

Vascular and metabolic profile of 5-year sustained hypertensive versus normotensive black South Africans

Melissa Maritz

22212337

B.Sc Physiology and Psychology

Honours B.Sc Physiology

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

Supervisor: Dr. CMT Fourie

Co-supervisor: Prof. JM van Rooyen

Co-supervisor: Prof. AE Schutte

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

November 2014

ACKNOWLEDGEMENTS

First and foremost thank you to the Lord my God, for carrying me through the process of writing this dissertation.

Thank you to my brilliant supervisors, Dr. CMT Fourie, Prof. JM van Rooyen and Prof. AE Schutte. You have given and continue to give me the ultimate gift of education.

Parents, Gerrit and Angelique, thank you for your love, support, understanding and helpfulness throughout this year.

Thank you to Maretha Botes for the language editing of this dissertation.

Thank you to the participants and staff of the PURE-SA study.

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

AFFIRMATION BY AUTHORS

The following researchers contributed to this study:

Mrs M Maritz

Responsible for literature research, statistical analyses, cleaning and processing of the PURE data and planning and writing of the manuscript.

Dr. CMT Fourie

Supervisor

Supervised the collection and statistical analyses of the data, as well as planning and writing of the dissertation.

Prof. JM van Rooyen

Co-supervisor

Collection and interpretation of the data, and gave recommendations for the planning and writing of the dissertation.

Prof. AE Schutte

Co-supervisor

Collection and interpretation of the data, and gave recommendations for the planning and writing of the dissertation.

This is a statement from the co-authors confirming their individual role in the study and giving permission that the article may form part of this study.

Dr. CMT Fourie

Prof. JM van Rooyen

Prof. AE Schutte

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
AFFIRMATION BY AUTHORS	iii
SUMMARY	vi
OPSOMMING	viii
PREFACE	x
LIST OF TABLES AND FIGURES	xi
LIST OF ABBREVIATIONS	xii
Chapter 1: Introduction	1
1.1 BACKGROUND	2
1.2 AIM	3
1.3 OBJECTIVES	3
1.4 HYPOTHESES	3
1.6 REFERENCES	4
Chapter 2: Literature Study	7
Introduction	8
1. Blood pressure	10
<i>1.1 Normal blood pressure</i>	10
<i>1.2 Hypertension</i>	10
<i>1.2.1 Dyslipidemia and hypertension</i>	10
<i>1.2.2 Hyperglycemia and hypertension</i>	11
<i>1.2.3 Renal function and hypertension</i>	12
<i>1.2.4 Health behaviours and hypertension</i>	13
2. Arterial structure and function	14
<i>2.1 Atherosclerosis and arteriosclerosis</i>	15
<i>2.2 Measures of arterial function</i>	18
<i>2.2.1 Pulse pressure</i>	18
<i>2.2.2 Pulse wave velocity</i>	18
<i>2.3 Measures of carotid structure and function</i>	18
<i>2.3.1 Carotid intima-media thickness</i>	19
<i>2.3.2 Central blood pressure</i>	19
<i>2.3.3 Young's elastic modulus</i>	20
<i>2.3.4 Carotid distensibility</i>	20
3. Inflammation	20
<i>3.1 Inflammation and hypertension</i>	20

3.2 Inflammation, arterial structure and function	21
4. Endothelial activation	22
4.1 Endothelial activation and hypertension	23
References.....	25
Chapter 3: Manuscript for publication	38
INSTRUCTIONS TO AUTHORS: <i>Journal of Hypertension</i>	39
Abstract	41
Introduction	42
Methods	43
Results	47
Discussion	53
References	57
Chapter 4: Concluding Remarks and Discussion of Main Findings	67
Introduction	68
Summary of main findings	68
Comparison to relevant literature	69
Discussion of main findings	69
Conclusion	70
Chance and confounding	70
Recommendations	71
Final remarks	71
References	73
Appendix A: Ethics approval.....	75
Appendix B: Turn it in documents.....	76

SUMMARY

Motivation

A close association exists between hypertension and arterial stiffness. Whether the increased arterial stiffness seen in hypertensives are due to structural or functional adaptations in the vasculature is uncertain. Hypertension is more common in blacks and they have an increased arterial stiffness and higher stroke prevalence than white populations. Arterial stiffening, or a loss of arterial distensibility, increases the risk for cardiovascular events, including stroke and heart failure, as it increases the afterload on the heart, as well as creating a higher pulsatile load on the microcirculation. The stiffness of the carotid artery is associated with cardiovascular events, like stroke, and all-cause mortality. Furthermore, carotid stiffness is independently associated with stroke, probably because stiffening of the carotid artery may lead to a higher pressure load on the brain. Inflammation, endothelial activation, dyslipidemia, hyperglycemia and health behaviours may also influence hypertension and arterial stiffness. Limited information is available on these associations in black South Africans. The high prevalence of hypertension and cardiovascular disease in blacks creates the need for effective prevention and intervention programs in South Africa.

Aim

We aimed to compare the characteristics of the carotid artery between 5-year sustained hypertensive and normotensive black participants. Furthermore, we aimed to determine whether blood pressure, conventional cardio-metabolic risk factors, markers of inflammation, endothelial activation and measures of health behaviours are related to these carotid characteristics.

Methodology

This sub-study forms part of the South African leg of the multi-national Prospective Urban and Rural Epidemiology (PURE) study. The participants of the PURE-SA study were from the North West Province of South Africa, and baseline data collection took place in 2005 (N=2010), while follow-up data was collected five years later, in 2010 (N=1288). HIV-free participants who were either hypertensive or normotensive (N=592) for the 5-year period, and who had complete datasets, were included in this sub-study. The study population thus consists of a group of 5-year sustained normotensive (n=241) and hypertensive (n=351) black participants.

Anthropometric measurements included height, weight, waist circumference and the calculation of body mass index (BMI). We included several cardiovascular measurements, namely brachial systolic- and diastolic blood pressure, heart rate, central systolic blood pressure, central pulse pressure and the carotid dorsalis-pedis pulse wave velocity. Carotid characteristics included distensibility, intima media thickness, cross sectional wall area, maximum and minimum lumen diameter. Biochemical

variables that were determined included HIV status, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, triglycerides, fasting glucose, glycated haemoglobin (HbA1c), creatinine clearance, interleukin-6, C-reactive protein, intracellular adhesion-molecule-1 and vascular adhesion molecule-1. Health behaviours were quantified by measuring γ -glutamyltransferase and by self-reported alcohol, tobacco and anti-hypertensive, anti-inflammatory and lipid-lowering medication use.

We compared the normotensive and hypertensive groups by using independent *t*-tests and chi-square tests. The carotid characteristics were plotted according to quartiles of central systolic blood pressure by making use of standard analyses of variance (ANOVA) and the analyses of co-variance (ANCOVA). Pearson correlations done in the normotensive and hypertensive Africans helped to determine covariates for the multiple regression models. We used forward stepwise multiple regression analyses with the carotid characteristics as dependent variables to determine independent associations between variables.

Results and Conclusion

The cardiovascular measures, including pulse wave velocity, were significantly higher in the hypertensive group (all $p \leq 0.024$). The lipid profile, markers of inflammation, endothelial activation and glycaemia, as well as health behaviours, did not differ between the hypertensives and normotensives after adjustments for age, sex, waist circumference, γ -glutamyltransferase, tobacco use and anti-hypertensive medication use. After similar adjustments, all carotid characteristics, except IMT, were significantly different between the groups (all $p \leq 0.008$). However, upon additional adjustment for cSBP, significance was lost.

The stiffness and functional adaptation seen in this study are not explained by the classic cardio-metabolic risk factors, markers of endothelial activation or health behaviours of the participants. The differences that exist in terms of arterial stiffness between the normotensive and hypertensive groups may be explained by the increased distending pressure in the hypertensive group. Despite their hypertensive status, it seems that there are no structural adaptations in these hypertensive Africans.

Keywords: ethnicity, black, large artery, stiffness, carotid distensibility, hypertension, central pressure

OPSOMMING

Motivering

Hipertensie en arteriële styfheid assosieer met mekaar. Onsekerheid bestaan oor die oorsaak van verhoogde arteriële styfheid in hipertensiewes, aangesien dit toegeskryf kan word aan funksionele of strukturele veranderinge, of beide, in die arteriële wand. Hipertensie kom meer in swart populasies voor en hulle het ook 'n verhoogde arteriële styfheid en 'n hoër beroerte-voorkoms as wit populasies. Arteriële verstywing verhoog die risiko vir beroertes en hartversaking, aangesien stywer arteries lei na 'n hoër nabelading op die hart, sowel as hoër polserende druk op die mikrosirkulasie. Die styfheid van die karotis arterie assosieer onafhanklik met kardiovaskulêre insidente, asook met alle-oorsaak mortaliteit. Karotis styfheid assosieer onafhanklik met beroerte, moontlik omdat verstywing van hierdie arterie 'n hoër druk op die brein plaas. Inflammasie, endoteel aktivering, dislipidemie, hiperglisemie en leefstyl faktore kan ook 'n moontlike invloed hê op die voorkoms van hipertensie en arteriële styfheid. Slegs beperkte informasie is beskikbaar ten opsigte van hierdie faktore in swart Suid-Afrikaners. As gevolg van die hoë voorkoms van hipertensie en kardiovaskulêre siektes in swart populasies, is effektiewe voorkomings- en behandelingsprogramme nodig in Suid-Afrika.

Doel

Die doel van hierdie studie is om die eienskappe van die karotis arterie tussen hipertensiewe en normotensiewe swart Suid-Afrikaners te vergelyk. Hierdie Afrikaner het of 'n normotensiewe, of 'n hipertensiewe bloeddruk-lesing in beide 2005 en 2010 gehad. Tweedens is die doel om te bepaal of bloeddruk, klassieke kardiovaskulêre risikofaktore, merkers van inflammasie en endoteel aktivering, of leefstyl faktore met die karotis eienskappe assosieer.

Metodologie

Hierdie sub-studie vorm deel van die Suid-Afrikaanse deel van die multi-nasionale *Prospective Urban and Rural Epidemiology (PURE) study*. Dit het deelnemers uit die Noordwes provinsie van Suid-Afrika ingesluit, waarvan die eerste data-opname in 2005 (N=2010) was, met 'n opvolg in 2010 (N=1288). Slegs die HIV-vrye deelnemers wat of normotensief of hipertensief was vanaf 2005- 2010 is in die studie-populasie van hierdie sub-studie ingesluit. Dus, die studie-populasie bestaan uit 'n groep swart deelnemers wat vir ten minste 5 jaar of normotensief (N=241) of hipertensief (N=351) was.

Antropometriese metings sluit in lengte, gewig, middel omtrek en die berekening van liggaamsmassa-indeks. Kardiovaskulêre metings, insluitend bragiale sistoliese- en diastoliese bloeddruk, hart tempo, sentraal sistoliese bloeddruk, sentrale polsdruk en die carotis dorsalis-pedis polsgolfsnelheid is bepaal. Die karotis eienskappe, insluitend die rekbaarheid, intima-media dikte, deursnee-wand area,

maksimum- en minimum lumen deursnee is bepaal. Die biochemiese veranderlikes wat bepaal is sluit in HIV-status, lae- en hoë-digtheid lipoproteïen cholesterol (LDL-C en HDL-C), trigliseriede, vastende glukose, gliseerde hemoglobien (HbA1c), kreatinien opruiming, interleukin-6, C-reaktiewe proteïen, intrasellulêre-adhesie molekuul-1 en vaskulêre-adhesie molekuul-1. Gesondheidsgedrag is gekwantifiseer deur die meting van γ -glutamietransferase (GGT), en deur die self-gerapporteerde gebruik van alkohol, tabak en anti-hipertensiewe, anti-inflammatoriese en lipied-verlaagende medikasie.

Onafhanklike *t*-toetse en chi-kwadraat toetse is gebruik om die verskil tussen die normotensiewe en hipertensiewe groepe te bepaal. Die *tendens p-waarde* tussen die kwartiele van die sentrale sistoliese bloeddruk en die karotis eienskappe is bepaal met gestandaardiseerde analise van variansie (ANOVA) en analise van ko-variensie (ANCOVA) toetse. Pearson korrelasies in die normotensiewe en hipertensiewe Afrikane het gehelp om ko-variate vir die meervoudige regressie model te bepaal. Onafhanklike assosiasies tussen veranderlikes is uitgewys met behulp van meervoudige regressie analyses. Die karotis eienskappe is die afhanklike veranderlikes in die meervoudige regressie analyses.

Resultate en Gevolgtrekking

Die kardiovaskulêre metings, insluitend die polsgolfsnelheid, was hoër in die hipertensiewe groep (alle $p \leq 0.024$). Die lipied profiel, merkers van inflammasie, endoteel aktivering en glisemie, sowel as die leefstyl faktore van die normotensiewe en die hipertensiewe groepe het nie verskil nadat aanpassings gemaak is nie. Die faktore waarvoor aangepas is sluit in: ouderdom, geslag, middel-omtrek, γ -glutamietransferase, tabak- en anti-hipertensiewe medikasie gebruik. Na soortgelyke aanpassings het al die eienskappe van die karotis arterie, behalwe die intima-media dikte, betekenisvol verskil ($p < 0.008$). Hierdie verskille het egter verdwyn na addisionele aanpassing vir sentrale sistoliese bloeddruk.

Die styfheid en funksionele adaptasie wat in die hipertensiewe groep gesien word, kan nie deur die klassieke kardio-metaboliese risiko faktore, merkers van inflammasie en endoteel aktivering, of die leefstyl faktore van hierdie deelnemers verduidelik word nie. Die verskil kan dalk verduidelik word deur die verhoogde wand druk in die hipertensiewe groep. Ten spyte van hul 5-jaar lange hipertensiewe status, kon geen strukturele veranderinge by hierdie hipertensiewe Afrikane gevind word nie.

Sleutelwoorde: etnisiteit, swart, elastiese arteries, styfheid, karotis uitrekbaarheid, hipertensie, sentrale druk

PREFACE

The article format that was used to complete this dissertation is an approved format and recommended by the North-West University. The dissertation is written in English, but includes an Afrikaans summary as required by the university. It consists of a manuscript which is ready to be submitted to a peer-reviewed journal, as well as an in depth literature review and an interpretation of the results. The chosen journal for the manuscript of this project is the *Journal of Hypertension*.

The layout of the dissertation is as follows:

- **Chapter 1:** An introductory chapter which consists of the motivation, background, aim, objectives and hypotheses of this study.
- **Chapter 2:** An in depth literature study of the relevant topics.
- **Chapter 3:** The research article, consisting of author instructions for the *Journal of Hypertension*, an abstract, and introduction, the research materials and methods, results, discussion, conclusion and acknowledgements.
- **Chapter 4:** The concluding remarks, a critical discussion of the findings and recommendations for future studies.

A reference list is provided at the end of each chapter, according to the style required by the *Journal of Hypertension*.

LIST OF TABLES AND FIGURES

Tables

Chapter 3:

- Table 1 - Characteristics of normotensive and hypertensive Africans
- Table 2 - Adjusted characteristics of normotensive and hypertensive Africans
- Table 3 - Carotid characteristics of normotensive and hypertensive Africans, additionally adjusted for cSBP
- Table 4 - Forward stepwise multiple regression analyses with CD and cIMT as dependent variables

Supplementary tables:

- Table 1 - Pearson correlations in normotensive Africans
- Table 2 - Pearson correlations in hypertensive Africans
- Table 3 - Forward stepwise multiple regression analyses with CSWA and Max LD as dependent variables

Figures

Chapter 2

- Figure 1 - Hypertension and commonly associated factors
- Figure 2 - Pathophysiological mechanisms of arterial remodelling

Chapter 3

- Figure 1 - Study population
- Figure 2 - Quartiles of central blood pressure plotted against measures of the carotid artery in the total group: (A) Unadjusted; (B) Adjusted

LIST OF ABBREVIATIONS

%CDT	---	Percentage carbohydrate deficient transferrin
AGE	---	Advanced glycation end-products
ANCOVA	---	Analyses of covariance
ANOVA	---	Analyses of variance
AST	---	Aspartate aminotransferase
bDBP	---	Brachial diastolic blood pressure
BMI	---	Body mass index
BP	---	Blood pressure
bSBP	---	Brachial systolic blood pressure
CD	---	Carotid distensibility
cdPWV	---	Carotid dorsalis-pedis pulse wave velocity
CHD	---	Coronary heart disease
cIMT	---	Carotid intima-media thickness
CKD	---	Chronic kidney disease
cPP	---	Central pulse pressure
CrCl	---	Creatinine clearance
cSBP	---	Central systolic blood pressure
CSWA	---	Cross-sectional wall area
CV	---	Cardiovascular
CVD	---	Cardiovascular disease
DBP	---	Diastolic blood pressure
EMP	---	Endothelial microparticles
ECM	---	Extracellular matrix
ESC	---	European Society of Cardiology
ESH	---	European Society of Hypertension
ET-1	---	Endothelin-1
GFR	---	Glomerular filtration rate

GGT	---	Gamma-glutamyltransferase
HbA1c	---	Glycosated hemoglobin
HDL-C	---	High-density lipoprotein-cholesterol
HR	---	Heart rate
hsCRP	---	High-sensitivity C-reactive protein
HUVEC's	---	Human umbilical vein endothelial cells
ICAM-1	---	Intracellular-adhesion molecule-1
IL-6	---	Interleukin-6
IMT	---	Intima-media thickness
LDL-C	---	Low-density lipoprotein-cholesterol
LGI	---	Low-grade inflammation
MAP	---	Mean arterial pressure
Max LD	---	Maximum lumen diameter
MI	---	Myocardial infarction
Min LD	---	Minimum lumen diameter
MMP	---	Matrix metalloproteinases
NO	---	Nitric oxide
Ox-LDL	---	Oxidized low-density lipoprotein
PP	---	Pulse pressure
PWV	---	Pulse wave velocity
RAGE	---	Receptor of advanced glycation end products
SBP	---	Systolic blood pressure
TC	---	Total cholesterol
TG	---	Triglycerides
VCAM-1	---	Vascular adhesion molecule-1
VSMC	---	Vascular smooth muscle cells
vWF	---	von Willebrand factor
WC	---	Waist circumference

Chapter 1: Introduction

1.1 BACKGROUND

“The emerging epidemic of non-communicable diseases in South Africa” [1]

Hypertension affects people around the world [2] and globally 17 million deaths a year can be attributed to cardiovascular disease [2]. Of these, more than half (9.4 million) are caused by the complications of hypertension [2]. An increase in blood pressure shortens a person’s life expectancy, as some of the most serious complications of hypertension include myocardial infarction (MI), stroke and kidney disease [3].

The similarities between the human immunodeficiency virus (HIV) epidemic and hypertension were highlighted in a recent article by Lloyd-Sherlock, where both are largely asymptomatic, easily diagnosed, need life-long management and treatment and can have serious and fatal outcomes [4]. Comparing hypertension to the HIV epidemic [4] captures the magnitude of the health threat that hypertension poses to the world population, and more specifically, to South Africa.

The disease burden from infectious diseases like HIV and tuberculosis remain high in South Africa. However, with the HIV epidemic in the process of stabilisation [5], more emphasis needs to be placed on the prevalence of non-communicable diseases like cardiovascular disease (CVD) and diabetes, which are on the increase [1].

It is known that hypertension is more prevalent in black than in white populations [6-8], and several studies also indicate that blacks have increased arterial stiffness compared to whites [9-12]. Both urban and rural populations suffer from the burden of non-communicable diseases [13]. Socio-economic status comes into play, especially in South Africa, with the poor urban communities suffering the largest incidence of non-communicable diseases [13].

Sustained hypertension may accelerate structural alterations in the arterial wall [14]. Although structural proteins determine the intrinsic stiffness of the arterial wall, the stiffness measured also depends on the instantaneous pressure exerted on the wall by the flowing blood [15]. An increase in arterial stiffness leads to increased systolic and pulse pressure, creating an increased pulsatile load on the microcirculation and afterload on the heart [16]. These changes in the circulation may lead to CVD, resulting in potentially fatal events like stroke and heart failure [16, 17]. Local stiffness of the carotid artery independently associates with cardiovascular events, such as stroke, and all-cause mortality [16].

Several factors associate with hypertension, including arterial stiffness (arteriosclerosis) [15], atherosclerosis [18], obesity [19, 20], a low socioeconomic status [6], urbanisation [8], excessive alcohol use [19], inflammation [21], endothelial activation [22], dyslipidaemia [23] and diabetes mellitus [24]. However, no one factor or mechanism has been identified as the main causative factor thus far, and discrepancies also exist among different ethnic populations in terms of these factors [6].

The high prevalence of hypertension and CVD in blacks creates the need for effective prevention and intervention programs in South Africa. This study will focus on several relevant aspects that may be important when investigating hypertension and arterial stiffness in black South Africans.

1.2 AIM

We aimed to compare the characteristics of the carotid artery, cardiometabolic risk factors, as well as markers of inflammation and endothelial activation between 5-year sustained hypertensive and normotensive black participants. Furthermore, we aimed to determine whether blood pressure, conventional cardio-metabolic risk factors, markers of inflammation, endothelial activation and measures of health behaviours are related to these carotid characteristics.

1.3 OBJECTIVES

The following objectives were identified for this study:

1. To determine the differences in the carotid artery characteristics (distensibility, intima media thickness, cross sectional wall area and lumen diameter) between hypertensive and normotensive Africans.
2. To determine the differences in metabolic variables, the markers of inflammation, endothelial activation and health behaviours between hypertensive and normotensive Africans.
3. To determine whether central blood pressure is associated with measures of carotid structure and function in the hypertensive and/or normotensive Africans..
4. To determine whether measures of carotid structure and function are related to metabolic variables, the markers of inflammation and endothelial activation and health behaviours in the hypertensive and/or normotensive Africans.

1.4 HYPOTHESES

Based on the aim and objectives, the following hypotheses have been formulated:

1. Hypertensive Africans have an increased carotid intima-media thickness, cross-sectional wall area and a larger lumen diameter, but lower carotid distensibility, than normotensive Africans.
2. The hypertensives have an adverse lipid profile, higher glucose levels, a higher alcohol intake (γ -glutamyltransferase), and higher levels of inflammatory (CRP, IL-6) and endothelial activation (ICAM-1, VCAM-1) markers than normotensives.
3. Central blood pressure is positively associated with carotid structure and function in both groups.
4. The measures of carotid structure and function are positively associated with markers of inflammation (CRP) and endothelial activation (ICAM) in the hypertensive group.

1.6 REFERENCES

1. Shisana O, Labadarios D, Rehle T, Simbayi L, Zuma K, Dhansay A, et al. South African National Health and Nutrition examination survey. Cape Town: HSRC Press; 2013.
2. World Health Organization (WHO). Global status report on noncommunicable diseases. 2013.
3. Hall JE. Guyton and Hall: Textbook of medical physiology. 12th ed. Philadelphia: Saunders Elsevier; 2011.
4. Lloyd-Sherlock P. Is hypertension the new HIV epidemic? *Int J Epidemiol*. 2014.
5. Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Pillay-van-Wyk V, et al. South African national HIV prevalence, incidence, behaviour and communication survey 2008: A turning tide among teenagers? Cape Town: HSRC Press; 2009.
6. Opie LH, Seedat YK. Hypertension in Sub-saharan African populations. *Circulation*. 2005;112:3562-8.
7. Schutte AE, van Vuuren D, van Rooyen JM, Huisman HW, Schutte R, Malan L, et al. Inflammation, obesity and cardiovascular function in African and Caucasian women from South Africa: The POWIRS study. *J Hum Hypertens*. 2006;20:850-9.
8. Van Rooyen JM, Kruger HS, Huisman HW, Wissing MP, Margretts BM, Venter CS, et al. An epidemiological study of hypertension and its determinants in a population in transition: The THUSA study. *J Hum Hypertens*. 2000;14:779-87.
9. Chaturvedi N, Bulpitt CJ, Leggetter S, Schiff R, Nihoyannopoulos P, Strain WD, et al. Ethnic differences in vascular stiffness and relations to hypertensive target organ damage. *J Hypertens*. 2004;22:1731-7.
10. Hefferman KS, Jae SY, Fernhall B. Racial differences in arterial stiffness after exercise in young men. *Am J Hypertens*. 2007;20:840-5.
11. Morris AA, Patel RS, Binongo JNG, Poole J, Mheid IA, Ahmed Y, et al. Racial differences in arterial stiffness and microcirculatory function between black and white Americans. *J Am Heart Assoc*. 2013;24:2:e002154.

12. Schutte AE, Huisman HW, Schutte R, Van Rooyen JM, Malan L, Malan NT, et al. Arterial stiffness profiles: Investigating various sections of the arterial tree of African and Caucasian people. *Clin Exp Hypertens*. 2011;33:511-7.
13. Mayosi BM, Flisher AJ, Lalloo UG, Sitas F, Tollman SM, Bradshaw D. The health burden of non-communicable diseases in South Africa. *Lancet*. 2009;374:14.
14. Benetos A, Adamopoulos C, Bureau J, Temmar M, Labat C, Bean K, et al. Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. *Circulation*. 2002;105:1202-7.
15. Cecelja M, Chowienzyk P. Role of arterial stiffness in cardiovascular disease. *J R Soc Med Cardiovasc Dis*. 2012;1:11.
16. Van Sloten TT, Schram MT, van den Hurk K, Dekker JM, Nijpels G, Henry RMA, et al. Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality. *J Am Coll Cardiol*. 2014;63:1739-47.
17. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. *Eur Heart J*. 2006;27:2588-605.
18. Alexander RW. Hypertension and the pathogenesis of atherosclerosis: Oxidative stress and the mediation of arterial inflammatory response: A new perspective. *Hypertension*. 199;25:155-61.
19. Schutte AE, Schutte R, Huisman HW, Van Rooyen JM, Fourie CMT, Smith W, et al. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in black South Africans? A 5-year prospective study. *Int J Epidemiol*. 2012;41:1114-23.
20. Peer N, Steyn K, Lombard C, Gwebushe N, Levitt N. A high burden of hypertension in the urban black population of Cape Town: The cardiovascular risk in black South Africans (CRIBSA) study. *PLoS ONE*. 2013;8:e78567.
21. Lakoski SG, Cushman M, Palmas W, Blumenthal R, D'Agostino RB, Herrington DM. The relationship between blood pressure and C-reactive protein in the multi-ethnic study of atherosclerosis (MESA). *J Am Coll Cardiol*. 2005;46:1869-74.

22. Preston RA, Ledford M, Materson BJ, Baltodano NM, Meman A, Alonso A. Effects of severe, uncontrolled hypertension on endothelial activation: Soluble vascular cell adhesion molecule-1, soluble intercellular adhesion molecule-1 and von willebrand factor. *J Hypertens*. 2002;20:871-7.
23. Osuji CU, Omejua EG, Onwubuya EI, Ahaneku GI. Serum lipid profile of newly diagnosed hypertensive patients in Nnewi, South-east Nigeria. *Int J Hypertens*. 2012;2012:7.
24. Ferrannini E, Cushman WC. Diabetes and hypertension: The bad companions. *The Lancet*. 2012;380:601-10.

Chapter 2: Literature Study

Introduction

In 1931 Paul Dudley White suggested that the increase in blood pressure is a compensatory mechanism to ensure optimal perfusion to target tissues [1]. Since then, many studies have shown that lowering blood pressure lowers the cardiovascular morbidity and mortality risk for hypertension of all degrees of severity and even in high-risk normotensive individuals [2].

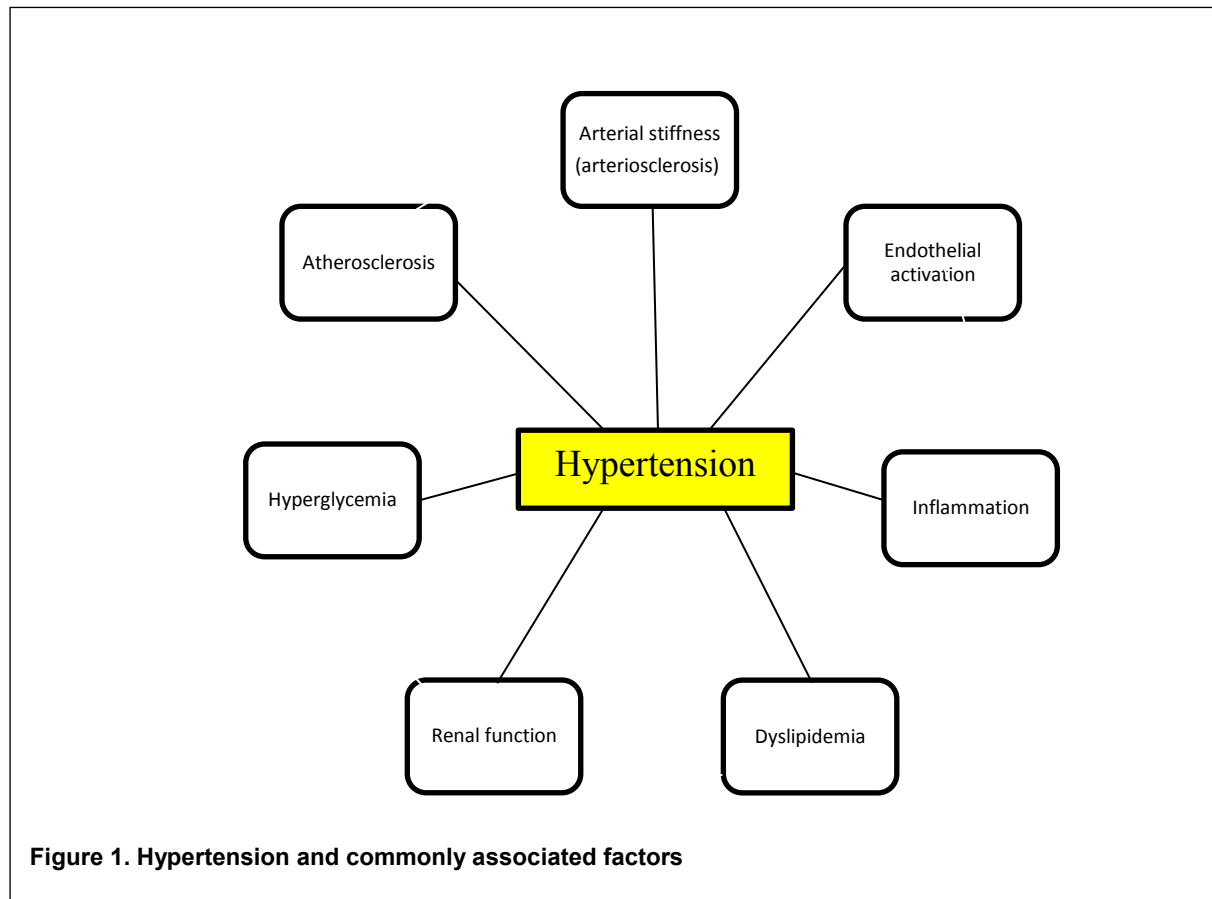


Figure 1 illustrates that hypertension, one of the most common cardiovascular (CV) risk factors [3], is associated with endothelial activation [4], inflammation [5-7], dyslipidemia [8], renal function [9, 10] hyperglycemia [11] atherosclerosis [12-14] and arterial stiffness [15, 16]. In recent years, the involvement of arterial stiffness in the development of cardiovascular disease (CVD) received great interest [17], as the stiffening of the vasculature leads to an increased systolic blood pressure (SBP), afterload on the heart and pulsatile load on the microcirculation, as well as decreased coronary perfusion [18]. Therefore, arterial stiffness may cause stroke, coronary heart disease (CHD) and heart failure [18].

Research comparing hypertensive to normotensive Africans in terms of arterial structure and function and the associations thereof with blood pressure, metabolic profile (lipids and glucose), inflammation and endothelial activation is limited. Therefore, a better understanding regarding these factors in

hypertensive versus normotensive Africans is needed. In light of the emerging epidemic of non-communicable diseases in South Africa [19], it is clear that hypertension and its associated factors need to be addressed in order to implement effective prevention and intervention strategies, as well as the needed health policies [19]. It has been proposed that the main contributing factors in terms of hypertension in Africans are modifiable [20], increasing the emphasis on the need for prevention and intervention.

The 2010 data of the PURE sub-study allows us to explore these differences and associations in hypertensive versus normotensive Africans. The participants to be included in this study have been either hypertensive or normotensive for two consecutive blood pressure measurements over a five-year interval (2005 and 2010), which creates the opportunity to investigate the mentioned markers after a long-term disease period. Therefore, this study will attempt to contribute to the on-going efforts of understanding hypertension in Africans.

1. Blood pressure

1.1 Normal blood pressure

Blood pressure is the force that blood exerts against a unit area of the vessel wall, and it is measured in millimetres mercury (mmHg) [9]. The body has the ability to regulate blood pressure, and this is a complex, dynamic process that aims to match tissue perfusion with metabolic demands [21].

The South African Hypertension Guidelines as well as the 2013 Guidelines of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) state that normal blood pressure is a systolic value of 120-129 mmHg, and/or a diastolic value of 80-84 mmHg [22, 23], while optimal blood pressure is a systolic value of <120 mmHg and a diastolic value of <80 mm Hg [23].

1.2 Hypertension

Arterial hypertension is a cardiovascular syndrome associated with structural and functional changes in the cardiovascular system that damage the vasculature, kidneys, heart and brain, leading to premature morbidity and death [21].

The South African Hypertension Guidelines of 2011 as well as the 2013 ESH and ESC Guidelines define hypertension as a systolic blood pressure of ≥ 140 mmHg, and/or a diastolic blood pressure (DBP) of ≥ 90 mmHg [22, 23].

Approximately 90 percent of all people with hypertension are said to have essential hypertension, meaning that the cause of the hypertension is unknown [9]. Well-documented evidence exist for the association between blood pressure and cardiovascular events like stroke, myocardial infarction, organ damage and mortality in both normotensive and hypertensive individuals, and this association is influenced by age [24].

1.2.1 Dyslipidemia and hypertension

‘Dyslipidemic hypertension’ refers to the common occurrence of hypertension together with hypercholesterolemia as risk factors for cardiovascular disease [8]. Dyslipidaemia is a broad term referring to abnormal levels of lipids (hydrophobic fat molecules such as cholesterol and fatty acids) and lipoproteins (molecules consisting of lipids and apolipoproteins) in the blood [25]. Lipid levels are attributable to genetic factors and/or environmental factors, like diet [25].

The assessment of an individual’s serum lipid levels may help to predict the occurrence of future cardiovascular events. According to the ESH/ESC guidelines, the following values are indicators of dyslipidaemia: total cholesterol (TC) >4.9 mmol/L, low-density lipoprotein (LDL-C) >3.0 mmol/L, high-density lipoprotein-cholesterol (HDL-C) <1 mmol/L for men and <1.2 mmol/L for women, and triglyceride (TG) >1.7 mmol/L [23].

High TC, TG and LDL-C levels are associated with the risk for cardiovascular events, while HDL-C is considered the 'good cholesterol', and associated with a cardio-protective role [26]. The role of HDL-C in reverse cholesterol transport is thought to be associated with its protective effect [27]. However, HDL-C may also possess some additional protective properties, including antioxidant, antithrombotic and anti-inflammatory effects [27].

Wang *et al.*, found that lipids associate with arterial stiffness; LDL-C associated with aortic stiffness and HDL-C associated inversely with aortic and peripheral stiffness in their study [28]. The oxidation of LDL-C is regarded as one of the first steps in the development of atherosclerosis [29], however, this process may also play a role in arterial stiffening [30]. Oxidized LDL-C could contribute to arterial stiffening by increasing collagen production in the vascular smooth muscle cells [31] and by limiting the bioavailability of nitric oxide (NO) [32].

In an African population from Nigeria, hypertensive participants had higher TC, TG and LDL-C levels compared to their normotensive counterparts [33]. However, studies from various parts of the world indicate that people of African descent have a favourable lipid profile [34]. A genetic factor (a haplotype of the Y-chromosome) seems to be responsible for a favourable lipid profile in an African population of men residing in the UK [35]. Black South Africans also exhibit a favourable lipid profile, with low TC and high HDL-C levels [36, 37], and are not prone to CHD [38].

1.2.2 Hyperglycemia and hypertension

Hypertension is present in two-thirds of diabetic cases, supporting the notion that high blood pressure develops along with hyperglycaemia [11]. Hypertension and dyslipidaemia are commonly co-morbid in patients with insulin resistance, thus suggesting that insulin resistance may be a mechanism that underlies their co-morbidity [39]. Insulin resistance may be caused by a combination of genetic and lifestyle factors, and is characterised by a deficient uptake of glucose by the tissues at a given concentration of insulin in the plasma [40]. Therefore, plasma glucose is increased due to the lack of insulin-signalling [39]. The pancreatic β -cell increases the production of insulin in response to the hyperglycaemia in the plasma, resulting in hyperinsulinaemia [39].

A fasting plasma glucose concentration of 5.6- 6.9 mmol/L is considered to be a risk factor other than blood pressure (BP) that influences prognosis and contributes to CV risk, while diabetes mellitus is diagnosed by a fasting plasma glucose concentration of ≥ 7.0 mmol/L on two separate measurements [23]. Chronic hyperglycaemia in the blood can be detected by glycosylated haemoglobin, or haemoglobin A1c (HbA1c) [41]. This biomarker detects the severity of the hyperglycaemia, enabling it to evaluate the diabetes-risk, or the risk for other vascular complications [41]. HbA1c differs from fasting plasma glucose in that it is a marker of long-term glucose levels in an individual [42]. Tropeano *et al.*, found that glycemia is a determinant of carotid intima-media thickness (cIMT) in hypertensive hyperglycemic patients [43]. It also seems that patients with abnormal insulin sensitivity

have an increased risk of developing arterial stiffness, even if they are not hypertensive or diabetic [44].

In insulin resistance the metabolism of glucose and lipids are altered, leading to the production of excess aldehydes like glyoxal and methylglyoxal [40]. These aldehydes react non-enzymatically with the amino- and sulfhydryl-groups of amino acids, to form advanced glycation end products (AGEs) [40]. AGEs act on a receptor of AGE (RAGE) [45]. RAGE is a type 1 transmembrane protein, and is part of the immunoglobulin superfamily of cell surface receptors [46]. This receptor is expressed in various tissue types, including the vascular tissue [45]. AGEs are thought to act directly, as well as on receptors to alter the functions of many proteins, including antioxidant and metabolic enzymes, calcium channels, lipoproteins and structural proteins [40]. These alterations are associated with inflammation, endothelial dysfunction and oxidative stress, and are characteristic of hypertension [40].

Plasma AGEs may also play a role in large artery remodelling, independent of blood pressure [47]. Studies have shown that the binding of RAGE to its ligands may result in the generation of oxidative stress and inflammatory responses, thus possibly contributing to the progression of atherosclerosis [48, 49].

The prevalence of diabetes is scarce in rural-dwelling Africans, but rising in Africans from an urban setting, attributed to the increased prevalence of obesity and impaired glucose tolerance in these Africans [50]. Koegelenberg *et al.*, showed that in black South Africans, blood pressure, fasting glucose and HbA1c increased over a period of five years, but no association could be found between the blood pressure and glycemic status of these participants [51].

1.2.3 Renal function and hypertension

Several different studies have suggested that “blood pressure goes with the kidney”, since a normotensive recipient of a kidney genetically programmed for hypertension will develop hypertension [10]. Impaired renal function in a hypertensive individual is a strong predictor of future CV events and death [52]. High blood pressure can damage the kidneys, producing renal destruction and eventually kidney failure [9].

The kidney is the major organ involved in salt homeostasis, and is important in the long-term regulation of blood pressure [10]. The long-term control of arterial BP is dependent on the body fluid volume, which is determined by the balance between fluid intake and output [9].

The glomerular filtration rate (GFR) is indicative of the level of renal function [53]. A decreased GFR indicates impaired renal functioning and will lead to the accumulation of creatinine in the serum [23]. Creatinine is a by-product of muscle metabolism and is cleared from the body almost exclusively by glomerular filtration; therefore, the creatinine clearance rate (CrCl) can also be used to assess the GFR

[9]. The rate of creatinine clearance by the kidneys can be calculated by using equations like the Cockcroft-Gault formula [54]. Therefore, the rate by which creatinine is cleared from the body is indicative of kidney function, although it should not be used as the only measurement of kidney function [53]. The reason for this is that there is a wide range of 'normal' serum creatinine levels, since the concentration of serum creatinine is affected by the production, secretion, and excretion of creatinine through mechanisms other than renal excretion [53]. Therefore, the GFR can decline to half its normal value before the serum creatinine will increase above the normal level. The cut-off values which indicate mild renal damage are as follows: serum creatinine $>132.6 \mu\text{mol/l}$ in men, and $>123.8 \mu\text{mol/l}$ in women [55], or a CrCl of $<60 \text{ mL/min/1.73 m}^2$ [56].

Stimuli like angiotensin II or salt cause a modest blood pressure elevation [57]. This first phase, or pre-hypertension, activates the T-cells of the immune system, bringing about an inflammatory response [57]. This encompasses the infiltration of T-cells and macrophages into the kidneys and vasculature, where they release cytokines and inflammatory mediators [57]. These inflammatory substances cause vascular and renal dysfunction, sodium retention, vasoconstriction, and vascular remodelling along with angiotensin II, catecholamines and sodium. Finally, the second phase, sustained hypertension develops [57].

1.2.4 Health behaviours and hypertension

Certain lifestyle changes are associated with a decrease in blood pressure: dietary salt restriction, weight reduction, regular consumption of fruits and vegetables, regular exercise, moderation of alcohol consumption and smoking cessation [23].

Excessive alcohol intake is associated with both increased blood pressure and risk of stroke [23, 58]. Hypertensive men should not consume more than 20-30g of ethanol per day, and hypertensive women not more than 10-20g per day [23]. However, light to moderate alcohol consumption has been linked to a reduction in the risk for atherosclerosis, and this protective effect may also extend to hypertensive individuals [58].

Evidence indicates that serum hepatic enzymes like aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) are elevated in adults who consume alcohol excessively [59].

Schutte *et al.*, showed that the five-year change from normal blood pressure to hypertension in a group of Africans were independently explained by baseline GGT, a marker of alcohol intake [20]. Furthermore, alcohol intake predicted bSBP and cross-sectional wall area (CSWA) at follow-up [20]. Zatu *et al.*, found that self-reported alcohol-use significantly predicted the change in blood pressure over five years, and that this was not true for the biochemical alcohol markers GGT and percentage carbohydrate deficient transferrin (%CDT) [60].

Smoking is a risk factor for CVD [23] and one of the leading causes of pro-atherosclerotic alterations in the structure and function of the cardiovascular system [61]. Long-term chronic smoking as well as acute smoking is associated with vascular dysfunction [62, 63]. The acute effects of smoking include a rise in blood pressure and heart rate [64]. Hypertension and smoking are risk factors for arterial stiffening, and when combined they exert an additive effect, increasing arterial stiffness even more [65].

Mechanisms by which smoking may exert its effects on the cardiovascular system include the production of superoxide anions, resulting in oxidative damage to the endothelium, decreased synthesis and bioavailability of NO and increased synthesis and bioavailability of endothelin-1 (ET-1) [61].

2. Arterial structure and function

The structure of the arterial wall consists out of three concentric zones: the tunica intima, media and adventitia [66]. The intima and media is separated by the internal elastic lamina, while the media and adventitia is separated by the outer elastic lamina [66]. The vascular endothelium is the predominant constituent of the intima, while the media consists of vascular smooth muscle cells, elastin and collagen [66]. The adventitia is a zone of collagen and some elastin tissues, which merges with the surrounding connective tissue made up of fibroblasts, nerves and small blood vessels [66]. Central and peripheral arteries differ in their composition, with the central arteries being more compliant and largely consisting of elastin, while the peripheral muscular arteries are stiffer and contain more collagen [66]. The thickness of the arterial intima-media layer (IMT) can be defined as the distance between the intima-lumen interface and the media-adventitia interface [67].

The vascular system is structured to perform two main functions: firstly, a dampening function which softens the pressure pulse created when the heart ejects blood, thereby ensuring continuous blood flow and constant organ perfusion [68]. This dampening function is mainly the responsibility of the large arteries [66]. Secondly, the vascular system provides a way for blood to flow throughout the body, while carrying oxygen and nutrients to the different tissues [68]. SBP in the aorta is composed of two parts: the outgoing pressure wave, generated by the contraction of the left ventricle, and the returning pressure wave from reflection sites in the periphery [2]. The reflected wave should return to the heart during diastole, to enhance diastolic filling and coronary perfusion [2]. If the wave returns to the heart during systole, it amplifies the afterload on the heart and increases the central aortic pressure [2]. The magnitude and time of wave-reflection depends on several factors, including: the stiffness of the aorta, the heart rate and the distance of the reflection sites from the heart [2].

The cells present in the arterial wall have the ability to respond to environmental stimuli, including mechanical stretch, vasoactive forces, and inflammatory and thrombotic mediators [69, 70]. The large

elastic arteries can accommodate changes in blood volume under normal physiologic conditions, but with large pressure increases the structure and function of the vessel wall are modified by vascular remodelling [69]. Vascular remodelling encompasses the activation of several intracellular pathways that modulate the migration, proliferation and death of vascular cells, as well as the synthesis and degradation of the extracellular matrix (ECM) [70].

2.1 Atherosclerosis and arteriosclerosis

Progressive, sub clinical arterial wall alterations precede cardiovascular events [71]. Atherosclerosis is a disease of the intima which is characterised by the accumulation of lipids and inflammatory cells, the migration of vascular smooth muscle cells, foam cell development, and the deposition of connective tissue and calcium [72]. Atherosclerotic plaque formation usually occurs in areas of turbulent flow and low shear stress [73].

Atherosclerotic CVD begins as asymptomatic lesions on the arterial walls [74]. The lesions manifest as fatty streaks in the endothelium of the artery [74]. An important event in the development of atherosclerosis is the expression of adhesion molecules, intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) on the endothelial surface [75]. This expression of the adhesion molecules, or endothelial activation, mediates the recruitment and attachment of leukocytes to the arterial wall [76]. Endothelial activation is discussed in detail subsequently in this literature review. An abnormal lipid metabolism and a chronic inflammatory state seems to be the underlying pathophysiology related to the development of atherosclerosis [13]. Furthermore, insulin resistance is associated with atherosclerosis [39]. Increasing levels of glycated hemoglobin, or HbA1c, which is a marker of chronic hyperglycemia in the blood [41], is associated with coronary atherosclerosis [77].

The thickening of the intima-media of the carotid artery is also regarded as an initial step in the development of atherosclerosis [78]. Continuous exposure to cardiovascular risk factors like hypertension, smoking and dyslipidaemia can cause the atherosclerotic lesions to develop into atherosclerotic plaques [74]. These plaques protrude into the arterial lumen, thus narrowing the lumen diameter of the artery and decreasing blood flow to target tissues, resulting in tissue ischemia [74]. Upon complete occlusion of the artery, tissue infarction is certain [74].

Carotid intima-media thickness (cIMT) has been found to be a predictor, as well as an indicator of the extent of coronary artery disease in black South Africans [79]. Atherosclerosis and coronary artery disease do not seem to be highly prevalent in black South Africans, or at least to a lesser extent than arteriosclerosis, hypertension and stroke [38, 80]. However, the westernisation of behaviour taking place in rural and urban areas of South Africa might lead to a greater incidence of coronary artery disease in Africans [20]. Nevertheless, congestive heart failure and stroke are still more common than coronary artery disease in black South Africans [81].

Arterial disease is often thought to refer to coronary atherosclerosis, but the term encompasses more widespread alterations of the arteries which results in arterial stiffening [82]. Arterial stiffness is also referred to as “arteriosclerosis” [82]. Arterial stiffness refers to the rigidity of the arterial wall [83], and the decreased ability of the artery to dilate, constrict and recoil in response to changes in blood pressure [72]. Arterial stiffness is recognised as an independent risk factor for mortality and morbidity relating to the cardiovascular system in hypertensive individuals [84]. As the large arteries stiffen, their dampening ability is lessened, leading to an increase in pulse pressure and a decrease in shear stress [66].

The arterial wall stiffens with age [85]. The most well-reported alterations that accompanies aging in the arterial wall includes an increase in lumen diameter together with wall thickening, and a decrease in the elastic properties of the large elastic arteries [85]. The main structural change associated with aging is the degeneration of the arterial media [85]. This increased arterial stiffness has been attributed to the continuous cycles of distension and elastic recoil of the arterial wall over the life-span, resulting in the fragmentation of elastin and the deposition of collagen [86]. Vascular ageing may be accelerated by cardiovascular risk factors like hypertension, or a genetic background that predisposes to it [87].

Hypertension is a haemodynamic disorder, and therefore exposes the arterial tree to increased pulsatile stress [2]. Circumferential stress is the intraluminal force of a large volume of blood that exerts a distending pressure on the vessel wall, while shear stress is the force exerted due to the friction of the blood on the vessel wall [69]. Shear stress displaces the endothelium and the inner layers of the arterial wall in the direction of the flow [88], and it also increases endothelial nitric oxide synthase expression and decreases ET-1 expression [69]. Therefore, shear stress is generally thought to be anti-atherosclerotic [89].

The elastic properties of the arterial wall are pressure-dependent [24], meaning arterial stiffness is increased at higher pressures without any structural change [90]. At low arterial pressures, the wall stress is supported by the compliant elastin fibres, while at high arterial pressures the strain shifts to the stiffer collagen fibres [66]. Therefore, systolic, diastolic and pulse pressures are associated with the structural characteristics of the elastic arteries [24]. Sustained hypertension may accelerate changes in the structure of the arterial wall [91]. Indeed, several studies have shown that central arterial stiffness is increased in hypertensive individuals when compared to normotensive individuals [92, 93]. Once structural change has taken place, blood pressure reduction may not have a large impact on arterial stiffness [91].

During the hypertensive state, the blood vessel wall is constantly subjected to high strain [70]. The arteries resist stretch the more they are subjected to it, theoretically because of the transferral of strain from the elastin to the stiffer collagen fibres [94]. Therefore, the resistance to stretch under low

pressure is mainly due to elastin, under physiologic conditions due to both elastin and collagen, and at high pressures mainly due to collagen [94].

The smaller arteries and arterioles undergo vascular smooth muscle cell (VSMC) hyperplasia, while the larger arteries undergo VSMC hypertrophy and ECM reorganisation in order to resist the high strain [70]. Vascular hypertrophy is visible as an increase in the arterial cross-sectional wall area (CSWA) [95]. In other words, arterial CSWA is a measure of the arterial volume or mass [96]. While vascular remodelling helps the blood vessel to resist the high strain and pressure, it also increases the stiffness of the vessel, leading to a decreased ability of the vessel to dampen the pulsatile nature of blood ejection from the heart [70]. Arterial remodelling may represent a compensatory mechanism by which the blood vessel can resist strain during blood pressure increases, but it also sets the stage for a progressive change in the vessel wall shape and composition, ultimately leading to several clinical complications like arterial fibrosis and stiffening, which is characteristic of hypertension [69, 70].

Arterial remodelling is driven by a variety of processes [97]. These include the fracture and degradation of elastin fibres, collagen deposition, the switching of the VSMC phenotype from the normal contractile phenotype to a migratory-proliferative phenotype, a secretory phenotype or an osteogenic phenotype [97, 98]. Furthermore, both VSMC phenotype switching and the degradation of the extracellular matrix (EMC) result in accelerated vascular calcification [97].

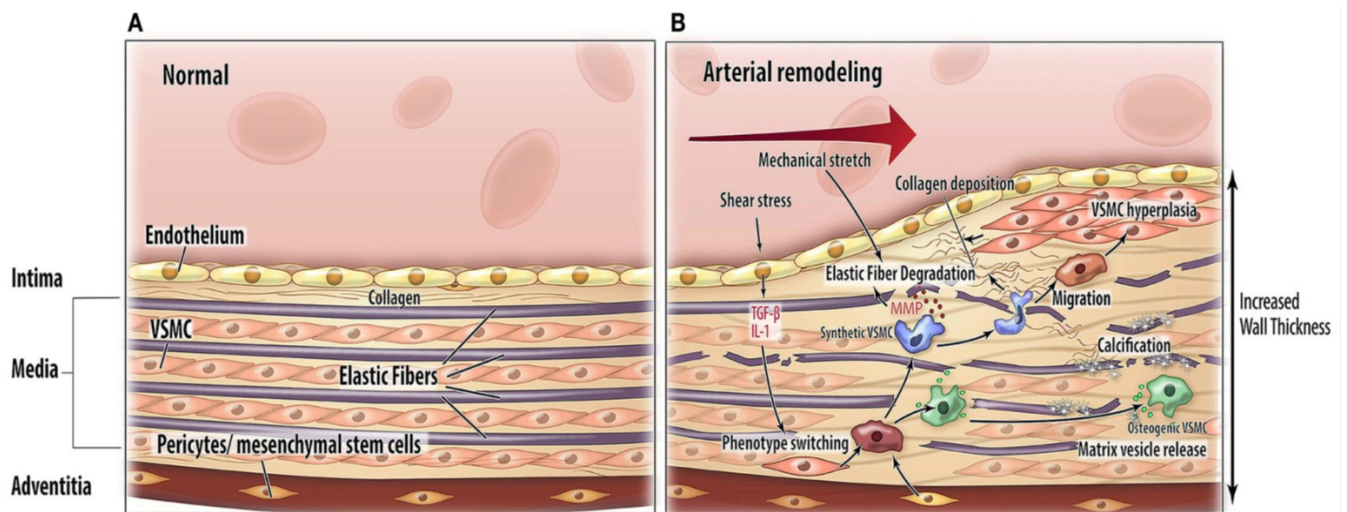


Figure 1. Pathophysiological mechanisms of arterial remodelling, Van Varik *et al.* [97].

A: The normal arterial wall. B: The arterial wall undergoing remodelling. Elastin fibre degradation, collagen deposition, VSMC phenotype switching and calcium deposition leads to the adaptation and thickening of the arterial wall.

Arterial stiffness and endothelial function stimulate the development of an atherosclerotic plaque, which may contribute to arterial stiffening, thus creating a link between atherosclerosis and arteriosclerosis [97].

When comparing a South African population of blacks and whites, it seems that arterial stiffness is associated with ageing in whites, but in blacks it associates more with blood pressure [99]. In addition, the muscular arteries of blacks were found to be stiffened even at a young age [99].

2.2 Measures of arterial function

2.2.1 Pulse pressure

Pulse pressure (PP) is calculated as the difference between the systolic and diastolic blood pressure [100]. PP depends on the cardiac output, the stiffness of the large arteries and pulse wave reflection [83]. A rise in PP is the major cause of the age-related increase in hypertension, and it has been attributed to arterial stiffening [72]. It is one of the simplest measures of arterial stiffness, but PP alone cannot be used to measure arterial stiffness accurately [83].

2.2.2 Pulse wave velocity

Pulse wave velocity (PWV) is a marker of arterial stiffness [72]. It is considered the gold standard measure of arterial stiffness [101]. PWV is the velocity by which the forward pressure pulse, created by the heart contracting and ejecting blood, travels along the arterial tree [72]. The velocity of the forward-travelling wave is influenced by the stiffness of the arterial walls; the stiffer the walls, the higher the velocity [83]. PWV is measured by determining the time that the pressure pulse takes to travel between two points in the body [72], and the gold standard for measuring PWV dictates that these two points are the carotid and femoral arteries [17]. However, other measurements of PWV also exist, including the carotid-dorsalis-pedis PWV, or the ankle-brachial PWV, both of which are more representative of the PWV in the peripheral arteries [102]

A carotid-femoral PWV threshold value of 10 m/s is suggested to be indicative of aortic changes in the middle-aged hypertensive patient [103]. According to Cecelja and Chowienczyk, PWV shows no association with the classical atherosclerosis risk factors, including smoking, gender and lipids [104].

One study found that PWV was 24% higher and carotid distensibility (CD) 47% lower in hypertensive than in normotensive subjects, supporting the notion that blood pressure and arterial stiffness are important determinants of each other [93]. Furthermore, it is suggested that not only the blood pressure, but also the structure of the arterial wall contributes to the level of stiffness in hypertensive individuals [93]. This statement is supported by the fact that this study found no significant reduction in PWV in hypertensive participants, even though their mean arterial pressure (MAP) had been reduced by the administration of nitroglycerin to a pressure similar to that of their normotensive counterparts [93].

2.3 Measures of carotid structure and function

Arterial stiffness can be measured at several arterial segments as well and by using different techniques [18]. Various measurement-sites are necessary, as stiffness differs along the arterial tree

[17, 18]. Recently, van Sloten *et al.*, showed that the stiffness of the carotid artery is associated with incident CV events and mortality [18]. The Atherosclerosis Risk in Communities (ARIC) study indicated that stiffening of the carotid artery associates more with cerebrovascular disease (stroke) than CHD, possibly because stiffness in this artery leads to a higher pressure-load on the brain [105].

Carotid atherosclerosis commonly occurs in the bulb region of the carotid artery, an area with low shear stress [37], while intimal thickening in the more proximal segments of the carotid artery could be the result of high shear stress, as in hypertension [37].

2.3.1 Carotid intima-media thickness

A widely accepted surrogate marker for the prediction of cardiovascular events involves the measurement of the carotid intima-media thickness (cIMT) [73]. CIMT is measured with B-mode ultrasound [73]. According to Bergmann, IMT should be regarded as a form of end-organ disease that is a precursor of atherosclerosis [106]. The measurement of IMT reflects not only early atherosclerosis, but also non-atherosclerotic compensatory remodelling of the arterial wall in response to hypertension [106] and shear stress, such as medial hypertrophy [71, 74]. Furthermore, age-related thickening of the intima and media of the common carotid arteries may also occur in the absence of atherosclerosis [73]. Carotid wall thickening, or an IMT of >0.9 mm is considered to be indicative of asymptomatic organ damage [23].

A variety of different techniques can be used to measure arterial stiffness, but presently these measurements are mostly utilised in a research setting rather than in clinical practice [83].

2.3.2 Central blood pressure

Measuring central BP in hypertensive patients is receiving interest because of its predictive value for CV events and the different effect that antihypertensive drugs have on central BP and brachial BP [23]. SBP is higher at the level of the radial arteries than the central arteries, but DBP and MAP differ only slightly [87]. Roman and colleagues demonstrated that central aortic pressure has a stronger relation to carotid hypertrophy and atherosclerosis than brachial blood pressure, thus supporting the theory that central pressures reflect the workload on the heart more accurately [107].

Central pulse pressure (cPP) is a reflection of the workload on the left ventricle, as well as the coronary and the cerebral vessels [108]. The consequence of arterial stiffening and therefore an increased cPP is explained by the effect on ventricular afterload [72]. Elevated central SBP can predispose to left ventricular hypertrophy [109], which is an independent predictor of cardiovascular death [110]. On the other hand, a decrease in DBP reduces coronary perfusion, leading to ischemia of the heart [111].

2.3.3 Young's elastic modulus

Young's elastic modulus represents the stiffness of the arterial wall at operating pressure [18]. It is calculated by a formula which includes the carotid diastolic lumen diameter, IMT and distensibility [112]. Bussy *et al.* found that Young's elastic modulus of the carotid artery was higher in young hypertensive individuals when compared to normotensive individuals, but it did not differ between middle-aged and older hypertensive and normotensive individuals [113]. These results point to an increased intrinsic stiffness of the arterial wall material in younger patients with essential hypertension [113]. Furthermore, these results indicate that adaptive mechanisms are at work in middle-aged and older hypertensives, as the intrinsic wall properties were unchanged compared with their counterparts [113].

2.3.4 Carotid distensibility

Carotid distensibility is a risk factor for CDV [114-116], and recently, Van Sloten *et al.*, showed that carotid stiffness indices are independently associated with CV events and mortality independent of CV risk factors [18].

Distensibility refers to the ability of the artery to expand and recoil in response to the pulsating flow of blood in the large arteries [117]. The CD gives information about the elastic properties of the arterial wall as a hollow structure [118], however, CD is only an estimation of the mean strain on the artery since the soft tissue around the artery also responds to a change in volume [119]. CD is calculated with a formula which includes the minimum and maximum lumen diameters, the difference between them and the central pulse pressure [120].

3. Inflammation

3.1 Inflammation and hypertension

The immune system plays the role of protecting the body from pathogens and toxins [121]. It has been suggested that an imbalance in the immune system leads to inflammation and contributes to hypertension [121, 122]. Hypertension may also play a role in inducing a pro-inflammatory state in the blood vessels, by damaging the arterial wall through high circumferential and shear stress [69].

The term 'low-grade inflammation' (LGI) has been used in recent years to describe a mechanism leading to disease in healthy individuals [123]. It is characterised by increased levels of pro-inflammatory cytokines and immune activation markers, as well as a raised white blood cell count [123]. These mediators may impair the ability of the endothelium to release NO, a vasodilating factor, resulting in endothelial dysfunction and at a later stage, hypertension [124]. Currently, C-reactive protein (CRP) is the gold standard for the measurement of LGI [123].

CRP is an acute phase protein produced by the liver in response to interleukin-6 (IL-6) and interleukin-1 β (IL-1 β) [124]. CRP is a non-specific marker of inflammation, in other words, a marker

of low-grade systemic inflammation in the body [125]. CRP appears to be a good marker of metabolic inflammation, associating with body mass index (BMI) and lipids [123]. In healthy subjects the CRP concentration is usually less than 1 µg/mL [75], and an elevated CRP concentration is associated with an increased risk for cardiovascular disease [124], diabetes [126] and certain types of cancer [127]. Hypertension is also associated with higher CRP-levels [6]. Lakoski *et al.*, found that hypertensive African-Americans had a 10-15% higher CRP-level than their normotensive counterparts [5]. This supports the idea that sub clinical inflammation, as indicated by higher CRP-levels, may be one of the mechanisms contributing to the higher risk for adverse events like MI in hypertensive patients [5].

IL-6, a pleiotropic cytokine, is synthesised by a wide range of cells, including immune, endothelial, smooth muscle and ischemic heart cells [128]. Its physiologic activity includes the mediation of a pro-inflammatory reactions, as well as a state of cytoprotection [128]. Chamarthi and colleagues found that the baseline IL-6 were higher in hypertensive than in normotensive participants [6]. Furthermore, it was shown that the concentration of IL-6 increased significantly after infusion of exogenous angiotensin (Ang II), supporting the theory that IL-6 is involved in the pathogenesis of Ang II-mediated hypertension [6]. IL-6 increases the production of interleukin-17 (IL-17) [129], which is thought to stimulate chemokine release and chemotaxis of other inflammatory cells to the vasculature, contributing to vascular pathology in the setting of hypertension [129].

3.2 Inflammation, arterial structure and function

Inflammatory diseases are associated with atherosclerosis [130], in fact, atherosclerosis itself is an inflammatory disease [13, 131]. Inflammatory cells like leukocytes are present in the atherosclerotic arterial wall [131]. Macrophages can amplify low-density lipoprotein cholesterol (LDL-C) modification and decrease the stability of advanced atherosclerotic plaques through the secretion of various cytokines [13]. Evidence suggests that the modification of LDL-C by the oxidation process is the stimulus for the inflammatory process that underlies atherosclerosis [13]. CRP is significantly associated with both carotid and femoral IMT [132]. Furthermore, CRP is associated with carotid plaques in a dyslipidemic population [133].

Accumulating evidence indicate that acute and chronic inflammation is associated with stiffness of the large arteries [134]. An increase in circulating inflammatory mediators may increase leukocyte infiltration into the arteries, and alter the VSMC phenotype [134]. The released matrix metalloproteinases (MMP) from these cell types can degrade elastin, pointing to one possible mechanism by which inflammation can lead to arterial stiffness [134, 135]. Furthermore, it is possible that the state of hydration and the proteoglycan composition is different in inflamed arteries compared to normal arteries, thus altering the biomechanical properties of large arteries [134].

Although CRP is a marker of inflammation, it is possible that it may also be a role-player in the process of arterial stiffening [136]. Long-term inflammation might play a role in the stiffening of the aorta [101]. This statement is supported by the findings of McEniery *et al.*, which shows that after twenty years, baseline- and follow-up CRP-levels were strongly related to aortic PWV [101]. Inflammation in the arterial wall of large arteries may exert its effects through decreasing the elasticity and increasing the stiffness of the artery [124]. The proposed role that inflammation plays in the stiffening process is by altering the composition of the arterial wall [137]. Inflammation increases the expression of vascular adhesion molecules [75], chemokines, growth factors, heat shock proteins, endothelin-1 and angiotensin-II [124]. These factors initiate a cascade of other processes, like eventual vascular fibrosis in the case of angiotensin-II-initiated pathways [136], possibly leading to arterial stiffness.

4. Endothelial activation

Endothelial function plays a critical role in the maintenance of vascular homeostasis [138]. The endothelium is constantly subjected to mechanical and biochemical stresses, for which it has a range of defence mechanisms [139]. However, various factors associated with cardiovascular disease can overwhelm these defence mechanisms and cause structural damage, thus impairing the function of the endothelium [139]. Endothelial dysfunction is characterised by alterations from its normal vasorelaxant, anti-coagulant, anti-platelet phenotype to one that is vasoconstrictive, procoagulant and platelet-activating [138].

The endothelium expresses several cell adhesion molecules, including intracellular-adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin and P-selectin [139]. Endothelial activation is defined as the expression of cell surface adhesion molecules, such as ICAM-1 and VCAM-1, on the endothelium [76]. ICAM-1 and VCAM-1 have molecular structures similar to that of immunoglobulins [138]. These molecules function as endothelial ligands for integrins that are expressed on leukocytes and platelets; therefore they facilitate the binding of leukocytes and platelets to the endothelial surface [138]. However, ICAM-1 and VCAM-1 are not endothelial specific; they are also expressed on several different cell types, including VSMC and monocytes [138]. Endothelial activation may be induced by proinflammatory cytokines (for example IL-6), hypercholesterolemia or smoking [76, 138]. Endothelial activation may also be induced by oxidised LDL [138].

A study that exposed human umbilical vein endothelial cell (HUVECs) to cyclic stress caused the migration of zyxin (a zinc finger protein), which acts to coordinate the expression of pro-inflammatory genes such as IL-8, VCAM and ICAM [89]. Continuous circumferential stress also increases the expression of the IL-6 gene through the NF κ B pathway in HUVECs [140]. The up-regulation of these genes indicate that mechanical stress, such as in hypertension [70], may induce an inflammatory response in endothelial cells resulting in the activation of adhesion molecules (ICAM,

VCAM) and cytokines leading to the increased permeability of the vascular endothelium [69]. Endothelial activation may also facilitate the recruitment and adhesion of leukocytes to the arterial wall [76].

4.1 Endothelial activation and hypertension

Endothelial activation may be an important mediator of hypertensive vascular injury [141]. In individuals with essential hypertension, IL-6, CRP and tumour necrosis factor- α (TNF- α) are increased and related to oxidative stress and endothelial activation [142]. NO donors decrease the expression of ICAM, VCAM and E-selectin in response to several pro-inflammatory cytokines [76].

At a concentration of 5 $\mu\text{g/mL}$, CRP has a pro-inflammatory effect, inducing high levels of adhesion cell expression (ICAM and VCAM) by human endothelial cells [75]. When the levels of endothelial activation markers increase they facilitate the migration and adhesion of inflammatory cells to the endothelium [138].

Shear stress is essential for endothelial function and pathophysiology [143]. The current perspective states that areas with laminar flow, high shear stress and low bidirectional oscillatory flow are atheroprotective, while areas with disturbed flow, reverse flow and low shear stress are more prone to the development of atherosclerosis [143].

Preston *et al.* demonstrated that the markers of endothelial activation are different between hypertensive and normotensive subjects; ICAM-1, VCAM-1 and a marker of vascular injury, the von Willebrand factor (vWF), were significantly higher in hypertensives than in normotensives [4]. This supports the finding that hypertension is associated with endothelial activation [4]. However, the authors did not find a difference in the markers of endothelial activation between patients with mild hypertension and with severe uncontrolled hypertension [4]. It seems that the expression of ICAM-1 and VCAM-1 are elevated even with mild increases in blood pressure, possibly indicating a low threshold for maximal endothelial activation, or that the injured vasculature in severe hypertension is unable to produce any more adhesion molecules [4]. The vascular injury associated with severe hypertension may depend more on other factors, like structural alterations of the vessel wall, than the activation of the endothelium [4].

Furthermore, other factors may be more important in activating the endothelium than hypertension *per se* [4]. Conditions commonly associated with hypertension like diabetes [11], hyperlipidaemia [8], smoking [64], and atherosclerosis are also associated with higher levels of endothelial activation markers, and the effect of hypertension may be less important when these concomitant conditions are also taken into account [4]. Therefore, the level of endothelial activation markers may depend more on the metabolic factors in the hypertensive environment than the level of blood pressure [141].

Endothelial microparticles (EMP) are small vesicles that are released by damaged or activated endothelial cells, and these particles are more likely to be directly influenced by hypertension [138].

Interestingly, Miller *et al.*, found that in a population from England, people of African origin have lower levels of ICAM, VCAM and P-selectin than people of European descent [144].

References

1. White PD. Heart disease. 1st ed. New York: McMillan; 1931.
2. Messerli FH, Williams B, Ritz E. Essential hypertension. *The Lancet*. 2007;370:591-603.
3. Ventura HO, Taler SJ, Strobeck JE. Hypertension as a hemodynamic disease: The role of impedance cardiography in diagnostic, prognostic, and therapeutic decision making. *Am J Hypertens*. 2005;18:26-43.
4. Preston RA, Ledford M, Materson BJ, Baltodano NM, Meman A, Alonso A. Effects of severe, uncontrolled hypertension on endothelial activation: Soluble vascular cell adhesion molecule-1, soluble intercellular adhesion molecule-1 and von willebrand factor. *J Hypertens*. 2002;20:871-7.
5. Lakoski SG, Cushman M, Palmas W, Blumenthal R, D'Agostino RB, Herrington DM. The relationship between blood pressure and C-reactive protein in the multi-ethnic study of atherosclerosis (MESA). *J Am Coll Cardiol*. 2005;46:1869-74.
6. Chamarthi B, Williams GH, Ricchiuti V, Srikumar N, Hopkins PN, Luther JM, et al. Inflammation and hypertension: The interplay of interleukin-6, dietary sodium and the renin-angiotensin system in humans. *Am J Hypertens*. 2011;24:13.
7. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. *Biomed Res Int*. 2014;2014:406960.
8. De Bacquer D, De Backer G. The prevalence of concomitant hypertension and hypercholesterolaemia in the general population. *Int J Cardiol*. 2006;110:217-23.
9. Hall JE. Guyton and Hall textbook of medical physiology. 12th ed. Philadelphia: Saunders Elsevier; 2011.
10. Pinto V, Pinho MJ, Soares-da-Silva P. Renal amino acid transport systems and essential hypertension. *The FASEB Journal*. 2013;27:2927-38.
11. Ferrannini E, Cushman WC. Diabetes and hypertension: The bad companions. *The Lancet*. 2012;380:601-10.
12. Alexander RW. Hypertension and the pathogenesis of atherosclerosis: Oxidative stress and the mediation of arterial inflammatory response: A new perspective. *Hypertension*. 1995;25:155-61.

13. Weber C, Noels H. Atherosclerosis: Current pathogenesis and therapeutic options. *Nature Med.* 2011;17:1410-22.
14. Li JJ, Chen JL. Inflammation may be a bridge connecting hypertension and atherosclerosis. *Med Hypotheses.* 2005;64:925-9.
15. Wagenseil JE, Mecham RP. Elastin in large artery stiffness and hypertension. *J Cardiovasc Transl Res.* 2012;5:246-73.
16. Payne RA, Wilkinson IB, Web DJ. Arterial stiffness and hypertension: Emerging concepts. *Hypertension.* 2010;55:9-14.
17. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. *Eur Heart J.* 2006;27:2588-605.
18. Van Sloten TT, Schram MT, van den Hurk K, Dekker JM, Nijpels G, Henry RMA, et al. Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality. *J Am Coll Cardiol.* 2014;63:1739-47.
19. Shisana O, Labadarios D, Rehle T, Simbayi L, Zuma K, Dhansay A, et al. South African National Health and Nutrition examination survey. Cape Town: HSRC Press; 2013.
20. Schutte AE, Schutte R, Huisman HW, Van Rooyen JM, Fourie CMT, Smith W, et al. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in black South Africans? A 5-year prospective study. *Int J Epidemiol.* 2012;41:1114-23.
21. Giles TD, Berk BC, Black HR, Cohn JN, Kostis JB, Izzo JL, et al. Expanding the definition and classification of hypertension. *J Clin Hypertens.* 2005;7:505-12.
22. Seedat YK, Rayner BL. The South African hypertension guidelines 2011. *S Afr Med J.* 2012;102:57-84.
23. Mancia G, Fagard R, Narkiewicz K, Rredon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC guidelines for the management of hypertension. *J Hypertens.* 2013;31:1281-357.
24. Nichols WW. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. *Am J Hypertens.* 2005;18:3S-10S.

25. Peterson AL, McBride PE. A review of guidelines for dyslipidemia in children and adolescents. *WMJ*. 2012;111:274-81.
26. Toth P. The "good-cholesterol"- high density lipoprotein. *Circulation*. 2005;111:e89-91.
27. Taskinen M. LDL-cholesterol, HDL-cholesterol or triglycerides- which is the culprit? *Diabetes Res Clin Pract*. 2003;61:S19-26.
28. Wang F, Ye P, Luo L, Xiao W, Qi L, Bian S, et al. Association of serum lipids with arterial stiffness in a population-based study in Beijing. *Eur J Clin Invest*. 2011;41:929-36.
29. Stocker R, Keaney JF. Role of oxidative modifications in atherosclerosis. *Physiol Rev*. 2004;84:1381-478.
30. Brinkley TE, Nicklas BJ, Kanaya AM, Satterfield S, Lakatta EG, Simonsick EM, et al. Plasma oxidized low-density lipoprotein levels and arterial stiffness in older adults: The health, aging, and body composition study. *Hypertension*. 2009;53:846-52.
31. Jimi S, Saku K, Uesugi N, Sakata N, Takebayashi S. Oxidized low-density lipoprotein stimulates collagen production in arterial smooth muscle cells. *Atherosclerosis*. 1995;116:15-26.
32. Hein TW, Liao JC, Kuo L. oxLDL specifically impairs endothelium-dependent NO-mediated dilation of coronary arterioles. *Am J Physiol*. 2000;278:H175-83.
33. Osuji CU, Omejua EG, Onwubuya EI, Ahaneku GI. Serum lipid profile of newly diagnosed hypertensive patients in Nnewi, South-east Nigeria. *Int J Hypertens*. 2012;2012:7.
34. Agyemang C, Addo J, Bhopal R, De Graft Aikins A, Stronks K. Cardiovascular disease, diabetes and established risk factors among populations of Sub-Saharan African descent in Europe: A literature review. *Global Health*. 2009;5:7.
35. Russo P, Siani A, Miller MA, Karanam S, Esposito T, Gianfrancesco F, et al. Genetic variants of Y chromosome are associated with a protective lipid profile in black men. *Arterioscler Thromb Vasc Biol*. 2008;28:1569-74.
36. Vorster HH. The emergence of cardiovascular disease during urbanisation of Africans. *Public Health Nutr*. 2002;5:239-43.

37. Fowkes FGR, Thorogood M, Connor MP, Lewando-Hundt G, Tzoulaki I, Tollman SM. Distribution of a subclinical marker of cardiovascular risk, the ankle brachial index, in a rural African population: SASPI study. *Eur J Cardiovasc Prev Rehabil*. 2006;13:964-9.
38. Silwa K, Wilkinson D, Hansen C, Ntyintyane L, Tibazarwa K, Becker A, et al. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto study): A cohort study. *Lancet*. 2008;371:915-22.
39. Semenkovich CF. Insulin resistance and atherosclerosis. *J Clin Invest*. 2006;116:1813-22.
40. Vasdev S, Gill V, Singal P. Role of advanced glycation end products in hypertension and atherosclerosis: Therapeutic implications. *Clin Biochem Biophys*. 2007;49:48-63.
41. Lyons TJ, Basu A. Biomarkers in diabetes: Hemoglobin A1c, vascular and tissue markers. *Transl Res*. 2012;159:303-12.
42. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. *J Clin Endocrinol Metab*. 2008;93:2447-53.
43. Tropeano A, Boutouyrie P, Katsahian S, Laloux B, Laurent S. Glucose level is a major determinant of carotid intima-media thickness in patients with hypertension and hyperglycemia. *J Hypertens*. 2004;22:2153-60.
44. Meng C, Sun M, Wang Z, Fu Q, Cao M, Zhu Z, et al. Insulin sensitivity and beta-cell function are associated with arterial stiffness in individuals without hypertension. *J Diabetes Res*. 2013;2013:151675.
45. Baumann M. Role of advanced glycation end products in hypertension and cardiovascular risk: Human studies. *J Am Soc Hypertens*. 2012;6:427-35.
46. González I, Romero J, Rodriguez BL, Perez-Castro R, Rojas A. The immunobiology of the receptor of advanced glycation end products: Trends and challenges. *Immunobiology*. 2013;218:790-7.
47. McNulty M, Mahmud A, Feely J. Advanced glycation end-products and arterial stiffness in hypertension. *Am J Hypertens*. 2007;20:242-7.

48. Lindsey JB, Cipollone F, Abdullah SM, McGuire DK. Receptor for advanced glycation end-products (RAGE) and soluble RAGE (sRAGE): Cardiovascular implications. *Diab Vasc Dis Res*. 2009;6:7-14.
49. Herold K, Moser B, Chen Y, Zeng S, Yan SF, Ramasamy R, et al. Receptor for advanced glycation end products (RAGE) in a dash to the rescue: Inflammatory signals gone awry in the primal response to stress. *J Leukocyte Biol*. 2007;82:204-12.
50. Peer N, Steyn K, Lombard C, Gwebushe N, Levitt N. A high burden of hypertension in the urban black population of Cape Town: The cardiovascular risk in black South Africans (CRIBSA) study. *PLoS ONE*. 2013;8:e78567.
51. Koegelenberg ASE, Kruger A, Towers GW, Schutte AE. Lack of association of glycated haemoglobin with blood pressure and subclinical atherosclerosis in black South Africans: A five-year prospective study. *JEMDSA*. 2013;18:148-53.
52. De Leeuw PW, Ruilope LM, Palmer CR, Brown MJ, Castaigne A, Mancina G, et al. Clinical significance of renal function in hypertensive patients at high risk. *Arch Intern Med*. 2004;164:2459-64.
53. Johnson CA, Levey AS, Coresh J, Levin A, Lau J, Eknoyan G. Clinical practice guidelines for chronic kidney disease in adults: Part II. glomerular filtration rate, proteinuria and other markers. *Am Fam Physician*. 2004;70:1091-7.
54. Cockcroft D, Gault M. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16:31-41.
55. Ruilope LM. The kidney as a sensor for cardiovascular risk in essential hypertension. *J Am Soc Nephrol*. 2002;13:S165-8.
56. Levey AS, Eckardt K, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: A position statement from kidney disease: Improving global outcomes (KDIGO). *Kidney Int*. 2005;67:2089-100.
57. Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, et al. Inflammation, immunity and hypertension. *Hypertension*. 2011;57:132-40.
58. Puddey IB, Beilin LJ. Alcohol is bad for blood pressure. *Clin Exp Pharmacol Physiol*. 2006;33:837-52.

59. Tsai J, Ford ES, Li C, Zhao G. Past and current alcohol consumption patterns and elevations in serum hepatic enzymes among US adults. *Addict Behav.* 2012;37:78-84.
60. Zatu MC, van Rooyen JM, Loots D, Wentzel-Viljoenb E, Greef M, Schutte AE. Self-reported alcohol intake is a better estimate of 5-year change in blood pressure than biochemical markers in low-resource settings: The PURE study. *J Hypertens.* 2014;32:749-55.
61. Rahman MM, Laher L. Structural and functional alterations of blood vessels caused by cigarette smoking: An overview of the molecular mechanisms. *Curr Vasc Pharmacol.* 2007;5:276-92.
62. Weil BR, Byrne SA, Baynard T, Kanaley JA. Vascular function is impaired early after the initiation of chronic cigarette smoking. *Artery Research.* 2009;3:111-4.
63. Esen AM, Barutcu I, Acar M, Degirmenci B, Kaya D, Turkmen M, et al. Effect of smoking on endothelial function and wall thickness of brachial artery. *Circ J.* 2004;68:1123-6.
64. Bang LE, Buttenschon L, Kristensen KS, Svendsen TL. Do we undertreat hypertensive smokers? A comparison between smoking and non-smoking hypertensives. *Blood Press Monit.* 2000;5:271-4.
65. Scallan C, Doonan RJ, Daskalopoulou SS. The combined effect of hypertension and smoking on arterial stiffness. *Clin Exp Hypertens.* 2010;32:319-28.
66. Nichols WW, O'Rourke MF. McDonald's blood flow in arteries: Theoretic, experimental and clinical principles. 5th ed. London: Edward Arnold; 2005.
67. Wikstrand J. Methodological considerations of ultrasound measurement of carotid artery intima-media thickness and lumen diameter. *Clin Physiol Funct Imaging.* 2007;27:341-5.
68. Steppan J, Barodka V, Berkowitz DE, Nyhan D. Vascular stiffness and increased pulse pressure in the aging cardiovascular system. *Cardiol Res Pract.* 2011;2011:8.
69. Anwar MA, Shalboub J, Lim CS, Gohel MS, Davies AH. The effect of pressure-induced mechanical stretch on vascular wall differential gene expression. *J Vasc Res.* 2012;49:463-78.
70. Lemariéa CA, Tharaux P, Lehoux S. Extracellular matrix alterations in hypertensive vascular remodelling. *J Mol Cell Cardiol.* 2010;48:433-9.
71. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004–2006–2011). *Cerebrovasc Dis.* 2012;34:290-6.

72. Cecelja M, Chowienczyk P. Role of arterial stiffness in cardiovascular disease. *J R Soc Med Cardiovasc Dis.* 2012;1:11.
73. Finn AV, Kolodgie FD, Virmani R. Correlation between carotid intimal/medial thickness and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2010;30:177-81.
74. Robertson CM, Fowkes FGR, Price JF. Carotid intima-media thickness and the prediction of vascular events. *Vasc Med.* 2012;17:239-48.
75. Pasceri V, Willerson JT, Edward THY. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation.* 2000;102:2165-8.
76. Liao JK. Linking endothelial dysfunction with endothelial activation. *J Clin Invest.* 2013;123:540-1.
77. Rivera JJ, Choi EK, Yoon YE, Chun EJ, Choi SI, Nasir K, et al. Association between increasing levels of hemoglobin A1c and coronary atherosclerosis in asymptomatic individuals without diabetes mellitus. *Coron Artery Dis.* 2010;21:157-63.
78. Koenig W, Khuseyinova N. Biomarkers of atherosclerotic plaque instability and rupture. *Arterioscler Thromb Vasc Biol.* 2007;27:15-26.
79. Holland Z, Ntintyane L, Gill G, Raal F. Carotid intima-media thickness is a predictor of coronary artery disease in south african black patients. *Cardiovasc J Afr.* 2009;20:237-9.
80. Opie LH, Seedat YK. Hypertension in Sub-saharan African populations. *Circulation.* 2005;112:3562-8.
81. Mayosi BM, Flisher AJ, Lalloo UG, Sitas F, Tollman SM, Bradshaw D. The health burden of non-communicable diseases in South Africa. *Lancet.* 2009;374:14.
82. London GM. Cardiovascular calcifications in uremic patients: Clinical impact on cardiovascular function. *J Am Soc Nephrol.* 2003;14:S305-9.
83. Mackenzie IS, Wilkinson IB, Cockcroft JR. Assessment of arterial stiffness in clinical practice. *Q J Med.* 2002;95:67-74.
84. Mahmud A, Feely J. Arterial stiffness and the renin-angiotensin-aldosterone system. *J Renin Angiotensin Aldosterone Syst.* 2004;5:102-8.

85. Lee H, Oh B. Aging and arterial stiffness. *Circ J*. 2010;74:2257-62.
86. O'Rourke MF, Nichols WW. Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. *Hypertension*. 2005;45:652-8.
87. Protogerou AD, Papaioannou TG, Blacher J, Papamichael CM, Lekakis JP, Safar ME. Central blood pressures: Do we need them in the management of cardiovascular disease? Is it a feasible therapeutic target? *J Hypertens*. 2007;25:265-72.
88. Bots ML, Hofman A, Grobbee DE. Increased common carotid intima-media thickness: Adaptive response or a reflection of atherosclerosis? Findings from the Rotterdam study. *Stroke*. 1997;28:2442-7.
89. Wójtowicz A, Babu SS, Li L, Gretz N, Hecker M, Cattaruzza M. Zyxin mediation of stress-induced gene expression in human endothelial cells. *Circ Res*. 2010;107:898-902.
90. Bergel DH. The static elastic properties of the arterial wall. *J Physiol*. 1961;156:446-57.
91. Benetos A, Adamopoulos C, Bureau J, Temmar M, Labat C, Bean K, et al. Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. *Circulation*. 2002;105:1202-7.
92. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension*. 2001;37:1236-41.
93. Stewart AD, Jiang B, Millasseau SC, Ritter JM, Chowienzyk PJ. Acute reduction of blood pressure by nitroglycerin does not normalize large artery stiffness in essential hypertension. *Hypertension*. 2006;48:404-10.
94. Roach MR, Burton AC. The reason for the shape of the distensibility curves of arteries. *Can J Biochem Physiol*. 1957;35:681-90.
95. Faraci FM. Surviving the remodel: The impact of hypertension during pregnancy. *Hypertension*. 2008;51:995-6.
96. Roman MJ, Kizer JR, Best LG, Lee ET, Howard BV, Shara NM, et al. Vascular biomarkers in the prediction of clinical cardiovascular disease: The strong heart study. *Hypertension*. 2012;59:29-35.

97. Van Varik BJ, Rennenberg RJMW, Reutelingsperger CP, Kroon AA, de Leeuw PW, Schurgers LJ. Mechanisms of arterial remodelling: Lessons from genetic diseases. *Front Genet.* 2012;3:290.
98. Gerthoffer WT. Mechanisms of vascular smooth muscle cell migration. *Circ Res.* 2007;100:607-21.
99. Schutte AE, Huisman HW, Schutte R, Van Rooyen JM, Malan L, Malan NT, et al. Arterial stiffness profiles: Investigating various sections of the arterial tree of african and caucasian people. *Clin Exp Hypertens.* 2011;33:511-7.
100. White WB. Systolic versus diastolic blood pressure versus pulse pressure. *Curr Cardiol Rep.* 2002;4:463-7.
101. McEniery CM, Spratt M, Munnery M, Yarnell J, Lowe GD, Rumley A, et al. An analysis of prospective risk factors for aortic stiffness in men: 20-year follow-up from the caerphilly prospective study. *Hypertension.* 2010;56:36-43.
102. Liang J, Li Y, Zhou N, Teng F, Zhao J, Zou C, et al. Synergistic effects of serum uric acid and cardiometabolic risk factors on early stage atherosclerosis: The cardiometabolic risk in Chinese study. *PLos One.* 2012;12:e51101.
103. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens.* 2012;30:445-8.
104. Cecelja M, Chowienczyk P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension. *Hypertension.* 2009;54:1328-36.
105. Yang EY, Chambless L, Sharrett AR, Virani SS, Liu X, Tang Z, et al. Carotid arterial wall characteristics are associated with incident ischemic stroke but not coronary heart disease in the atherosclerosis risk in communities (ARIC) study. *Stroke.* 2012;43:103-8.
106. Bergmann SR. Carotid plaque measurement is superior to IMT invited editorial comment on: Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: A meta-analysis. Inaba, Y. Chen, J.A. Bergmann, S. R. M.D. *Atherosclerosis.* 2012;220:34-5.

107. Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, et al. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: The strong heart study. *Hypertension*. 2007;50:197-203.
108. Roman MJ, Devereux RB, Kizer JR, Okin PM, Lee ET, Wang W, et al. High central pulse pressure is independently associated with adverse cardiovascular outcome. *J Am Coll Cardiol*. 2009;54:1730-4.
109. Wang KL, Cheng HM, Chuang SY, Spurgeon HA, Ting C, Lakatta EG, et al. Central or peripheral systolic or pulse pressure: Which best relates to target organs and future mortality? *J Hypertens*. 2009;27:461-7.
110. Brown DW, Giles WH, Croft JB. Left ventricular hypertrophy as a predictor of coronary heart disease mortality and the effect of hypertension. *Am Heart J*. 2000;140:848-56.
111. Namasivayam M, Adji A, O'Rourke MF. Influence of aortic pressure wave components determined noninvasively on myocardial oxygen demand in men and women. *Hypertension*. 2011;57:193-200.
112. Leone N, Ducimetière P, Gariépy J, Courbon D, Tzourio C, Dartigues J, et al. Distension of the carotid artery and risk of coronary events: The three-city study. *Arterioscler Thromb Vasc Biol*. 2008;28:1392-7.
113. Bussy C, Boutouyrie P, Lacolley P, Challande P, Laurent S. Intrinsic stiffness of the carotid wall material in essential hypertension. *Hypertension*. 2000;35:1049-54.
114. Simons PCG, Algra A, Bots ML, Grobbee DE, van der Graaf Y, for the SMART Study Group. Common carotid intima-media thickness and arterial stiffness: Indicators of cardiovascular risk in high-risk patients the SMART study (second manifestations of ARterial disease). *Circulation*. 1999;100:951-7.
115. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, et al. Multi-ethnic study of atherosclerosis: Objectives and design. *Am J Epidemiol*. 2002;156:871-81.
116. Van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, et al. Association between arterial stiffness and atherosclerosis: The Rotterdam study. *Stroke*. 2001;32:454-60.

117. Kawasaki T, Sasayama S, Yagi S, Asakawa T, Hirai T. Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovasc Res.* 1987;21:678-87.
118. Tropeano A, Boutouyrie P, Pannier B, Joannides R, Balkestein E, Katsahian S, et al. Brachial Pressure-Independent reduction in carotid stiffness after long-term angiotensin-converting enzyme inhibition in diabetic hypertensives. *Hypertension.* 2006;48:80-6.
119. Golemati S, Lever MJ, Bharath AA, Sassano A, Dhanjil S, Nicolaides AN. Carotid artery wall motion estimated from B-mode ultrasound using region tracking and block matching. *Ultrasound Med Biol.* 2003;29:387-99.
120. Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J, et al. Clinical applications of arterial stiffness, task force III: Recommendations for user procedures. *Am J Hypertens.* 2002;15:445-52.
121. Kassan M, Wecker A, Kadowitz P, Treback M, Matrougui K. $CD_4^+ CD_{25}^+$ Foxp3 regulatory tcell and vascular dysfunction in hypertension. *J Hypertens.* 2013;31:1939-43.
122. Harrison DG, Guzik TJ, Goronzy J, Weyand C. Is hypertension an immunologic disease? *Curr Cardiol Reports.* 2008;10:464-9.
123. Eugen-Olsen J. suPAR- a future risk marker in bacteremia. *J Intern Med.* 2011;270:29-31.
124. Boos CJ, Lip GYH. Elevated high-sensitivity C-reactive protein, large arterial stiffness and atherosclerosis: A relationship between inflammation and hypertension. *J Hum Hypertens.* 2005;19:511-3.
125. Kruger R, Schutte R, Huisman HW, Hindersson P, Olsen MH, Eugen-Olsen J, et al. NT-proBNP, C-reactive protein and soluble uPAR in a bi-ethnic male population: The SAfrEIC study. *PLoS ONE.* 2013;8:e58506.
126. Dehghan A, Kardys I, de Maat MPM, Uitterlinden AG, Sijbrands EJG, Bootsma AH, et al. Genetic variation, C-reactive protein levels, and incidence of diabetes. *Diabetes.* 2007;56:872-8.
127. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. *J Clin Oncol.* 2009;27:2217-24.
128. Guo Y, Lip GYH, Apostolakis S. Inflammation in arterial fibrillation. *J Am Coll Cardiol.* 2012;60:2263-70.

129. Harrison DG, Marvar PJ, Titze JM. Vascular inflammatory cells in hypertension. *Front Physiol.* 2012;3:128.
130. Roman MJ, Devereux RB, Schwartz JE, Lockshin MD, Paget SA, Davis A, et al. Arterial stiffness in chronic inflammatory diseases. *Hypertension.* 2005;46:194-9.
131. Galkina E, Ley K. Leukocyte influx in atherosclerosis. *Curr Drug Targets.* 2007;8:1239-48.
132. Boaz M, Chernin G, Schwartz I, Kotzir Z, Schwartz D, Agbaria A, et al. C-reactive protein and carotid and femoral intima media thickness: Predicting inflammation. *Clin Nephrol.* 2013;80:449-55.
133. Blackburn R, Giral P, Bruckert E, André J, Gonbert S, Bernard M, et al. Elevated C-reactive protein constitutes an independent predictor of advanced carotid plaques in dyslipidemic subjects. *Arterioscler Thromb Vasc Biol.* 2001;21:1962-8.
134. McEniery CM, Wilkinson IB. Large artery stiffness and inflammation. *J Hum Hypertens.* 2005;19:507-9.
135. Gkaliagkousi E, Douma S. The pathogenesis of arterial stiffness and its prognostic value in essential hypertension and cardiovascular disease. *Hippokratia.* 2009;13:70-5.
136. Park S, Iakatta Eg. Role of inflammation in the pathogenesis of arterial stiffness. *Yonsei Med J.* 2012;53:258-61.
137. Stehouwer CDA, Henry RMA, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: A pathway to cardiovascular disease. *Diabetologia.* 2008;51:527-39.
138. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. *Clin Chem Acta.* 2006;368:33-47.
139. Marsh SA, Coombes JS. Exercise and the endothelial cell. *Int J Cardiol.* 2003;99:165-9.
140. Kobayashi S, Nagino M, Komatsu S, Naruse K, Nimura Y, Nakanishi M, et al. Stretch-induced IL-6 secretion from endothelial cells requires NF- κ B activation. *Biochem Biophys Res Commun.* 2003;308:306-12.
141. Preston RA, Jy W, Jimenez JJ, Mauro LM, Horstman LL, Valle M, et al. Effects of severe hypertension on endothelial and platelet microparticles. *Hypertension.* 2003;41:211-7.

142. Cottone S, Mule G, Nardi E, Vadala A, Guarneri M, Briolotta C, et al. Relation of C-reactive protein to oxidative stress and to endothelial activation in essential hypertension. *Am J Hypertens*. 2006;19:313-8.
143. Shyy JY. Extracellular matrix differentiating good flow versus bad flow. *Circ Res*. 2009;104:931.
144. Miller MA, Sagnella GA, Kerry SM, Strazzullo P, Cook DG, Cappuccio FP. Ethnic differences in circulating soluble adhesion molecules: The Wadsworth Heart and Stroke study. *Clin Sci*. 2003;104:591-8.

Chapter 3: Manuscript for publication

*Carotid characteristics of 5-year sustained hypertensive
black South Africans*

INSTRUCTIONS TO AUTHORS: *Journal of Hypertension*

Title page

- Full title (20 words)
- Short title
- Names of all authors
- Affiliations of all authors
- Sources of any support for all authors, for the work in the form of grants, equipment, drugs or a combination of these
- Disclosure of funding from any of the following: National Institutes of Health, Wellcome trust, Howard Hughes Medical Institute and other(s)
- Statement on potential conflicts of interest
- Name and address of author responsible for correspondence concerning the manuscript
- Word count: Including references, excluding tables and legends.
- Number of tables
- Number of figures
- Number of supplementary digital content files.

Abstract

- No more than 250 words
- The abstract should state the Objective(s) of the study, basic Methods (selection of study participants, observational and analytical methods), main Results (specific data and their statistical significance), and the principal Conclusions
- The abstract should be followed by a list of 3-10 key words or short phrases

Text

- Introduction
- Methods (including ethical and statistical information)
- Results
- Discussion (including a conclusion)

Tables

- Assign a Arabic numeral, e.g. (Table 3) and a brief title
- Place explanatory matter in the footnote
- Identify statistical measures of variation

References

- Numbered in the order in which they first appear in text
- Assigned with Arabic numerals given in brackets, e.g. [17]
- Include the names of all authors if 7 or fewer, when eight or more, list the first 6 names followed by et al
- Include the full title and source information
- Journal names abbreviated according to MEDLINE
- E.g. Journal article: Zhou M-S, Schulman IH, Raij L. Vascular inflammation, insulin resistance, and endothelial dysfunction in salt-sensitive hypertension: role of nuclear factor kappa B activation. *J Hypertension* 2010; 28:527-535
- E.g. Book: Katz AM, Konstam MA. Heart failure. Pathophysiology, Molecular Biology, and Clinical Management. Philadelphia: Lippincott Williams & Wilkins; 2008



Carotid characteristics of 5-year sustained hypertensive black South Africans

Short title: Carotid characteristics of hypertensive Africans

Melissa MARITZ,^a Carla MT FOURIE,^a Johannes M VAN ROOYEN,^a Aletta E SCHUTTE^a

^aHypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa

Conflict of Interest: All authors declare no conflict of interest. **Source of Funding:** Funding was obtained from the South African National Research Foundation (NRF), the Medical Research Council (MRC), SANPAD (South Africa-Netherlands Research Program on Alternatives in Development) and PHRI (Population Health Research Institute).

Word count: 4314

Tables: 4

Figures: 2

Supplemental data: 3 tables.

Corresponding author:

Dr. CMT Fourie

Hypertension in Africa Research Team (HART)

North-West University

Private Bag X6001

Potchefstroom

2520

South Africa

Tel: +2718 299 2080

Fax: +27 18 285 2432

E-mail: carla.fourie@nwu.ac.za

Abstract

Objectives: Increased arterial stiffness in hypertensives may be due to structural or functional adaptations of the vasculature. More information regarding large artery alterations in hypertensive Africans is needed. We therefore compared the carotid characteristics between 5-year sustained hypertensive and normotensive blacks. We also investigated the association of the carotid characteristics with cardiometabolic risk factors, markers of inflammation, endothelial activation and health behaviours.

Methods: We included HIV-free Africans who were either hypertensive (N=351) or normotensive (N=241) during baseline (2005) and follow-up (2010) data collection. Carotid characteristics, including the intima-media thickness (IMT), distensibility, Young's elastic modulus, cross-sectional wall area and lumen diameter were assessed with B-mode ultrasound. Brachial and central systolic blood pressure (cSBP) were measured, and blood samples were used to determine metabolic, inflammatory and endothelial activation markers, and health behaviours.

Results: Cardiovascular measures (all $p \leq 0.024$) and carotid characteristics, except IMT, were significantly different between the normotensive and hypertensive groups (all $p \leq 0.008$) after adjustments. However, upon additional adjustment for cSBP, all significance was lost. Metabolic, inflammatory and endothelial activation markers, as well as health behaviour, were not different between the two groups. Carotid distensibility associated with glycosated hemoglobin ($p=0.018$) and IMT with low-density lipoprotein ($p=0.005$) in the hypertensive group.

Conclusions: Increased distending pressure resulting from higher BP in the hypertensives may explain the differences in the carotid characteristics, such as lower distensibility between the hypertensive and normotensive groups. Therefore, it seems that functional, but no structural adaptations have taken place despite being hypertensive for at least 5 years.

Key Words: ethnicity, black, large artery, stiffness, distensibility, hypertension, central pressure

Introduction

Hypertension is an important contributor to the global epidemic of cardiovascular disease (CVD) [1], and the burden of CVD is more evident among black populations [2-5]. Arterial stiffness has been increasingly implicated in the development of CVD, resulting in stroke, coronary heart disease and heart failure [6, 7]. Various studies have shown that compared to whites, blacks have increased arterial stiffness [8-10]. We previously showed that in black Africans, arterial stiffness associates more with blood pressure than with age [11].

Increased arterial stiffness in hypertensives have been attributed to arterial wall remodelling, or structural and functional alterations in the arterial wall in response to injury, disease or ageing [12]. Atherosclerosis, which involves thickening of the intima [13], and arteriosclerosis, or stiffening of the large arteries [14], is associated with general cardiovascular [12], metabolic [15] and inflammatory disease [16]. Arterial remodelling may be a compensatory mechanism by which the artery resists strain during blood pressure increases, but it also predisposes the vessel to a progressive change in the wall shape and composition, ultimately leading to several clinical complications like arterial fibrosis and stiffening, which is characteristic of hypertension [17, 18].

Recently, Van Sloten *et al.*, reported that the stiffness of the carotid artery independently associates with incident cardiovascular (CV) events and all-cause mortality [7]. The ARIC (Atherosclerosis Risk in Communities) study reported an independent association between carotid stiffness and stroke, possibly because stiffening of the carotid artery might lead to a high pressure load on the brain [19].

It is unclear whether the increased arterial stiffness seen in hypertensives are due to the increased distending pressure characteristic of hypertension, or to intrinsic structural adaptations in the arterial wall [20]. Information on the carotid characteristics, functional adaptation and structural wall alterations in hypertensive Africans are limited. Therefore, we compared the characteristics of the carotid artery between five-year sustained hypertensive and normotensive black participants. We also determined whether conventional cardio-metabolic risk factors, markers of inflammation, endothelial activation or measures of health behaviours are related to these carotid characteristics.

Methods

Study population

This sub-study forms part of the South African leg of the multi-national Prospective Urban and Rural Epidemiology (PURE) study [21]. The participants of the PURE-SA study were from the North West Province of South Africa, and baseline data collection took place in 2005 (N=2010), while follow-up data was collected five years later, in 2010 (N=1288). The study population for this sub-study is described in Figure 1. Only the HIV-free black participants with two consecutive (2005, 2010) blood pressure measurements in either the hypertensive or normotensive range (N=592) were included in this sub-study. Thus, the study population consists of a group of 5-year sustained normotensive (n=241) and hypertensive (n=351) black Africans.

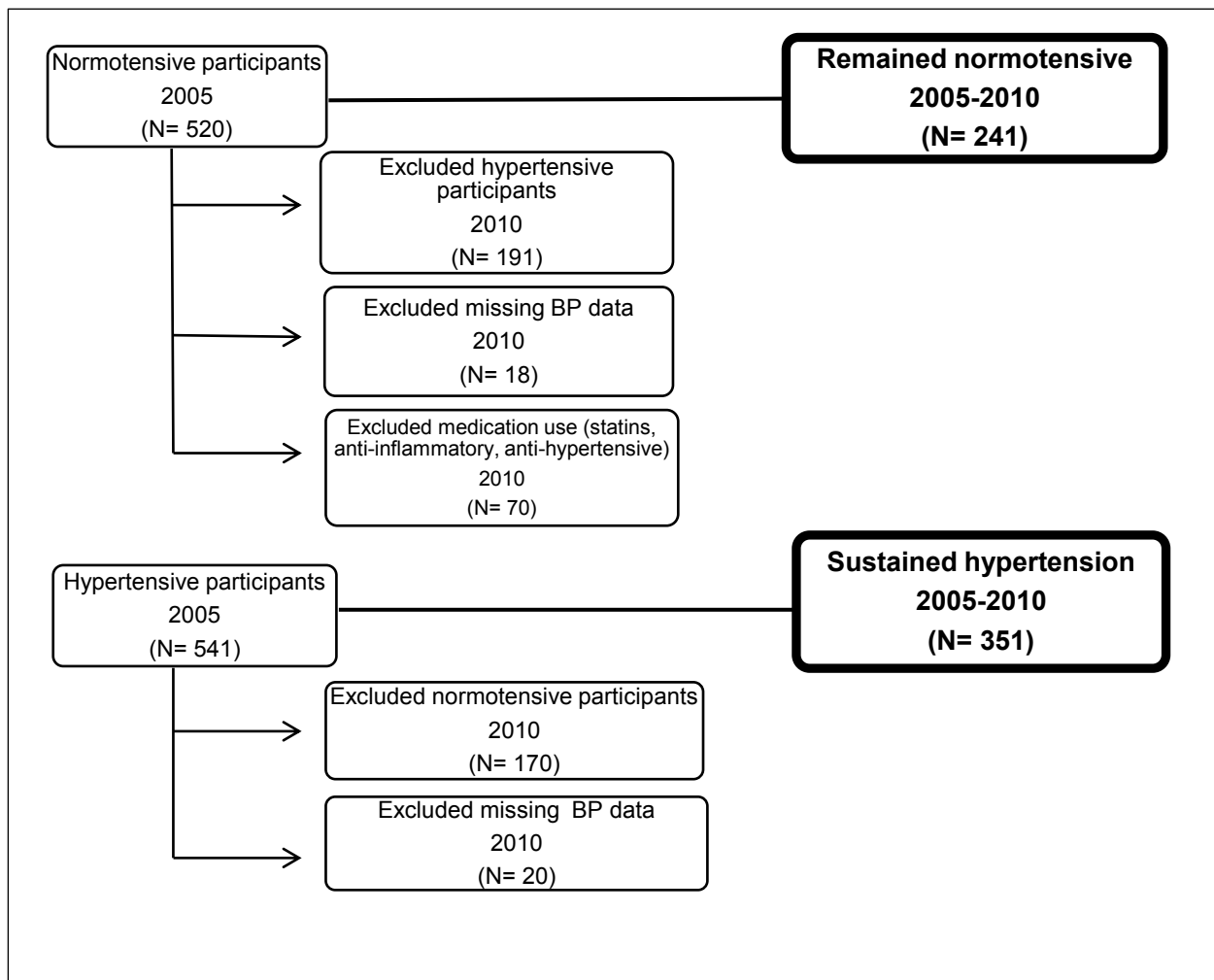


Figure 1. Study population. All participants were HIV-free. BP, blood pressure.

Ethical considerations

The Health Research Ethics Committee of the North-West University approved the protocol of the PURE-SA study, as well as this sub-study (Ethics number: NWU-00016-10-A1), and it complies with the Declaration of Helsinki (2013). Participants completed structured demographic, lifestyle, physical activity and socio-economic questionnaires. Trained African field workers (from the communities) assisted in the collection of the data. Before measurements commenced, all procedures were explained to the participants in their home language. The participants then gave written informed consent. Locality refers to urban or rural locality.

Anthropometric measurements

Height (Invicta Stadiometer IP 1465, Leicester, UK), weight (Precision Health Scale, A & D Company, Tokyo, Japan) and waist circumference (WC) (Holtain unstretchable metal tape) were measured using standardised methods and calibrated instruments [22]. Body mass index (BMI) was calculated with the formula: weight (kg)/height (m²).

Cardiovascular measurements

The validated OMRON HEM-757 (Omron Healthcare, Kyoto, Japan) device was used to measure blood pressure. Each participant was fitted with the correct cuff size. After a resting period of ten minutes, the brachial systolic (bSBP) and diastolic blood pressure (bDBP) were measured on the right upper arm, followed by a five-minute resting period and a second blood pressure measurement. The blood pressure of the second measurement was used for analysis. The participant was seated in a relaxed upright position with legs uncrossed during the measurements. The 2013 ESH and ESC Guidelines define hypertension as a SBP of ≥ 140 mmHg, and/or a DBP of ≥ 90 mmHg, and the participants were classified as hypertensive or normotensive accordingly [23].

The central systolic blood pressure (cSBP) was measured with the OMRON 9000AI device (Omron Healthcare, Kyoto, Japan), and we used the second systolic peak as measure of cSBP (reflected wave). The central pulse pressure (cPP) was calculated by subtracting the bDBP from the cSBP.

The pulse wave velocity (PWV) was measured non-invasively on the left side of each participant in a supine position. The Complior SP device (Artech-Medical, Pantin, France) used superficial pulses to measure the PWV. The measurement was taken from the carotid-dorsalis pedis (cdPWV) section of the arterial tree.

The carotid characteristics were measured non-invasively with the SonoSite Micromaxx ultrasound system (SonoSite, Inc., Bothell, WA, USA) using a 6-13 MHz linear array transducer. A minimum of two optimal angles were used from images of the right and left common carotid arteries. These images were then measured by a single reader according to protocols, digitised and imported into the automated software of the Artery Measurements System (Gothenburg, Sweden). The carotid intima-media thickness (cIMT) was analysed on a good quality image of a maximum 10 mm segment. The borders of the inner diameter of the blood vessel and the near and far wall of the intima-media were identified by an automated function of the program, but user intervention was possible. Approximately one hundred discrete measurements were used to obtain the mean cIMT and the carotid diameter.

A single reader also used B-mode ultrasonography to analyse video-clips of the carotid artery in order to determine the systolic/maximum and diastolic/minimum lumen diameters (LD).

The carotid distensibility (CD) coefficient was calculated according to the following formula: $((2 \times \Delta LD \times dLD) + (\Delta LD)^2) / (cPP \times dLD^2)$. LD, lumen diameter; ΔLD , systolic LD- diastolic LD; dLD, diastolic LD; cPP, central pulse pressure [24].

Young's elastic modulus was calculated according to the following formula: $dLD / (cIMT \times \text{distensibility})$. cIMT, average carotid intima media thickness across heart cycle; ΔLD , delta lumen diameter; dLD, diastolic lumen diameter [25].

Cross-sectional wall area (CSWA) was calculated according to the following formula: $[3.14 \times (LD/2 + cIMT_f) \times (LD/2 + cIMT_f)] - [3.14 \times (LD/2) \times (LD/2)]$. LD- lumen diameter, cIMT_f- carotid intima media thickness of the far wall.

Blood, serum and plasma samples

Participants were asked to fast for at least eight hours and blood samples were taken with a sterile winged infusion set and syringes from the ante-brachial vein. The preparation of the serum and plasma was done according to standardised methods, snap frozen on dry ice and stored in the laboratory at -80°C. In the cases of blood collection in a rural area, serum and plasma was snap frozen and stored at -20°C for not more than five days. The serum was then transported to the laboratory and stored at -80°C for further analysis.

Biochemical analysis

An enzymatic colorimetric method (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) was used to assess the quantitative aspect of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), triglycerides (TG) and gamma-glutamyltransferase (GGT) and creatinine levels in serum samples.

The Friedewald formula was used to calculate the quantitative aspect of low-density lipoprotein cholesterol (LDL-C): $LDL-C = TC - HDL-C - VLDL-C$ (where $VLDL-C = 0.456 \times TG$, VLDL-C: very low density lipoprotein-cholesterol) [26]. The estimated creatinine clearance (CrCl) was calculated with the Cockcroft-Gault formula: $CrCl \text{ in ml/min} = [(140 - \text{age}) \times (\text{weight in kg}) \times (0.85 \text{ if female})] / (72 \times \text{creatinine in mmol/l})$ [27].

A particle enhanced turbidimetric assay (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN) was used to analyse high sensitivity C-reactive protein (hsCRP) levels in serum. Fasting glucose (fGluc) levels were determined in fluoride plasma with an enzymatic reference method with hexokinase (Cobas Integra 400 Roche® Clinical System, Roche Diagnostics, Indianapolis, IN). The glycosylated haemoglobin (HbA1c) were determined from EDTA whole blood with ion-exchange high-performance liquid chromatography (D-10 Haemoglobin testing system, Bio-Rad #220-0101).

Serum interleukin-6 (IL-6) was determined with an electro-chemiluminescence immunoassay (Cobas e411 Analyzer, Roche, Basel, Switzerland).

Serum intercellular adhesion molecule 1 (sICAM-1) and serum vascular cell adhesion molecule 1 (sVCAM-1) were assessed by sandwich ELISAs (human sICAM-1 and human sVCAM-1 assay, IBL, Hamburg, Germany).

Statistical analysis

Statistical analyses were performed with Statistica® 12 (StatSoft, Inc., Tulsa, OK, USA). Descriptive statistics, including the mean and standard deviation, were performed on data with a normal distribution. If the data was not normally distributed, it was logarithmically transformed and presented as the geometric mean and the 5th and 95th percentiles. Differences between normotensives and hypertensives were determined by independent t-tests (with the distinguishing factor being either normotensive or hypertensive for 5 years) or with Chi-square tests for categorical variables. We compared the groups with ANCOVA while adjusting for age, sex, WC, GGT, tobacco and anti-hypertensive medication use. Finally, we plotted quartiles of cSBP against carotid features, and compared carotid features using ANOVA and ANCOVA while adjusting for cSBP. We used single and partial linear regression analyses to determine associations between carotid measures and cardio-metabolic risk factors, health behaviours, inflammation and endothelial activation. Multiple regression analyses were done to determine independent associations between the carotid characteristics and cardio-metabolic risk factors, with the dependent variables including CD and IMT, while CSWA and max LD are shown in supplementary table 4. The following co-variates were included in the regression model: locality, sex, age, WC, cSBP, HR, LDL-cholesterol, HbA1c, CrCl, CRP, ICAM-1, GGT, tobacco use and for the hypertensive group only, anti-hypertensive medication use.

Results

The characteristics of normotensive and hypertensive Africans are shown in Table 1. The hypertensive Africans were older, had higher BMI and WC, and a larger percentage were from an urban area than the normotensives. All the cardiovascular measurements, as well as carotid

characteristics, glycemic and inflammatory markers, ICAM, TG, GGT and self-reported alcohol use were significantly higher in the hypertensive group.

TABLE 1. Characteristics of normotensive and hypertensive Africans

	Normotensive 241	Hypertensive 351	<i>p</i>
N			
Men, N (%)	90 (37.3)	129 (36.8)	0.88
Urban, N (%)	78 (32.4)	178 (50.7)	<0.001
Age, years	52.8 ± 8.93	59.0 ± 10.0	<0.001
<i>Anthropometry</i>			
Waist circumference, cm	78.7 ± 11.9	84.7 ± 13.5	<0.001
Body mass index, kg/m ²	24.7 ± 6.87	26.6 ± 7.85	0.003
<i>Cardiovascular measures</i>			
Brachial SBP, mm Hg	119 ± 11.9	157 ± 22.4	<0.001
Brachial DBP, mm Hg	78.9 ± 7.23	100 ± 12.5	<0.001
Heart rate, bpm	61.8 ± 14.7	67.8 ± 17.9	<0.001
Central SBP, mm Hg	116 ± 12.8	150 ± 22.2	<0.001
Central PP, mm Hg	37.4 ± 11.8	50.9 ± 20.4	<0.001
Carotid dorsalis-pedis PWV, m/s	8.25 ± 1.35	10.0 ± 1.89	<0.001
<i>Carotid characteristics</i>			
Distensibility x 10 ⁻³ , 1/kPa	4.72 ± 2.00	3.02 ± 1.83	<0.001
Young's elastic modulus x 10 ³ , kPa	2.17 ± 1.07	3.72 ± 2.20	<0.001
Intima media thickness, mm	0.68 ± 0.13	0.77 ± 0.17	<0.001
Cross-sectional wall area, mm ²	14.2 ± 4.41	17.4 ± 5.30	<0.001
Lumen diameter maximum, mm	6.18 ± 0.77	6.60 ± 0.85	<0.001
Lumen diameter minimum, mm	5.74 ± 0.72	6.22 ± 0.83	<0.001
<i>Lipids</i>			
HDL-cholesterol, mmol/l	1.45 ± 0.65	1.51 ± 0.60	0.30
LDL-cholesterol, mmol/l	2.86 ± 1.17	2.96 ± 1.14	0.29
Triglycerides, mmol/l	1.09 [0.57, 2.11]	1.20 [0.59, 2.89]	0.039
<i>Glycemia</i>			
Glucose, mmol/l	4.86 [3.91, 6.06]	5.27 [3.96, 7.90]	<0.001
HbA1c (%)	5.94 [5.30, 6.80]	6.08 [5.20, 7.80]	0.038
<i>Inflammatory markers</i>			
Interleukin-6, pg/ml	3.39 [0.75, 23.5]	4.43 [0.75, 20.0]	<0.001
C-reactive protein, mg/l	2.96 [0.21, 32.6]	3.84 [0.38, 28.4]	0.022
<i>Adhesion molecules</i>			
Intracellular adhesion molecule-1, pg/ml	286 ± 92.7	321 ± 111	<0.001
Vascular adhesion molecule-1, pg/ml	732 [442, 1388]	762 [443, 1735]	0.23
<i>Renal function</i>			
Creatinine clearance, ml/min	91.4 [53.7, 163]	91.6 [47.7, 164]	0.94
<i>Health behaviours</i>			
γ-glutamyltransferase, U/l	33.7 [11.9, 167]	52.7 [13.6, 347]	<0.001
Self-reported alcohol intake, N, total (%)	60/220 [27.3]	159/336 [47.3]	<0.001
Self-reported tobacco use, N, total (%)	117/229 [51.1]	159/341 [46.6]	0.56
Anti-hypertension medication, N, total (%)	-	124/351 [35.3]	
Lipid-lowering medication, N, total (%)	-	5/351 [1.4]	
Anti-inflammatory medication, N, total (%)	-	21/351 [5.9]	

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

Table 2 reports the characteristics after adjustments for age, sex, WC, GGT, tobacco and anti-hypertensive medication use. All carotid characteristics, except IMT, remained significantly different.

However, the inflammatory and glycemic markers, lipids and adhesion molecules did not differ significantly (similar results were obtained when adjusting only for age, sex and WC).

TABLE 2. Adjusted characteristics of normotensive and hypertensive Africans

	Normotensive	Hypertensive	<i>p</i>
N	241	351	
<i>Cardiovascular measures</i>			
Brachial SBP, mm Hg	120 ± 1.34	155 ± 1.14	<0.001
Brachial DBP, mm Hg	79.2 ± 0.74	99.8 ± 0.64	<0.001
Heart rate, bpm	62.7 ± 1.22	66.8 ± 1.05	0.017
Central SBP, mm Hg	117 ± 1.40	148 ± 1.18	<0.001
Central PP, mm Hg	38.4 ± 1.24	49.4 ± 1.05	<0.001
Carotid dorsalis-pedis PWV, m/s	8.87 ± 0.14	9.37 ± 0.12	0.024
<i>Carotid characteristics</i>			
Carotid distensibility × 10 ⁻³ , 1/kPa	4.34 ± 0.20	3.38 ± 0.18	0.003
Young's Elastic Modulus × 10 ³ , kPa	2.49 ± 0.20	3.35 ± 0.18	0.008
Intima-media thickness, mm	0.72 ± 0.01	0.73 ± 0.01	0.42
Cross-sectional wall area, mm ²	14.8 ± 0.33	16.7 ± 0.28	<0.001
Lumen diameter maximum, mm	6.24 ± 0.07	6.51 ± 0.06	<0.001
Lumen diameter minimum, mm	5.80 ± 0.07	6.12 ± 0.05	<0.001
<i>Lipids</i>			
HDL-cholesterol, mmol/l	1.46 ± 0.04	1.50 ± 0.03	0.47
LDL-cholesterol, mmol/l	2.93 ± 0.08	2.90 ± 0.07	0.81
Triglycerides, mmol/l	1.16 [1.08, 1.24]	1.11 [1.05, 1.19]	0.50
<i>Glycemia</i>			
Glucose, mmol/l	5.01 [4.86, 5.16]	5.10 [4.97, 5.23]	0.43
HbA1c, %	6.05 [5.93, 6.15]	5.99 [5.89, 6.08]	0.44
<i>Inflammatory markers</i>			
Interleukin-6, pg/ml	3.62 [3.19, 4.11]	4.05 [3.64, 4.51]	0.22
C-reactive protein, mg/l	3.41 [2.84, 4.08]	3.18 [2.72, 3.72]	0.62
<i>Adhesion molecules</i>			
Intracellular adhesion molecule-1, pg/ml	293 ± 7.17	311 ± 6.11	0.07
Vascular adhesion molecule-1, pg/ml	748 [706, 792]	747 [711, 785]	0.98
Creatinine clearance, ml/min	90.8 [87.8, 94.0]	92.3 [89.6, 95.0]	0.54

Data are arithmetic mean ± SEM or geometric mean (5th and 95th percentile intervals) for logarithmically transformed. N, number of participants; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; HbA1c, glycated haemoglobin. Data adjusted for age, sex, WC, GGT, tobacco use and anti-hypertensive medication use.

The carotid characteristics plotted against quartiles of cSBP are shown in Figure 2. All the carotid characteristics changed significantly with increasing cSBP before and after adjustments were made.

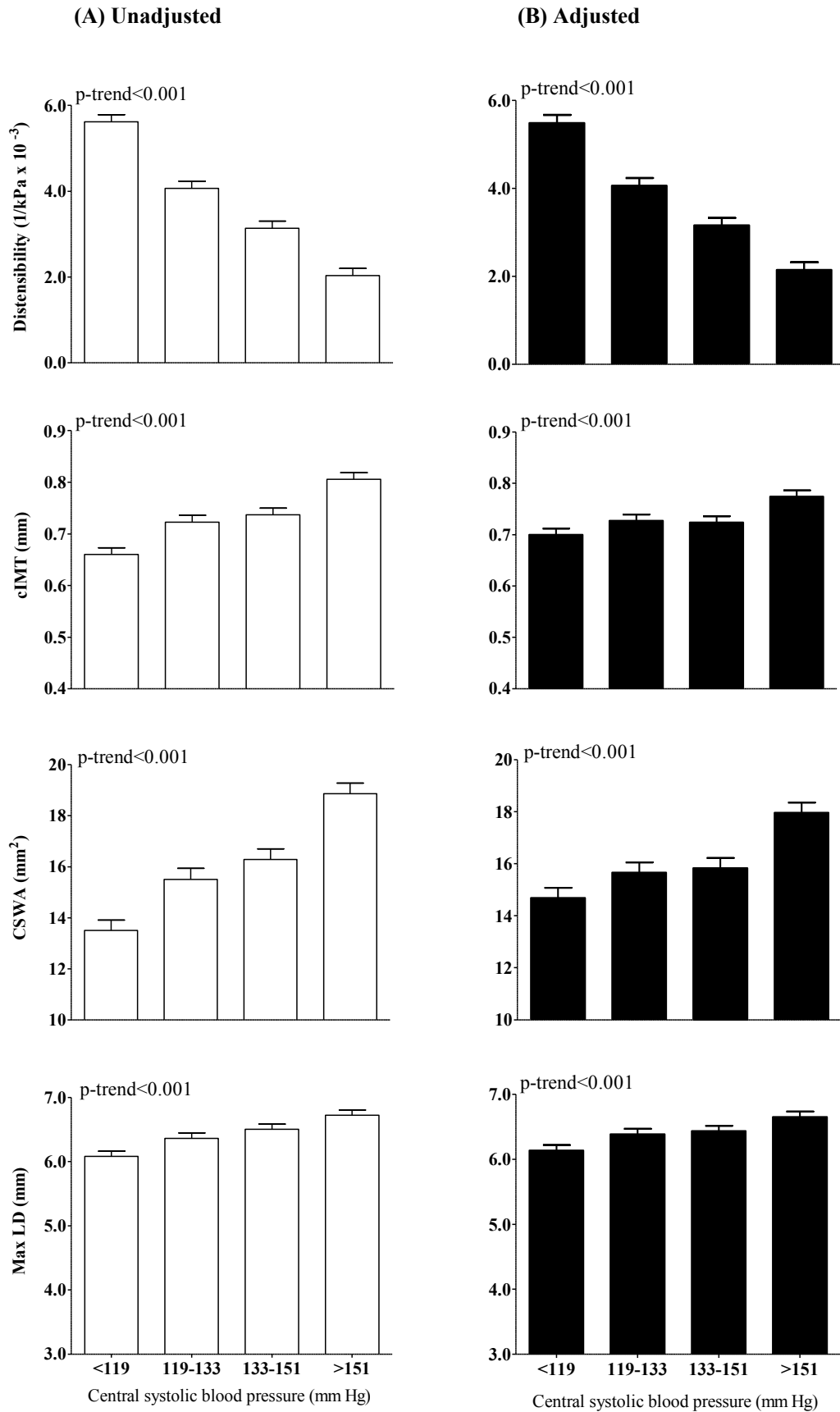


FIGURE 2 . Quartiles of central systolic blood pressure plotted against measures of the carotid artery in the total group (N= 592); (A) unadjusted; (B) adjusted for age, sex, waist circumference, GGT, tobacco use and antihypertensive medication use. cIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; Max LD, maximum lumen diameter.

We finally adjusted for cSBP when comparing the carotid characteristics between the normotensive and the hypertensive groups (Table 3), resulting in no significant differences between the groups.

TABLE 3. Carotid characteristics of normotensive and hypertensive Africans, additionally adjusted for cSBP

	Normotensive (n=241)	Hypertensive (n=351)	p
<i>Carotid characteristics</i>			
Distensibility x 10 ⁻³ , 1/kPa	3.77 ± 0.17	3.95 ± 0.15	0.50
Young's elastic modulus x 10 ³ , kPa	3.04 ± 0.16	2.80 ± 0.14	0.34
Intima-media thickness, mm	0.71 ± 0.01	0.73 ± 0.01	0.21
Cross-sectional wall area, mm ²	15.2 ± 0.36	16.2 ± 0.31	0.09
Lumen diameter maximum, mm	6.30 ± 0.07	6.41 ± 0.07	0.35
Lumen diameter minimum, mm	5.85 ± 0.08	6.02 ± 0.06	0.13

Data are arithmetic mean ± SEM. Data adjusted for age, sex, WC, GGT, tobacco use, anti-hypertensive medication use and central systolic blood pressure.

Pearson correlations in normotensive and hypertensive Africans are shown in Supplementary Table 1 and 2, respectively. Table 4 reports the forward stepwise multiple regression analyses performed in both the normotensive and hypertensive groups with the CD and cIMT as dependent variables. CD associated with age in the hypertensive group (p=0.009) and with cSBP in both groups (p<0.001). CD associated negatively with HbA1c in the hypertensive group only (p=0.018). IMT strongly associated with age in both groups (p<0.001), while LDL-C (p=0.005) and cSBP (p=0.016) were positively associated with IMT in the hypertensive group only.

Supplementary Table 3 reports the forward stepwise multiple regression analyses performed in the normotensive and hypertensive groups with CSWA and Max LD as the dependent variables. CSWA associated with male sex and age in both groups (p<0.001), while LDL-C (p=0.026) and cSBP (p<0.001) associated with CSWA in the hypertensive group only. Max LD associated with male sex and cSBP in both the normotensive and hypertensive groups. Max LD also associated negatively with LDL-C in the normotensives and hypertensives (p=0.027; p=0.045), respectively.

TABLE 4. Forward stepwise multiple regression analyses with CD and cIMT as dependent variables

	Normotensive (N=241)		Hypertensive (N=351)	
	β (95% CI)	p	β (95% CI)	p
Distensibility (1/kPa)				
Adjusted R²	0.27		0.37	
Locality (urban)	-0.16 (-0.31, -0.01)	0.031		
Age, years	-0.14 (-0.29, 0.003)	0.058	-0.20 (-0.35, -0.05)	0.009
Waist circumference, cm			0.22 (0.06, 0.39)	0.007
Central SBP, mm Hg	-0.44 (-0.59, -0.29)	<0.001	-0.56 (-0.68, -0.45)	<0.001
Heart rate, bpm	-0.10 (-0.25, 0.04)	0.16	-0.12 (-0.23, -0.01)	0.030
LDL-Cholesterol, mmol/l			0.08 (-0.03, 0.19)	0.18
HbA1c (%)			-0.14 (-0.26, -0.02)	0.018
CrCl, ml/min			-0.10 (-0.28, 0.07)	0.24
ICAM-1, pg/ml	0.11 (-0.04, 0.25)	0.16		
Tobacco use (no/yes)	-0.14 (-0.28, 0.003)	0.058		
cIMT, mm				
Adjusted R²	0.25		0.35	
Locality (urban)			-0.10 (-0.20, -0.01)	0.030
Sex (male)	0.26 (0.13, 0.39)	<0.001	0.25 (0.15, 0.36)	<0.001
Age, years	0.33 (0.21, 0.46)	<0.001	0.44 (0.34, 0.54)	<0.001
Waist circumference, cm	0.14 (0.01, 0.28)	0.033	0.08 (-0.02, 0.19)	0.13
Central SBP, mm Hg			0.12 (0.02, 0.22)	0.016
Heart rate, bpm	-0.08 (-0.20, 0.05)	0.21		
LDL-Cholesterol, mmol/l	0.07 (-0.05, 0.19)	0.29	0.15 (0.05, 0.25)	0.005
HbA1c (%)			0.07 (-0.03, 0.18)	0.17
C-reactive protein, pg/ml	0.16 (0.03, 0.30)	0.017	0.06 (-0.04, 0.16)	0.27
ICAM-1, pg/ml	0.12 (-0.01, 0.24)	0.079		
γ -glutamyl transferase, U/l			-0.08 (-0.18, 0.02)	0.12
Tobacco use (no/yes)			-0.08 (-0.18, 0.02)	0.11
Anti-HT meds (no/yes)			-0.10 (-0.19, -0.003)	0.044

Data expressed as beta-values and 95% confidence intervals, p-values obtained with forward stepwise multiple regression analyses. cIMT, carotid-intima media thickness; CSWA, cross-sectional wall area; Max LD, maximum lumen diameter; SBP, systolic blood pressure; LDL, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; CrCl, creatinine clearance; ICAM-1, intracellular adhesion molecule-1; Anti-HT meds, anti-hypertensive medication. Included in each model: locality, age, sex, WC, HR, cSBP, LDL-C, HbA1c, CRP, ICAM, CrCl, GGT, tobacco and anti-hypertensive medication use.

Discussion

The most prominent finding of this study is that decreased carotid distensibility and increased carotid cross-sectional wall area of 5-year sustained hypertensive Africans are purely dependent on cSBP. Indeed, we observed significant differences for all carotid measurements between the hypertensives and the normotensives prior to adjustment for cSBP. Our findings are consistent with evidence that indicate the importance of blood pressure as a determinant of arterial stiffness [20]. Increased stiffness has been found to be due to the increased distending pressure that accompanies hypertension, suggesting a functional adaptation, and not necessarily structural alterations of the arterial wall [28, 29].

IMT was similar between the two groups after adjustments, thus suggesting that no structural alterations in terms of arterial remodelling have taken place in the hypertensive Africans. IMT is an important marker of the atherosclerotic burden of the carotid artery [30], but it may also indicate non-atherosclerotic compensatory remodelling of the arterial wall in response to hypertension [31]. However, neither of these seems to be the case in our population. On the other hand, IMT was associated with cSBP in the total and hypertensive groups; showing a similar trend to the findings of Wang *et al.* [32].

These results indicate that vascular injury has not yet taken place in this African population. If structural adaptations were present, the reduction of blood pressure may not have an impact on arterial stiffness [33]. However, it seems that only functional adaptations have taken place in these Africans, thus suggesting that a reduction in blood pressure may reduce the arterial stiffness of the hypertensives.

Arterial stiffening increases systolic blood pressure (SBP) and decreases diastolic blood pressure (DBP), resulting in inadequate coronary perfusion, an increased afterload on the heart, as well as an increased pulsatile load on the microcirculation [7]. Since blood pressure is modifiable [2], prevention and intervention strategies may lower the burden of hypertension and stroke in Africans significantly.

However, the treatment and control of hypertension in low income countries, like South Africa, is inadequate [34].

When evaluating different contributors to carotid measures, we confirmed the well-known association between central arterial stiffness and age [35]. Age and blood pressure are associated with molecular changes in the arterial wall, like thinning, splitting and disorientation of the arrangement of the elastic fibres and lamellae [36], which may explain the increase in arterial stiffness associated with ageing and hypertension.

Neither the glucose nor the HbA1c differed between our groups, but we found that CD was associated with HbA1c in the hypertensive group only. Although we did not observe structural differences in carotid IMT after adjustment for cSBP, the positive link of CD with HbA1c in the hypertensives suggest a role for glucose metabolism in arterial alteration- although not yet detectable with ultrasound. Henry *et al.*, found that carotid stiffness is associated with type 2 diabetes mellitus, but that arterial stiffening takes place even before the onset of diabetes, when only an impaired glucose tolerance is seen [37]. In insulin resistance or impaired glucose tolerance, the metabolism of glucose and lipids are altered, leading to the production of excess aldehydes like glyoxal and methylglyoxal, eventually resulting in the formation of advanced glycation end products [38]. Advanced glycation end products are thought to act directly and on receptors to alter the functions of many proteins, including antioxidant and metabolic enzymes, calcium channels, lipoproteins and structural proteins [38], indicating a possible mechanism by which abnormal glucose tolerance may contribute to arterial stiffening.

Our findings indicate that inflammation and endothelial activation (as indicated by the adhesion molecules) do not play a major role in the mediation of central arterial stiffness in this population. These results are unexpected in light of previous findings, which indicate that acute and chronic inflammation are associated with stiffness of the large arteries [39], and that endothelial activation may be an important mediator of hypertensive vascular injury [40].

No differences were found in the lipid levels between the hypertensive and normotensive groups. These results may be explained by the fact that Africans are not prone to coronary heart disease [41], and generally exhibit a favourable lipid profile [42] of low TC levels and high levels of protective HDL-C [42, 43]. However, our findings do show the commonly found association between IMT and LDL-C [44, 45] in the hypertensive group only. It may thus be possible that a period longer than five years is needed in order for structural alterations to take place, and that LDL-C might be a key factor in the process.

The findings of this study should be interpreted in context of its limitations and strengths. Our study population consisted of individuals from specific urban and rural areas in the North West Province of South Africa, and may not be representative of the whole population. We were not able to use echotracking techniques to determine local arterial stiffness; however, the procedures of ultrasound assessment are standardised [46] and were performed by a single reader in a large study population. Due to the cross-sectional study design, causality cannot be inferred. Although the results were consistent after several adjustments, we cannot exclude residual confounding.

In conclusion, we suggest that the increased distending pressure and higher circumferential stress resulting from higher blood pressure may explain the stiffness and functional adaptation in the hypertensive group. Despite their hypertensive status, there are no detectable structural adaptations or arterial remodelling in these Africans. The classic cardio-metabolic risk factors, markers of endothelial activation and health behaviour do not seem to play a visible role in the mediation of carotid stiffness in this population.

Funding:

This work was financially supported by SANPAD (South Africa-Netherlands Research Program on Alternatives in Development), PHRI (Population Health Research Institute), the MRC (Medical Research Council) of South Africa, the South African NRF (National Research Foundation) (GUN numbers 2069139 and FA2006040700010), the North-West University, Roche Diagnostics. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF do not accept any liability in regard thereto.

Acknowledgements

The authors would like to thank all supporting staff and the participants of the PURE study and in particular:

1. **PURE-South Africa:** The PURE-NWP-SA research team, field workers and office staff in the Africa Unit for Transdisciplinary Health Research (AUTHeR) and the Hypertension in Africa Research Team (HART), Faculty of Health Sciences, North-West University, South Africa.
2. **PURE International:** Dr. S Yusuf and the PURE project office staff at the Population Health Research Institute (PHRI), Hamilton Health Sciences and McMaster University. ON, Canada.

Conflict of Interest:

No conflict of interest declared.

References

1. Stewart S, Libhaber E, Carrington M, Damasceno A, Abbasi H, Hansen C, et al. The clinical consequences and challenges of hypertension in urban-dwelling black africans: Insights from the heart of soweto study. *Int J Cardiol.* 2011;146:22-7.
2. Schutte AE, Schutte R, Huisman HW, Van Rooyen JM, Fourie CMT, Smith W, et al. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in black south africans? A 5-year prospective study. *Int J Epidemiol.* 2012;41:1114-23.
3. Opie LH, Seedat YK. Hypertension in sub-saharan african populations. *Circulation.* 2005;112:3562-8.
4. Seedat YK. Hypertension in black south africans. *J Hum Hypertens.* 1999;13:96-103.
5. Seedat YK, Rayner BL. The south african hypertension guidelines 2011. *S Afr Med J.* 2012;102:57-84.
6. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. *Eur Heart J.* 2006;27:2588-605.
7. Van Sloten TT, Schram MT, van den Hurk K, Dekker JM, Nijpels G, Henry RMA, et al. Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality. *J Am Coll Cardiol.* 2014;63:1739-47.
8. Chaturvedi N, Bulpitt CJ, Leggetter S, Schiff R, Nihoyannopoulos P, Strain WD, et al. Ethnic differences in vascular stiffness and relations to hypertensive target organ damage. *J Hypertens.* 2004;22:1731-7.
9. Hefferman KS, Jae SY, Fernhall B. Racial differences in arterial stiffness after exercise in young men. *Am J Hypertens.* 2007;20:840-5.
10. Morris AA, Patel RS, Binongo JNG, Poole J, Mheid Ia, Ahmed Y, et al. Racial differences in arterial stiffness and microcirculatory function between black and white Americans. *J Am Heart Assoc.* 2013;2.
11. Schutte AE, Huisman HW, Schutte R, Van Rooyen JM, Malan L, Malan NT, et al. Arterial stiffness profiles: Investigating various sections of the arterial tree of African and Caucasian people. *Clin Exp Hypertens.* 2011;33:511-7.

12. van Varik BJ, Rennenberg RJMW, Reutelingsperger CP, Kroon AA, de Leeuw PW, Schurgers LJ. Mechanisms of arterial remodelling: Lessons from genetic diseases. *Front Genet.* 2012;3:290.
13. Cecelja M, Chowienczyk P. Role of arterial stiffness in cardiovascular disease. *J R Soc Med Cardiovasc Dis.* 2012;1:11.
14. London GM. Cardiovascular calcifications in uremic patients: Clinical impact on cardiovascular function. *Journal of the American Society of Nephrology.* 2003 September 01;14(suppl 4):S305-9.
15. Della-Morte D, Gardener H(1), Denaro F(1), Sacco RL(1), Rundek T(1), Boden-Albala B, et al. Metabolic syndrome increases carotid artery stiffness: The northern Manhattan study. *Int J Stroke.* 2010;5:138-44.
16. Roman MJ, Devereux RB, Schwartz JE, Lockshin MD, Paget SA, Davis A, et al. Arterial stiffness in chronic inflammatory diseases. *Hypertension.* 2005;46:194-9.
17. Anwar MA, Shalboub J, Lim CS, Gohel MS, davies AH. The effect of pressure-induced mechanical stretch on vascular wall differential gene expression. *J Vasc Res.* 2012;49:463-78.
18. Lemariéa CA, Tharaux P, Lehoux S. Extracellular matrix alterations in hypertensive vascular remodelling. *J Mol Cell Cardiol.* 2010;48:433-9.
19. Yang EY, Chambless L, Sharrett AR, Virani SS, Liu X, Tang Z, et al. Carotid arterial wall characteristics are associated with incident ischemic stroke but not coronary heart disease in the atherosclerosis risk in communities (ARIC) study. *Stroke.* 2012;43:103-8.
20. Stewart AD, Jiang B, Millasseau SC, Ritter JM, Chowienczyk PJ. Acute reduction of blood pressure by nitroglycerin does not normalize large artery stiffness in essential hypertension. *Hypertension.* 2006;48:404-10.
21. Teo K, Chow C, Vaz M, Rangarajan S, Yusuf S. The prospective urban rural epidemiology (PURE) study: Examining the impact of societal influences on chronic non-communicable diseases in low-, middle- and high-income countries. *Am Heart J.* 2009;518:1-7.
22. Marfell-Jones M, Olds T, Stewart A, Carter L. International standards for anthropometric assessment. International Society for the Advancement of Kinanthropometry (ISAK); 2006.
23. Mancia G, Fagard R, Narkiewicz K, Rredon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC guidelines for the management of hypertension. *J of Hypertens.* 2013;31:1281-357.

24. Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J, et al. Clinical applications of arterial stiffness, task force III: Recommendations for user procedures. *Am J Hypertens*. 2002 5;15:445-52.
25. Leone N, Ducimetière P, Gariépy J, Courbon D, Tzourio C, Dartigues J, et al. Distension of the carotid artery and risk of coronary events: The three-city study. *Arterioscler Thromb Vasc Biol*. 2008;28:1392-7.
26. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.
27. Cockcroft D, Gault M. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16:31-41.
28. Bussy C, Boutouyrie P, Lacolley P, Challande P, Laurent S. Intrinsic stiffness of the carotid wall material in essential hypertension. *Hypertension*. 2000;35:1049-54.
29. Laurent S, Caviezel B, Beck L, Girerd X, Billaud E, Boutouyrie P, et al. Carotid artery distensibility and distending pressure in hypertensive humans. *Hypertension*. 1994;23:878-83.
30. Bauer M, Caviezel S, Teynor A, Erbel R, Mahabadi AA, Schmidt-Trucksass A. Carotid-intima media thickness as a biomarker for subclinical atherosclerosis. *Swiss Med Wkly*. 2012;142:w13705.
31. Bergmann SR. Carotid plaque measurement is superior to IMT invited editorial comment on: Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: A meta-analysis- Inaba Y, Chen JA, Bergmann SR. *Atherosclerosis*. 2012;220:34-5.
32. Wang KL, Cheng HM, Chuang SY, Spurgeon HA, Ting C, Lakatta EG, et al. Central or peripheral systolic or pulse pressure: Which best relates to target organs and future mortality? *J Hypertens*. 2009;27:461-7.
33. Benetos A, Adamopoulos C, Bureau J, Temmar M, Labat C, Bean K, et al. Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. *Circulation*. 2002;105:1202-7.
34. Lloyd-Sherlock P, Minicuci N, Beard J, Ebrahim S, Chatterji S. Hypertension among older adults in low and middle income countries: Prevalence, awareness and control. *Int J Epidemiol*. 2014.

35. Benetos A, Waeber B, Izzo J, Mitchell G, Resnick L, Asmar R, et al. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: Clinical applications. *American Journal of Hypertension*. 2002;15:1101-8.
36. Laurent S, Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. *Hypertension*. 2007;49:1202-6.
37. Henry RMA, Kostense PJ, Spijkerman AMW, Dekker JM, Nijpels G, Heine RJ, et al. Arterial stiffness increases with deteriorating glucose tolerance status: The Hoorn study. *Circulation*. 20;107:2089-95.
38. Vasdev S, Gill V, Singal P. Role of advanced glycation end products in hypertension and atherosclerosis: Therapeutic implications. *Clin Biochem Biophys*. 2007;49:48-63.
39. McEniery CM, Wilkinson IB. Large artery stiffness and inflammation. *J Hum Hypertens*. 2005;19:507-9.
40. Preston RA, Jy W, Jimenez JJ, Mauro LM, Horstman LL, Valle M, et al. Effects of severe hypertension on endothelial and platelet microparticles. *Hypertension*. 2003;41:211-7.
41. Silwa K, Wilkinson D, Hansen C, Ntyintyane L, Tibazarwa K, Becker A, et al. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto study): A cohort study. *Lancet*. 2008;371:915-22.
42. Vorster HH. The emergence of cardiovascular disease during urbanisation of Africans. *Public Health Nutr*. 2002;5:239-43.
43. Fowkes FGR, Thorogood M, Connor MP, Lewando-Hundt G, Tzoulaki I, Tollman SM. Distribution of a subclinical marker of cardiovascular risk, the ankle brachial index, in a rural African population: SASPI study. *Eur J Cardiovasc Prev Rehabil*. 2006;13:964-9.
44. Yang C, Sun Z, Li Y, Ai J, Sun Q, Tian Y. The correlation between serum lipid profile with carotid intima-media thickenss and plaque. *BMC Cardiovasc Discord*. 2014;14:181.
45. Sun P, Dwyer KM, Merz CNB, Sun W, Johnson CA, Shircore AM, et al. Blood pressure, LDL cholesterol, and intima-media thickness: A test of the “Response to injury” hypothesis of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2000;20:2005-10.

46. Caviezel S, Dratva J, Schaffner E, Schindler C, Zemp Stutz E, de Groot E, et al. Sex-specific associations of cardiovascular risk factors with carotid stiffness – results from the SAPALDIA cohort study. *Atherosclerosis*. 2014;235:576-84.

SUPPLEMENTARY TABLE 1. Pearson correlations in normotensive Africans

Normotensives (N= 241)	CD	IMT	CSWA	Max LD
Age, years	-0.19 p=0.018	0.37 p<0.001	0.32 p<0.001	0.08 p=0.27
Sex, (male) 1	-0.10 p=0.18	0.25 p<0.001	0.27 p<0.001	0.38 p<0.001
Waist circumference, cm	0.08 p=0.30	0.11 p=0.080	0.13 p=0.046	0.02 p=0.73
Body mass index	0.12 p=0.11	-0.01 p=0.84	-0.005 p=0.93	-0.08 p=0.27
<i>Cardiovascular measures</i>				
Brachial SBP, mm Hg	-0.17 p=0.033	0.11 p=0.091	0.14 p=0.03	0.22 p=0.004
Brachial DBP, mm Hg	0.07 p=0.38	-0.05 p=0.41	-0.007 p=0.91	0.17 p=0.031
Heart rate, bpm	0.01 p=0.8	-0.07 p=0.29	-0.008 p=0.90	-0.12 p=0.14
Central SBP, mm Hg	-0.46 p<0.001	0.11 p=0.10	0.14 p=0.040	0.22 p=0.007
Central PP, mm Hg	-0.567 p<0.001	0.16 p=0.016	0.17 p=0.014	0.27 p=0.001
<i>Cardiometabolic risk factors</i>				
Total cholesterol, mmol/l	0.007 p=0.92	0.15 p=0.028	0.11 p=.082	-0.07 p=0.37
HDL-cholesterol, mmol/l	0.07 p=0.39	0.03 p=0.65	0.06 p=0.31	0.14 p=0.075
LDL-cholesterol, mmol/l	0.11 p=0.18	0.11 p=0.090	0.07 p=0.29	-0.17 p=0.028
Triglycerides, mmol/l	-0.07 p=0.38	0.08 p=0.21	0.06 p=0.33	0.04 p=0.57
Glucose, mmol/l	0.07 p=0.34	0.15 p=0.021	0.18 p=0.006	-0.08 p=0.26
HbA1c (%)	0.09 p=0.27	0.02 p=0.68	0.03 p=0.58	-0.10 p=0.21
Interleukin-6, pg/ml	0.02 p=0.69	0.11 p=0.10	0.13 p=0.057	0.09 p=0.23

C-reactive protein, mg/l	-0.08 p=0.28	0.17 p=0.009	0.17 p=0.010	0.08 p=0.27
Creatinine clearance, ml/min	-0.80 p<0.001	-0.11 p=0.09	-0.04 p=0.51	0.09 p=0.25
<i>Health behaviour</i>				
γ-glutamyltransferase, U/l	-0.07 p=0.36	0.16 p=0.013	0.21 p=0.002	0.30 p<0.001
Self-reported alcohol intake, N, total (%)	0.04 p=0.55	0.08 p=0.23	0.16 p=0.018	0.35 p<0.001
Self-reported tobacco use, N, total (%)	0.008 p=0.91	0.008 p=0.90	-0.004 p=0.95	0.08 p=0.29
<i>Endothelial Activation</i>				
Intracellular-adhesion molecule-1, pg/ml	-0.04 p=0.62	0.19 p=0.003	0.15 p=0.020	0.20 p=0.010
Vascular adhesion molecule-1, pg/ml	-0.12 p=0.13	0.06 p=0.35	0.009 p=0.88	0.02 p=0.76

Data expressed as r-values, p-values obtained with Pearson correlations. Bold text indicates significant p-values. CD, carotid distensibility; IMT, intima-media thickness; CSWA, cross-sectional wall area; Max LD, maximum lumen diameter; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SUPPLEMENTARY TABLE 2. Pearson correlations in hypertensive Africans

Hypertensives (N=351)	CD	IMT	CSWA	Max LD
Age, years	-0.26 p<0.001	0.51 p<0.001	0.46 p<0.001	0.05 p=0.43
Sex, (male)	-0.04 p=0.45	0.13 p=0.014	0.17 p=0.002	0.02 p<0.001
Waist circumference, cm	0.12 p=0.067	0.15 p=0.004	0.14 p=0.010	0.09 p=0.14
Body mass index	0.10 p=0.11	0.05 p=0.28	0.03 p=0.48	0.01 p=0.77
<i>Cardiovascular measures</i>				
Brachial SBP, mm Hg	-0.32 p<0.001	0.26 p<0.001	0.29 p<0.001	0.11 p=0.065
Brachial DBP, mm Hg	0.05 p=0.39	-0.03 p=0.49	-0.01 p=0.85	0.04 p=0.51
Heart rate, bpm	-0.03 p=0.59	-0.12 p=0.030	-0.10 p=0.076	-0.04 p=0.54
Central SBP, mm Hg	-0.56 p<0.001	0.24 p<0.001	0.27 p<0.001	0.12 p=0.067
Central PP, mm Hg	-0.64 p<0.001	0.30 p<0.001	0.32 p<0.001	0.12 p=0.054
<i>Cardiometabolic risk factors</i>				
Total cholesterol, mmol/l	0.02 p=0.73	0.11 p=0.038	0.07 p=0.19	-0.18 p=0.004
HDL-cholesterol, mmol/l	0.04 p=0.51	-0.14 p=0.008	-0.14 p=0.008	-0.08 p=0.20
LDL-cholesterol, mmol/l	0.22 p=0.001	0.18 p=0.001	0.13 p=0.017	-0.18 p=0.005
Triglycerides, mmol/l	0.02 p=0.69	0.06 p=0.027	0.06 p=0.22	0.09 p=0.12
Glucose, mmol/l	-0.03 p=0.60	0.06 p=0.22	0.08 p=0.14	0.02 p=0.75
HbA1c (%)	-0.03 p=0.57	0.14 p=0.009	0.12 p=0.033	0.01 p=0.81
Interleukin-6, pg/ml	-0.05 p=0.37	0.04 p=0.43	0.06 p=0.27	0.14 p=0.023

C-reactive protein, mg/l	-0.02 p=0.75	0.09 p=0.095	0.11 p=0.043	0.08 p=0.19
Creatinine clearance, ml/min	-0.73 p<0.001	-0.20 p<0.001	-0.17 p=0.002	0.05 p=0.36
<i>Health behaviour</i>				
γ-glutamyltransferase, U/l	0.02 p=0.75	-0.17 p=0.002	-0.14 p=0.012	0.09 p=0.15
Self-reported alcohol intake, N, total (%)	-0.07 p=0.26	-0.10 p=0.075	-0.03 p=0.64	0.18 p=0.004
Self-reported tobacco use, N, total (%)	-0.04 p=0.50	-0.04 p=0.38	0.02 p=0.71	0.09 p=0.14
<i>Endothelial Activation</i>				
Intracellular-adhesion molecule-1, pg/ml	0.07 p=0.26	-0.06 p=0.23	-0.05 p=0.35	0.10 p=0.11
Vascular adhesion molecule-1, pg/ml	0.07 p=0.27	0.11 p=0.046	0.07 p=0.18	0.05 p=0.41

Data expressed as r-values, p-values obtained with Pearson correlations. Bold text indicates significant p-values. CD, carotid distensibility; IMT, intima-media thickness; CSWA, cross-sectional wall area; Max LD, maximum lumen diameter; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SUPPLEMENTARY TABLE 3. Forward stepwise multiple regression analyses with CSWA and Max LD as dependent variables

	Normotensive (N=241)		Hypertensive (N=351)	
	β (95% CI)	p	β (95% CI)	p
CSWA, mm²				
Adjusted R²	0.23		0.32	
Locality (urban)			-0.11 (-0.21, -0.01)	0.022
Sex (male)	0.29 (0.15, 0.42)	<0.001	0.26 (0.15, 0.37)	<0.001
Age, years	0.29 (0.17, 0.42)	<0.001	0.43 (0.30, 0.57)	<0.001
Waist circumference, cm	0.18 (0.05, 0.31)	0.008	0.02 (-0.12, 0.17)	0.77
Central SBP, mm Hg			0.18 (0.08, 0.28)	<0.001
LDL-Cholesterol, mmol/l			0.12 (0.01, 0.23)	0.026
HbA1c (%)			0.08 (-0.02, 0.19)	0.13
Creatinine clearance, ml/min			0.11 (-0.05, 0.26)	0.18
C-reactive protein, pg/ml	0.16 (0.02, 0.29)	0.025	0.09 (-0.01, 0.20)	0.090
γ -glutamyl transferase, U/l	0.11 (-0.02, 0.24)	0.10	-0.08 (-0.18, 0.02)	0.11
Anti-hypertension medication (yes)			-0.12 (-0.22, -0.02)	0.013
Max LD, mm				
Adjusted R²	0.27		0.10	
Locality (urban)			-0.08 (-0.21, 0.05)	0.21
Sex (male)	0.26 (0.10, 0.42)	0.001	0.22 (0.08, 0.36)	0.002
Age, years	0.13 (-0.03, 0.29)	0.12		
Waist circumference, cm			0.14 (-0.005, 0.28)	0.061
Central SBP, mm Hg	0.20 (0.05, 0.35)	0.010	0.14 (0.007, 0.27)	0.039
Heart rate, bpm	-0.09 (-0.24, 0.06)	0.22		
LDL-Cholesterol, mmol/l	-0.16 (-0.30, -0.02)	0.027	-0.14 (-0.2, -0.003)	0.045
Creatinine clearance, ml/min	0.21 (0.05, 0.37)	0.011		
C-reactive protein, pg/ml			0.09 (-0.05, 0.23)	0.23
ICAM-1, pg/ml	0.16 (0.01, 0.30)	0.037	0.08 (-0.05, 0.21)	0.25
γ -glutamyl transferase, U/l	0.17 (0.03, 0.32)	0.023		

Data expressed as beta-values and 95% confidence intervals, p-values obtained with forward stepwise multiple regression analyses. CSWA, cross-sectional wall area; Max LD, maximum lumen diameter; SBP, systolic blood pressure; LDL, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; ICAM-1, intracellular adhesion molecule-1. Included in each model: Locality, age, sex, WC, HR, cSBP, LDL-C, HbA1c, CRP, ICAM, CrCl, GGT, tobacco and anti-hypertensive medication use.

Chapter 4: Concluding Remarks and Discussion of Main Findings

Introduction

In this chapter, the main findings are summarised, compared to the relevant literature, discussed and concluded in order to point out the differences in the carotid characteristics between normotensive and hypertensive Africans, and to elucidate the associations between the carotid characteristics, classic cardiometabolic risk factors, endothelial activation and health behaviours. Future recommendations are also given.

Summary of main findings

The main objective was to investigate the differences in the carotid characteristics, as well as the association thereof with cardiometabolic risk factors, inflammation, endothelial activation and health behaviours, in 241 normotensive and 351 hypertensive black South Africans.

Hypothesis 1: Hypertensive Africans have an increased carotid intima-media thickness, cross-sectional wall area and a larger lumen diameter, but lower carotid distensibility, than normotensive Africans

Hypothesis 3: Central blood pressure is positively associated with carotid structure and function.

Our most prominent finding is that the carotid characteristics (except IMT) were increased in the hypertensive group, but after adjustment for central systolic blood pressure (cSBP) all differences were eliminated. Thus, our findings suggest that arterial stiffness will not be present in the hypertensives if their blood pressure is reduced. Based on these findings, we partially accept our first hypotheses, as only arterial function (carotid distensibility), and not the structure (cIMT) differed between the normotensive and hypertensive group. Furthermore, carotid distensibility (CD) and intima-media thickness (cIMT) associated with cSBP, thus enabling us to accept the third hypotheses, since the increased carotid stiffness of the hypertensive group is strongly related to cSBP.

Hypothesis 2: The hypertensives have an adverse lipid profile, higher glucose levels, a higher alcohol intake (γ -glutamyltransferase), and higher levels of inflammatory (CRP, IL-6) and endothelial activation (ICAM-1, VCAM-1) markers than normotensives.

Hypothesis 4: The measures of carotid structure and function are positively associated with markers of inflammation (CRP) and endothelial activation (ICAM).

After adjustments, the lipid profile, glycemic and inflammatory state, degree of endothelial activation and health behaviours were similar between the normotensive and hypertensives. Therefore, we reject our second hypothesis.

Even though no differences were found between the normotensive and hypertensive groups, we found that CD is associated with glycated haemoglobin (HbA1c) and IMT with low-density lipoprotein-cholesterol (LDL-C) in the hypertensive group only. Therefore, we partially accept our forth

hypotheses, since the metabolic measures (LDL-C and HbA1c) showed an association with carotid distensibility and cIMT.

Comparison to relevant literature

Literature regarding the carotid characteristics in hypertensive black South Africans is limited, which gives this study a unique edge. The findings of this study is similar to those of Laurent *et al.*, [1] and Bussy *et al.*, [2] who both found that increased arterial stiffness in the hypertensive group can be attributed to the increased distending pressure associated with hypertension, and not structural alterations of the arterial wall.

In contrast to our findings, Stewart *et al.*, found that reducing the blood pressure of hypertensive patients to normotensive levels did not reduce their arterial stiffness, suggesting structural alterations in the arterial wall [3]. Our results indicate very clearly that cSBP is mostly responsible for the increased carotid stiffness in the hypertensive group, however, structural alterations is a possibility if these participants remain hypertensive for longer periods.

We did not find associations between CD and C-reactive protein (CRP), or cIMT and CRP in the hypertensive group. This finding is in contrast to that of Schroff *et al.*, who showed that there is a relationship between inflammation, indicated by CRP and vascular stiffness, indicated by the β -stiffness index of the carotid artery [4].

Discussion of main findings

In 1929 Donnison commented on the low prevalence of high blood pressure among black people in a hospital in Kenya [5]. However, today, 85 years after the publication of Donnison's article, hypertension is more prevalent in black than in white populations [6-8]. Furthermore, it has been shown that blacks have increased arterial stiffness when compared to their white counterparts [9, 10], and that they are more prone to strokes, heart failure and kidney failure than whites [8].

Arterial stiffness is higher in hypertensives than in normotensives [11]. Our findings indicate that the carotid stiffness is increased in hypertensive versus normotensive Africans, which might increase the risk for cardiovascular events and stroke [12, 13] in the hypertensive group. We also suggest, as was found in The Framingham Heart Study, that the increased arterial stiffness of the hypertensives may further exacerbate hypertension, resulting in a vicious cycle of hypertension and increasing arterial stiffness [14].

The fact that no structural changes were indicated is somewhat surprising, since these participants have been hypertensive for at least five years. On the other hand, this also suggests that the increased carotid stiffness in this hypertensive African population is probably treatable. Unfortunately,

medication that specifically targets blood pressure reduction in the central arteries is not available yet [13]. Although standard anti-hypertensive medication is readily available, the challenge remains awareness and control. Blood pressure is modifiable [15], and the cornerstone of blood pressure management is a healthy lifestyle [16]. Blood pressure reduction must take place in this population, whether it is by means of drastic lifestyle changes, conventional anti-hypertensive medication or both.

Classic cardiovascular risk factors do not seem to play a major role in the mediation of carotid stiffness in our population. These results are unexpected, as dyslipidaemia, diabetes mellitus, a high body mass index and smoking have been implicated in the accelerated development of arterial stiffness [17]. However, our multiple regression results indicate that carotid distensibility is associated with glycated haemoglobin, and that IMT is associated with LDL-C in the hypertensive group. It may thus be possible that a period longer than five years is needed in order for structural alterations to take place in the hypertensive group, and that glycated haemoglobin and LDL-C will be key factors in the process.

Conclusion

Although differences exist in terms of carotid structure and function between normotensive and hypertensive Africans, it may be accounted for by the increased distending pressure in the hypertensive group. Despite their hypertensive status, there were no indication of structural adaptations in these Africans. The classic cardio-metabolic risk factors, markers of inflammation, endothelial activation and health behaviour did not differ between the normotensive and hypertensive groups, and also seemed to play only a minor role in the mediation of carotid stiffness in this population at this stage of disease development.

Chance and confounding

It is important to reflect on the factors that could have confounded the results of this study.

The sensitivity and resolution of B-mode ultrasound may have been too low to pick up the finer details of the carotid artery, as would have been possible with echotracking techniques.

The possibility of white-coat hypertension cannot be excluded. The participants may not have been comfortable with having their blood pressure taken, or with the person that took the measurement. This could have led to a higher blood pressure than normal. We did not do follow-up blood pressure measurements between 2005 and 2010. Our participants were selected from targeted populations, and are thus not generalizable to the general population.

One out of approximately every 20 statistical findings is due to chance. Statistical results were interpreted from a physiological perspective and statistical significance may not necessarily always indicate physiological significance.

Recommendations

Recommendations to future research:

- A population with a larger age-range should be included in future studies, to assess the intrinsic stiffness of a younger and also an older group of Africans.
- A study population including black and white groups would enable the researcher to investigate differences between the two ethnic groups.
- More variables, including oxidized-LDL, endothelial micro particles (EMC) and advanced glycation end products (AGE) should be analysed to better establish relationships between hypertension, carotid stiffness, inflammation, endothelial activation and the metabolic profile.
- The gold-standard PWV measurement (carotid-femoral PWV) should be measured since only the carotid-dorsalis pedis PWV measurements were available for this study.
- The carotid characteristics should be measured using echotracking techniques, which have advantages over B-mode ultrasound.
- Follow-up studies should continue to measure carotid characteristics, which will enable the researchers to plan longitudinal studies, and to infer causality between variables.
- Future studies should aim to elucidate whether anti-hypertensive medication (as well as which type of anti-hypertensive medication) will lower the carotid stiffness in hypertensive Africans.

Final remarks

Although our study indicated that the carotid stiffness of the hypertensive group may be reduced by means of blood pressure reduction, this may not be easily achieved in South Africa.

Sub-Saharan Africa is currently undergoing an epidemiological transition characterised by urbanisation and a shift from traditional to more Western lifestyles [18, 19]. This transition is evident in South Africa, where highly urbanised lifestyles in industrialised cities meet more traditional lifestyles in rural areas [20]. The resulting increase in cardiovascular disease is difficult to control properly, since the low- and middle income countries in this region do not have the resources that is needed to contain both communicable diseases (such as HIV-AIDS), and non-communicable diseases, such as cardiovascular disease [22].

Overall, undetected and untreated hypertension in South African blacks is leading to an epidemic of advanced forms of cardiovascular disease [18]. Therefore, the development of community-based education, prevention, screening and intervention programs catered to the average black South African is urgently needed [18]. The best approach seems to be population-based and linked to prevention and lifestyle changes [16].

References

1. Laurent S, Caviezel B, Beck L, Girerd X, Billaud E, Boutouyrie P, et al. Carotid artery distensibility and distending pressure in hypertensive humans. *Hypertension*. 1994;23:878-83.
2. Bussy C, Boutouyrie P, Lacolley P, Challande P, Laurent S. Intrinsic stiffenss of the carotid wall material in essential hypertension. *Hypertension*. 2000;35:1049-54.
3. Stewart AD, Jiang B, Millasseau SC, Ritter JM, Chowienczyk PJ. Acute reduction of blood pressure by nitroglycerin does not normalize large artery stiffness in essential hypertension. *Hypertension*. 2006;48:404-10.
4. Schroff GR, Chen Y, Duprez DA, Bart BA. Relationship between carotid artery stifness index, BNP and high-sensitivity CRP. *J Hum Hypertens*. 2009;23:783-7.
5. Donnison CP. Blood pressure in the African native: its bearing upon the ætiology of hyperplasia and arteriosclerosis. *The Lancet*. 1929;213:6-7.
6. Van Rooyen JM, Kruger HS, Huisman HW, Wissing MP, Margretts BM, Venter CS, et al. An epidemiological study of hypertension and its determinants in a population in transition: The THUSA study. *J Hum Hypertens*. 2000;14:779-87.
7. Seedat YK. Hypertension in black South Africans. *J Hum Hypertens*. 1999;13:96-103.
8. Opie LH, Seedat YK. Hypertension in Sub-saharan African populations. *Circulation*. 2005;112:3562-8.
9. Hefferman KS, Jae SY, Fernhall B. Racial differences in arterial stiffness after exercise in young men. *Am J Hypertens*. 2007;20:840-5.
10. Morris AA, Patel RS, Binongo JNG, Poole J, Mheid IA, Ahmed Y, et al. Racial differences in arterial stiffness and microcirculatory function between black and white americans. *J Am Heart Assoc*. 2013;2:e002154.
11. Franklin SS. Arterial stiffness and hypertension: A two-way street? *Hypertension*. 200;45:349-51.
12. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. *Eur Heart J*. 2006;27:2588-605.

13. Van Sloten TT, Schram MT, van den Hurk K, Dekker JM, Nijpels G, Henry RMA, et al. Local stiffness of the carotid and femoral artery is associated with incident cardiovascular events and all-cause mortality. *J Am Coll Cardiol*. 2014;63:1739-47.
14. Franklin SS, Gustin W, Wong ND, Larson MG, Weber MA, Kannel WB, et al. Hemodynamic patterns of age-related changes in blood pressure: The Framingham Heart study. *Circulation*. 1997;96:308-15.
15. Schutte AE, Schutte R, Huisman HW, Van Rooyen JM, Fourie CMT, Smith W, et al. Are behavioural risk factors to be blamed for the conversion from optimal blood pressure to hypertensive status in black South Africans? A 5-year prospective study. *Int J Epidemiol*. 2012;41:1114-23.
16. Seedat YK, Rayner BL. The South African Hypertension Guidelines 2011. *S Afr Med J*. 2012;102:57-84.
17. Cecelja M, Chowienczyk P. Role of arterial stiffness in cardiovascular disease. *J R Soc Med Cardiovasc Dis*. 2012;1:11.
18. Stewart S, Libhaber E, Carrington M, Damasceno A, Abbasi H, Hansen C, et al. The clinical consequences and challenges of hypertension in urban-dwelling black Africans: Insights from the Heart of Soweto study. *Int J Cardiol*. 2011;146:22-7.
19. BeLue R, Okoror TA, Iwelunmor J, Taylor K, Degboe AN, Agyemang C, et al. An overview of cardiovascular risk factor burden in Sub-saharan African countries: A socio-cultural perspective. *Global Health*. 2009;5:12.
20. Sliwa K, Wilkinson D, Hansen C, Ntyintyane L, Tibazarwa K, Becker A, et al. Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto study): A cohort study. *Lancet*. 2008;371:915-22.
21. Mayosi BM, Flisher AJ, Lalloo UG, Sitas F, Tollman SM, Bradshaw D. The health burden of non-communicable diseases in South Africa. *The Lancet*. 2009;374:14.
22. Yach D, Hawkes C, Gould L, Hofmann KJ. The global burden of cardiovascular diseases: Overcoming impediments to prevention and control. *JAMA*. 2004;291:2616-22.

Appendix A: Ethics approval



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

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: <http://www.nwu.ac.za>

Dr CMT Fourie
Physiology

Faculty of Health Sciences
Tel: 018-299 2092
Fax: 018-299 2088
Email: Minrie.Greeff@nwu.ac.za

21 August 2014

Dear Dr CMT Fourie

Ethics Application: NWU-00016-10-A1 "PURE study"

Thank you for amending your application. All ethical concerns have now been addressed. The sub-study "Vascular and metabolic profiles of 5-year sustained hypertensive versus normotensive black South Africans" have been approved for inclusion under this umbrella project until 31/12/2015.

Yours sincerely

Prof Minrie Greeff
Health Research Ethics Committee Chairperson

Original details: Prof Minrie Greeff(10187308) C:\Users\113210572\Documents\ETIEK\2010 ETHICS\NWU-00016-10-A1 (CMT Fourie-M Maritz) - Approval letter.docm
21 August 2014

File reference: NWU-00016-10-A1 (CMT Fourie-M Maritz)

Appendix B: Turn it in documents



Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author: MELISSA MARITZ
Assignment title: Post-graduate reports
Submission title: 22212337:Turn_it_in_dokument.do...
File name: ts_131832e4-c263-496f-9448-020..
File size: 2.34M
Page count: 43
Word count: 14,246
Character count: 85,645
Submission date: 13-Nov-2014 03:45PM
Submission ID: 477249755

Methodology

A clear association exists between hypertension and arterial stiffness. Whether the increased arterial stiffness seen in hypertensives are due to structural or functional adaptations in the vasculature is uncertain. Hypertension is more common in blacks and they have an increased arterial stiffness and higher stroke prevalence than white populations. Arterial stiffening, or a loss of arterial distensibility, increases the risk for cardiovascular events, including stroke and heart failure, as it increases the afterload on the heart, as well as creating a higher pulsatile load on the microcirculation. The stiffness of the carotid artery is associated with cardiovascular events, like stroke, and all-cause mortality. Furthermore, carotid stiffness is independently associated with stroke, probably because stiffening of the carotid artery may lead to a higher pressure load on the brain. Inflammation, endothelial activation, dyslipidaemia, hyperglycaemia and health behaviours may also influence hypertension and arterial stiffness. Limited information is available on these associations in black South Africans. The high prevalence of hypertension and cardiovascular disease in blacks creates the need for effective prevention and intervention programs in South Africa.

Aim

We aimed to compare the characteristics of the carotid artery between 5-year sustained hypertensive and normotensive black participants. Furthermore, we aimed to determine whether blood pressure, conventional cardiovascular risk factors, markers of inflammation, endothelial activation or measures of health behaviours are related to these carotid characteristics.

Methodology

This sub-study forms part of the South African leg of the multi-national Prospective Urban and Rural Epidemiology (PURE) study. The participants of the PURE-SA study were from the North West Province of South Africa, and baseline data collection took place in 2008 (N=2010), while follow-up data was collected five years later, in 2013 (N=1280). HIV-free participants who were either hypertensive or normotensive (N=992) for the 5-year period, and who had complete datasets, were included in this sub-study. The study population thus consists of a group of 5-year sustained normotensive (n=241) and hypertensive (n=351) black participants.

Anthropometric measurements included height, weight, waist circumference and the calculation of body mass index (BMI). We included several cardiovascular measurements, namely brachial systolic and diastolic blood pressure, heart rate, central systolic blood pressure, central pulse pressure and the carotid-femoral pulse wave velocity. Carotid characteristics included distensibility, intima-media thickness, cross-sectional wall area, maximum and minimum lumen diameter. Biochemical variables that were determined included HIV status, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, triglycerides, fasting glucose, glycated haemoglobin (HbA1c), creatinine

ORIGINALITY REPORT

4%

SIMILARITY INDEX

2%

INTERNET SOURCES

6%

PUBLICATIONS

1%

STUDENT PAPERS

PRIMARY SOURCES

1**"Abstract Book 2008", Diabetologia, 09/2007**

Publication

1%**2****etheses.bham.ac.uk**

Internet Source

1%**3****Sudesh Vasdev. "Role of Advanced Glycation End Products in Hypertension and Atherosclerosis: Therapeutic Implications", Cell Biochemistry and Biophysics, 07/31/2007**

Publication

1%**4****Cecelja, M., and P. Chowienczyk. "Role of arterial stiffness in cardiovascular disease", JRSM Cardiovascular Disease, 2012.**

Publication

1%**5****Gafane, L F, R Schutte, I M Kruger, and A E Schutte. "Large artery stiffness and carotid intima-media thickness in relation to markers of calcium and bone mineral metabolism in African women older than 46 years", Journal of Human Hypertension, 2014.**

Publication

1%**6****Constans, J.. "Circulating markers of endothelial function in cardiovascular****1%**