Arterial stiffness and its association with advanced glycation end-products in 6-8 year old boys: The ASOS study

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Dissertation submitted in fulfilment of the requirements for the degree *Master of Science* in Physiology at the Potchefstroom Campus of the North-West University

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September 2016

PREFACE

This dissertation comprises five chapters and forms part of the Magister Scientiae program in Physiology. Chapter 1 contains a motivation to elucidate the purpose of the study. Chapter 2 is a literature review concerning the vascular system, development of arterial stiffness, pathophysiology and factors that contribute to the development of arterial stiffness, as well as cardiovascular outcomes of arterial stiffness. Chapter 3 contains the methodology of the study. Chapter 4 includes the research article written according to the instructions of the Journal of Hypertension. The final chapter (chapter 5) summarises the main findings of the study, and includes a reflection on the hypotheses. All references at the end of each chapter are indicated according to the style of the designated journal.

ACKNOWLEDGEMENTS

I would like to extend my appreciation and express thanks to the following people who contributed in making this study possible.

- Dr. R Kruger, my supervisor, for his wisdom, statistical advice and commitment to this study regardless of his professional commitments. His passion and exceptional mentorship have inspired me. I am grateful for his encouragement and believing in me.
- Prof. AE Schutte, my co-supervisor, for her intellectual and accommodative input. For her support, kindness and making me feel welcome in her presence at all times.
- Ms. CS du Plooy, for her hard work and assisting with the process of data collection. I truly appreciate her taking time from her own studies to assist in the data collection of the ASOS study.
- My parents, for their sacrifices, support and unconditional love.
- My beloved siblings. No words can express the love and appreciation I have for them. For their love, encouragement and support.
- All the participants that took part in this study.
- The North-West University Strategic Research Fund; National Research Foundation/Department of Science and Technology South African Research Chairs Initiative; South African Medical Research Council (MRC); the South African Sugar Association (SASA) and the South African National Research Foundation (NRF).

CONTRIBUTION OF AUTHORS

Miss. GG Mokwatsi: responsible for data collection and capturing of data into final database, literature review, design and planning of the manuscript, statistical analyses, interpretation of results and writing of all sections of this dissertation and manuscript.

Dr. R Kruger: Principle Investigator of the Arterial Stiffness in Offspring Study (ASOS) responsible for the design and conceptualisation of all processes regarding the larger study. Intellectual and technical input, data collection, evaluation of statistical analyses, manuscript and dissertation. Supervised writing of the manuscript and initial design and planning of the dissertation and manuscript.

Prof. AE Schutte: Co-investigator of the ASOS project, intellectual and technical input, evaluation of statistical analyses and initial design and planning of the dissertation and manuscript.

The following statement from the co-authors confirms their individual involvement in this study and give their permission that the relevant research article may form part of this dissertation:

Hereby, I declare that I approved the abovementioned dissertation and that my role in this study (as stated above) is representative of my contribution towards the manuscript and supervised postgraduate study. I also give my consent that this manuscript may be published as part of the *Magister Scientiae* dissertation of Gontse Gratitude Mokwatsi.

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Dr. R Kruger Prof. AE Schutte

SUMMARY

Motivation

Early vascular changes are suggested to develop prematurely in the black population even in the absence of vascular disease, therefore increasing their risk for hypertension and arterial stiffness development. Different factors are known to contribute to the development of arterial stiffness, including biological ageing, adiposity and advanced glycation end-products (AGEs). AGEs are formed through non-enzymatic oxidation and glycation of free amino acid groups of lipids, nucleic acids and proteins. Formation of AGEs is stimulated by hyperglycemia and is associated with conditions such as diabetes mellitus. AGEs have received scientific interest regarding their role in arterial stiffness and cardiovascular related diseases such as type 2 diabetes mellitus. Information regarding the influence of AGEs on arterial stiffness in children is scant, and no previous comparative studies regarding the contribution of body composition and AGEs on arterial stiffness development in black and white children have been conducted.

Aim

To compare different estimates of arterial stiffness in 6–8 year old black and white South African boys and investigate the links between arterial stiffness indices, body composition and advanced glycation end-products (AGEs).

Methodology

We included 40 black and 41 white South African boys aged from 6–8 years in this study. This study obtained approval from the Provincial Department of Education and the Health Research Ethics Committee of the North-West University (NWU-00007-15-A1). We excluded obese children and those using any chronic medication, with type 1 diabetes mellitus, renal disease or cancer. AGEs, specifically pentosidine, in urine was analysed by a trained biochemist. Trained postgraduate students measured blood pressure in triplicate and continuous arterial blood pressure with participants in a sitting position. The SonoSite MicroMaxx (SonoSite Micromaxx, Bothell, WA) and a 6-13 MHz linear array probe were used to determine the carotid artery distensibility. Pulse wave velocity (PWV) was determined across various sections (carotid-radial; carotid-dorsalis pedis; carotid-femoral) of the arterial tree in duplicate. Anthropometric measurements included body height, weight, hip, waist and neck circumferences and were measured in triplicate. Body mass index z-scores were used to classify body composition of the boys according to appropriate age, height and weight cut-offs.

Results

Age and body composition were comparable between the groups except for white boys with higher neck circumference (p=0.003) and waist-to-hip ratio (p<0.0001) than black boys. Pentosidine levels were higher in black boys (p=0.039), as well as diastolic blood pressure (p=0.001), mean arterial pressure (p=0.003) and total peripheral resistance (p=0.044) compared to white boys. After adjusting for mean arterial pressure, carotid-to-radial pulse wave velocity, carotid-to-femoral pulse wave velocity and carotid-to-dorsalis pedis pulse wave velocity (all p<0.002) as well as carotid intima-media thickness (p=0.007) were higher in black compared to white boys. Correlations between measures of arterial stiffness and body composition were evident in white boys only. Carotid-to-femoral PWV correlated inversely with BMI (r =–0.32; p=0.049), only in black boys. Pentosidine inversely correlated with body composition variables including body mass index (p=0.015), body surface area (p=0.017), weight (p=0.018), waist circumference (p=0.022) and hip circumference (p=0.010) in black boys only. Arterial stiffness indices did not correlate with AGEs in any group.

General conclusion

In conclusion, pulse wave velocity of black boys was higher in all sections of the arterial tree, along with higher diastolic blood pressure, intima-media thickness and AGEs, suggesting that early arterial changes are already present in young black boys. This phenotype may have an impact on the increasing trend of hypertension in the black population of South Africa.

Keywords: advanced glycation end-products, arterial stiffness, body composition, pentosidine, pulse wave velocity

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LIST OF ABBREVIATIONS

AGE: Advanced glycation end-products

ASOS: Arterial Stiffness in Offspring Study

BMI: Body mass index

BPM: Beats per minute

cfPWV: Femoral pulse wave velocity

cm: Centimeters

crPWV: Radial pulse wave velocity

CVD: Cardiovascular disease

Cωk: Windkessel arterial compliance

DBP: Diastolic blood pressure

dpPWV: Dorsalis pedis pulse wave velocity

ECM: Extracellular matrix

g/ml: Grams per milliliter

HIV: Human immunodeficiency virus

Kg: Kilogram

kg/m^2: Kilograms per meter squared

kPa: Kilopascal

L: Liter

L/m: Liters per minute

Log: Logarithm

m^2: Square meter

m/s: Meters per second

mg/L: Milligrams per liter

mL: Milliliter

ml/min: Milliliters per minute

mg/mmol: Milligram per millimole

mm: Millimeter

mmHg: Millimeters Mercury

mL/mmHg: Milliliter per millimeter mercury

MU:	Medical unit
n:	Number of
p:	Probability value
PWV:	Pulse wave velocity
r:	Regression coefficient
SE:	Standard error
SBP:	Systolic blood pressure
SD:	Standard deviation
TPR:	Total peripheral resistance
vs:	Versus
WC:	Waist circumference

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CHAPTER 1

Introduction

1.1 General introduction and motivation

In South Africa hypertension and arterial stiffness have a high prevalence in the black adult population compared to the white population [1,2]. It was suggested that arterial stiffness may develop earlier in the black population, a term coined as early vascular aging [2,3]. Factors that may contribute to this phenomenon include urbanisation, low socio-economic background, limited health care access and unhealthy lifestyle choices [4,5]. Furthermore, parents with an elevated cardiovascular disease risk have an impact on their offspring's risk for developing cardiovascular disease in early adulthood [6,7]. It therefore seems that cardiovascular disease originates in childhood due to risk factors that may initiate early endothelial dysfunction and increased arterial wall stiffness [8].

Arterial stiffness is a leading cause of cardiovascular mortality and is closely associated with atherosclerosis and age-related changes in the arterial structure [9-11]. Arterial stiffness is associated with several conditions that relate to cardiovascular disease, such as hypertension, diabetes mellitus and dyslipidaemia [12-14]. Studies that examined arterial stiffness in children are limited and those that were conducted in Europe and Australia reported that arterial stiffness increases in children with elevated blood pressure, those who are obese or have a low level of physical activity and high fat intake or lack of breast-feeding during infancy [15-18].

Hypertension may increase arterial stiffness by altering the mechanical properties of arteries through extracellular matrix remodelling, thereby reducing their compliance and distensibility [19]. Obese children with elevated blood pressure are at increased risk for developing arterial stiffness in early adulthood and subsequent atherosclerosis [8,20,21]. It is also important to mention that arterial stiffness due to other factors can lead to the occurrence of hypertension [22,23].

Advanced glycation end products (AGEs) gained increasing scientific interest for their contribution to arterial stiffness. AGEs are stimulated by hyperglycaemia and are elevated in diabetic patients due to insulin resistance [24]. AGEs are synthesised through the non-enzymatic glycation of lipids, nucleic acids and proteins; they also play an important role in the development of arterial stiffness through the AGE-AGE intermolecular covalent bonds or cross-linking formed with extracellular matrix proteins of the arterial wall [13,14,25-27]. This cross-linking alters the elastic properties of the arterial wall, leading to extracellular matrix remodelling and as a result reducing arterial compliance [13,18]. AGEs are implicated in the pathology of conditions such as hypertension and atherosclerosis and also serve as

important predictors of complications including arterial stiffness, myocardial abnormalities and atherosclerotic plaque formation [14,15,25,26].

Body composition is inversely associated with AGEs in adults [27]. Semba *et al*. found that AGE concentration is low in obese compared to lean participants due to a scavenger receptor that is expressed by adipocytes, which binds to AGEs and facilitates their endocytosis and degradation, leading to low concentrations of AGEs in obese people [27]. This phenomenon is also true for children. Several studies that were conducted in children reported that obese children had low AGE concentrations compared to lean children, despite the presence of insulin resistance in obese children [28-30]. Enhanced glomerular filtration rate evident in obese individuals also increases renal removal of AGE peptides that are filtered by the glomeruli, decreasing AGE concentration in obese individuals [29].

Current knowledge indicates that the black population is subjected to early vascular aging, increased blood pressure and arterial stiffness [2,31,32]. A previous study focusing on arterial function and stiffness in children conducted in South Africa included children between 10–15 years, and it was found that arterial compliance was already compromised in the black group with normal or elevated blood pressure compared to the white group [33]. In the present study, the question is whether these changes will be observed in an even younger black population (6–8 years old) compared to their white counterparts with a comparable socio economic status. The previously mentioned study did not make use femoral pulse wave velocity and this current study is going to use femoral pulse wave velocity to assess arterial stiffness. To the best of our knowledge, no comparative study has been conducted to investigate arterial stiffness and its association with AGEs and body composition in black and white boys aged from 6–8 years.

Data for this study was obtained from the Arterial Stiffness in Offspring Study (ASOS), which included a total of 81 participants, including black (n=40) and white (n=41) boys from 6–8 years from Potchefstroom in the North-West province of South Africa.

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CHAPTER 2

Literature overview

2.1 INTRODUCTION

Central arterial stiffness is increasingly recognised as an independent predictor of cardiovascular disease events and all-cause mortality and is closely associated with atherosclerosis and age-related changes in the arterial structure [1-9]. Factors such as diabetes mellitus, dyslipidaemia, aging, unhealthy lifestyle habits as well as obesity have been proven to accelerate arterial stiffness in the adult population [7,10-13]. Increased arterial stiffness may also be caused by compounds known as advanced glycation end-products (AGEs) proven to play an essential role in the development of cardiovascular disease such as heart failure, myocardial infarction, coronary artery disease and stroke [14-17]. However, these links are not known in children.

Arterial stiffness is elevated in black compared to white South Africans, and may develop at a younger age as hypothesised in previous studies [18,19]. In children, factors such as obesity, elevated blood pressure, low levels of physical exercise, high fat intake and lack of breast-feeding during infancy have been associated with arterial stiffness [20-23]. There are limited studies regarding the development of arterial stiffness along with factors that influence its development in South African children. This study, therefore, aims to investigate different arterial stiffness measures and the associations thereof with AGEs in a young black and white South African male population from 6–8 years of age.

In order to achieve this aim, all relevant literature will be provided in this chapter to underline the physiology of the processes involved in early vascular changes leading to arterial stiffness.

2.2 ARTERIAL STIFFNESS

2.2.1 The vascular system, early vascular aging and arterial stiffness

2.2.1.1 The vascular system

Large elastic arteries are important for effective cardiac function by serving as elastic reservoirs to ensure adequate blood flow to tissues and organs according to their metabolic requirements [24,25]. It also enables the arterial tree to undergo volume changes with minor changes in arterial pressure [24,25]. Elastic arteries store a portion of blood flow from the left ventricle during systole and discharge it during diastole [24]. This helps to reduce the load on the heart and to also minimise systolic flow while maximising diastolic flow in the arterioles [24]. This is known as the Windkessel effect [24].

Vessel wall properties including arterial compliance and distensibility enable the Windkessel properties of arteries [24,26]. Compliance and distensibility accommodate augmented volume with small changes in arterial pressure [24,26]. Compliance is defined as the absolute change in volume for a given pressure change and it indicates the buffering ability of an artery [26]. Arterial distensibility is characterised by the relative change in volume for a given pressure change, and it indicates arterial wall elasticity [26]. The equations of the above mentioned vessel wall properties are shown in the box below.

Arterial compliance = $\frac{\Delta \mathrm{V}}{\Delta P \times \mathrm{V}}$

Arterial distensibility = $\frac{\Delta V}{\Delta P}$

V – volume; P – pressure; Δ – change [27]

The vessel wall contains the extracellular matrix (ECM) which provides a structural framework essential in the functional properties of arteries [24,28,29]. Figure 1 shows the vascular wall containing three layers embedded in the ECM, namely the *tunica intima* (inner layer), *tunica media* (middle layer) and *tunica adventitia* (outer layer) [24,29,30]. Each layer plays an essential role in the vascular system. The *tunica intima* layer comprises of internal elastic lamina, fibrocollagenous tissue and a single layer of endothelial cells [29]. The *medial layer* consists of vascular smooth muscle cells (VSMCs) important for depositing ECM proteins. Two of these important proteins namely elastin and collagens are essential in giving arteries their elastic properties [24,29-33]. The *adventitia* contains fibroblasts and consists of external elastic lamina embedded between two fibrocollagenous layers [24,29]. Elastin is the most abundant protein and is important for the elasticity of arteries and

regulation of arterial compliance [24,29,31-33]. Collagens are essential for structural support to local cells and prevention of rupture of vessel walls in response to volume changes [24,29,31-33].

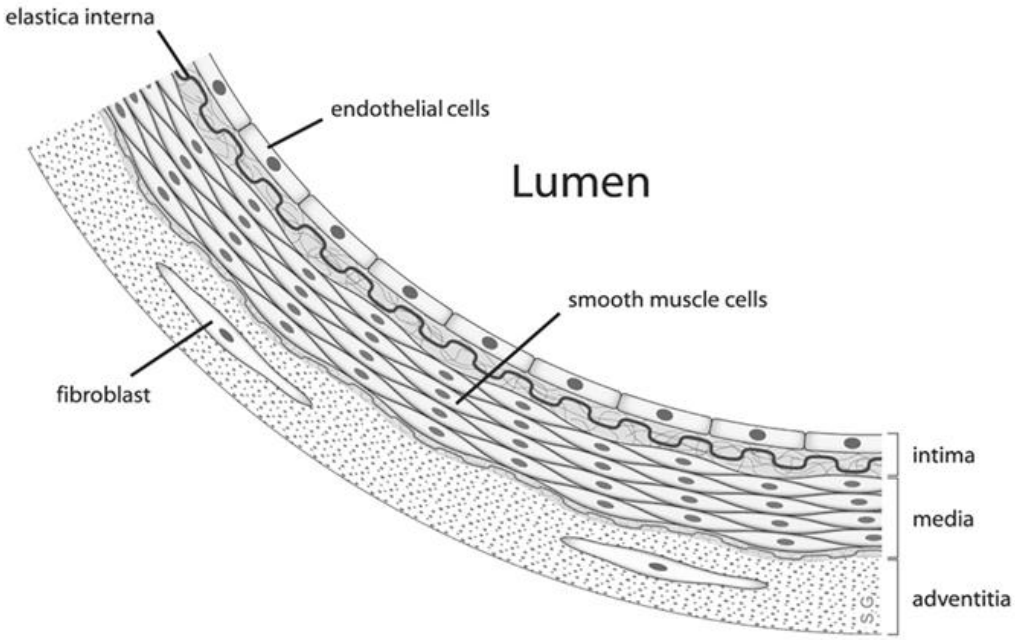

Figure 1. A cross-sectional view of the arterial wall [34]

2.2.1.2 Early vascular aging and arterial stiffness

Arteries gradually stiffen with chronological age in healthy individuals [4,11,19,35]. This phenomenon is termed biological aging [36]. This process causes changes in vascular structure and function including decreased arterial compliance and increased stiffness of arteries [36]. Vascular aging is accelerated in susceptible individuals, resulting in premature aging of arteries, a concept known as early vascular aging [36]. A South African study has shown that early vascular aging may have a higher prevalence in black South African children [37]. Age-dependent factors such as shortened telomere length, vascular remodelling and diabetes are associated with early vascular aging [7,35,38]. Early vascular aging is also associated with an increased risk for organ damage, cardiovascular events and mortality as shown in Figure 2 [36,38,39].

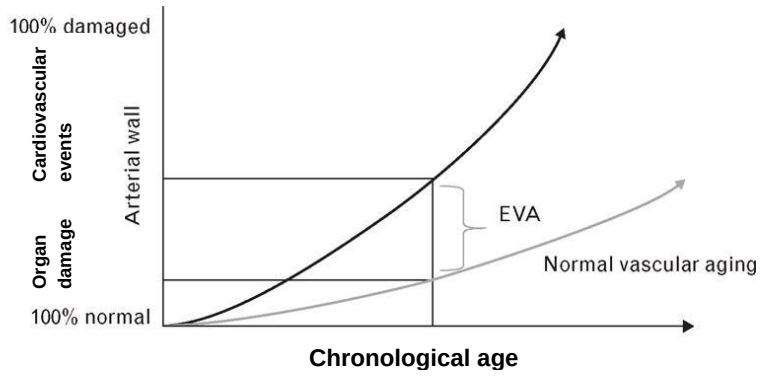

Figure 2. Graphic illustration of biological versus early vascular aging, adapted from Kotsis *et al.* [38]

Advancing age results in medial degeneration which promotes biological aging and stiffness [7,35], by which the accumulation of collagen is increased and elastin breakdown is enhanced, leading to ECM remodelling and potential early vascular aging [7,29,40]. Another marker associated with early vascular aging is telomere length [36]. Telomeres are specialised threadlike structures of the nucleic acid, which form at the end of the deoxyribonucleic acid helix to protect genetic material [36]. Telomeres shorten with every cellular reproduction, and they are regulated by the activity of the enzyme, telomerase transcriptase, essential for mending the decreased length of telomeres [38]. Shortened telomere length is a predictor of mortality risk in older individuals and is also associated with early vascular aging [36,39].

Apart from telomere length, age-related changes in the function of beta-cells and insulin resistance are also associated with early cardiovascular events [36]. This may be due to the impact of increased compounds known as advanced glycation end-products (AGEs), which are stimulated by hyperglycaemia [3,36,41]. AGEs play an important role in the process of arterial stiffness [36,42]. They form irreversible cross-links with elastin and collagen, which are essential for giving arteries their elastic properties [3,24,30,41-45]. These cross-links alter the properties of the arterial wall leading to early vascular aging and resultant reduction of arterial compliance [3,23]. Arteries lose their elasticity when the ratio of collagen and elastin is altered during vascular injury, resulting in the manifestation of vascular pathologies [29,30].

Arteries also react to changes in chemical and physical conditions, adapting to the new surroundings through vascular ECM remodelling [28]. ECM remodelling alters the function and structure of the vessel wall to accommodate new settings such as chronic elevated blood pressure [28]. Smaller ECM proteins and components such as laminins, fibrilin,

fibulins, integrins and matrix metalloproteinase (MMPs) are also involved in ECM alterations and partly linked to arterial stiffness [24,29,46,47].

Blood pressure determines arterial wall stretch and shear stress [25]. Elevated blood pressure causes tension on the arterial wall, initiating the response of smaller arteries to the force through VSMC hypertrophy and ECM remodelling as shown in Figure 3. This allows arteries to withstand the increased pressure load [25,28]. Remodelling results in arterial stiffness and decreased arterial compliance and distensibility reducing arterial elastic properties [28,48].

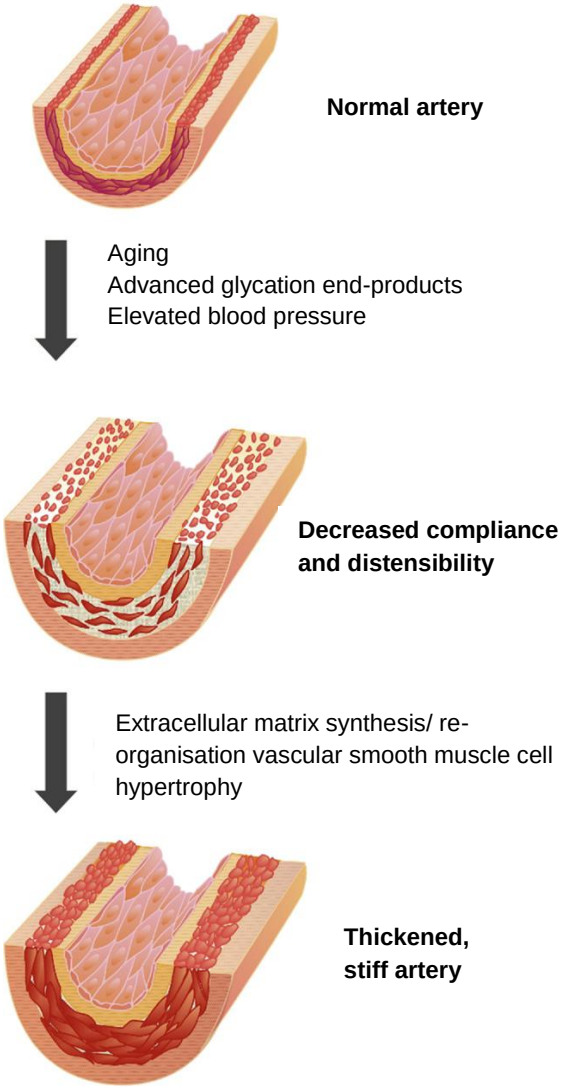

Figure 3. Schematic presentation of arterial remodelling [28]

2.2.2 Other factors contributing to arterial stiffness

2.2.2.1 Ethnicity

Hypertension is highly prevalent in black compared to white populations globally [49-52]. It is also known that the black population on a global scale has a high prevalence of arterial stiffness that is evident from a young age compared to their white counterparts [49,53-57]. This phenomenon may be caused by factors such as genetic variances, urbanisation and limited health care access [49,56,58,59].

The renin-angiotensin aldosterone system (RAAS) is one of the main blood pressure regulatory mechanisms [60]. Various genetic variations of RAAS also influence the development of arterial stiffness [61]. Low renin hypertension and a lower activity of the RAAS system is a well-known phenomenon in the black population [62]. Due to this reason, antihypertensive medications such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARBs) are not as effective in black than in white populations [62]. It was proposed that the angiotensin II type I receptor gene regulates aortic stiffness and is also associated with hypertension and arterial stiffness in black adults [61,63,64]. The presence of angiotensin II type I receptor allele influences the activity of the receptor which in turn regulates angiotensin II activity [41,64,65]. Angiotensin II influences arterial stiffness development through different mechanisms including hypertrophy and cell death [41,61,64,65]. There is no adequate information regarding the influence of genetic variations on the development of arterial stiffness in children.

2.2.2.2 Sex

Sex differences in arterial stiffness are influenced by various factors such as height, sex steroids and the function of the heart [66-71]. Arterial stiffness generally has a higher prevalence in older women compared to men [66,67]. Body height is related to aortic length and wave reflection arrives later during diastole in taller individuals, causing decreased systolic pressure and pulse pressure [67,72]. This phenomenon is applicable to men as they are on average taller than women who have shorter aortic lengths permitting early return of the reflected wave during systole [67,72]. This causes elevation of systolic pressure and pulse pressure [67,72]. Amplified pulse pressure and systolic pressure are associated with an increase in arterial stiffness [72].

Arterial diameter is also associated with the development of arterial stiffness [67]. Women generally have smaller arterial diameter which increases their prevalence of arterial stiffness compared to their height-matched men [67]. Women also have a prolonged time to the

systolic peak and longer ejection time than men of the same height, leading to a difference between men and women in their left ventricular outflow [67]. The prolonged outflow and ventricular contraction in women delays systole, enabling the reflected wave to reach the heart during systole, amplifying systolic pressure and pulse pressure which then contribute to amplified stiffness of arteries [67,72]. There seems to be no sufficient evidence regarding the influence of body height, aortic length and arterial diameter on the development of arterial stiffness in children.

A study conducted in children proved that young (pre-puberty) girls have a higher prevalence of arterial stiffness compared to their age-matched male counterparts [69]. However, it is also important to note that evidence indicates that stiffness and blood pressure of boys increases from puberty onwards [69]. This trend may be influenced by sex steroids, namely oestrogen and testosterone, which have an impact on blood pressure and prevalence of arterial stiffness in men and women [68-71]. Due to very low concentrations of oestrogen with less effective actions in girls during childhood (pre-puberty) and the elderly (post-menopause), women have a higher prevalence of arterial stiffness due to the diminished protective effects of oestrogen on the vasculature [68-71]. Oestrogen increases blood flow and improves endothelial dilation by inducing the nitric oxide synthase gene to increase nitric oxide bioavailability [69,70]. It also regulates the elastin/collagen ratio by increasing the ratio [29,71].

2.2.2.3 Lifestyle exposures and body composition

Lifestyle exposures such as poor diet (with high salt, saturated fats and low antioxidants) and physical inactivity contribute to the development of arterial stiffness in both children and adults [12,20-23,73-76]. Unhealthy diets with a high salt content have been proven to increase stiffness of arteries through the increased activation of the RAAS [77,78]. Both angiotensin II and aldosterone promote the proliferation and growth of VSMCs leading to hypertrophy and stiffness of arteries [41,65].

Physical inactivity is associated with endothelial dysfunction which, in turn, promotes tissue damage and stiffness [20,29,60,79]. Exercise increases elastin content and decreased calcium content which both prevent vascular remodelling and the development of arterial stiffness [7,11,23,29]. Low physical activity is also associated with the prevalence of obesity which has been proven to contribute to the development of arterial stiffness in children and adults [80-85]. Obesity is the most important factor leading to arterial stiffness in children [86,87]. The global burden of obesity is increasing independent of age and ethnicity and may

be attributed to genetic and non-genetic risk factors [13,88]. Adult obesity is shown to have its origin in childhood and children with obese parents are more likely to become obese in adulthood [89]. Obesity has increased among South African children and adults [90-92] with the highest obesity prevalence of 42% recently recorded in women above 20 years of age [93]. It was also reported that in South Africa, 13.5% of men older than 20 years are obese [93]. Approximately 7% of South African men younger than 20 years are obese whereas 9.6% women are obese [93].

Obesity imposes adverse effects on the vasculature leading to increased cardiovascular risk [82,88,94]. Furthermore, it has been reported that obesity is associated with hyperglycaemia which leads to insulin resistance [89]. Expanded adipose tissue in obese individuals expresses adipocytokines, hormones, growth factors and cytokines [13,81]. These factors induce alterations in insulin sensitivity, renal handling of sodium and water as well as elevated angiotensin II activity which all contribute to the development of arterial stiffness [13,81].

Increased hemodynamics (total blood volume and cardiac output) are associated with metabolic requirements of excess weight [94]. These altered hemodynamics and other characteristics of excess body weight such as dyslipidaemia and insulin insensitivity stimulate VSMC proliferation through the generation of reactive oxygen species and protein kinase C [95]. Proliferation of vascular smooth muscle cells may lead to endothelial dysfunction, increased vessel wall thickness and ultimately arterial stiffness [94,95].

2.2.3 Pathophysiological development of arterial stiffness

Arterial stiffness develops from complex pathways that are associated with alterations in the cellular and structural elements of the vascular wall [28,65]. These changes are influenced by numerous factors including hormones, hemodynamic factors, AGEs and chronological age [28,65].

2.2.3.1 Blood pressure

Arterial stiffness measurements are dependent on blood pressure due to the influence of blood pressure on arterial function [96-98]. Adults with elevated blood pressure experience increased accumulation of collagen and elastin breakdown of the ECM leading to vascular remodelling, reduced arterial compliance and increased stiffness of arteries [28,29,65]. Elevated blood pressure is also associated with the overexpression of pro-inflammatory molecules such as monocyte chemoattractant protein-1, interleukin 6 and macrophage

colony-stimulating factor [29,41,65]. These molecules influence the production of MMPs that participate in vascular ECM degradation by creating a less effective collagen and elastin molecules, leading to ECM remodelling and stiffness of arteries [29,41,65]. Previous reports have shown that elevated blood pressure in children predicts arterial stiffness in young adulthood [21,99].

2.2.3.2 Chronological age

Aging is a dominant determinant of functional and structural changes of the arterial wall [7,100,101]. Aging contributes to arterial functional and structural changes through various mechanisms such as hypertrophy and ECM accumulation [7,10,11,29, 40,102]. These changes increase arterial wall thickness and decreased distensibility and compliance of arteries causing elevated pulse wave velocity (PWV) [35,101,103]. Apart from distensibility, the intima-media thickness of arteries increases by two-to-threefold from the age of 20 years and is associated with luminal dilation and increased wall stiffness [29,35]. It has also been reported that elastin degrades with age [29,104]. This leads to increased collagen turn over and extracellular matrix remodelling which contributes to the stiffness of arteries [7,29,40].

Aging results in hypertrophy of VSMCs through increased expression of adhesion molecules, migration of medial VSMCs and proliferation [7,11,29]. This leads to eccentric thickening of arteries which is characterised by increased luminal dilation [7,11,29].

2.2.3.3 Advanced glycation end-products

2.2.3.3.1 Biochemistry and sources of advanced glycation end-products

Advanced glycation end-products (AGEs) are compounds that are formed through nonenzymatic glycation of lipids, nucleic acids or proteins [3,42,43,105]. The synthesis of AGEs occurs over a period of weeks and is stimulated by hyperglycaemia [42]. High amounts of AGEs are found in patients with diabetes (children and adults) due to insulin resistance [42,106], but also in non-diabetic individuals due to unhealthy diet. Apart from diabetes-related complications, AGEs are also implicated in the pathology of conditions such as hypertension and atherosclerosis [15,37-39,99]. AGEs accumulate in blood, blood vessels, urine as well as the skin [38,96] and were shown to predict myocardial relaxation abnormalities, endothelial dysfunction and atherosclerotic plaque formation [38,96]. Serum levels of AGEs are not only dependent on endogenous production [42,105]. They can also be influenced by exogenous sources such as overheating of foods, caramel production,

coffee roasting and bread baking among other exogenous sources [42,105]. Overheating of foods results in nonenzymatic browning, otherwise known as the Maillard reaction which adds colour, flavour and aroma to food [42,105].

AGEs are formed via three pathways, as shown in Figure 4, which are named the Maillard reaction, polyol (alcohol containing multiple hydroxyl groups) pathway and lipid peroxidation and oxidation of glucose [42,105].

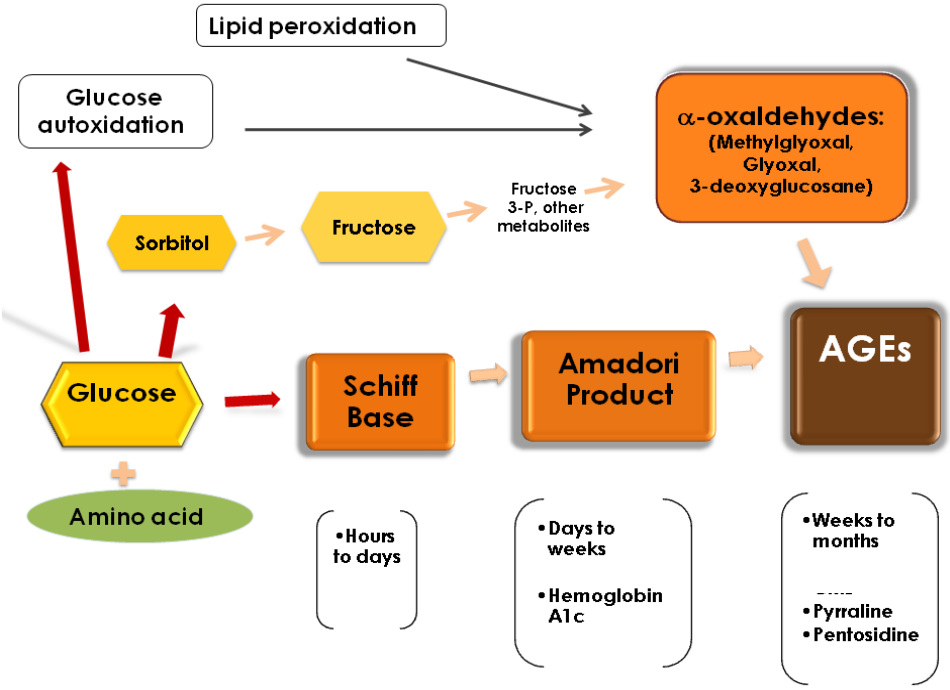

Figure 4. Formation of advanced glycation end-products (AGEs) [105]

Glucose reacts nonenzymatically with a free amino acid, lipid or deoxyribonucleic acid in the Maillard reaction to form a Schiff base [42,105]. The Schiff base undergoes chemical rearrangement to form the Amadori products which also undergo rearrangements to form AGEs [42,105]. Peroxidation of lipids and autoxidation of glucose form dicarbonyl derivatives known as alpha-oxaldehydes which react with monoacids to produce AGEs [42,105]. In the polyol pathway glucose is converted to sorbitol which is then converted into fructose whose metabolites are converted into alpha-oxaldehydes that react with monoacids to produce AGEs [42,105].

Various types of AGEs such as pentosidine, N-Ɛ-(carboxyethyl)lysine, pypraline and pyrraline are formed through the previously mentioned pathways [42,106]. Pentosidine is regarded as the most stable and best characterised AGE and it also has oxidative properties [42]. Normal serum levels of pentosidine in healthy individuals is 0.0007 μg/ml [107]. Serum levels of pentosidine are found at high levels in children with type 1 diabetes and chronic

kidney disease [106,108]. Pentosidine is associated with cardiovascular states such as arterial stiffness and heart failure [42,109].

2.2.3.3.2 Importance of advanced glycation end-products in the development of arterial stiffness

AGEs play an essential role in the development of arterial stiffness [36,42]. AGEs increase the production of reactive oxygen species which lead to the accumulation of oxidising agents which cause vascular damage through cross-linking with ECM proteins (collagen and elastin) and oxidation [42,43]. AGE-induced oxidation quenches nitric oxide through the reduction in nitric oxide synthase half-life in the endothelium causing reduced vasodilation and endothelial dysfunction driving the development of arterial stiffness [41,43,44,105,110]. Elevated levels of reactive oxygen species are linked with arterial stiffness in children and the black adult population [49,111]. Increased reactive oxygen species cause oxidative stress [49,79]. Oxidative stress is characterised by a cascade of cellular reactions that promote vascular injury through endothelial cell apoptosis as well as the oxidation of lipids and proteins [49,60,79]. Vascular injury induces endothelial dysfunction which drives consecutive tissue damage with resultant ECM remodelling that promotes stiffness of arteries [29,49,60,79].

AGEs accumulate on stable and long lived proteins including ECM proteins such as collagen and elastin, vitronectin and laminin [3,41-43,105]. They alter the elastic properties of proteins through AGE-AGE intermolecular covalent bonds or cross-linking [3,41].

AGEs also have inflammatory effects that lead to the development of arterial stiffness [44]. High AGE content stimulates the overexpression of transforming growth factor beta which influences ECM remodelling via various pathways including increased ECM synthesis [44]. In general the previously described AGE-related processes were evidenced in adults or diseased populations. Currently there is inadequate information regarding the synthesis and contribution of AGEs in the development of arterial stiffness in children.

2.2.4 Arterial stiffness in different segments of the arterial tree

Arterial stiffness can be quantified in different segments of the arterial tree including (i) the overall arterial system (measured in the entire circulation), (ii) in a specific region/ segment (measured in a segment of the arterial tree) or (iii) at a local site (measured in a small section of one blood vessel) [112-115]. Arterial elasticity can be quantified by means of indices such as pulse wave velocity (PWV), Windkessel arterial compliance and arterial distensibility in children and adults [103,112-119].

2.2.4.1 Arterial distensibility

By using high-resolution ultrasound arterial distensibility can be quantified non-invasively [112,113,116,120]. Ultrasound imaging in humans is restricted to superficial arteries such as the carotid artery [116]. Several derivatives can be obtained from ultrasound clips such as distensibility coefficient, compliance, β-stiffness index, Young's elastic modulus and Peterson's elastic modulus [121]. Formulas of the previously mentioned ultrasound clip derivatives are shown on the box below.

Distensibility = $\frac{\Delta D}{\Delta P \times D}$

Compliance = $\frac{\Delta D}{\Delta P}$

β-stiffness index: β = $\frac{\ln(\frac{Ps}{Pd})}{(Ds-Dd)/Dd}$

Young's elastic modulus = $\frac{(\Delta P \times D)}{\Delta D \times h}$

Peterson's elastic modulus = $\frac{(\Delta P \times D)}{\Delta D}$

P – pressure; D – diameter; h – wall thickness; s – systolic; d – diastolic [113].

As previously mentioned, aging results in alterations of the arterial wall structure and function, causing a decrease in arterial distensibility [36,120].

Distensibility is expressed by the distensibility coefficient, incremental elastic modulus and beta stiffness index to determine local stiffness of arteries [120]. The distensibility coefficient describes relative changes in diameter for a defined pressure whereas incremental elastic modulus provides the pressure force needed to result in vessel distortion [120]. Stiffness index beta is used to express the integral rigidity of a vessel [120]. Arterial distensibility is regarded as a sensitive indicator of arterial functional changes and it decreases with age in both children and adults [120].

2.2.4.2 Pulse wave velocity

Pulse wave velocity (PWV) is defined as the speed at which the forward pressure wave generated by the heart is transmitted from the aorta and reflected from the peripheral sites back to the heart [113,122]. The forward pressure wave and reflected wave are shown on Figure 5 below. PWV is measured between two points at arterial sites of major physiological importance such as the aorta [103,113-115]. PWV can be measured between different arterial points such as between the carotid and radial artery, carotid artery and femoral artery as well as between the carotid artery and the dorsalis pedis artery [118,123]. PWV can be assessed by measuring the distance at which the pressure wave travels between two points on the surface of the skin and also making use of mechanotransducers that are placed directly on the skin [124,125].

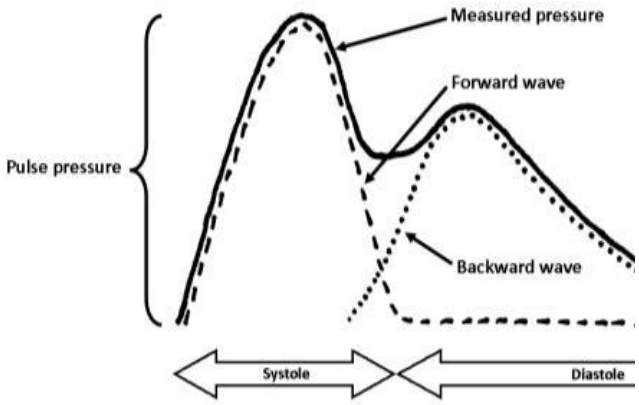

Figure 5. The pulse wave of central blood pressure [126]

The proposed threshold for carotid-to-femoral PWV in adults is 10 m/s and the higher the PWV, the stiffer the arteries [127-129]. Various studies conducted in countries such as Greece and Hungary have proposed different carotid-to-femoral PWV threshold values in children, however, there are no known values identified for South Africa [130-132].

All arterial sites have potential interest in arterial stiffness, however, the aorta is a major vessel of interest when determining regional arterial stiffness because the thoracic and abdominal aorta makes the largest contribution to the arterial buffering function [114].

2.2.4.2.1 Carotid-to-radial pulse wave velocity

Carotid-to-radial PWV is measured between the radial artery in the wrist and the carotid artery, as shown in Figure 6 [133].

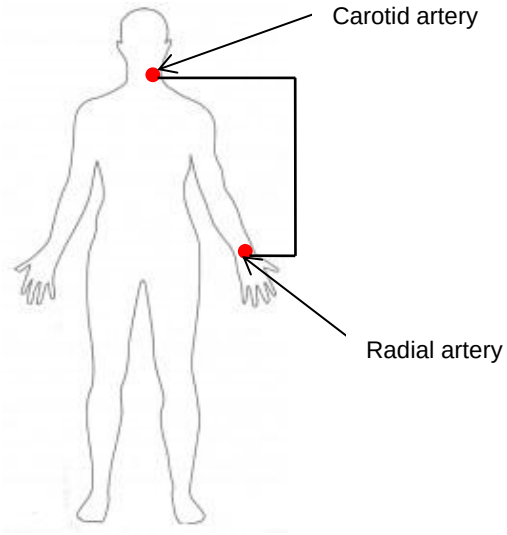

Figure 6. Carotid and radial pulse sites for placement of sensor to determine pulse wave velocity [134]

This section of the arterial tree consists mainly of muscular arteries and it is known that these arteries do not stiffen significantly with advancing age as typically seen in the elastic conduit arteries [10,135]. Healthy offspring of type 2 diabetes parents have been proven to have higher carotid-to-radial PWV [136]. This phenomenon is caused by insulin resistance that children inherit from their parents [136]. Insulin resistance influences basal arterial tone and thus stiffness of arteries in absence of risk factors [136]. Arterial stiffness results in elevated values of carotid-to-radial PWV which has been proven to predict the severity of coronary artery disease and may also be used as a surrogate of atherosclerosis [137].

2.2.4.2.2 Carotid-to-femoral pulse wave velocity

Carotid-to-femoral PWV is considered as the “golden standard” measurement of arterial stiffness because it represents aortic stiffness and has been proven to be the only pulse wave measurement to independently predict outcome [114,125]. Carotid-to-femoral PWV is measured between the carotid artery and the femoral artery as shown in Figure 7 [124,138].

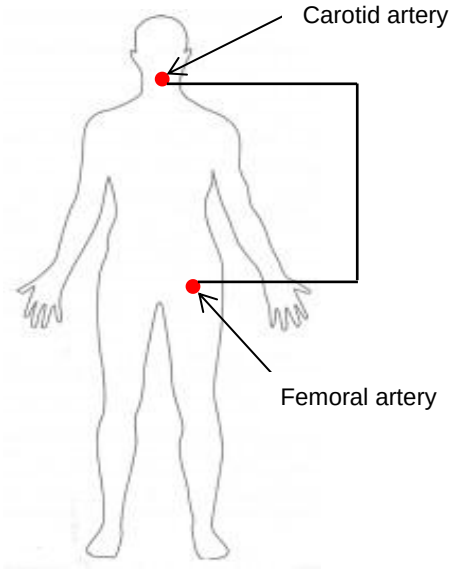

Figure 7. Carotid-to-femoral pulse wave velocity [134]

It has been reported that elastic arteries such as the aorta stiffen with age [129,139]. High values of carotid-to-femoral PWV are influenced by age and height in children and adults [125,140]. As previously indicated, biological aging results in ECM remodelling and arterial stiffness which causes an increase in carotid-to-femoral PWV values [29,103]. Individuals that are shorter are proven to have higher PWV values due to the early return of the reflected pressure wave during systole [67,72]. Early return of the pressure wave increases PWV [140].

2.2.4.2.3 Carotid-to-dorsalis pedis pulse wave velocity

Carotid-to-dorsalis pedis PWV is measured between the carotid artery and the dorsalis pedis artery in the foot as demonstrated in Figure 8.

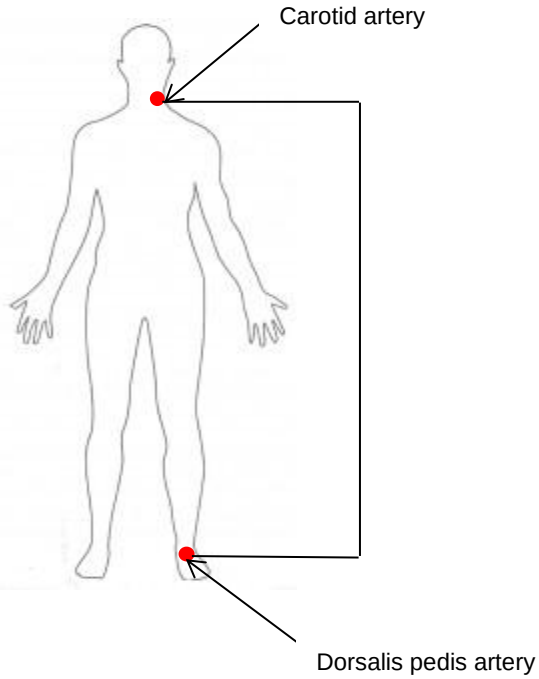

Figure 8. Carotid-to-dorsalis pedis pulse wave velocity [134]

As previously indicated, carotid-to-dorsalis pedis PWV encompasses mixed segments of the arterial tree and thus represents the stiffness of central elastic and peripheral muscular arteries [141]. Aging has been proven to increase wall thickness and diameter of elastic arteries, while decreasing their distensibility leading to higher values of carotid-to-dorsalis pedis PWV [142]. The proposed threshold value of carotid-to-dorsalis pedis PWV in Japanese boys is 9,47 m/s, however, there are no known values at an international level [130].

2.2.4.3 Windkessel arterial compliance

Windkessel arterial compliance is defined as the increase in volume for a given change in pressure [27]. It reflects systemic arterial stiffness and the volume component of arteries in adults and children [27,113,114,117]. Altered arterial compliance has been indicated to independently predict cardiovascular outcome in patients with varying degrees of cardiovascular risk [143]. Compliance of arteries decreases with age independent of cardiovascular disease [144]. Regular aerobic exercise can assist in restoring some loss of central arterial compliance in children and middle-aged men, as well as older men and women independent of body composition and arterial pressure [37,145-147].

2.2.5 Importance of arterial stiffness in cardiovascular outcomes

Aortic stiffness, as measured by carotid-to-femoral PWV, has independent predictive value for cardiovascular mortality and events such as stroke and myocardial infarction independent of systolic blood pressure, sex and age [1,6,8,148-151]. Cross-sectional and follow-up studies with large and small cohorts, conducted mostly in European countries, found that large artery stiffness predicts cardiovascular outcome in adults with varying degrees of cardiovascular risk [1,6,148-153]. The varying degrees of cardiovascular risk include the following: very high risk (end-stage renal disease and diabetes) [1]; medium risk (hypertension) [148]; and low risk (general population and healthy elderly individuals) [1,148]. For every one standard deviation increase in aortic PWV, cardiovascular risk increases by 16-20% in adults [151,153]. Thus, arterial stiffness may be presented as an important biomarker of cardiovascular risk [149]. Given the predictive value of aortic PWV, identifying ways to prevent or decrease arterial stiffness to avert cardiovascular events is essential [2]. A better physiological understanding on the early development of arterial stiffness in the youth may aid in better future interventions to prevent or delay cardiovascular disease onset or early vascular aging.

2.3 Aims and objectives

To the best of our knowledge, there is limited ethnic-specific information regarding the early development of arterial stiffness in the different sections of the arterial tree in black and white populations. Furthermore, it is unknown whether AGEs are linked to arterial stiffness development in children. This study, therefore, aims to compare different estimates of arterial stiffness in 6–8 year old black and white boys and to investigate the links between arterial stiffness indices, body composition and AGEs in these children.

The objectives are:

- To determine whether ethnic differences in arterial stiffness indices, blood pressure, AGEs and body composition are evident among black and white boys of similar age;
- To evaluate the relationship between several measures of arterial function and body composition in both ethnicities and
- To explore the relationship of measures of arterial function and body composition with urinary and dermal AGEs respectively.

2.4 Hypotheses

- Arterial stiffness and blood pressure will be higher in black compared to white boys of similar age, whereas body composition (determined by body mass index (BMI) z-scores) and AGEs are comparable between the two ethnicities.
- Positive relationships exist between measures of arterial stiffness and body composition in both groups.
- Measures of arterial function and body composition relate adversely to urinary and dermal AGEs in both groups.

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CHAPTER 3

Methods

3.1 Study design

The Arterial Stiffness in Offspring Study (ASOS) was a cross-sectional observational study conducted from April to October 2015 in Potchefstroom in the North-West Province of South Africa. This study planned to include a total of 240 participants (Figure 1), comprising of 40 black and 41 white boys from 6–8 years along with their biological parents. Participants were recruited from four primary schools in the city of Potchefstroom. During the recruitment phase it became evident that we would not be able to collect data from each biological parent of each child since some parents (n=36) were not involved or parents were divorced or deceased. Based on the set aims, the present sub-study only included the 81 children from the larger study.

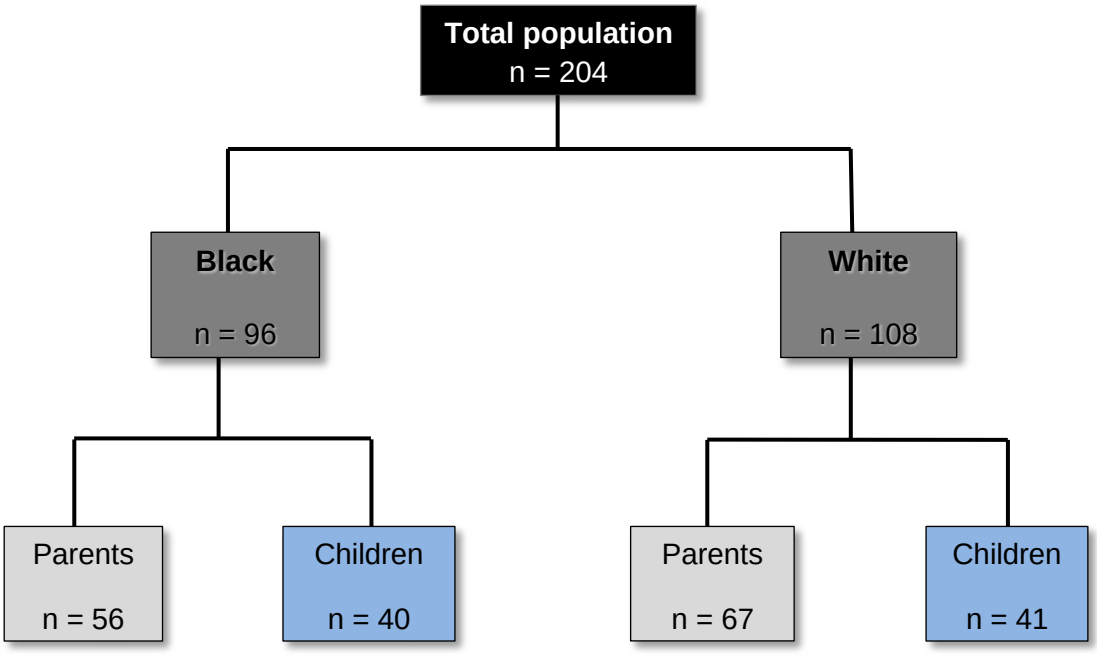

Figure 1. An illustration of the total study population of the larger Arterial Stiffness in Offspring Study (ASOS)

We applied the following exclusion criteria: children aged <6 and >8 years; girls (to exclude hormonal influences of unknown onset of pubescence); obese children (whose body mass index z-score is greater than 95th percentile, as indicated by the World Health Organization) and those using any chronic medication, or with self-reported type 1 diabetes mellitus, renal disease or cancer. Information on human immunodeficiency virus (HIV) status of participants was not acquired and no HIV tests were performed. We included apparently healthy black and white boys aged from 6–8 years in the study.

3.2 Materials and methods

3.2.1 Organisational procedures

ASOS obtained approval to do the research from the Provincial Department of Education (Appendix A) and the Health Research Ethics Committee of the North-West University, Potchefstroom Campus (NWU-00007-15-A1) (Appendix B). We set up research stations in a secured private room provided by the schools, and performed measurements on at least 2 – 4 children each day in preselected time slots. We gave children general health questionnaires, consent/assent and permission forms as well as cooler boxes with a urine specimen collection kit at their schools to take home the day before their scheduled participation date. Parents and children collected spot urine samples on the morning of participation in the privacy of their own homes. Urine samples were taken back to the schools along with the questionnaires and consent/assent and permission forms where a research assistant collected them. Urine was taken to the laboratory for further handling. When children arrived at the research stations, we introduced them to the research environment after which we thoroughly explained all the procedures to them again. Anthropometric measurements were taken after which we performed cardiovascular measurements. Each participant received refreshments after the completion of measurements. After the completion of the study, we gave each participant feedback in the form of a report with their basic health information.

3.2.2 Recruitment

Once permission was obtained, we contacted schools localised in Potchefstroom and scheduled a meeting with each school principal. The Principle Investigator of the study (Dr. Ruan Kruger) presented the project and discussed the purpose of the project along with the details of each measurement. The school principal identified a school teacher not directly involved with the particular children and the same discussion took place with these teachers as potential mediators. Once we established a relationship with the school principal and selected teacher, we obtained permission from the school principal in order to gain access to school registers. School registers were anonymised for the unbiased selection of the children from each school. We sent hand-out letters as an invitation to an information session to the parents and their children fitting the inclusion criteria. We made Powerpoint presentations for parents, after parents meeting at the schools, explaining the purpose and objectives of the study, as well as the measurements that were going to be performed. We gave parents information sheets regarding the study at the end of the information session, to make an informed decision regarding participation. We gave parents a period of two weeks to decide if they wanted to participate. We informed parents and their children who agreed to

participate on which date they would take part after scheduling and liaising with the schools. Parents and their children had to complete informed consent/assent and permission forms before we commenced with basic health measurements.

3.2.3 Urine handling and biochemical analyses

All measurements of this study were non-invasive, hence we did not obtain blood samples from the participants. We provided participants with a urine specimen collection kit, which were clearly marked for children and their parents as well as a cooler box with a gel ice pack and instructions on how to collect the urine sample. We asked parents and their children to provide urine samples early in the morning before consuming any fluids in the comfort of their home. Parents helped their children where necessary. In order to keep urine samples at low temperature, we instructed parents to keep the urine bottles inside the cooler box with a frozen ice pack. We prepared urine samples of children and their parents according to standard procedures and stored at –80°C until it were analysed. Once all samples were collected, a trained biochemist determined urinary albumin and creatinine concentrations in duplicate using the Cobas Integra® 400 plus (Roche Diagnostics Mannheim, Germany), with intra-assay variability of 1.9% and inter-assay variability of 2.2% for albumin; and an intra-assay variability of 1.4% and inter-assay variability of 2.5% for creatinine.

Various types of AGEs such as N-Ɛ-(carboxymethyl)lysine, N-Ɛ-(carboxyethyl)lysine, pentosidine, pypraline, pyrraline, glucosepane and hydroimidazolone accumulate in human fluids and tissue (such as saliva, urine, blood, blood vessels and skin) [1-5]. AGEs such as N-Ɛ-(carboxymethyl)lysine, pentosidine and pyrraline are found in urine [6,7]. Pentosidine is regarded as the best characterised and stable AGE, and we therefore decided to quantify pentosidine in the urine samples [2,8]. A biochemist quantified pentosidine in duplicate using the Human Pentosidine enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, Inc., San Diego, CA, USA), with an intra-assay variability of 8.9% and inter-assay variability of 13.1%. Pentosidine levels were quantified using the sandwich enzyme immunoassay technique. Standards and samples were pipetted into the wells and any pentosidine present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for pentosidine was added to the wells. Avidin conjugated Horseradish Peroxidase was added to the wells, and following a wash procedure to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of pentosidine bound.

3.2.4 Anthropometric measurements

A trained research assistant measured body height, weight, hip, waist and neck circumferences according to standard procedures [9]. We measured circumferences in triplicate with a Lufkin® Executive thinline 2mm steel tape (Apex Tool Group B.V.; AK Emmen, Netherlands). We used a Seca 813 digital scale to measure weight, and measured height with a Seca 213 stadiometer (Birmingham, United Kingdom). In order to get the most accurate measurements we requested the boys to remove some clothes and shoes [10,11]. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

We used BMI z-scores for the assessment of adiposity since BMI of children changes with age during childhood due to different growth patterns occurring between boys and girls [12,13]. We used thresholds derived from a child growth reference, which is used to classify the BMI z-scores of children according to their age and sex [13]. We used an international growth reference developed from the World Health Organisation (WHO) Multicentre Growth Reference Study (MGRS) (Figure 2) and compared the children's BMI z-scores with the thresholds of the child growth reference to see whether their BMI z-scores are above or below the defined thresholds [14,15]. Children whose z-scores were less than 2 standard deviations were classified as lean [13]. Those whose standard deviation was more than +1 and less than +2 were classified as overweight, whereas those whose z-scores were greater than a standard deviation of +2 were regarded as obese [13].

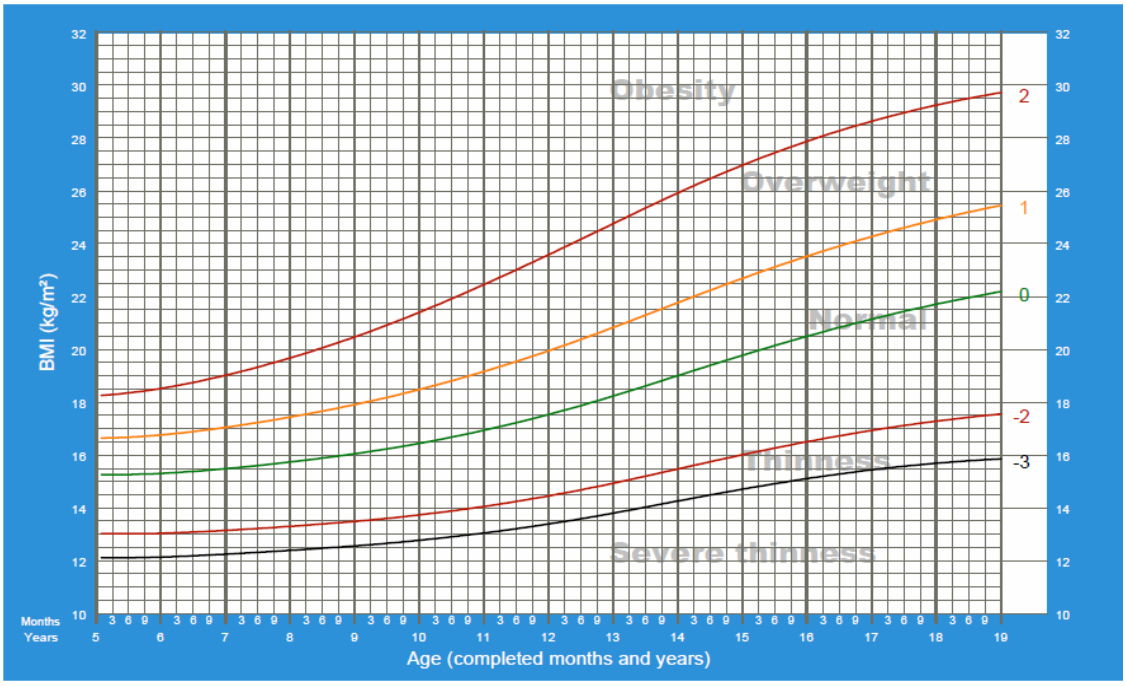

Figure 2. The body composition guidelines of the World Health Organization, according to appropriate age, height and weight cut-offs [16]

3.2.5 Cardiovascular measurements

We used an Omron 705IT device model HEM-759-E (Omron Healthcare, Tokyo, Japan), clinically validated (by both the European Society of Hypertension and the British Hypertension Society) in children [17] to determine blood pressure and heart rate. We also chose this particular model since it is a product endorsed by the Southern African Society of Hypertension. Blood pressure monitoring was performed with the participant in a sitting position in a comfortable chair and the paediatric cuff attached to the left upper arm [17]. We measured blood pressure in triplicate after a resting period of at least 5 minutes. We classified the blood pressure of the children according to the reference values provided by the National Institutes of Health in the United State of America. Blood pressure was classified as follows:

- Children whose systolic blood pressure was <90th percentile were considered to have normal blood pressure.
- Those whose systolic blood pressure was between the 90th percentile and 95th percentile were classified with high-normal blood pressure.
- Systolic blood pressure greater than the 95th percentile was classified as stage 1 hypertension.

We acquired continuous arterial blood pressure recordings using the validated Finometer device and processed the captured data with the Beatscope v1.1 software (Finapres Medical Systems, Amsterdam, the Netherlands) which is validated in the South African context [18]. The Finometer device was connected with a finger cuff placed on the middle phalanx of the middle left finger and a cuff was also placed on the left upper arm with the participant in sitting position. The device was calibrated before a 7 minute continuous measurement of cardiovascular data was taken, based on the vascular unloading technique of Peñáz [19-22]. During the measurement a return-to-flow systolic calibration was executed after 2 minutes to provide an individual subject-level adjustment of the finger arterial pressure with the brachial artery pressure [23]. The cardiovascular data processed included systolic and diastolic blood pressure, mean arterial pressure, heart rate, total peripheral resistance and arterial compliance.

The SonoSite MicroMaxx (SonoSite Micromaxx, Bothell, WA) and a 6-13 MHz linear array probe were used to determine the carotid artery distensibility by recording a 6 second cineloop of the 3-lead ECG guided pulsatile artery on both sides of the neck while participants were in supine position [24]. We calculated the carotid intima-media thickness from at least two optimal angles on both left and right side. We used the Artery Measurement Systems (AMS) software (Gothenburg, Sweden) to analyse images and

cineloops as well as quantifying the carotid intima-media thickness and the derived luminal diameter of the common carotid arteries [25,26]. This data was used to calculate strain, distensibility, compliance, β-stiffness index, Peterson's elastic modulus and Young's elastic modulus [24] which are explained in chapter 2. These variables are considered as carotid stiffness parameters and they function as local measures of arterial stiffness [24].

We determined pulse wave velocity (PWV) in various sections (carotid-to-radial; carotid-to-femoral and carotid-to-dorsalis pedis) of the arterial tree with the validated Complior SP Acquisition System (Artech Medical, Pantin, France) [27]. An alternative, validated device called the SphygmoCor system (AtCoR Medical, West Ryde, Australia) can also be used to measure PWV and is currently recommended for measuring the femoral PWV [28]. However, we chose to use the Complior SP device due to its robust functionality to determine PWV in different sections of the arterial tree. This device allows a direct measurement of the pressure wave velocity and does not make use of any mathematical algorithms and derivatives. We measured distances between the pulsated sites on the right side of each participant using a Lufkin® Executive thinline 2mm steel tape (Apex Tool Group B.V.; AK Emmen, Netherlands) with participants in supine position and 80% of these distances were used as the pulse wave travelled distance [26,29]. We placed sensors directly in pulsatile regions to measure PWV [30].

We used the DiagnOptics AGE Reader, Standard Unit & Software v2.4.0.1 (DiagnOptics Technologies, Groningen, The Netherlands) to non-invasively assess the accumulation of advanced glycation end-products in the skin [31]. We assessed the accumulation of AGEs on the right forearm of participants using autofluorescence of ultraviolet light and absorbency [32]. Three readings were taken and the average was calculated automatically by the AGE reader [31]. We could not determine the autoflourescence value on very dark skin and therefore our aim to compare dermal AGEs between black and white boys was not achievable and is discussed as a limitation in our reflective chapter (chapter 5). This may have been due to the low skin reflection of less than 6% of dark skinned people [31].

3.2.6 Statistical analyses

With the consultation of a biostatistician we the used the G*power v3.1.9.2 software† to compute the power for this study at a probability value of ≤0.05 to determine significance. We performed our statistical analyses using the IBM® SPSS® Statistics, Version 22 software (IBM Corporation, Armonk, New York). We tested all variables used in the statistical analysis for normality by visual inspection of histograms and also reviewing the coefficients of skewness and kurtosis. In the case of non-Gaussian distribution we performed a logarithmic transformation for each skewed variable (pentosidine, albumni-to-creatinine ratio, beta

stiffness index, Peterson's elastic modulus and Young's elastic modulus). We performed the comparison of mean values between groups using independent T-tests and ANCOVA (with adjustment for mean arterial pressure), and also performed Chi-square tests to compare proportions. Pulse wave velocity varies due to actions of factors such as mean arterial pressure [30]. It is, therefore, important to adjust for mean arterial pressure when comparing mean values. Pearson and partial correlations were performed to determine the potential link between body composition and measures of arterial function as well as AGEs.

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CHAPTER 4

Manuscript

Ethnic differences of arterial stiffness in 6-8 year old black and white boys

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Statement of financial support

This publication was supported by grants from the North-West University Strategic Research Fund, National Research Foundation/Department of Science and Technology South African Research Chairs Initiative, South African Medical Research Council (MRC) and the South African Sugar Association (SASA) as well as the South African National Research Foundation (NRF). Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and therefore the National Research Foundation does not accept any liability in regard thereto.

Conflicts of interest

The authors report that they have no conflict of interest.

Word count: 3086 words

Number of tables: 3

Number of figures: 1

Number of supplementary digital content files: 5

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Abstract

Objectives: To compare different estimates of arterial stiffness in black and white boys and investigate the links between arterial stiffness indices, body composition and advanced glycation end-products (AGEs).

Methods: We included 40 black and 41 white boys aged from 6–8 years, and measured pulse wave velocity in different arterial sections. Systemic arterial compliance was estimated with the Finometer device and arterial distensibility estimated based on ultrasound cineloops. Anthropometric data was collected and urinary pentosidine was measured as a marker of AGEs.

Results: Black boys displayed increased pulse wave velocity (carotid-to-radial ($p=0.002$), carotid-to-femoral ($p<0.0001$), carotid-to-dorsalis pedis ($p=0.008$), diastolic blood pressure ($p=0.001$) and carotid intima-media thickness (cIMT) ($p=0.007$) compared to white boys. Despite higher pentosidine in black boys ($p=0.039$), arterial stiffness indices did not correlate with pentosidine in any group. However, pentosidine correlated negatively with body composition variables including body mass index ($p=0.015$), body surface area ($p=0.017$), weight ($p=0.018$), waist circumference ($p=0.022$) and hip circumference ($p=0.010$) in black boys only. Arterial stiffness indices related inversely to body composition in white boys, but femoral pulse wave velocity correlated inversely with body mass index ($r=–0.32$; $p=0.049$) in black boys.

Conclusion: At an age as young as 6 years old we found higher arterial stiffness in all sections of the arterial tree, along with higher diastolic blood pressure, cIMT and AGEs in black compared to white boys of similar age. This phenotype in black children underlines the increasing trend of hypertension incidence among black populations.

Keywords: advanced glycation end-products, arterial stiffness, black, blood pressure, body composition, ethnicity, pentosidine, pulse wave velocity

Introduction

Arterial stiffness is associated with arterial structural changes and predicts myocardial infarction, stroke, heart failure as well as all-cause mortality [1-4]. Arterial stiffness is known to be elevated in black adults [5-8], and is suggested to develop earlier in black populations along with elevated blood pressure compared to white groups of similar age [9,10]. Nilsson *et al*. defined the premature aging of arteries which may be caused by risk factors such as ethnicity, age, medical or family history of cardiovascular disease or diabetes, obesity, elevated blood pressure and physical inactivity as early vascular aging (EVA) [11]. EVA can lead to the stiffening of arteries through various mechanisms including medial degeneration and extracellular matrix remodeling [3,12,13]. A South African study conducted among children from four ethnic groups (aged 10–15 years) revealed reduced arterial compliance among the black children [14]. However, no previous studies have compared very young black and white children using carotid-to-femoral pulse wave velocity to determine if increased arterial stiffness is already evident in black children younger than 10 years of age.

Several studies have reported that arterial stiffness development in children may be caused by cardiovascular disease risk factors such as adiposity and hyperglycemia [11,15,16], however, limited evidence exist in children from South Africa. Expanded adipose tissue of obese individuals expresses hormones, growth factors as well as cytokines which may cause elevation of angiotensin II which can contribute towards arterial stiffness development [17,18]. Numerous studies have also indicated the involvement of advanced glycation end-products (AGEs) towards the development of arterial stiffness [19-21]. AGEs accumulate on long-living proteins such as collagen and elastin of the extracellular matrix, forming irreversible cross-links with the proteins [20,21]. The cross-links alter elastic properties of the proteins, leading to extracellular matrix remodeling and stiffening of arteries [22,23].

To contribute to our understanding regarding EVA and the contribution of body composition and AGEs towards arterial stiffness development, we compared different estimates of arterial stiffness in 6-8 year old black and white South African boys and investigated the links between arterial stiffness indices, body composition and AGEs.

Methods

Study design and population demographics

The Arterial Stiffness in Offspring Study (ASOS) was a cross-sectional observational study conducted during 2015. We obtained approval to conduct the study from the Provincial Department of Education and the Health Research Ethics Committee of the North-West University (NWU-00007-15-A1). A total of 81 participants, comprising of 40 black and 41

white boys from 6–8 years were included, from Potchefstroom in the North-West Province, South Africa. We included healthy black and white boys, and excluded obese children and those using any chronic medication, or self-reported type 1 diabetes mellitus, renal disease or cancer.

Basic procedures

Participants were recruited from schools localised in the city of Potchefstroom. Participating schools circulated recruitment letters to each child within the age range of the planned study. Parents of children who fulfilled the inclusion criteria were invited to an information session held at the school premises. Parents received information regarding the study and were given a period of two weeks to make an informed decision. All participants received permission, consent and/or assent forms that had to be completed before measurements were taken. Each participant received refreshments after the completion of measurements. After the completion of the study each participant received feedback on their basic health information.

Biochemical sampling and clinical procedures

Parents and children were provided with sealable urine cups the day before their scheduled participation date. They were required to collect a midstream urine sample on the morning of participation in the privacy of their own homes before any meal or fluids were taken. Urine samples were collected at the schools in the morning along with the questionnaires and signed consent/assent and permission forms. Urine samples were prepared according to standard procedures and stored at –80°C for future biochemical analyses.

Urinary albumin and creatinine were determined using the Cobas Integra® 400 plus (Roche Diagnostics Mannheim, Germany), with intra-assay variability of 1.9% and inter-assay variability of 2.2% for albumin; and an intra-assay variability of 1.4% and inter-assay variability of 2.5% for creatinine. The determination of AGEs, specifically pentosidine, in urine were analysed using the Human Pentosidine enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, Inc., San Diego, CA, USA) with an intra-assay variability of 8.9% and inter-assay variability of 13.1%.

Cardiovascular measurements

Participants were required to rest for at least five minutes prior blood pressure recordings. Blood pressure was measured in triplicate with a validated Omron HEM-759-E (750IT) device (Omron Healthcare, Tokyo, Japan) on the upper left arm at heart level. Continuous arterial blood pressure was recorded with the Finometer device (Finapres Medical Systems, Amsterdam, the Netherlands) with participants in a sitting position. The cardiovascular data

collected with the Finometer was processed using the Beatscope v1.1 (Finapres Medical Systems, Amsterdam, the Netherlands) software to provide systolic and diastolic blood pressure, mean arterial pressure, heart rate, total peripheral resistance and arterial compliance.

The SonoSite MicroMaxx (SonoSite Micromaxx, Bothell, WA) and a 6-13 MHz linear array probe were used to determine the carotid artery distensibility by recording a six second cineloop of the 3-lead ECG guided pulsatile artery on both sides of the neck. The carotid intima-media thickness (cIMT) was also calculated from at least two optimal angles on both left and right side. The Artery Measurement System (AMS) software was used to quantify the cIMT and the derived luminal diameter of the common carotid arteries. This data was used to calculate strain, distensibility, compliance, β-stiffness index, Peterson's elastic modulus and Young's elastic modulus [24].

We determined pulse wave velocity (PWV) across various sections (carotid-radial; carotid-dorsalis pedis; carotid-femoral) of the arterial tree in duplicate, using the Complior SP Acquisition System (Artech Medical, Pantin, France). The distances between the pulsated sites were measured using a Lufkin® Executive thinline 2mm steel tape (Apex Tool Group B.V.; AK Emmen, Netherlands), and 80% of these distances were used as pulse wave travelled distance [25].

The DiagnOptics AGE Reader, Standard Unit & Software v2.4.0.1 (DiagnOptics, the Netherlands) was used to non-invasively assess the accumulation of advanced glycation end-products (AGEs) in the skin using autofluorescence of ultraviolet light on the forearm, in triplicate.

Anthropometric measurements

Anthropometric measurements included body height, weight, hip, waist and neck circumferences, measured in triplicate according to standard procedures [26]. Circumferences were measured with a Lufkin® Executive thinline 2mm steel tape (Apex Tool Group B.V.; AK Emmen, Netherlands) [27]. Body weight was measured using the Seca 813 digital scale, and height as measured with a Seca 213 stadiometer (Birmingham, United Kingdom). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. BMI z-scores were used to classify body composition of the boys according to appropriate age, height and weight cut-offs.

Statistical analyses

All statistical analyses were performed with IBM® SPSS® Statistics, Version 22 software (IBM Corporation, Armonk, New York). All variables were tested for normality by visual inspection and skewness and kurtosis coefficient. In the case of non-Gaussian distribution, a logarithmic transformation was performed for each skewed variable (pentosidine, albumni-to-creatinine ratio, beta stiffness index, Peterson's elastic modulus and Young's elastic modulus). The comparison of mean values between groups was performed using independent T-tests and analysis of covariance (ANCOVA) upon adjustment for mean arterial pressure. Chi-square tests were performed to compare proportions. Pearson and partial correlations were performed to determine whether relationships exist between measures of arterial stiffness, body composition and AGEs.

Results

The general characteristics of the study population are provided in Table 1. Body composition was comparable between the groups, except for white boys with higher neck circumference ($p=0.003$) and waist-to-hip ratio ($p<0.0001$) than their black counterparts. According to the body composition guidelines of the World Health Organization [28], our groups were classified as 81% healthy weight, whereas 3% were underweight, 10% overweight and 6% obese (Supplementary Figure 1), with no differences between black and white boys. Pentosidine levels were higher in black boys ($p=0.039$), whereas albumin-to-creatinine ratio was similar. Black boys had higher diastolic blood pressure ($p=0.001$), mean arterial pressure ($p=0.003$) and total peripheral resistance ($p=0.044$) compared to white boys. In the black boys carotid-to-radial pulse wave velocity, carotid-to-femoral pulse wave velocity and carotid-to-dorsalis pedis pulse wave velocity (all $p\leq0.002$) as well as carotid intima-media thickness ($p=0.007$) were higher compared to white boys, after adjusting for mean arterial pressure. Arterial compliance and markers of carotid stiffness were similar in the groups.

We performed Pearson (Supplementary Table 1) and partial (Table 2) correlations (adjusting for age and mean arterial pressure) of measures of arterial function with body composition in black and white boys. Body composition related more strongly with arterial function indices in white boys. Carotid-to-femoral pulse wave velocity showed a significant negative relationship with body mass index in black boys only. Carotid intima-media thickness related positively to body surface area in black boys. We obtained an inverse relationship of strain with all body composition variables in white boys only. The same trend was evident with the compliance coefficient in white boys only. Distensibility coefficient correlated inversely with body mass index (in both groups) as well as with body surface area and waist circumference in white

boys only. Beta stiffness index correlated positively with body surface area and waist circumference in white boys only. Positive correlations were also obtained between Peterson's elastic modulus and body mass index (in both groups), as well as with body surface area and waist circumference in white boys only. Another local stiffness indicator, Young's elastic modulus, correlated positively with all body composition variables in white boys only.

We explored the associations of pentosidine, a measure of AGEs, with body composition and measures of arterial function (Table 3), whilst adjusting for age and mean arterial pressure. We found that pentosidine related negatively with body mass index, body surface area, weight, waist circumference and hip circumference in black boys only ($p<0.05$). We found no correlation between pentosidine and arterial stiffness indices in any group.

In exploratory analyses (Figure 1), body mass index, systolic blood pressure (both unadjusted), femoral pulse wave velocity and carotid intima-media thickness (both adjusted for mean arterial pressure) were plotted by age tertiles. By doing so, a significant positive trend was observed for carotid intima-media thickness with increasing age ($p=0.008$) in black boys only. We also found significantly higher femoral pulse wave velocity values in black boys for age tertiles 1 ($p=0.007$) and 2 ($p=0.009$), as well as in carotid intima-media thickness for age tertiles 1 ($p=0.050$) and 3 ($p=0.008$).

Discussion

With previous studies indicating early vascular changes in the black population [10], our study is the first to demonstrate that black boys from 6–8 years have higher pulse wave velocity (throughout the arterial tree), diastolic blood pressure, carotid intima-media thickness and AGEs, when compared to white boys of the same age. We therefore support the notion of early onset manifestations of vascular changes in the black population of South Africa as previously described.

In adults elevated systolic blood pressure predicts cardiovascular disease [29] where elevated systolic blood pressure plays an important role in vascular remodeling through collagen accumulation and elastin breakdown of the extracellular matrix remodeling leading to increased arterial stiffness [13,30,31]. Our results show that diastolic blood pressure is higher in black boys compared to white boys. It has been reported that diastolic blood pressure may be a better measure of cardiovascular risk than systolic blood pressure or pulse pressure in children [32,33]. In children the pulse wave is reflected during diastole resulting in an increased mean diastolic blood pressure affecting the afterload on the heart

[32,34]. Increased diastolic blood pressure increases venous return leading to an increase in end-diastolic blood pressure and afterload in the left ventricle [35,36]. Elevated afterload is associated with left ventricular hypertrophy which has detrimental effects on the heart, including heart failure [35]. Thus, diastolic blood pressure may be a better predictor of cardiovascular risk in children [32,34] which is also demonstrated by accompanying increased arterial stiffness in black children of the present study.

Carotid intima-media thickness was shown to provide a graded measure of vascular damage [37] and vascular damage may contribute towards arterial stiffness development through mechanisms such as extracellular matrix remodeling [31]. In our study both pulse wave velocity and carotid intima-media thickness were higher in black than white boys. Studies have shown that aging plays an essential role in arterial stiffness development even in the absence of cardiovascular disease [38]. However, different segments of the arterial tree respond differently to aging [38]. Elastic arteries are more affected by advancing age than muscular arteries [39]. The response difference may be caused by differences in the distribution of collagen and elastin in both elastic and muscular arteries [39]. Carotid-to-radial pulse wave velocity represents stiffness of muscular arteries which do not stiffen with age, but may stiffen with factors such as insulin resistance [40,41]. Carotid-to-dorsalis pedis pulse wave velocity represents the stiffness of central elastic and peripheral muscular arteries due to the mixed segment of the arterial tree encompassed in this section [42]. Carotid-to-femoral pulse wave velocity represents stiffness of elastic arteries which stiffen with age [25,43,44]. Carotid-to-femoral pulse wave velocity provides an accurate and robust assessment of arterial stiffness and is considered the “golden standard” measurement of arterial stiffness [44,45]. Arterial stiffness causes elevation of blood pressure and mean arterial pressure, which are all risk factors for cardiovascular events (such as myocardial infarction, stroke and heart failure) as well as isolated hypertension and elevated pulse pressure [1-4,46]. Studies have indicated that increased arterial stiffness during childhood has implications on blood pressure in early adulthood [46].

To explain the elevated pulse wave velocity in black boys, we explored if pulse wave velocity relates to body composition and AGEs. We hypothesised that arterial stiffness, represented by pulse wave velocity would relate positively to AGEs, measured as pentosidine, and body composition. However, we found no associations of pentosidine with arterial stiffness. We found inverse relations between arterial stiffness indices and body composition in white boys, but femoral pulse wave velocity correlated inversely with body mass index in black boys. We also fond inverse relations between AGEs and body composition in black boys only. This occurrence was shown by several studies [47,48], indicating normal weight children having higher AGE levels compared to obese children due to a scavenger receptor

that is expressed by adipocytes which binds to AGEs and facilitates their endocytosis and degradation, leading to low concentrations of AGEs in obese children [47-49].

We can only speculate on the early differences observed in arterial stiffness, diastolic blood pressure, carotid intima-media thickness and AGEs among 6-8 year old boys. Early stiffening of arteries in black boys may be due to factors such as genetic predisposition, poor nutrition during first days of life or low birth weight and renin-angiotensin system disparities. Although we did not have the data to support this, other studies have shown that the presence of angiotensin II receptor allele in blacks influences the activity of the receptor which in turn regulates angiotensin II activity [30,46,50]. Angiotensin II contributes towards arterial stiffness development through various mechanisms including hypertrophy and cell death [30,46,50,51]. Previous reports indicated black South Africans consumed a poor diet high in fat, with decreased carbohydrates and fibre [52,53]. In addition, several studies have shown that nutrition during the first thousand days of life, from the beginning of a woman's pregnancy until the child's second year, is essential for future vascular health in offspring [54,55]. Poor nutrition during these crucial days of life may result in infants born with a low birth weight. A study of 9 year old Swedish children with low birth weight demonstrated impaired endothelial function and a tendency of increased carotid stiffness [56]. Although we demonstrated comparable carotid arterial distensibility estimates between black and white boys, we found a link between body composition and adverse arterial stiffness in both black and white boys (Supplementary Table 1).

Our small observational study did not obtain blood samples to quantify other markers of AGEs and biomarkers of endothelial and vascular dysfunction. Additionally, we did not assess carotid stiffness by means of echotracking. However, this was the first study to investigate ethnic differences in arterial stiffness indices (including femoral PWV) among a homogenous group of very young (6 to 8 year old) black and white South African boys.

In conclusion we highlighted an early vascular compromise in black boys as indicated by their increased arterial stiffness, diastolic blood pressure, carotid wall thickness and AGEs which may have significant implications for cardiovascular disease development in adult life.

Acknowledgements

The authors are grateful towards the participants, research assistants who helped with data collection, the North-West University Strategic Research Fund, National Research Foundation/Department of Science and Technology South African Research Chairs Initiative, South African Medical Research Council (MRC), the South African Sugar Association (SASA) and the South African National Research Foundation (NRF). Any opinion, findings

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

Declaration of interest

The authors report that they have no conflict of interest.

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Table 1. Phenotypic characteristics of black and white boys.

	Black n = 40	**White** n = 41	**p-value**
Age (years)	7.30 ± 0.69	7.27 ± 0.81	0.85
Body composition			
Body mass index (kg/m^2)	16.5 ± 2.08	16.0 ± 1.69	0.32
Body surface area (m^2)	0.95 ± 0.11	0.94 ± 0.12	0.68
Weight (kg)	26.2 ±5.00	25.7 ± 5.01	0.62
Height (cm)	126 ± 6.37	126 ± 7.14	0.93
Waist circumference (cm)	57.0 ± 5.67	58.0 ± 5.76	0.40
Neck circumference (cm)	26.3 ± 1.52	27.4 ± 1.73	0.003
Hip circumference (cm)	69.0 ± 6.46	67.1 ± 5.64	0.16
Waist-to-hip ratio	0.83 ± 0.04	0.86 ± 0.04	<0.0001
Biochemical analyses			
Pentosidine (g/ml)	11.4 (9.36 – 13.4)	10.2 (8.16 – 12.2)	0.039
Albumin-to-creatinine ratio (mg/mmol)	0.63 (-1.47 – 2.73)	0.63 (-1.49 – 2.75)	0.96
Cardiovascular profile			
Blood pressure measurements			
Systolic blood pressure (mmHg)	105 ± 11.0	102 ± 7.34	0.18
Diastolic blood pressure (mmHg)	69.4 ± 8.96	62.9 ± 7.79	0.001
Pulse pressure (mmHg)	36.1 ± 7.48	39.6 ± 7.23	0.033
Heart rate (bpm)	81.8 ± 9.63	83.1 ± 9.46	0.53
Mean arterial pressure (mmHg)	81.4 ± 9.0	76.1 ± 6.7	0.003
Total peripheral resistance (MU)	3.43 ± 1.48	2.77 ± 1.42	0.044
Aortic characteristic impedance (mMU)	192 ± 79.2	167 ± 87.0	0.19
Blood pressure classification			
Normal blood pressure, n (%)	27 (67.5)	39 (95.1)	0.25
High-normal blood pressure, n (%)	8 (20)	2 (4.9)	0.22
Stage 1 hypertension, n (%)	5 (12.5)	–	
Arterial stiffness indices			
Systemic arterial stiffness			
Arterial compliance (mL/mmHg)	0.79 ± 0.68	0.97 ± 0.79	0.26
Regional arterial stiffness			
Carotid radial PWV (m/s)*	9.72 ± 1.72	8.21 ± 1.82	0.002
Carotid femoral PWV (m/s)*	5.01 ± 0.68	4.42 ± 0.62	<0.0001
Carotid dorsalis pedis PWV (m/s)*	5.49 ± 0.62	5.01 ± 0.63	0.008
Local carotid stiffness			
Intima-media thickness (mm)*	0.47 ± 0.05	0.44 ± 0.05	0.007
Strain (%)	0.12 ± 0.05	0.12 ± 0.04	0.91
Distensibility coefficient (10^{-3}/kPa)	0.007 ± 0.003	0.007 ± 0.003	0.37
Compliance coefficient (mm^2/kPa)	0.13 ± 0.047	0.13 ± 0.054	0.68
Beta stiffness index	3.96 (1.84 – 6.08)	4.51 (2.39 – 6.63)	0.24
Peterson's elastic modulus (kPa)	339 (337 – 341)	364 (362 – 366)	0.53
Young's elastic modulus (kPa)	1714 (1712 – 1716)	2004 (2002 – 2006)	0.22

Values are arithmetic mean ± SD, geometric mean (5th and 95th percentiles) or number of participants. * – adjusted for mean arterial pressure. Abbreviations: PWV – pulse wave velocity.

Table 2. Partial correlations between measures of arterial function and body composition of black and white boys.

	Body mass index (kg/m^2)	Body surface area (m^2)	Waist circumference (cm)
Pulse pressure (mmHg)			
Black (n = 36)	r = 0.20; p = 0.22	r = 0.29; p = 0.08	r = 0.25; p = 0.23
White (n = 37)	r = 0.04; p = 0.83	r = 0.07; p = 0.66	r = 0.09; p = 0.60
Total peripheral resistance			
Black (n = 36)	r = –0.31; p = 0.06	r = 0.003; p = 0.99	r = –0.23; p = 0.17
White (n = 37)	r = –0.08; p = 0.62	r = –0.11; p = 0.51	r = –0.11; p = 0.51
Arterial compliance (mL/mmHg)			
Black (n = 36)	r = 0.12; p = 0.48	r = –0.08; p = 0.62	r = 0.05; p = 0.77
White (n = 37)	r = 0.12; p = 0.46	r = 0.16; p = 0.33	r = 0.16; p = 0.34
Carotid to radial Pulse wave velocity (m/s)			
Black (n = 36)	r = –0.24; p = 0.14	r = –0.03; p = 0.85	r = -0.11; p = 0.51
White (n = 37)	r = 0.12; p = 0.48	r = 0.17; p = 0.29	r = 0.29; p = 0.08
Carotid to femoral pulse wave velocity (m/s)			
Black (n = 36)	**r = –0.32; p = 0.049**	r = –0.22; p = 0.19	r = –0.21; p = 0.22
White (n = 37)	r = 0.16; p = 0.34	r = 0.27; p = 0.09	r = 0.28; p = 0.08
Carotid to dorsalis pedis pulse wave velocity (m/s)			
Black (n = 36)	r = 0.29; p = 0.86	r = -0.18; p = 0.27	r = -0.02; p = 0.92
White (n = 37)	r = 0.19; p = 0.25	r = 0.28; p = 0.08	r = 0.31; p = 0.06
Carotid intima-media thickness (mm)			
Black (n = 40)	r = 0.23; p = 0.17	**r = 0.37; p = 0.024**	r = 0.21; p = 0.20
White (n = 41)	r = 0.25; p = 0.12	r = 0.18; p = 0.29	r = 0.16; p = 0.34
Strain (%)			
Black (n = 36)	r = –0.29; p = 0.08	r = –0.06; p = 0.73	r = –0.14; p = 0.40
White (n = 37)	**r = –0.37; p = 0.022**	**r = –0.44; p <0.01**	**r = –0.38; p = 0.016**
Distensibility coefficient (10^{-3}/kPa)			
Black (n = 36)	**r = –0.39; p = 0.017**	r = –0.22; p = 0.19	r = –0.31; p = 0.06
White (n = 37)	**r = –0.39; p = 0.014**	**r = –0.46; p <0.01**	**r = –0.41; p = 0.010**
Compliance coefficient (mm^2/kPa)			
Black (n = 36)	r = –0.28; p = 0.09	r = –0.13; p = 0.42	r = –0.20; p = 0.24
White (n = 37)	**r = –0.34; p = 0.034**	**r = –0.41; p <0.01**	**r = –0.37; p = 0.019**
Beta stiffness index			
Black (n = 36)	r = 0.25; p = 0.13	r = 0.05; p = 0.77	r = 0.13; p = 0.43
White (n = 37)	r = 0.31; p = 0.053	**r = 0.39; p = 0.014**	**r = 0.36; p = 0.023**
Peterson's elastic modulus (kPa)			
Black (n = 36)	**r = 0.33; p = 0.045**	r = 0.12; p = 0.46	r = 0.21; p = 0.20
White (n = 37)	**r = 0.37; p = 0.020**	**r = 0.46; p <0.01**	**r = 0.44; p <0.01**
Young's elastic modulus (kPa)			
Black (n = 36)	r = 0.32; p = 0.051	r = 0.08; p = 0.62	r = 0.21; p = 0.22
White (n = 37)	**r = 0.32; p = 0.046**	**r = 0.42; p <0.01**	**r = 0.40; p = 0.013**

Adjustments applied for age and mean arterial blood pressure.

Table 3. Partial correlations of pentosidine with measures of arterial function and body composition in black and white boys.

	Pentosidine (g/ml)
Body mass index (kg/m^2)	
Black (n = 36)	**r = –0.39; p = 0.015**
White (n = 37)	r = 0.28; p = 0.08
Body surface area (m^2)	
Black (n = 36)	**r = –0.39; p = 0.017**
White (n = 37)	r = 0.12; p = 0.48
Waist to hip ratio	
Black (n = 36)	r = 0.02; p = 0.89
White (n = 37)	r = –0.17; p = 0.29
Weight (kg)	
Black (n = 36)	**r = –0.38; p = 0.018**
White (n = 37)	r = 0.15; p = 0.36
Height (cm)	
Black (n = 36)	r = –0.25; p = 0.14
White (n = 37)	r = –0.05; p = 0.75
Waist circumference (cm)	
Black (n = 36)	**r = –0.37; p = 0.022**
White (n = 37)	r = 0.06; p = 0.74
Neck circumference (cm)	
Black (n = 36)	r = –0.24; p = 0.15
White (n = 37)	r = 0.003; p = 0.98
Hip circumference (cm)	
Black (n = 36)	r **= –0.41; p = 0.010**
White (n = 37)	r = 0.16; p = 0.32
Pulse pressure (mmHg)	
Black (n = 36)	r = –0.15; p = 0.39
White (n = 37)	r = 0.11; p = 0.51
Total peripheral resistance	
Black (n = 36)	r = –0.09; p = 0.59
White (n = 37)	r = –0.21; p = 0.19
Arterial compliance (mL/mmHg)	
Black (n = 36)	r = 0.10; p = 0.55
White (n = 37)	r = 0.25; p = 0.13
Carotid to radial pulse wave velocity (m/s)	
Black (n = 36)	r = –0.16; p = 0.35
White (n = 37)	r = 0.04; p = 0.80
Carotid to femoral pulse wave velocity (m/s)	
Black (n = 36)	r = 0.11; p = 0.53
White (n = 37)	r = –0.11; p = 0.52
Carotid to dorsalis pedis pulse wave velocity (m/s)	
Black (n = 36)	r = –0.18; p = 0.27
White (n = 37)	r = –0.05; p = 0.78
Carotid intima-media thickness (mm)	
Black (n = 36)	r = –0.10; p = 0.55
White (n = 37)	r = 0.21; p = 0.19
Strain (%)	
Black (n = 36)	r = 0.05; p = 0.75
White (n = 37)	r = 0.22; p = 0.19
Distensibility coefficient, 10^{-3}/kPa	
Black (n = 36)	r = 0.07; p = 0.66
White (n = 37)	r = 0.10; p = 0.55
Compliance coefficient (mm^2/kPa)	
Black (n = 36)	r = 0.05; p = 0.77
White (n = 37)	r = 0.14; p = 0.40
Beta stiffness index	
Black (n = 36)	r = –0.09; p = 0.61
White (n = 37)	r = –0.10; p = 0.56
Peterson's elastic modulus (kPa)	
Black (n = 36)	r = –0.11; p = 0.53
White (n = 37)	r = –0.11; p = 0.49
Young's elastic modulus (kPa)	
Black (n = 36)	r = –0.09; p = 0.59
White (n = 37)	r = –0.14; p = 0.39

Adjusted for age and mean arterial pressure.

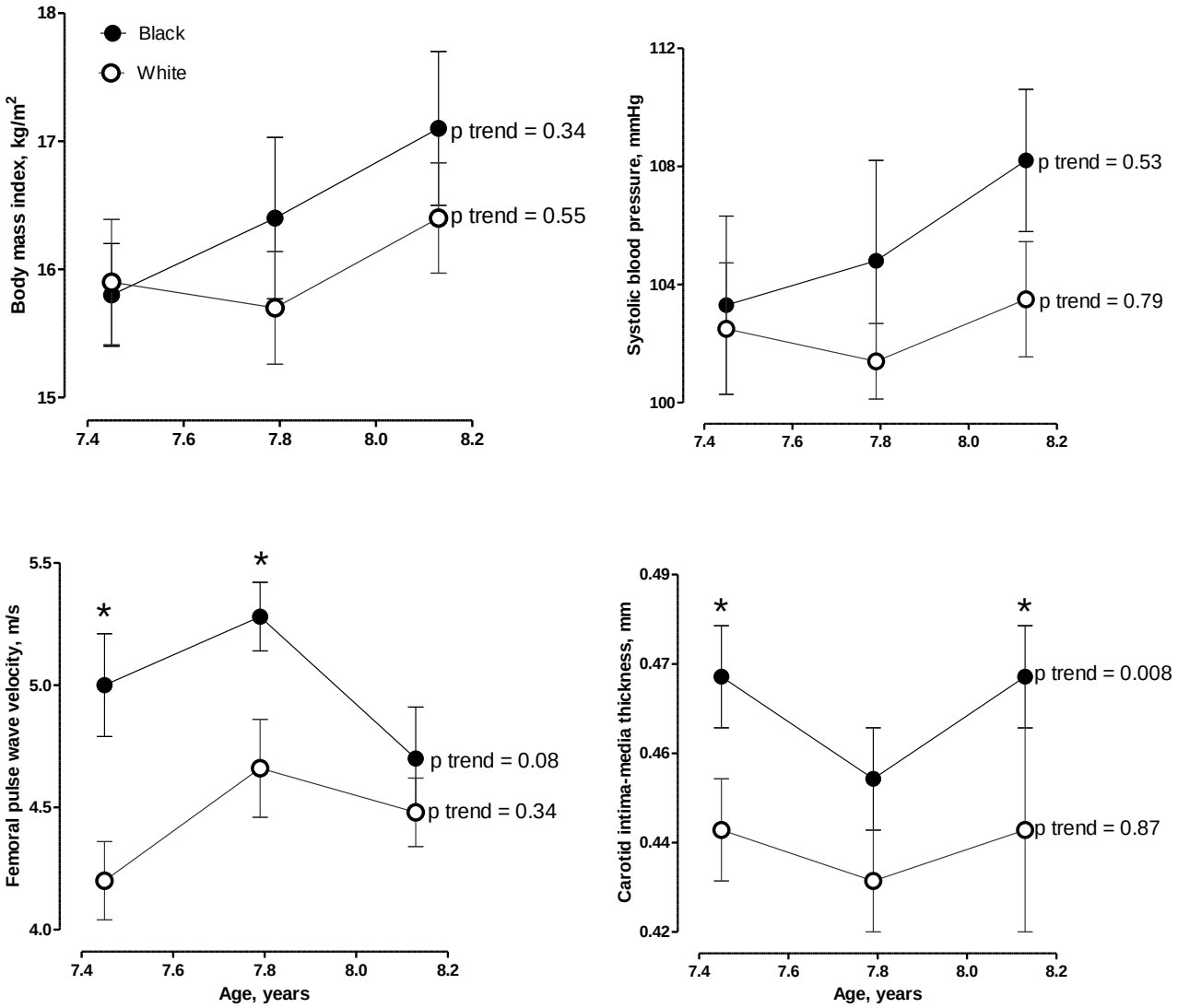
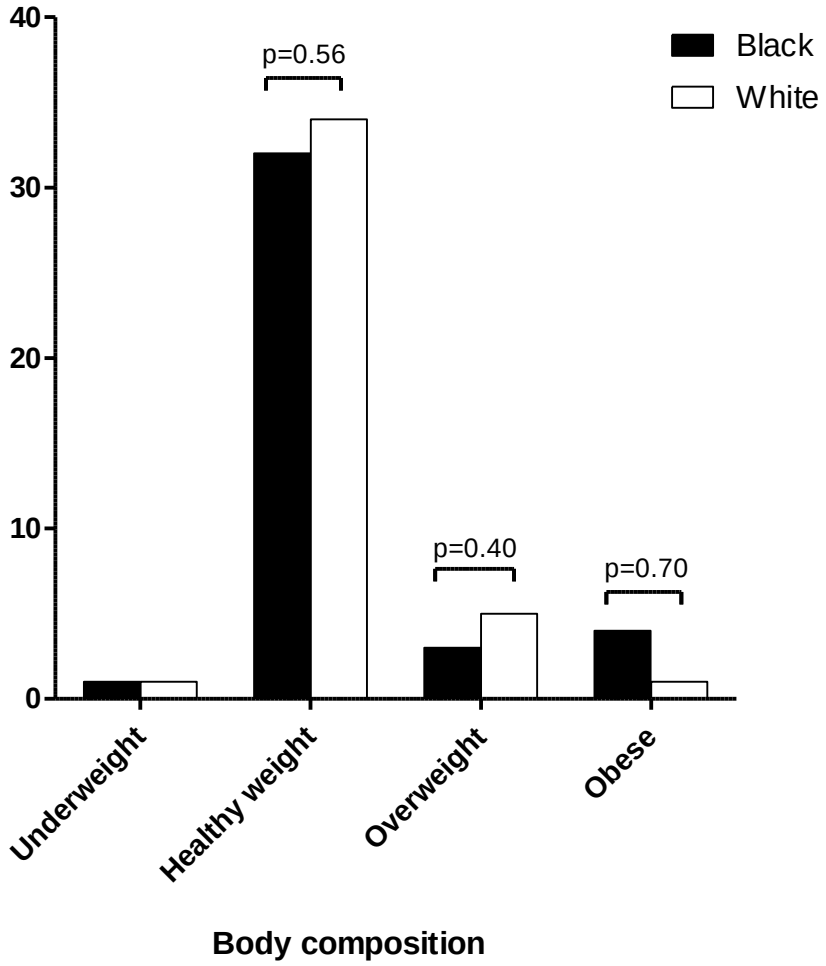


Figure 1. Body mass index, systolic blood pressure (both unadjusted), femoral pulse wave velocity and carotid intima-media thickness (both adjusted for mean arterial pressure) by age tertiles in black and white boys. *P* denotes significance for trend; * indicates difference between ethnicities within an age group ($p<0.05$). Values are presented as mean ± S.E.



Supplementary Figure 1. Body mass index values of black and white boys according to body composition categories.

Supplementary Table 1. Unadjusted correlations of several measures of arterial function with body composition in black and white boys.

	Body mass index (kg/m^2)	Body surface area (m^2)	Waist to hip ratio	Weight (kg)	Height (cm)	Waist circumference (cm)	Neck circumference (cm)	Hip circumference (cm)
Pulse pressure (mmHg)								
Black (n = 40)	r = 0.22; p =0.17	**r = 0.31; p = 0.049**	r = 0.17; p = 0.29	r = 0.30; p = 0.06	r = 0.28; p = 0.08	r = 0.27; p = 0.09	**r = 0.39; p = 0.013**	r = 0.21; p = 0.19
White (n = 41)	r = 0.05; p = 0.74	r = 0.13; p = 0.44	r = 0.05; p = 0.73	r = 0.18; p = 0.47	r = 0.15; p = 0.37	r = 0.19; p = 0.46	r = 0.13; p = 0.40	r = 0.11; p = 0.51
Total peripheral resistance								
Black (n = 40)	**r = –0.34; p = 0.034**	r = –0.08; p = 0.61	r = –0.21; p = 0.19	r = –0.14; p = 0.38	r = 0.20; p = 0.21	r = –0.27; p = 0.09	r = –0.23; p = 0.15	r = –0.19; p = 0.23
White (n = 41)	r = –0.09; p = 0.58	r = –0.15; p = 0.35	r = –0.05; p = 0.77	r = –0.15; p = 0.36	r = –0.15; p = 0.34	r = –0.13; p = 0.41	r = 0.63; p = 0.70	r = –0.12; p = 0.47
Arterial compliance (mL/mmHg)								
Black (n = 40)	r = 0.17; p = 0.31	r = –0.003; p = 0.98	r = 0.04; p = 0.79	r = 0.04; p = 0.81	r = –0.17; p = 0.30	r = 0.11; p = 0.49	r = 0.08; p = 0.63	r = 0.10; p = 0.56
White (n = 41)	r = 0.13; p = 0.43	r = 0.16; p = 0.31	r = 0.12; p = 0.46	r = 0.16; p = 0.31	r = 0.16; p = 0.33	r = 0.16; p = 0.31	r = 0.15; p = 0.93	r = 0.11; p = 0.49
rPWV (m/s)								
Black (n = 40)	r = –0.20; p = 0.23	r = –0.12; p = 0.94	r = –0.007; p = 0.97	r = –0.06; p = 0.73	r = 0.17; p = 0.28	r = –0.07; p = 0.68	r = 0.08; p = 0.65	r = –0.07 ; p = 0.65
White (n = 41)	r = 0.13; p = 0.42	r = 0.24; p = 0.13	r = 0.21; p = 0.20	r = 0.23; p = 0.16	r = 0.27; p = 0.89	**r = 0.32; p = 0.040**	**r = 0.37; p = 0.017**	r = 0.25; p = 0.11
fPWV (m/s)								
Black (n = 40)	r = –0.27; p = 0.09	r = –0.20; p = 0.22	r = 0.56; p = 0.73	r = –0.22; p = 0.18	r = –0.07; p = 0.69	r = –0.17; p = 0.31	r = –0.05; p = 0.76	r = –0.20; p = 0.21
White (n = 41)	r = 0.16; p = 0.32	**r = 0.34; p = 0.031**	r = 0.002; p = 0.99	**r = 0.32; p = 0.044**	**r = 0.38; p = 0.013**	r = 0.31; p = 0.052	r = 0.21; p = 0.18	**r = 0.35; p = 0.025**
dpPWV (m/s)								
Black (n = 40)	r = 0.01; p = 0.96	r = –0.21; p = 0.20	r = 0.19; p = 0.24	r = –0.18; p = 0.28	**r = –0.33; p = 0.040**	r = –0.04; p = 0.81	r = 0.02; p = 0.93	r = –0.14; p = 0.41
White (n = 41)	r = 0.19; p = 0.23	r = 0.26; p = 0.10	r = 0.15; p = 0.36	r = 0.26; = 0.11	r = 0.27; p = 0.09	r = 0.30; p = 0.53	r = 0.30; p = 0.06	r = 0.26p = 0.10
Carotid intima media thickness (mm)								
Black (n = 40)	r = 0.28; p = 0.08	r = 0.41; p = 0.01	r = 0.08; p = 0.65	**r = 0.40; p = 0.011**	r = 0.40; p = 0.01	r =0.28 ; p = 0.09	**r = 0.44; p = 0.005**	r = 0.27; p = 0.09
White (n = 41)	r = 0.25; p = 0.17	r = 0.17; p = 0.28	r = 0.051; p = 0.75	r = 0.19; p = 0.25	r = 0.07; p = 0.66	r = 0.16; p = 0.32	r = 0.12; p = 0.46	r = 0.17; p = 0.30
Strain (%)								
Black (n = 40)	r = –0.31; p = 0.06	r = –0.09; p = 0.58	r = –0.04; p = 0.82	r = –0.13; p = 0.44	r = 0.12; p = 0.45	r = –0.17; p = 0.30	r = –0.16; p = 0.32	r = –0.17; p = 0.30
White (n = 41)	**r = –0.36; p = 0.022**	**r = –0.37; p = 0.017**	r = –0.11; p = 0.51	**r = –0.38; p = 0.014**	r = –0.29; p = 0.07	**r = –0.36; = 0.023**	r = –0.26; = 0.11	**r = –0.36; p = 0.023**
Distensibility coefficient (10^{-3}/kPa)								
Black (n = 40)	**r = –0.40; p = 0.010**	r = –0.25; p = 0.12	r = –0.18; p = 0.27	r = –0.28; p = 0.08	r = –0.03; p = 0.87	**r = –0.33; p = 0.038**	**r = –0.37; p = 0.020**	r = –0.28; p = 0.09
White (n = 41)	**r = –0.39; p = 0.012**	**r = –0.41; p = <0.01**	r = –0.10; p = 0.55	**r = –0.42; p = <0.01**	**r = –0.33; p = 0.036**	**r = –0.40; p = 0.011**	**r = –0.32; p = 0.045**	**r = –0.40; p <0.01**
Compliance coefficient (mm^2/kPa)								
Black (n = 40)	**r = –0.32; p = 0.045**	r = –0.21; p = 0.21	r = –0.08; p = 0.63	r = –0.23; p = 0.16	r = –0.02; p = 0.91	r = –0.25; p = 0.12	r = –0.21; p = 0.19	r = –0.24; p = 0.14
White (n = 41)	**r = –0.33; p = 0.033**	**r = –0.35; p = 0.027**	r = –0.12; p = 0.47	**r = –0.36; p = 0.022**	r = –0.27; p = 0.09	**r = –0.35; p = 0.026**	r = –0.25; p = 0.12	**r = –0.34; p = 0.031**
Beta stiffness index								
Black (n = 40)	r = 0.27; p = 0.10	r = 0.89; p = 0.59	r = 0.10; p = 0.54	r = 0.12; p = 0.47	r = –0.09; p = 0.58	r = 0.16; p = 0.33	r = 0.19; p = 0.25	r = 0.13; p = 0.43
White (n = 41)	**r = 0.31; p = 0.046**	**r = 0.37; p = 0.018**	r = 0.15; = 0.34	**r = 0.37; p = 0.017**	**r = 0.33; p = 0.038**	**r = 0.36; p = 0.020**	r = 0.20; p = 0.20	**r = 0.34; p = 0.032**
Peterson's elastic modulus (kPa)								
Black (n = 40)	**r = 0.36; p = 0.025**	r = 0.17; p = 0.29	r = 0.11; p = 0.49	r = 0.20; p = 0.21	r = –0.05; p = 0.76	r = 0.25; p = 0.17	r = 0.28; p = 0.08	r = 0.22; p = 0.17
White (n = 41)	**r = 0.38; p = 0.016**	**r = 0.44; p = <0.01**	r = 0.16; p = 0.33	**r = 0.44; p = <0.01**	**r = 0.38; p = 0.013**	**r = 0.44; p = <0.01**	r = 0.30; p = 0.58	**r = 0.42; p = <0.01**
Young's elastic modulus (kPa)								
Black (n = 40)	**r = 0.34; p = 0.033**	r = 0.12; p = 0.47	r = 0.12; p = 0.45	r = 0.16; p = 0.34	r = –0.11; p = 0.48	r = 0.23; p = 0.15	r = 0.25; p = 0.13	r = 0.20; p = 0.23
White (n = 41)	**r = 0.38; p = 0.037**	**r = 0.40; p = <0.01**	r = 0.14; p = 0.39	**r = 0.40; p = <0.01**	**r = 0.37; p = 0.019**	**r = 0.40; p = 0.010**	r = 0.28; p = 0.07	**r = 0.39; p = 0.013**

Supplementary Table 2. Partial correlations of several measures of arterial function with body composition, adjusted for age and mean arterial blood pressure in black and white boys.

	Waist to hip ratio	Weight (kg)	Height (cm)	Neck circumference (cm)	Hip circumference (cm)
Pulse pressure (mmHg)					
Black (n = 36)	r = 0.19; p = 0.25	r = 0.28; p = 0.09	r = 0.26; p = 0.12	**r = 0.39; p = 0.017**	r = 0.18; p = 0.27
White (n = 37)	r = 0.04; p = 0.79	r = 0.07; p = 0.68	r = 0.08; p = 0.61	r = 0.08; p = 0.62	r = 0.08; p = 0.65
Total peripheral resistance					
Black (n = 36)	r = –0.28; p = 0.09	r = –0.07; p = 0.69	r = 0.30; p = 0.07	r = –0.21; p = 0.21	r = –0.11; p = 0.50
White (n = 37)	r = –0.06; p = 0.70	r = –0.11; p = 0.50	r = –0.10; p = 0.54	r = 0.10; p = 0.55	r = –0.08; p =0.64
Arterial compliance (mL/mmHg)					
Black (n = 36)	r = 0.07; p = 0.66	r = –0.04; p = 0.82	r = –0.24; p = 0.15	r = 0.03; p = 0.85	r = 0.01; p = 0.94
White (n = 37)	r = 0.11; p = 0.50	r = 0.16; p = 0.33	r = 0.16; p = 0.33	r = -0.01; p = 0.96	r = 0.11; p = 0.52
rPWV (m/s)					
Black (n = 36)	r = –0.24; p = 0.89	r = –0.08; p = 0.61	r = 0.18; p = 0.28	r = 0.04; p = 0.81	r = –0.11; p = 0.51
White (n = 37)	r = 0.22; p = 0.18	r = 0.17; p = 0.32	r = 0.20; p = 0.23	**r = 0.34; p = 0.034**	r = 0.20; p = 0.22
fPWV (m/s)					
Black (n = 36)	r = 0.03; p = 0.86	r = –0.24; p = 0.14	r = –0.05; p = 0.75	r = –0.09; p = 0.60	r = –0.24; p = 0.16
White (n = 37)	r = 0.04; p = 0.80	r = 0.26; p = 0.11	r = 0.31; p = 0.06	r = 0.19; p = 0.26	r = 0.30; p = 0.07
dpPWV (m/s)					
Black (n = 36)	r = 0.18; p = 0.29	r = –0.15; p = 0.36	r = -0.31; p = 0.06	r = 0.03; p = 0.86	r = -0.11; p = 0.52
White (n = 37)	r = 0.15; p = 0.35	r = 0.27; p = 0.10	r = 0.31; p = 0.06	r = 0.31; p = 0.053	r = 0.27; p = 0.10
Carotid intima media thickness (mm)					
Black (n = 40)	r = 0.07; p = 0.69	**r = 0.35; p = 0.034**	**r = 0.37; p = 0.024**	**r = 0.40; p = 0.014**	r = 0.21; p = 0.21
White (n = 41)	r = 0.07; p = 0.70	r = 0.19; p = 0.25	r = 0.05; p = 0.75	r = 0.12; p = 0.45	r = 0.16; p = 0.34
Strain (%)					
Black (n = 36)	r = –0.05; p = 0.77	r = –0.10; p = 0.56	r = 0.15; p = 0.37	r = –0.14; p = 0.40	r = –0.14; p = 0.41
White (n = 37)	r = –0.11; p = 0.51	**r = –0.44; p = <0.01**	**r = –0.40; p = 0.013**	r = –0.30; p = 0.07	**r = –0.39; p = 0.014**
Distensibility coefficient (10^{-3}/kPa)					
Black (n = 36)	r = –0.20; p = 0.24	r = –0.25; p = 0.13	r = 0.01; p = 0.91	**r = –0.35; p = 0.030**	r = –0.24; p = 0.14
White (n = 37)	r = –0.09; p = 0.57	**r = –0.46; p = <0.01**	**r = –0.41; p = 0.010**	**r = –0.34; p = 0.036**	**r = –0.43; p = <0.01**
Compliance coefficient (mm^2/kPa)					
Black (n = 36)	r = –0.20; p = 0.48	r = –0.16; p = 0.34	r = 0.05; p = 0.77	r = –0.18; p = 0.29	r = –0.16; p = 0.33
White (n = 37)	r = –0.11; p = 0.50	**r = –0.41; p = <0.01**	**r = –0.37; p = 0.021**	r = –0.28; p = 0.09	**r = –0.37; p = 0.019**
Beta stiffness index					
Black (n = 36)	r = 0.12; p = 0.47	r = 0.08; p = 0.63	r = –0.13; p = 0.45	r = 0.17; p = 0.31	r = 0.09; p = 0.60
White (n = 37)	r = 0.16; p = 0.34	**r = 0.39; p =0.015**	**r = 0.37; p = 0.020**	r = 0.21; p = 0.21	**r = 0.34; p = 0.036**
Peterson's elastic modulus (kPa)					
Black (n = 36)	r = 0.13; p = 0.44	r = 0.16; p = 0.35	r = –0.09; p = 0.59	r = 0.25; p = 0.14	r = 0.17; p = 0.30
White (n = 37)	r = 0.16; p = 0.34	**r = 0.46; p = <0.01**	**r = 0.43; p = <0.01**	r = 0.30; p = 0.06	**r = 0.42; p <0.01**
Young's elastic modulus (kPa)					
Black (n = 36)	r = 0.14; p = 0.42	r = 0.12; p = 0.46	r = –0.15; p = 0.38	r = 0.22; p = 0.18	r = 0.16; p = 0.34
White (n = 37)	r = 0.14; p = 0.41	**r = 0.41; p = <0.01**	**r = 0.40; p = 0.011**	r = 0.28; p = 0.08	**r = 0.38; p = 0.016**

Supplementary Table 3. Unadjusted correlations of several measures of arterial function and body composition with pentosidine in black and white boys.

	Pentosidine (g/ml)
Body mass index (kg/m^2)	
Black (n = 40)	**r = –0.35; p = 0.027**
White (n = 41)	r = 0.26; p = 0.10
Body surface area (m^2)	
Black (n = 40)	**r = –0.34; p = 0.033**
White (n = 41)	r = 0.07; p = 0.78
Waist to hip ratio	
Black (n = 40)	r = 0.02; p = 0.90
White (n = 41)	r = –0.19; p = 0.23
Weight (kg)	
Black (n = 40)	**r = –0.34; p = 0.034**
White (n = 41)	r = 0.10; p = 0.52
Height (cm)	
Black (n = 40)	r = –0.22; p = 0.17
White (n = 41)	r = –0.08; p = 0.61
Waist circumference (cm)	
Black (n = 40)	**r = –0.32; p = 0.044**
White (n = 41)	r = 0.03; p = 0.85
Neck circumference (cm)	
Black (n = 40)	r = –0.20; p = 0.22
White (n = 41)	r = –0.05; p = 0.78
Hip circumference (cm)	
Black (n = 40)	**r = –0.35; p = 0.026**
White (n = 41)	r = 0.14; p = 0.39
Pulse pressure (mmHg)	
Black (n = 40)	r = –0.13; p = 0.42
White (n = 41)	r = 0.07; p = 0.65
Total peripheral resistance	
Black (n = 40)	r = –0.10; p = 0.53
White (n = 41)	r = –0.23; p = 0.15
Arterial compliance (mL/mmHg)	
Black (n = 40)	r = 0.12; p = 0.47
White (n = 41)	r = 0.22; p = 0.17
rPWV (m/s)	
Black (n = 40)	r = –0.13; p = 0.42
White (n = 41)	r = 0.04; p = 0.80
fPWV (m/s)	
Black (n = 40)	r = 0.02; p = 0.88
White (n = 41)	r = –0.05; p = 0.74
dpPWV (m/s)	
Black (n = 40)	r = –0.18; p = 0.26
White (n = 41)	r = –0.04; p = 0.82
Carotid intima media thickness (mm)	
Black (n = 40)	r = –0.09; p = 0.59
White (n = 41)	r = 0.23; p = 0.15
Strain (%)	
Black (n = 40)	r = 0.41; p = 0.80
White (n = 41)	r = 0.20; p = 0.21
Distensibility coefficient (10^{-3}/kPa)	
Black (n = 40)	r = 0.06; p = 0.72
White (n = 41)	r = 0.10; p = 0.52
Compliance coefficient (mm^2/kPa)	
Black (n = 40)	r = 0.02; p = 0.89
White (n = 41)	r = 0.15; p = 0.36
Beta stiffness index	
Black (n = 40)	r = –0.07; p = 0.66
White (n = 41)	r = –0.09; p = 0.57
Peterson's elastic modulus (kPa)	
Black (n = 40)	r = –0.08; p = 0.61
White (n = 41)	r = –0.12; p = 0.47
Young's elastic modulus (kPa)	
Black (n = 40)	r = –0.08; p = 0.65
White (n = 41)	r = –0.15; p = 0.36

Supplementary Table 4. Partial correlations of dermal AGEs with measures of arterial function and body composition in white boys.

	Pentosidine (g/ml) n = 35
Body mass index (kg/m^2)	r = –0.01; p = 0.94
Body surface area (m^2)	r = –0.08; p = 0.66
Waist to hip ratio	r = 0.30; p = 0.07
Weight (kg)	r = –0.08; p = 0.66
Height (cm)	r = –0.08; p = 0.62
Waist circumference (cm)	r = 0.05; p = 0.79
Neck circumference (cm)	r = –0.10; p = 0.57
Hip circumference (cm)	r = –0.12; p = 0.48
Pulse pressure (mmHg)	r = 0.10; p = 0.56
Total peripheral resistance	r = 0.15; p = 0.38
Arterial compliance (mL/mmHg)	r = –0.15; p = 0.37
Carotid to radial pulse wave velocity (m/s)	r = 0.09; p = 0.59
Carotid to femoral pulse wave velocity (m/s)	r = 0.11; p = 0.54
Carotid to dorsalis pedis pulse wave velocity (m/s)	r = –0.06; p = 0.73
Carotid intima-media thickness (mm)	r = 0.12; p = 0.50
Strain (%)	r = 0.12; p = 0.47
Distensibility coefficient, 10^{-3}/kPa	r = 0.09; p = 0.61
Compliance coefficient (mm^2/kPa)	r = 0.12; p = 0.49
Beta stiffness index	r = 0.03; p = 0.85
Peterson's elastic modulus (kPa)	r = –0.02; p = 0.89
Young's elastic modulus (kPa)	r = –0.03; p = 0.84

Adjusted for age and mean arterial pressure.

CHAPTER 5

Summary of main findings and final conclusion

5.1. Introduction

In this conclusive chapter, a summary of the main findings from the research article are stated. The results from the article are also explained and compared to the relevant literature. Conclusions are drawn and recommendations are made to researchers investigating arterial stiffness in children.

5.2. Summary of the main findings

This study aimed to assess different estimates of arterial stiffness and investigated the links thereof with body composition and advanced glycation end-products (AGEs) in 6–8 year old black and white South African boys.

We hypothesised that:

i. Arterial stiffness and blood pressure will be higher in black compared to white boys of similar age, whereas body composition (determined by body mass index (BMI) z-scores) and AGEs are comparable between the two ethnicities.
ii. Positive relationships exist between measures of arterial stiffness and body composition in both groups.
iii. Measures of arterial function and body composition relate adversely to urinary and dermal AGEs in both groups.

Our first hypothesis was partially accepted as we demonstrated higher diastolic blood pressure; carotid to radial, carotid to femoral and carotid to dorsalis pedis pulse wave velocity and carotid intima-media thickness among black boys compared to white boys. Systolic blood pressure and body composition were similar between the two groups. However, AGEs (urinary pentosidine) were higher in black than white boys.

Our second hypothesis was also partially accepted since carotid distensibility coefficient (inversely) and Peterson's elastic modulus (positively) correlated with body mass index in both groups. These were the only estimates of arterial stiffness to relate with body composition in both groups. This hypothesis is also partially rejected since all other measures of carotid stiffness correlated to measures of body composition in white boys only. Femoral pulse wave velocity correlated inversely with body mass index and carotid intima-media thickness positively with body surface area in black boys only.

With respect to our third hypothesis, we found that the dermal AGE Reader (DiagnOptics Technologies, Groningen, The Netherlands) was unable to determine the autoflourescence

value for most of the black boys and therefore our aim to determine dermal AGEs was not achieved. As a result, we could also not include correlations between dermal AGEs, arterial stiffness indices and body composition variables for black boys as part of the research manuscript due to missing data. We performed partial correlations of dermal AGEs with arterial stiffness indices and body composition variables in white boys (Supplementary Table 4), and no significant correlations were evident. In addition, urinary pentosidine as measure of AGEs was measured in all participants. We found no relationship of urinary pentosidine with any of the arterial stiffness indices. We did however observe inverse correlations between AGEs and some of body composition variables (body mass index, body surface area, weight, waist and hip circumference) in black boys only. Therefore, our third hypothesis is partially accepted. This hypothesis is also in part rejected since no correlations were found between AGEs and measures of arterial function in any group.

5.3 Comparison to relevant literature

When the results from this study are compared with results from other population groups, it is evident that certain findings confirm and others contradict previous observations. The confirming findings were that diastolic blood pressure is a more important component of blood pressure in children than in adults. Nürnberger *et al.* proposed that the pulse wave reflecting during diastole results in increased mean diastolic blood pressure in children [1]. In addition, pulse wave velocity in all sections of the arterial tree, which reflects arterial stiffness, is higher in the black population compared to their white counterparts. It has been proposed by several studies that arterial stiffness is elevated in black adults [2-4] and may develop earlier in black populations [5]. We are the first to show elevated pulse wave velocity (especially femoral PWV) as early as 6 years old in a black South African population sample compared to white children. Several studies have reported that adiposity and hyperglycaemia may contribute towards the development of arterial stiffness in children [6-8]. However, in this study we found an inverse correlation between femoral pulse wave velocity and body mass index in black boys, supporting the potential curvilinear observations from other studies [9,10]. We found higher pentosidine levels in black compared to white boys, suggesting early reduced arterial compliance and increased endothelial dysfunction [11]. However, arterial compliance did not differ significantly between our groups and no associations between arterial stiffness indices and pentosidine were evident.

5.4 Chance and confounding

It is important to reflect on some of the factors that might have affected the results in this study. There are some methodological issues that could have caused weaknesses, and therefore, might have influenced the outcomes of this study. The number of participants included in this study was limited to only 81 participants, however, power calculations indicated a number of 36 participants per group to be sufficient in testing our hypotheses. It would be ideal if our findings are confirmed in other similar population samples. In addition to AGE levels, pentosidine is the most stable biomarker of AGEs obtained in urine, but we did not have blood samples to verify the other AGEs in the circulation along with other biomarkers of vascular/endothelial function. Studies have recommended the use echotracking for its accuracy in the assessment of carotid artery stiffness [12,13], however, carotid distensibility assessment by means of echotracking was not done in this study, but standard B-mode ultrasonography. We used an availability sample of boys willing to participate, which may have been encouraged from parents who were concerned about their own health and thus may be healthier than the general population. The so-called white coat effect may also have influenced our results as some of the children might have been new to the cardiovascular methodology used in data collection, including blood pressure monitoring. Overall, this was a highly controlled study consisting of healthy participants, all selected from the same school to ensure similar socio-economic class. It is also the first study to show ethnic differences in all sections of the arterial tree at ages as early as 6 years.

5.4.1 Confounders

The confounders in this study included a narrow age range (6–8 years) and mean arterial pressure. As girls were excluded, sex was not a confounder. The possible influence of confounders in the associations between arterial stiffness indices, body composition and AGEs was kept to the minimum, with adjustments made where necessary in the statistical analyses. More adjustment could have overshadowed our results and caused further power issues in our small sample. Future studies embarking on the understanding of arterial stiffness, AGEs and body composition in children are encouraged to recruit large samples in order to accommodate additional confounding variables. Understanding the physiological mechanisms is therefore crucial when interpreting the associations observed in this study.

5.5 Discussion of main findings

Previous studies conducted in the United States of America [3,4] and South Africa [14] have indicated elevated arterial stiffness in black adults. Previous studies suggested earlier development of hypertension and arterial stiffness in the black population [5,15]. Our study supports this concept and showed higher arterial stiffness in black children at ages as young as 6 years. Our study aimed to assess arterial stiffness indices and investigate its link with body composition as well as with AGEs (related to adiposity and hyperglycaemia) [6-8] in black and white South African boys in order to explore potential novel mechanisms possibly contributing to early vascular changes in the black population. We showed elevated arterial stiffness along with increased diastolic blood pressure and intima-media thickness in black boys, indicating an early vascular compromise which may have a detrimental cardiovascular impact in early adulthood. The lack of associations of arterial stiffness with body composition and AGEs in black boys indicate that there may be other factors we did not consider that may contribute to arterial stiffness in the young black population. Although the findings cannot be expanded to the whole black South African population, it adds knowledge to the burden of non-communicable diseases in South Africa and could provide a reference for future studies to investigate other potential contributing factors.

5.6 Final conclusions

In conclusion, our study indicates that young healthy black boys have higher arterial stiffness in all sections of the arterial tree compared to white boys of the similar age. Furthermore, these black boys also have higher diastolic blood pressure, carotid intima-media thickness and pentosidine suggesting a phenotype of premature cardiovascular disease burden. These findings suggest that vascular changes are already present as early as 6 years of age in the black population which may have implications in the early onset and development of hypertension and arterial stiffness in early adulthood.

5.7 Recommendations

Relatively larger population samples of children of both sexes and ethnicities and of wider age range are needed to investigate the links between arterial stiffness, body composition and AGEs. The influence of genetic predisposition and poor diet during first days of life could provide insightful information on whether angiotensin II activity and low birth weight can contribute towards arterial stiffness development in black individuals. More reliable

measures of carotid arterial stiffness such as echotracking are needed. A call on clinical or epidemiological studies are emphasised to explore the various possible mechanisms to identify the most prominent earliest risk factors that may shed light on this premature cardiovascular burden among the black population. New knowledge on such contributors would be invaluable in implementing cardiovascular prevention programmes in children and young adults. Studies should also be developed to investigate nutrition and dietary intake from school tuck shops and lunch boxes to determine the potential contribution of harmful food that may cause an early rise in blood pressure or trigger extracellular matrix turnover known to play a part in arterial stiffness development and subsequent cardiovascular complications.

5.8 References

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Appendix A: Approval from the Provincial Department of Education for the ASOS study

Education and Sport Development
Department of Education and Sport Development
Departement van Onderwys en Sport Ontwikkeling
Lefapha la Thuto le Tlhabololo ya Metshameko
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DR KENNETH KAUNDA DISTRICT

OFFICE OF THE DISTRICT DIRECTOR

16 January 2015

Dr R Kruger
Project Leader: ASOS-study
North West University – Potchefstroom Campus

PERMISSION TO CONDUCT RESEARCH "ON CARDIOVASCULAR HEALTH OF SCHOOL CHILDREN AND THEIR PARENTS IN POTCHEFSTROOM PUBLIC SCHOOLS" TLOKWE AREA OFFICE - DR KENNETH KAUNDA DISTRICT

The above matter refers.

Permission is hereby granted to you to conduct your research in the schools in Tlokwe Area Office - Dr Kenneth Kaunda District under the following provisions:

- **The activity you undertake at the schools should not tamper with the normal process of learning and teaching; and will take place after school hours.**
- **You inform the principals of your identified schools of your impending visit and activity;**
- **You provide my office with a report in respect of your findings from the research; and**
- **You obtain prior permission from this office before availing your findings for public or media consumption.**

Wishing you well in your endeavour.

Thanking you

MR H MOTARA
DISTRICT DIRECTOR
DR KENNETH KAUNDA DISTRICT

cc *Ms S S Yssel – Area Manager: Tlokwe*

Appendix B: Ethics approval for the ASOS study and sub-study

NORTH-WEST UNIVERSITY
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Faculty of Health Sciences
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Email: Minrie.Greeff@nwu.ac.za

16 March 2015

Dr R Kruger
Physiology

Dear Dr Kruger

ETHICS APPLICATION: NWU-00007-15-S1 (R KRUGER-GG MOKWATSI) "THE ARTERIAL STIFFNESS IN OFFSPRING STUDY (ASOS)"

Thank you for amending your application. All ethical concerns have now been addressed and ethical approval is granted until 31/10/2018

Please note that any changes to the approved application must be submitted to the Health Research Ethics Committee for approval before implementation.

Yours sincerely

Prof Minrie Greeff
HREC Chairperson

Original details: (10187308) C:\Users\13210572\Documents\HREC\HREC - Applications\HREC - Applications 01 - February 2015\NWU-00007-15-S1 (R Kruger-GG Mokwatsi)\NWU-00007-15-S1 (R Kruger-GG Mokwatsi) - Approval letter\NWU-00007-15-S1 (R Kruger-GG Mokwatsi) - Approval letter.docm
16 March 2015

File reference: 9.1.5.3

Appendix C: Confirmation of the editing of the dissertation

DECLARATION

I, Clarina Vorster, Language editor and Translator, and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of the dissertation of ms GG Mokwatsi, student of the North-West University, Potchefstroom Campus (student number 22368590).

Title of the dissertation: Arterial stiffness and its association with advanced glycation end-products in 6-8 year old boys: The ASOS study

C Vorster _____ 1 April 2016

C Vorster Date

9 Lanyon Street

Potchefstroom

2520

082 440 4102

Appendix D: Turn it in originality report

Turnitin Originality Report

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Appendix E: Solemn Declaration

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

Higher Degree Administration

SOLEMN DECLARATION

1 Solemn Declaration by student

I, Gontse Gratitude Mokwatsi

hereby declare that the thesis/dissertation/article entitled

Arterial stiffness and its association with advanced glycation end-products in 6-8 year old boys: The ASOS study

which I herewith submit to the North-West University, Potchefstroom campus, in compliance/partial compliance with the requirements set for the MSc Physiology qualification, is my own work and has been language edited and has not been submitted to any other university.

I understand and accept that the copies submitted for examination are the property of the North-West University.

Student Signature

University

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Declaration of Commissioner of Oaths

Declared before me on this 14 day of March 2016

Commissioner of Oaths

NWU

PLEASE NOTE: If a thesis/dissertation/mini-dissertation/article of a student is submitted after the deadline for submission, the period available for examination is limited. No guarantee can therefore be given that (should the examiners' reports be positive) the degree will be conferred at the next applicable graduation ceremony. It may also imply that the student would have to re-register for the following academic year.

2 Solemn Declaration of supervisor/promoter

The undersigned hereby declares that:

- the student is granted permission to submit his/her thesis/dissertation for examination purposes; and
- the student's work was tested by Turnitin, and a satisfactory report has been obtained.

Signature of supervisor/promoter

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Date