDATION FOR FUTURE STUDIES

The baseline data from the African-PREDICT study was used for this dissertation. As a result of the cross-sectional design of this study, it is not possible to determine the pathophysiological effects of apolipoproteins over time. Our study did not include the data of the full apolipoprotein profile (*Apo AII, Apo CII, Apo CIII, and Apo E*), some of which may have additional effects on the vascular structure and function that could not be evaluated. Young, apparently healthy black and white individuals without pre-existing diseases were recruited for the African-PREDICT study and, thus, allowed investigation of the study population without the influence of underlying pathology. The African-PREDICT study included study participants from only one province in South Africa. As such, our results may not reflect the true effect of apolipoproteins on vascular structure and function of the South African population in its entirety. A large sample of black and white South African adults was included, which provided us with adequate statistical power. However, the possibility of chance should be considered. Residual confounding is possible even though several covariates were included in the multiple regression analyses. The following are recommendations for future research into the relationship between apolipoproteins and arterial stiffness:

- ? Longitudinal studies are required to identify changes in apolipoproteins of individuals as they age and how these changes contribute to pathophysiological changes within the vasculature over time.
- ? Research in larger study populations, including individuals from multiple regions of South Africa, is needed.
- ? Investigation of the effect of other apolipoproteins on the vasculature is needed.

4. CONCLUSION

Levels of Apo B, Apo A-1 and the Apo B/Apo A-1 ratio were higher in the white group when compared to the black group. Positive associations were found between markers of vascular structure and function and Apo B in the black group, while no associations were evident in the white group. Our findings show that apolipoproteins, specifically Apo B, may play an important role in the development of early changes within the vasculature in young, black South Africans.

APPENDIX C

CERTIFICATE OF LANGUAGE EDITING

This is to certify that

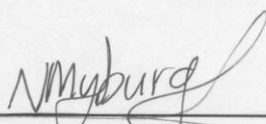
Dimoné Botha's

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