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THE DETECTION OF END-ORGAN CHANGES FOR PREDICTING PREMATURE VASCULAR EVENTS

Andrea Jeanine Kölkenbeck-Ruh

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.
Johannesburg, South Africa.

03/09/2019

DECLARATION

I, Andrea Jeanine Kölkenbeck-Ruh, declare that the work included in this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. The work contained in this thesis has not been submitted before for any degree or examination in this University, or any other University.



Andrea Jeanine Kölkenbeck-Ruh

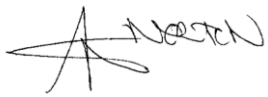
Signed on 3rd day of September 2019 in Parktown.

I certify that the studies included in this thesis have the approval of the Human Research Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics clearance numbers M16-04-01, M17-04-01 and M14-04-29.



Andrea Jeanine Kölkenbeck-Ruh

Signed on 3rd day of September 2019 in Parktown.



Prof G. Norton (Supervisor)



Prof A. Woodiwiss (Supervisor)



Prof M. Hale (Supervisor)

I dedicate this thesis to my parents, brother and husband.

ABSTRACT

Several cardiovascular events including stroke and critical limb ischaemia (CLI) often occur in developing countries at an age where the event is considered to be chronologically premature (often with events noted in teenagers or young adults). Because risk stratification depends so heavily on age, identifying those at a high risk in young adults is fraught with uncertainty. One approach to enhancing risk prediction is to assess end-organ measures. However, the efficacy of end-organ measures in risk prediction over a young adult age range is uncertain. In the present thesis I therefore explored the possible role of several end-organ measures that may be employed to enhance risk prediction for stroke or CLI over a young adult age.

Although chronic kidney disease (CKD) as determined from estimated glomerular filtration rate (eGFR) is recommended for affordable risk prediction by current guidelines, the equations to derive eGFR may not perform well in black South Africans. In the present thesis I demonstrated that in 1152 black South Africans, 294 and 186 with CLI and stroke respectively, in whom 37 % of events were premature, and 672 age-sex-matched controls from a randomly selected community sample, that eGFR was not associated with the odds of the event occurring in young adults. However, carotid intima-media thickness (IMT) was strongly and independently associated with the odds of the event occurring at a young adult age. These data suggest that while the current identification of CKD is unlikely to significantly add to the prediction of non-cardiac arterial events, such as stroke and CLI in young adults, IMT may enhance the prediction of these events at a young adult age.

Hypertension is thought to play an important role in contributing to premature events, The extent to which IMT is determined by aspects of blood pressure load is nevertheless unknown. In 1384 randomly-selected individuals from a community sample I demonstrated that independent of confounders including age, mean arterial pressure (MAP), and forward and backward wave pressures showed associations with indices of cardiac and vascular end-organ changes that were organ and age-specific. Backward wave pressures, but not MAP, showed strong independent relations with left ventricular hypertrophy in individuals younger than 50 years of ages and backward wave and MAP with pulse wave velocity (PWV) in those at any age. However, only MAP (younger than 50 years) and forward wave pressure (older than 50 years), showed independent relations with carotid IMT. Importantly, alternative risk factors failed to associate with IMT beyond age, These data suggest that IMT is primarily an index of the adverse effects of either the steady-state or pulsatile components of an increased blood pressure.

Although carotid IMT predicts arterial events, there is nevertheless considerable uncertainty as to the role of IMT in risk prediction beyond the identification of atherosclerotic plaque. In 54 patients with CLI and amputations I showed that histological thrombotic as opposed to atherosclerotic occlusion occurs more frequently at a younger age and is associated with less carotid plaque but similar age-adjusted increases in IMT. In 952 black South Africans, 473 with CLI or stroke and 479 age-sex-matched controls from a randomly selected Johannesburg community sample, I subsequently demonstrated that, over a younger age range, in contrast to stroke where carotid plaque was markedly increased, patients with CLI failed to show an increased carotid plaque whilst increases in carotid IMT were striking. Thus, carotid IMT adds to carotid plaque in a complementary fashion in associations with arterial vascular events at a younger adult age.

In conclusion, the results provided in the present thesis have advanced our understanding of how to enhance risk prediction for cardiovascular events occurring at a young adult age (premature events). In this regard, I show that whilst eGFR is too insensitive to predict risk for an early event, IMT, which is determined by the most important risk factor for events at this age (hypertension), is strongly associated with events at a young adult age and that IMT complements the assessment of carotid plaque to enhance risk prediction over this age range.

PUBLICATIONS

The following publications have arisen from this work to-date:

1. Kolkenbeck-Ruh A., Woodiwiss A.J., Naran R., Sadiq E., Robinson C., Motau T.H., Monareng T., Mabena P., Manyatsi N., Gazwa P.Z., Abdool-Carrim T., Majane O.H.I, Veller M., Modi G., Norton G.R. (2019). Carotid Intima-media thickness, but not chronic kidney disease independently associates with non-cardiac arterial vascular events in South Africa. *Journal of Hypertension*. 37(4), 795-804. doi: 10.1097/HJH.0000000000001921.

2. Kolkenbeck-Ruh A., Motau T.H., Naran R., Libhaber C.D., Sareli P., Norton G.R., Woodiwiss A.J. (2019). Organ-specific, age-dependent associations of steady-state pressures and pulsatile pressure wave components with end-organ measures. *American Journal of Hypertension*. 32(3), 272-281. doi: 10.1093/ajh/hpy171.

3. Kolkenbeck-Ruh A., Woodiwiss A.J., Monareng T., Sadiq E., Mabena P., Robinson C., Motau T.H., Stevens B., Manyatsi N., Tiedt S., Dembskey R., Abdool-Carrim T., Veller M., Cassimjee I., Modi G., Hale M., Norton G.R. (2019). Complementary impact of carotid intima media thickness with plaque in associations with non-cardiac arterial vascular events. *Angiology*: In press.

PRESENTATIONS

The following presentations have arisen from this work:

Oral presentations:

Oral presentation at the Stroke and Hypertension Congress, 3-5 August, Protea Hotel Stellenbosch, Western Cape, SA, 2018. Presentation Title: Carotid Intima-media thickness, but not chronic kidney disease independently associates with non-cardiac arterial vascular events in South Africa.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisors, Professor's Gavin Norton, Angela Woodiwiss and Martin Hale for their mentorship, guidance and constant support throughout the duration of my PhD. The invaluable skills and knowledge that you have imparted through the years will never be forgotten and have added to my research etiquette, professionalism as well as my own personal growth. I would like to thank members of the Cardiovascular Pathophysiology and Genomics Research Unit (CPGRU) as well as the School of Anatomical Pathology and NHLS for their assistance in data collection and the memorable moments shared in the lab and wards. To Candice your companionship and moral support proved precious throughout the PhD journey. I would also like to thank the National Research Foundation/Innovation Scholarship as well as the University of the Witwatersrand Postgraduate Scholarship for financially supporting this project. To all my family, especially to my mom, dad and brother (Byron), thank you for your constant support and encouragement and allowing me to pursue my dreams. Lastly, to my rock, pillar of strength and husband, Scott, after all the degrees and distance, we have come a long way! Thank you for your continuous support and love that have allowed me to reach my goals.

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LIST OF ABBREVIATIONS

Alx	augmentation index
BMI	body mass index
β-coeff	Beta coefficient
BP	blood pressure
CB	carotid bifurcation
CI	confidence intervals
CKD	chronic kidney disease
CKD-EPI	chronic kidney disease epidemiology collaboration
CLI	critical limb ischaemia
CVD	cardiovascular disease
DBP	diastolic blood pressure
DM	diabetes mellitus
ECA	external carotid artery
eGFR	estimating glomerular filtration rate
$HbA1_c$	percentage glycated haemoglobin
HDL	high density lipoprotein
ICA	internal carotid artery
IMT	carotid intima-media thickness
LDL	low density lipoprotein
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass index
MAP	mean arterial pressure
MDRD	modification of diet in renal disease
MESA	multiethnic study in atherosclerosis
MI	myocardial infarction
OR	odds ratio
Pa	aortic augmentation pressure
Pb	backward (reflected) wave pressure
Pf	forward wave pressure
PP	pulse pressure
PPc	central aortic pulse pressure
PTH	parathyroid hormone

PWV	pulse wave velocity
RM	reflected wave magnitude
SD	standard deviation
SBP	systolic blood pressure
SEM	standard error of the mean
SOWETO	south west township
TOAST	trial of org 10172 in acute stroke treatment

PREFACE

As compared to other diseases, cardiovascular disease accounts for the highest proportion of deaths and hospitalisation in the world today. Although through public health measures and modern medicine, deaths from cardiovascular disease in developed nations are on the decline, this is offset by a dramatic increase in these deaths in developing nations. Indeed, most deaths from cardiovascular disease in the world today now occur in developing nations, such as those in sub-Saharan Africa. Of particular concern is that in developing nations the lower average age of several cardiovascular events including the arterial events, stroke and critical limb ischaemia, is at a younger age, often 10-20 years younger than those events in other countries. Indeed, almost a third of all of these events occur at a chronologically premature age (less than 50 and 55 years for men and women respectively). In this regard, a far greater loss of disability adjusted life years occurs when events affect those at a young age. Yet little is understood as to how best to detect risk in young adults. Although cardiovascular risk stratification is well established, almost without exception these approaches have been developed in those at an older age and hence have evolved to account for events at a middle to older age. Because age is considered a primary driver of events, risk prediction at a younger adult age is difficult at best.

Several risk factors for stroke and critical limb ischaemia are well recognized, including smoking, hypertension and diabetes mellitus, however, as will be highlighted in the present thesis, risk factors may not be present in about 20 % of cases where events occur at a young adult age and only one risk factor may be present in a further 40 % of cases. Consequently, if relying on risk assessment using conventional risk factors alone, a significant proportion of those experiencing chronologically premature arterial events may have been erroneously perceived to carry a relatively low overall risk at the time of the event. Although risk prediction may be enhanced by the identification of changes in end-organ measures, whether at a younger age sufficient time has occurred for risk factors to markedly modify end-organ measures is unknown. In the present thesis I therefore explored the extent to which alterations in several end-organ measures occur in those with stroke or critical limb ischaemia over the full adult age range. Further, in a community-based study I evaluated the impact of risk factors on these end organ measures over the full adult age range.

In chapter 1 of the present thesis I review the current understanding and controversies related to premature vascular events and risk prediction thereof, thus arguing in favour of conducting the studies described herein. In chapters 2 - 4, I describe each of the studies,

their results and the implications thereof. These chapters are constructed as semi-independent chapters and each includes its own abstract, introduction, methods, results and discussion sections. The present thesis concludes with a chapter (chapter 5), which provides a summary of the findings reported; highlights the novelty of these findings and places them in context of our current understanding of the mechanisms responsible for premature vascular arterial events. In support of this thesis, the data provided in chapter 2 has been published in the *Journal of Hypertension* (Kolkenbeck-Ruh et al 2019); chapter 3 has been published in the *American Journal of Hypertension* (Kolkenbeck-Ruh et al 2019); and chapter 4 is currently in press in the journal *Angiology* (Kolkenbeck-Ruh et al 2019).

CHAPTER 1

INTRODUCTION

1.1. Introduction

Over the past two decades the global pattern and burden of disease has evolved with a shift from communicable to noncommunicable disease (McAloon et al 2016). Cardiovascular disease is presently the principle cause of death due to noncommunicable disease and is considered a major barrier to sustainable human development (Roth et al 2015) in high as well as middle-and low-income countries (Bhatia et al 2006; Bradshaw et al 2010; Borlaug and Redfield, 2011; Bowry et al 2015; Mozaffarian et al 2015). Over 17 million people presently die from cardiovascular disease per year, representing 31 % of all global deaths (WHO, 2014) with a trend for an increase expected to continue in years to follow (McAloon et al 2016). Furthermore, the burden of cardiovascular disease is greatly contributing to life years lost due to disability (Roth et al 2017). Disability adjusted life years account for the years of life, particularly healthy and functional life, lost due to a disease (McAloon et al 2016; Roth et al 2017). In this regard, cardiovascular disease accounted for 393.8 million (14.4 %) of total disability adjusted life years lost in 2012 which increased from 352.8 million (12.3 %) in 2000 (McAloon et al 2016).

In the present thesis I explored the possibility of enhancing the prediction (risk) of the development of several cardiovascular events that occur in Africa at a young adult age. Thus, in the present chapter I critically review the evidence and provide arguments to support the importance and highlight the novelty of this question. In this regard in the present chapter I first highlight the evidence to show that cardiovascular events are a major burden to healthcare systems not only in high-income countries, but also in middle-and low-income countries which characterise the African continent. I further underscore that several of these events frequently occur at a young adult age and that the burden of chronologically early (premature) events poses a major challenge to healthcare systems. I subsequently discuss the possible evidence or the lack thereof to support a role for unique risk factors that may explain these events at a young adult age. As I highlight that identification of conventional risk factors is likely to be self-limiting when attempting to select those at risk for an early event for more careful management, I thus argue that an important approach to identifying young adults with risk factors who are at particular risk of events may be to assess intermediate cardiovascular phenotypes (end-organ changes). However, I further suggest that low cost end organ measures currently recommended by guidelines for measurement in low and intermediate-income countries may be limited in their efficacy, but that further work, conducted as part of the present thesis, is required to address this point. I then justify the selection of more costly vascular end-organ measures,

but also underscore the limitations of these measurements which prompted me to perform further studies as part of the present thesis to better understand their use.

1.1.1. Importance of arterial disease as a cause of cardiovascular events

Of all cardiovascular events, diseases of the coronary arteries (coronary artery disease [CHD] or ischaemic heart disease [IHD]) and arteries to the central nervous system (stroke) which largely result in occlusion and hence disruption of the arterial supply to these regions are the two most common causes. In this regard, an estimated 7.4 million and 6.7 million deaths occur annually from CHD and stroke respectively (WHO, 2014; McAloon et al 2016) and the prevalence rates are predicted to increase. Indeed, it is estimated that of the 16 million first ever strokes and 5.7 million stroke deaths that occurred globally in 2005, this could increase to 23 million first ever and 7.8 million stroke deaths by 2030 (Mensah, 2007). Importantly, lower limb peripheral arterial disease which involves occlusion and hence disruption of the arterial supply to the legs, is the third most common cause of cardiovascular morbidity (Fowkes et al 2013). In this regard, it is estimated that globally more than 202 million people have peripheral arterial disease (Ganta et al 2018; Shu and Santulli, 2018) and that peripheral arterial disease affects between 8-12 million adults in the United States of America (USA) alone with an overall prevalence rate of 12 % in the adult population (Steffen et al 2008; Dua and Lee, 2016). As with CHD and stroke, the global prevalence of peripheral arterial disease has increased. Indeed, an estimated increase by 24 % (164 million to 202 million) occurred from 2000 to 2010 (Ganta et al 2018). The increase in high-income countries has occurred mainly in Europe with 40.5 million cases reported in 2010 (Ganta et al 2018).

In Africa, several other cardiovascular disorders are important causes of events at a younger age, including infectious diseases (rheumatic heart disease and infective endocarditis) and alternative causes of heart failure (idiopathic dilated cardiomyopathy and hypertensive heart disease). However, they are not attributed to arterial vascular pathology and hence other than hypertensive heart disease do not offer the possibility of risk prediction. As the present thesis focusses on enhancing risk prediction for cardiovascular events at a younger age, these disorders will not be further considered. Moreover, although CHD is a frequent presentation at a younger age in low and middle-income countries, presentation at a young age in populations of African ancestry in Africa is infrequent. Thus, in the present thesis I will focus the discussion on mainly stroke and peripheral arterial disease, arterial vascular disorders that as will be discussed frequently present a young age

in groups of African descent living in Africa. Mention will nonetheless be made of CHD in several sections as the risk factors and pathophysiology as well as risk prediction of CHD are similar to stroke and peripheral arterial disease.

1.1.2. Increasing burden of cardiovascular disease in low and middle-income countries

Cardiovascular disease is not uniformly distributed across the globe. Regardless of a decrease since 2000, death due to cardiovascular disease is the highest in Europe, with the mortality rate for cardiovascular events in Europe (47.9 %) being higher than the 2012 global average (McAloon et al 2016). However, the distribution of deaths due to cardiovascular events in Europe differs across European regions with 59.6 % of deaths in the Russian Federation being strikingly higher than the 30.5 % of deaths in the United Kingdom for example (McAloon et al 2016). Regional variations in death rates are largely attributed to socio-economic status with a lower status carrying a higher death rate. In this regard, over 75 % of cardiovascular deaths take place in low to middle-income countries (McAloon et al 2016). Of the 17 million global deaths that occurred in those under the age of 70 years due to noncommunicable disease in 2012, 82 % were in low to middle-income countries, and more than 37 % were caused by cardiovascular disease. Importantly, all low to middle-income countries demonstrated an increase in cardiovascular disease mortality from 2000-2012, whilst over the same period the proportion of deaths due to cardiovascular disease in high income countries decreased by 5.5 % (McAloon et al 2016). The impact of socio-economic status affects all arterial vascular conditions to a similar degree including diseases in the peripheral vasculature. In this regard of the fifth of a billion people that are estimated to have peripheral arterial disease, those living in low to middle-income countries are disproportionally affected (Ganta et al 2018). This is largely attributed to an increasing prevalence rate in low to middle-income countries. Indeed, from 2000 to 2010 the proportion of individuals with peripheral arterial disease increased by 29 % in low to middle-income countries as compared to 13 % in high income countries (Ganta et al 2018).

1.1.3. Arterial disease is an increasing burden in Africa

Africa being the home to more than one billion people, most of whom live in low to middle-income countries, is a major contributor to the global burden of cardiovascular disease (Keates et al 2017). Importantly, ethnic disparities contribute to cardiovascular

disease and this may play an important role in events in Africa. In this regard, as compared to Americans of European ancestry, the likelihood of peripheral arterial disease is 50 % greater in African Americans and 55 % lower among those of Chinese origins (Steffen et al 2008). Indeed, those of African descent are at a two to three times greater risk of peripheral arterial disease than non-Hispanic whites, even after adjusting for other cardiovascular risk factors (Ganta et al 2018). With African ancestry being an important risk factor for cardiovascular disease, it is not surprising that cardiovascular disease in Africa is a major contributor to the global burden. Indeed, it is estimated that one million deaths per year may be attributed to cardiovascular disease in sub-Saharan Africa (Keates et al 2017). This constitutes 5.5 % of global cardiovascular related deaths; 11.3 % of all deaths in Africa (Keates et al 2017); and 38 % of all noncommunicable disease-related deaths in Africa (Keates et al 2017). In sub-Saharan Africa, age adjusted standardized prevalence rates for stroke alone are 981 per 100 000 and a three-year fatality rate 84 % (Sarfo et al 2015). The prevalence of peripheral arterial disease in sub-Saharan Africa is also higher than that in high-income countries (Ganta et al 2018). In this regard, prevalence rates for peripheral arterial disease have been reported as being 24 % in South western Uganda (Okello et al 2014), 19 % in Ethiopia (Hagos et al 2017), 26 % in Ghana (Yeboah et al 2016), and 5.5 %, 15 % and 32.4 % in Benin, (West Africa, Bangui (Central Africa) and Brazzaville (Central Africa) respectively (Guerchet et al 2012; Amidou et al 2018). Importantly, there has been a notable increase in cardiovascular disease in Africa with countries such as Cameroon, reporting increases in admissions for stroke from 34 % in 1999-2000 to 84 % in 2011-2012 (Keates et al 2017).

In South Africa, stroke is listed as the fifth and CHD the eighth most common causes of death (Bradshaw et al 2010). Moreover, peripheral arterial disease is a common finding in South Africa affecting more than 29 % of outpatients in poor communities (Paul et al 2007). In South Africa, the prevalence for peripheral arterial disease increases from 8.6 % in those aged less than 40 years to 39.7 % in those aged older than 70 years (Keates et al 2017) and in those attending hospital outpatient departments, the prevalence of peripheral arterial disease is 29.3 % (Paul et al 2007). As with other African countries, in South Africa, the prevalence of cardiovascular disease is increasing (Steyn et al 2006; Bradshaw et al 2010; Rayner et al 2010; Mozaffarian et al 2015).

1.2. Prevalence of arterial disease at a younger age in those of African descent

Age is the strongest risk factor for arterial events with striking increases in mortality caused by events in those older than 60 years of age (McAloon et al 2016). However, the distribution of types of events across age groups is not uniform. In this regard, two thirds of the global burden of stroke is in those younger than 70 years of age (Connor et al 2007; Mensah, 2007; Nkoke et al 2015; Lekoubou et al 2016; Maillet et al 2017; Nkusi et al 2017). Importantly, populations in Africa differ in the age distribution of arterial events, with mortality from vascular disease being the lowest in older age groups as compared to other populations, reflecting the burden of vascular disease in younger age groups in Africa (Ngoungou et al 2012; McAloon et al 2016). In this regard, in Sub-Saharan Africa an increase in the incidence of stroke has occurred in younger individuals (less than 65 years of age) (Connor et al 2007; Sarfo et al 2015). It is estimated that the incidence of stroke occurring in individuals less than 65 years of age is 280 per 100 000 and 363 per 100 000 in South Africa and Zimbabwe respectively (Connor et al 2007). In many African countries, the lower age of stroke has been present for many years. Indeed, in central Ghana the low mean age of stroke has changed little with time over the recent past varying from 59 years to 64 years over a period from 1983 to 2013 (Sarfo et al 2015). Similar low mean ages for stroke have been observed in other African countries. Indeed, the mean age of 59 years was noted in Rwanda, with 27.1 % of patients with stroke being less than 50 years of age and 35.4 % between 50-64 years (Nkusi et al 2017), 59 years in Nigeria (Keates et al 2017), and well below 65 years in Cameroon (Nkoke et al 2015; Lekoubou et al 2016). Strikingly, 13 % of strokes in Africa may occur in individuals less than 45 years of age (Keates et al 2017). In this regard, South Africa is no exception with the average age of hospitalizations for stroke in referral hospitals in South Africa being 45-55 years (Connor et al 2009; Brand et al 2012), with events occurring in those as young as 18 years of age. In short, age-specific stroke incidence is much higher in younger age groups (less than 65 years of age), in sub-Saharan Africa as compared to populations in high-income nations (Connor et al 2007).

Peripheral arterial disease is a strong age-dependant condition (Johansson et al 2010) with nearly 20 % of adults aged 70 years and older carrying the disease (Dua and Lee, 2016). In European and North American countries most of those with peripheral arterial disease are older than 65 years of age. Relatively uncommon among young people, the prevalence of peripheral arterial disease affects a substantial proportion of the elderly with more than 20 % occurring in those 80 years of age or older (Shu and Santulli, 2018). In the

USA, the prevalence of peripheral arterial disease is 4.8 %, 12 %, and 22 % for men and women aged 60-69 years, 70-79 years, and greater than 80 years respectively (Steffen et al 2008). However, the mean age of African-Americans with peripheral arterial disease is 56.3 years (Collins et al 2017). Thus, the onset of peripheral arterial disease is also chronologically premature in individuals of African ancestry (less than 60 years). In this regard, similar low mean ages for peripheral arterial disease are reported in Africa. Indeed, in Nigeria, two separate studies noted that the prevalence of peripheral arterial disease in individuals less than 65 years of age was 52.5 % and 40 % (Oyelade et al 2012; Ogbera et al 2015). South African is again no exception with the average age of hospitalizations for critical limb ischaemia (the end stage of peripheral arterial disease) in referral hospitals in South Africa being 45-55 years (Brand et al 2012) with events occurring in those as young as 18 years of age.

1.2.1. Impact of cardiovascular events at a younger age

As there is considerable evidence to suggest that some cardiovascular events, including stroke and critical limb ischaemia occur in populations of African origins and in Africa at a much lower mean age than in the rest of the world, consideration should be given to the consequences of these events. Those who experience a stroke at a young age have an increased chance of survival and a better outcome as compared to older persons (Ezejimofor et al 2017). However, the impact of stroke at a young as compared to older age on disability adjusted life years is far greater. Indeed, the occurrence of a premature stroke (below age 50 years) results in considerable loss of potential and productivity with notable effects on personal wealth and the wealth of the country (Nakibuuka, 2015). Stroke is well recognized as resulting in a decreased physical activity and major emotional and psychological effects with a considerable reduction in the quality of life. Stroke at any age markedly reduces the chance of being fully employable (Muli and Rhoda, 2013). Only 25 % of patients with ischaemic stroke eventually return to their jobs after the stroke, whilst 70 % of patients remain disabled and are unemployable (Reshi et al 2017). The loss of jobs at a young age to those with families has a major impact on social structures limiting the ability of families to generate income, and feed and educate children. Hence, the disabling impact of stroke in the young affects not only the individual but is a major threat to immediate dependents such as children or spouses and to the economy due to loss of effectiveness and productivity at a working age (Smajlovic, 2015).

Similarly, the survival rate of young individuals with peripheral arterial disease is higher compared to older individuals however, the impact at a young age on disability adjusted life years is far greater. Young patients who survive peripheral arterial disease have to face considerable life changing adaptations particularly when lower limb amputation has occurred (Koureas et al., 2017). Pain is the major feature of living with peripheral arterial disease along with impaired mobility (Aquarius et al 2006; Regensteiner et al 2008; Koureas et al., 2017; Wu et al 2017). Thus, learning to live with both poses a challenge for the patient as well as their families. Even in cases where amputation does not occur, the patients mobility becomes increasingly restricted leading to an increase in help needed for activities of daily living and subsequently a decrease in their productively at work that, ultimately, may lead to the inability to work completely (Koureas et al., 2017; Wu et al 2017). This in turn affects their own and their families financial support and in the greater scheme the economy, particularly in middle to low-income countries. In addition, it restricts their ability to participate in social activities, resulting in them becoming progressively isolated, which may lead to psychosocial issues including depression (Aquarius et al 2006; Koureas et al., 2017). The decrease in mobility reduces blood circulation thus increasing the risk for future amputation and further negatively affecting their own and their loved-one's lives. Moreover, amputation has an increased chance of infection, post operation, and the recovery and adaptation to the loss of the limb is far greater as the patient's dependence on loved-ones or caregivers increases significantly (Koureas et al., 2017). Furthermore, as peripheral arterial disease is primarily of atherosclerotic aetiology, risk for other vascular events such as stroke and MI increases (Barochiner et al 2014; Wu et al 2017). Therefore, there is a high probability that the patient will be on medications such as statins and anti-coagulants (Anderson et al 2013) for the rest of their life.

1.3. Enhancing risk prediction for chronologically premature arterial events in Africa

The higher burden of cardiovascular events at a younger as compared to older age and reports that some vascular events, including stroke and critical limb ischaemia occur in populations of African origins and in Africa at a much lower mean age than in the rest of the world, raises the question of how best to prevent these events at such a young age. Without question risk prediction and subsequent appropriate intervention is a well-established approach to prevention of vascular events. However, almost without exception

studies that have contributed to knowledge on how best to risk predict have been conducted in those at an older age where the risk for events is high. Consequently, there is little knowledge on whether similar approaches as those established in older groups apply equally well to younger groups. Of importance, those with moderate risk factors at a young age seldom achieve a strikingly high-risk score as the strongest risk factor in several risk-scoring systems that is age places them at an intermediate risk. Thus, risk prediction in the young to middle aged is fraught with uncertainty. In the presence of risk factors that are severe, such as severe or refractory hypertension, risk prediction at a young adult age is much more obvious. However, those with events at a young adult age seldom present either as hypertensive emergency or as refractory hypertensives. To appropriately risk predict at a younger age consideration must be given to what may account for differences in the age of events?

Whether events in younger groups are attributed to different risk factors or their combinations and hence different pathophysiological processes, or the same risk factors which have been more severe, have occurred from an earlier age and have not been controlled is unknown. If events in younger groups are attributed to different risk factors or their combinations then these risk factors require early identification and management. If these events are attributed to the same risk factors which are more severe or have occurred from an earlier age and with little control, then appropriate approaches to detecting those who have had these risk factors from an earlier age and with little control require identification. In this regard, the presence of preclinical cardiovascular damage (end organ changes) is generally employed to detect those who have had these risk factors for longer and with little control. In the following sections, I will therefore address the issues of the possibility of age-specificity of the risk factors, particularly in groups of African descent that may account for vascular events at an early age and consequently the role of detecting preclinical cardiovascular damage and whether current approaches may be appropriate at a young adult age in African populations.

1.3.1. Age-specificity of risk factors for vascular events with a focus on groups of African descent

Before identifying whether certain risk factors or their combinations are age-specific, as premature events occur more frequently in groups of African ancestry, the question as to whether there are ethnic disparities in risk factors or their effects that may cause chronologically earlier events in one as opposed to another ethnic group, first

requires consideration. In this regard, for several decades it has been recognised that groups of African origins are predisposed to cardiovascular events (Wolf et al 1991, D'Agostino et al 1994). This predisposition to events may indeed account for the earlier presentation of several events in groups of Africa origins. Standard Framingham Heart Study risk factors (including the modifiable conventional risk factors hypertension, diabetes mellitus, smoking, and dyslipidaemia) are estimated to account for about 40 % of the excess risk of cardiovascular disease, including stroke and peripheral arterial disease, in people of African ancestry (Wolf et al 1991, D'Agostino et al 1994). Indeed, across all ages an increased prevalence of risk factors for cardiovascular disease is observed in African Americans and in black Africans living in African countries such as South Africa (Griffiths and Sturm, 2011). In addition, socioeconomic factors (low-income families, limited secondary or tertiary education levels, lack of access to private healthcare) may account for an additional 10 % of the ethnic disparity in cardiovascular disease (Wolf et al 1991; D'Agostino et al 1994). However, about 50 % of the ethnic disparity in cardiovascular disease risk, particularly stroke and peripheral arterial disease, in people less than 65 years of age remains unexplained (Howard et al 2011). Are there marked differences in the risk factors for events at different ages and are these differences ethnic specific?

There is some evidence to support a role for age-specificity of risk factors for arterial events. What is this evidence? Although haemorrhagic stroke in those below 45 years of age is more likely to be caused by drug abuse, aneurysms, bleeding disorders, and cavernomas than in older age groups, hypertension is still the strongest risk factor for haemorrhagic or ischaemic stroke in both younger and older age groups (Smajlovic, 2015). Importantly, there appears to be a strikingly greater impact of blood pressure on stroke in groups of African ancestry at a young age. Indeed, in those less than 65 years of age, a 10 mm Hg increase in systolic blood pressure results in a 3 times greater chance of developing stroke in groups of African than European ancestry (Howard et al 2013; Dua and Lee, 2016). In this regard, most of those in low to middle-income countries who present with a stroke at a younger age are unaware of the presence of hypertension (Ezejimofor et al 2017). Of note, a strikingly high proportion of young persons with stroke may have diabetes mellitus (Habib et al 2018). Indeed, diabetes mellitus has been reported to account for 87.3 % of ischaemic stroke in those between 18 to 49 years of age with hypertension (44.4 %), dyslipidaemia (23.5 %) and smoking (31 %) playing a much smaller role in that study (Habib et al 2018). Nevertheless, in Africa the role of diabetes mellitus may be less important, accounting for only 8.5 % of all strokes irrespective of age (Deresse and Shaweno, 2015). In a large study (n=3944) conducted across 12 countries in Europe,

smoking was the leading and dyslipidaemia was the second leading risk factor for stroke (46 %) at a young age (Putaala et al 2012). However, in groups of African ancestry in Africa increased total or low-density lipoprotein (LDL) cholesterol concentrations seldom account for vascular events. Whether smoking plays a particularly important role in events in the young in Africa is uncertain. Importantly, non-conventional risk factors such as inflammation, which are associated with vascular aging (arteriosclerosis and atherosclerosis), may be greater in those of African as compared to European ancestry (Howard et al 2011) and this may play a role in the development of chronologically premature vascular events in Africa. Although possible causes of cardiovascular disease in young adults include a genetic predisposition to cardiovascular disease (stroke and peripheral arterial disease), pregnancy and the use of oral contraceptives (Lawrence, 2010), the factors responsible for the earlier predisposition of stroke and peripheral arterial disease in the majority of those with these arterial events in young adults is unknown.

1.3.2. A role for the detection of preclinical cardiovascular disease in the young in Africa

There are several possibilities that may explain chronologically premature cardiovascular events some of which have been alluded to above. As unique differences in risk factors are unlikely to explain marked discrepancies in the age that events occur in African populations, although the identification of risk factors is critical to risk prediction, because age is a strong driver of higher risk scores, their presence alone may not confer a significant risk score in the young. Nonetheless, the detection of preclinical cardiovascular disease (end organ changes) may refine the ability to detect those at particular risk. However, several issues may limit the ability of end-organ measures to enhance risk prediction in the young in Africa. What are these considerations?

An important question is whether chronologically premature events occur because of an enhanced response to known risk factors (and hence a similar degree of end-organ change in younger as compared to older persons), earlier cardiovascular damage because of unmanaged risk factors (and hence a similar degree of end-organ change in younger as compared to older persons), or because of a predisposition to events despite a lower degree of cardiovascular damage (and hence a reduced degree of end-organ damage in younger as compared to older persons)? Indeed, occlusion of arteries produced by atherosclerosis is considered pathologically as atherosclerotic, thrombotic or mixed with thrombotic occlusion occurring in the presence of less advanced atheroma where the extent of the

vascular lesion is less obvious. In this regard, there is some evidence to suggest that patients with premature events have limited atheromatous lesions as compared to those with events occurring later in life (Collet et al 2006; Speidl et al 2007; Pineda et al 2009; Milgrom et al 2018). Rather, a contribution of pro-thrombotic conditions has been demonstrated to play a more important role (Collet et al 2006; Speidl et al 2007; Pineda et al 2009; Milgrom et al 2018). If the latter applies, which may indeed characterise events in the young where less time has occurred for vascular damage, then end organ changes may be less evident. Thus, an important consideration when risk assessing using end organ changes is whether the measurement is sufficiently sensitive in the young. In addition, as events at a younger age in Africa are mainly important in those of African descent, a further consideration is whether the ability of the assessment to detect risk is not limited in this ethnic group for reasons other than stated above. Indeed, as will be discussed some end organ measures in groups of African ancestry are limited in their ability to detect the changes required. Moreover, whether the end organ measure appropriately detects risk in response to the major risk factors in groups of African origins is a further critical consideration. For example, hypertension is likely to cause cardiac hypertrophy while smoking is far less likely to do so, if at all. In the present thesis, I considered the possibility of enhancing risk detection using several low- and subsequently relatively high-cost approaches to enhancing risk detection at a younger age. *In the following section, I will describe the evidence either for or against the use of these measures in Africa beyond cost. In so doing I will highlight the evidence that is still outstanding that is required to justify or refute the use of these end organ measures at a young age in Africa and hence provide the arguments for performing the studies conducted in the present thesis*.

1.3.2.1. Low cost end organ measures and their use in Africa

Presently guideline committees in South Africa recommend only the use of end organ measures obtained from routine clinical assessments performed as part of the normal clinical work-up. In this regard, only the use of electrocardiographic criteria for left ventricular hypertrophy (ECG-LVH) detection and estimated glomerular filtration rate (eGFR) are recommended in those with the most important risk factor for vascular events and that is hypertension (Seedat and Rayner, 2012). However, our group have demonstrated that ECG-LVH detection is markedly limited in groups of African ancestry (Maunganidze et al 2014). Indeed, because obesity attenuates ECG voltages, in both overweight and obese individuals and that this particularly obvious in groups of African ancestry, ECG criteria

are unable to adequately detect the presence of LVH (Maunganidze et al 2014). Although members of our group have developed several approaches to improving the performance of ECG voltages for the detection of LVH (Maunganidze et al 2014; Robinson et al 2016, the sensitivity for LVH detection remains remarkably low (Maunganidze et al 2014; Robinson et al 2016), In addition, in early observations, our group have noted that little echocardiographic LVH occurs in patients with either stroke or critical limb ischaemia (CLI), the end-point of peripheral arterial disease, and hence the sensitivity for event detection is likely to be very low (Naran, unpublished observations). Consequently, ECG-LVH is unlikely ton offer an appropriate end organ assessment that will enhance risk prediction for chronologically premature vascular events in Africa. What of the use of eGFR? In this regard, several issues must be considered.

1.3.2.1.1. Estimated glomerular filtration rate for enhancing risk prediction

The identification of chronic kidney disease (CKD) from a reduced glomerular filtration rate (GFR) estimated (eGFR) from serum creatinine concentrations enhances the prediction of arterial vascular events beyond conventional risk factors (Mahmoodi et al 2012; Matsushita et al 2015). Consequently, CKD identified from a reduced eGFR is included in guidelines for risk prediction (Mancia et al 2013). Risk factor-related renal injury results in a decrease in glomerular filtration rate (the best overall measure of kidney function) (Chang and Kramer, 2011; Sud and Naimark, 2016; Hirakawa et al 2017). What are the explanations for the utilisation of eGFR to enhance risk assessments? The kidney is a well-recognised target for damage by several cardiovascular risk factors. Hypertension and diabetes mellitus have long been identified as important determinants of CKD (Haroun et al 2003; Chang and Kramer, 2011; Kazancioglu, 2011; Sud and Naimark, 2016). Systemic hypertension increases intraglomerular capillary pressure leading to damage and scarring of the glomeruli in the kidney (glomerulosclerosis) and a decrease in glomerular filtration rate (Maclsaac et al 2014). Glomerular damage alters filtration processes and allows protein and other micronutrients to enter the urine thus further decreasing the GFR. Similar to hypertension, diabetes mellitus is a strongly related risk factor for CKD (Kazancioglu, 2011). The activation of metabolic, inflammatory and haemodynamic pathways characterize pathophysiological abnormalities in diabetes mellitus, resulting in diabetic nephropathy (Maclsaac et al 2014). Metabolic pathways begin with prolonged poorly controlled blood glucose concentrations that lead to increased secretion of profibrotic cytokines (transforming growth factor and connective tissue growth factor) and

non-enzymatic glycosylation that causes glycation of the glomerulus and the generation of advanced glycation end products (AGEs) (Maclsaac et al 2014). These changes also cause glomerulosclerosis (Lea and Nicholas, 2002; Kazancioglu, 2011; Maclsaac et al 2014). Furthermore, diabetes mellitus may cause constriction of the efferent arterioles and subsequently dilation of the afferent arterioles resulting in capillary hypertension (Lea and Nicholas, 2002). This, similar to prolonged systemic hypertension, also results in glomerulosclerosis (Lea and Nicholas, 2002; Kazancioglu, 2011). In addition, concurrent changes within the glomerulus including thickening of the basement membrane and the mesangial cells, result in an increase in mesangial matrix, which progressively expands within and engulfs the glomerulus thus extensively reducing filtration. In addition to diabetes mellitus and hypertension, smoking, a recognized modifiable cardiovascular risk factor, is known to increase the risk for CKD (Haroun et al 2003; Orth and Hallan, 2008; Kazancioglu, 2011). Smoking affects the kidney through a variety of mechanisms (Orth and Hallan, 2008). Endothelial cell dysfunction, activation of growth factors such as angiotensin II and endothelin-1, oxidative stress, increased clotting of platelets, impaired lipoprotein and glycosaminoglycan metabolism all are produced by smoking which ultimately damage the kidney. Of note, nicotine induces mesangial cell proliferation thus reducing filtration (Orth and Hallan, 2008) and increases blood pressure thus further promoting progression of CKD (Haroun et al 2003; Orth and Hallan, 2008; Chang and Kramer, 2011; Kazancioglu, 2011; Sud and Naimark, 2016).

In contrast to CKD indexing a composite of the long-term effects of several conventional risk factors, changes in renal function can occur beyond conventional risk factors. In this regard, renal damage may occur as a manifestation of endothelial dysfunction and chronic inflammation. As the kidneys receive 25 % of the entire blood volume and have no antioxidant abilities such as noted in the liver, the glomerulus of the kidney is a vulnerable target for persistent damage caused by inflammatory molecules (Mihai et al 2018). Circulating inflammatory cytokines activate endothelial cells and leukocytes within the microvessels in the glomerulus resulting in a local amplification of inflammatory factors in conjunction with compromised endothelial barrier function (endothelial dysfunction) and receptor-mediated vasoreactivity as well as activation of the coagulation system (Mihai et al 2018). These inflammation-mediated alterations cause irreversible tubular injury, scarring of the glomerulus and ultimately nephron failure resulting in a reduced eGFR and subsequently CKD (Mihai et al 2018). Thus, the use of eGFR may not only index the adverse effects of conventional risk factors but has the added advantage in that it also indexes the adverse effects of non-conventional risk factors.

Renal function is not simply an index of the extent of damage produced by conventional or non-conventional risk factors. Kidney dysfunction contributes to vascular changes through several mechanisms (Chang and Kramer, 2011; Sud and Naimark, 2016; Hirakawa et al 2017). Chronic kidney disease is associated with a range of complex physiological and metabolic changes all of which may produce vascular damage. These include but are not limited to alterations in protein, lipid, and mineral metabolism, a greater chance of metabolic acidosis, increases in inflammation and oxidative stress and changes in homocysteine metabolism (Kovacic et al 2003; Kraut & Kurtz, 2005; Cibulka and Racek, 2011; Slee, 2012; Raikou et al 2016). Metabolic acidosis enhances protein catabolism and muscle wasting resulting in the formation of homocysteine (Gerhard and Duell, 1999; Munter et al 2004; Nitz et al 2019). Auto-oxidation of homocysteine results in damage to vascular endothelial cells and promotes the oxidation of LDL thus stimulating athero and thrombogenesis. Concurrently, high homocysteine concentrations induce proliferation of smooth muscle cells, contributing to the progression of atherosclerosis (Gerhard and Duell, 1999; Busch et al 2004; Ducloux et al 2000; Muntner et al 2004). Diseased kidneys also cannot sufficiently hydroxylate 25-hydroxycholecalciferol, which is a precursor of calcitriol. This deficit of calcitriol causes inadequate absorption of calcium and hence hypocalcemia and hyperphosphatemia, a direct cause of vascular calcification (Cibulka and Racek, 2011). Furthermore, hypocalcemia and hyperphosphatemia lead to hyperparathyroidism which decreases endothelial nitric oxide synthase and modifies endothelial cell activity (Rashid et al 2009). The occurrence of parathyroid hormone-related vascular disease may be due to changes in the expression of inflammatory factors involved in the development of atherosclerosis such as interleukin-6 (Rashid et al 2009). Thus, kidney damage, independent of the traditional cardiovascular risk factors, is an independent risk factor for cardiovascular disease including stroke, coronary artery disease and peripheral arterial disease (Best et al 2002; Manjunath et al 2003; Matsushita et al 2015).

1.3.2.1.1.1. Possible limitations of the use of eGFR for risk prediction in Africa

Despite substantial evidence to support the use of eGFR in risk prediction (Mahmoodi et al 2012; Matsushita et al 2015), the value of risk predicting using eGFR in groups of African ancestry in Africa is uncertain. Several formulae have been developed for assessing estimated glomerular filtration rate (Michels et al 2010; Lamb et al 2014) with a more systematic approach than that used to obtain previous equations resulting in the derivation of the recent Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)

equation (Levey et al 2009). The CKD-EPI equation has been demonstrated to perform well with minimum bias across a range of GFR values in many ethnic groups (Stevens et al 2011). In addition, the CKD-EPI equation more accurately categorizes individuals regarding clinical risk (Matsushita et al 2010; White et al 2010; Matsushita et al 2012; Wang et al 2014; Parsh et al 2015) than the Modification of Diet in Renal Disease (MDRD) study equation, the equation predating the CKD-EPI equation. However, because the relationship between creatinine and GFR varies between ethnicities, neither the MDRD, nor the CKD-EPI equations perform as well in African as they do in Caucasian populations (Earley et al 2012; Lamb et al 2014), and the use of an ethnicity coefficient may not improve on performance in some African populations (Eastwood et al 2010; Van Deventer et al 2011; Stevens et al 2011). Moreover, as discussed arterial events in Africa are often premature, and hence occur at an age prior to when CKD more often occurs. Thus, even if eGFR effectively predicted events in older age groups, this would not address the question of appropriate risk prediction in young adults. Although in black South Africans eGFR derived from the CKD-EPI and MDRD equations is associated with several alternative target organ measures, with more consistent associations noted for CKD-EPI-derived eGFR (Booysen et al 2016), whether estimates of glomerular dysfunction are independently associated with cardiovascular events across the adult lifespan in Africa, is unknown. Hence, as part of the present thesis, I *aimed to determine the independent association of creatinine concentrations, estimated glomerular filtration rate or chronic kidney disease with arterial events that frequently occur across the adult lifespan in Africa (critical limb ischaemia and stroke) and to compare the associations with that of carotid intima-media thickness, a well-accepted index of arterial end-organ changes*. These data have been published in the *Journal of Hypertension* (Kolkenbeck-Ruh et al 2019) and are described in chapter 2 of the present thesis.

1.3.2.2. Higher cost end organ assessments

Importantly, guideline committees in South Africa do not mention the use of several additional end organ assessments that are advocated by committees in high-income countries. The exclusion of these measures from guidelines in South Africa is based purely on the cost and the limited evidence for efficacy in low to middle-income countries. Nonetheless, there may be an argument for the use of these end organ measures in specific individuals to prevent events at a younger age. Indeed, if low-cost end organ assessments have little use, the consideration should be given to employing higher cost approaches.

However, the utility of these higher cost assessments is also predicated on the adequacy for determining the impact of the risk factors assessed and for detecting the risk of events at a young age in Africa. In this regard, several higher cost end organ assessments have been identified for use in high-income countries. In the present thesis I selected to explore the role of several ultrasound indexes of atherosclerosis the major vascular change responsible for arterial events including stroke and peripheral arterial disease. In the following section I will justify my choice of these assessments, highlight the evidence to support the use of these assessments and underscore the outstanding questions in the field that prompted me to perform the studies described in the present thesis.

1.3.2.2.1. Why assess atherosclerotic end organ changes?

Arterial events including stroke and critical limb ischaemia are the end-points of mainly occlusion of arteries caused by several pathophysiological processes, the dominant of which is atherosclerosis. Atherosclerosis is the consequence of a variety of changes that occur in the walls of the arterial system. These changes occur in response to constant mechanical forces in the form of stretch and shear stress created by the pulsatile nature of blood flow (Nazemi et al 1990; Lehoux and Tedgui, 2003; Papaioannou and Stefanadis, 2005; Cecchi et al 2011). Alterations in shear stress transform the arterial wall to restore normal stress levels, but in the presence of risk factors such as hypertension, diabetes mellitus, increases in LDL cholesterol concentrations, smoking, inflammatory and a variety of alternative factors, pathological changes in the arterial wall occur (Nazemi et al 1990; Lehoux and Tedgui, 2003; Papaioannou and Stefanadis, 2005; Cecchi et al 2011; Weber and Noels, 2011). The most important pathological change to the arterial wall is the formation of focal elevated lesions within the tunica intima, representing atherosclerotic plaque. Atherosclerotic plaque formation in large and medium size arteries is the major cause of vascular events responsible for CHD and most peripheral arterial disease. Less common causes of CHD and peripheral arterial disease include inflammatory disorders of the arterial wall (vasculitis) and non-inflammatory arteriopathies, such as fibromuscular dysplasia (Kullo and Rooke, 2016). Atheroma is also a major determinant of stroke. In this regard, although haemorrhagic stroke, stroke caused by small vessel disease (lacunar) and cardio-embolic stroke is often mediated through mechanisms that are non-atherosclerotic in nature (for example small vessel damage caused by hypertension or increases in aortic stiffness and a reduction in the impedance mismatch between the aorta and distal vessels in lacunar disease), atherosclerotic stroke, some small vessel stroke (atheroma in distal large

arteries occluding the orifice of small arteries) and a significant proportion of cardio-embolic stroke (caused by CHD) is attributed to atherosclerosis, Thus, the assessment of atherosclerotic end organ changes may provide an important index that heralds the onset of vascular events at a young age.

1.3.2.2.2. Carotid intima-media thickness in risk prediction

Progressive and subclinical arterial wall alterations precede cardiovascular clinical events. The first morphological changes and abnormalities of the arterial wall can be visualized by ultrasonography (Touboul et al 2012). It is widely accepted that carotid intima-media thickness increases as the vasculature ages and this may in-part index atherosclerotic changes (Pignoli et al 1986). Measurement of carotid intima-media thickness with B-mode ultrasound is a non-invasive, sensitive and reproducible technique for identifying, quantifying and grading atherosclerotic burden and cardiovascular disease (Stein et al 2008). It is a well validated and widely used research tool that has translated into clinical practice which could be used as an additional risk measurement in cardiovascular disease risk assessment (Stein et al 2008). In this regard, the thickness of the carotid intima and media is well recognized as enhancing risk prediction beyond conventional risk factors (Breton et al 2011). Indeed, a number of studies indicate that increases in intima-media thickness are associated with vascular events (Salonen and Salonen, 1993; Chambless et al 1997; O'Leary et al 1999; Chambless et al 2000; Kitamura et al 2004; van der Meer et al 2004; Rosvall et al 2005; Lorenz et al 2006; Lorenz et al 2007; Stein et al 2008; Polak et al 2011; Touboul et al 2012; Naqvi and Lee, 2014; Bots et al 2016; Centurion, 2016; Øygarden, 2017) and that this occurs across ethnic groups (Villines et al 2017). These studies which include large samples and report on relative risk adjusted for risk factors demonstrate that an increase in carotid intima-media thickness predicts the risk of **myocardial infarction** (Salonen and Salonen, 1993; Chambless et al 1997; O'Leary et al 1999; van der Meer et al 2004; Rosvall et al 2005; Lorenz et al 2006; Øygarden, 2017), **coronary heart disease** (Geroulakso et al 1994; Chambless et al 1997; Hodis et al 1998; O'Leary et al 1999; Iglesias del Sol et al 2002; Raso et al 2002; Wang et al 2003; Kablak-Ziembicka et al 2004; Rosvall et al 2005; Lorenz et al 2006; Goldberger et al 2010; Simon et al 2010), **stroke** (O'Leary et al 1999; Chambless et al 2000; Kitamura et al 2004; Vemmos et al 2004; Lorenz et al 2006; Lee et al 2007; Stein et al 2008; Den Ruijter et al 2012) **or a combination of these** (Chambless et al 1997; O'Leary et al 1999; Rosvall et al 2005; Lorenz et al 2006) **as well as peripheral arterial disease** (Bots et al

1994; Burke et al 1995; Allan et al 1997; Cheng et al 2002; Pradeepa et al 2014). Of clinical importance, there is an approximately 11 % increase in cardiovascular disease with each 0.1 mm incremental increase of carotid intima-media thickness (Lorenz et al 2007; Øygarden, 2017). All the above reported observational studies relating carotid intima-media thickness values to cardiovascular events used B-mode measurements usually averaged over at least a 1 cm segment around the carotid bifurcation as described in the Mannheim consensus (Touboul et al 2012). However, most of the studies failed to indicate if the area measured was free from plaque. Furthermore, some studies measured and recorded the mean and maximum carotid intima-media thickness (Polak et al 2011). Therefore, much of the positive data may be because carotid intima-media thickness was specifically measured at points of obvious plaque. However, of importance, the above studies indicate a strong relationship between carotid intima-media thickness and cardiovascular disease beyond conventional cardiovascular risk factors. These studies therefore highlight the importance of carotid intima-media thickness as an end organ measure that adds additional information beyond traditional risk factors (Stein et al 2008).

Albeit that the measurement of carotid intima-media thickness may have several limitations, the fact that it is in-part an assessment of the most common vascular abnormality (atheroma) responsible for arterial events at any age and that it has been demonstrated to predict vascular events caused by atherosclerosis suggests that it may be of value in risk predicting in those at risk of an event when a young adult. However, as discussed in aforementioned sections, the assumption that intima-media thickness measurements adequately predict events in the young is nevertheless predicated on a further assumption that atherosclerosis in the young causing the event is just as advanced as in the elderly. As discussed above however, there is some evidence to suggest that patients with premature events have limited atheromatous lesions as compared to those with events occurring later in life (Collet et al 2006; Speidl et al 2007; Pineda et al 2009; Milgrom et al 2018). Rather, a contribution of pro-thrombotic conditions may play a more important role (Collet et al 2006; Speidl et al 2007; Pineda et al 2009; Milgrom et al 2018). If the latter applies, which may indeed characterise events in the young where less time has occurred for vascular damage, then intima-media thickness changes may be less evident. Thus, an important consideration when risk assessing using intima-media thickness measurements is whether the measurement is sufficiently sensitive in the young. Hence, as part of the present thesis, I *aimed to determine the independent association of carotid intima-media thickness with arterial events that frequently occur across the adult lifespan in Africa (critical limb ischaemia and stroke)*. These data have been published in the *Journal of*

Hypertension (Kolkenbeck-Ruh et al 2019) and are described in chapter 2 of the present thesis.

1.3.2.2.3. Hypertension and carotid intima-media thickening

It is widely accepted that hypertension is a major risk factor for cardiovascular disease and accounts for almost 8 million deaths per year worldwide (Cooper et al 2015). In groups of African ancestry living in developed countries, hypertension is the strongest and most consistent established risk factor for cardiovascular disease, including stroke and peripheral arterial disease (Gillum, 1999). Importantly, the adverse haemodynamic effects of blood pressure are mediated by both steady-state (mean arterial pressure) and pulsatile (pulse pressure) components. Whilst steady-state pressure changes are indexed by diastolic blood pressure, systolic blood pressure is employed as the surrogate index of the adverse effects of pulsatile pressures. As opposed to increases in steady-state pressures, which largely occur from early to middle age, increases in pulse pressure are thought to play an important role in the pathogenesis of hypertension after midlife (Cooper et al 2015). However, the adverse impact of pulse pressure on cardiovascular structure and function occurs in any form of hypertension irrespective of associated changes in diastolic blood pressure and these effects are noted at any age. Moreover, the large artery vascular changes (arteriosclerosis as opposed to atherosclerosis) responsible for increases in pulse pressure are often attributed to alternative (other than hypertension) risk factors such as diabetes mellitus and smoking, major risk factors for CLI in the young in African populations (Brand et al 2012). Thus, the damage caused by increases in pulsatile as opposed to steady-state pressure load may not only apply to the elderly but also to the young and these changes could account for the risk of premature events. It may therefore be important that the end organ assessment employed for risk prediction in the young adequately indexes the impact of both steady-state and pulsatile components of hypertension. What then are the determinants of pulsatile load and is it likely that vascular events or associated vascular end organ assessments that I propose for risk evaluation in Africa occur at a premature age through both steady-state as well as pulsatile loading conditions?

Central arterial pulse pressure (such as in the proximal aorta where pulse pressure is generated) and hence systolic BP (SBP) is not the same as brachial pulse pressure or SBP. In this regard, pulse pressure amplification occurs to a varying degree when moving from the aorta to the periphery. Beyond mean arterial pressure, several factors influence central arterial pulse pressure differently from the peripheral arteries. As the central arterial

pulse is closer to end organs than the peripheral pulse, the determinants of aortic pulse pressure may therefore be pathophysiologically more relevant than peripheral pressures for the pathogenesis of cardiovascular disease (Vlachopoulos[a] et al 2010; Vlachopoulos[b] et al 2010). One of the fundamental determinants of increases in central arterial pulse pressure is arteriosclerosis (as opposed to atherosclerosis), where through fragmentation of aortic elastic tissue, fibrosis, alterations in the cross-linked properties of collagen and calcification of the aortic wall increases in aortic stiffness occur. In the regard, an increased aortic stiffness and changes in aortic diameter enhance aortic characteristic impedance (resistance to flow in a pulsatile system in the absence of wave reflection) and thus the pressure generated by aortic ejection (forward travelling pressure wave). Forward travelling pressure waves (forward waves) are nevertheless rapidly transmitted to the periphery where they meet points of tapering of vessels (such as branch points or beds with a high resistance). At these points they are reflected back and these reflected waves travel back up the arterial tree to meet the forward travelling wave. Backward travelling pressure waves (backward waves) then summate with the forward travelling pressure waves to augment the arterial pulse. If the backward travelling wave returns to the central aorta earlier the overlap between the forward and backward travelling waves and hence the summation of these waves, will be greater. Through vasoconstriction of peripheral arteries and arterioles the magnitude of the backward travelling wave will increase and also augment the forward travelling wave to a greater extent. Thus, an increase in the magnitude of aortic backward (reflected) wave pressures or the speed of wave reflection augment arterial pulse pressure. Understanding the relative roles of mean arterial pressure, forward wave pressure and reflected wave pressures in mediating cardiovascular events will provide better insights into the pathophysiological mechanisms responsible for both events and end organ measures. Presently however, there is conflicting evidence regarding the relative roles of mean arterial pressure, forward wave and reflected wave pressures in mediating cardiovascular damage.

Meta-analyses, including an individual patient meta-analysis have demonstrated the ability of increases in aortic stiffness, a fundamental determinant of forward wave pressure, to predict events beyond brachial blood pressure (Vlachopoulos[a] et al 2010; Vlachopoulos[b] et al 2010; Ben-Shlomo et al 2014). Moreover, in the Framingham Heart Study, an increased aortic stiffness (Mitchell et al 2010) and an enhanced forward wave pressure (Cooper et al 2015), but not the reflected wave, predicted events. In contrast however, in the Multiethnic Study in Atherosclerosis (MESA) a more important role of the reflected wave as compared to forward wave pressure in mediating cardiovascular events was noted (Chirinos et al 2012; Zamani et al 2014). Nevertheless, in the MESA, a more striking

impact of reflected waves was noted for heart failure as opposed to alternative events driven by arterial changes, including myocardial infarction or stroke. Consequently, it is possible that whilst the reflected wave, which is wasted energy, largely determines damage to the myocardium, forward wave pressure, which indexes alterations in characteristic impedance and hence arteriosclerotic vascular pathology, is more closely associated with arterial damage. As reflected waves increase across the adult lifespan, whilst forward wave pressure increases over 50 years of age (Namasivayam et al 2009; Hodson et al 2017), differential effects of mean arterial pressure, reflected wave and forward wave pressure may therefore be age-dependent. It is therefore possible that the major haemodynamic factor responsible for premature vascular events is increases in reflected waves, whilst events that occur later in life are accounted for more by increases in forward wave pressures. Alternatively, irrespective of age, as it indexes arteriosclerotic changes, increases in forward wave pressures may determine vascular events and vascular changes such as increases in carotid intima-media thickness, whilst increases in reflected wave pressures determine cardiac changes. The uncertainty in this regard, thus prompted me to further evaluate these possibilities. When selecting an end organ measure that predicts risk beyond conventional risk factors it is essential that it closely reflects the impact of the risk factors that cause the event. Hence, as part of the present thesis, *I aimed to determine whether steady-state, forward wave and reflected wave pressures show differential associations with cardiac (mass) and large artery (carotid intima-media thickness) end-organ changes and whether these effects are age-dependent.* These data have been published in the *American Journal of Hypertension* (Kolkenbeck-Ruh et al 2019) and are described in chapter 3. Importantly, as I have demonstrated in chapter 2 that premature arterial events are strongly associated with increases in carotid intima-media thickness, the data in chapter 3 therefore provide insights into the determinants (risk factors) of that vascular end organ change (intima-media thickness) that is markedly altered in patients who experience arterial events early in adult life.

1.3.2.2.4. Carotid plaque as opposed to carotid intima-media thickness

As highlighted in preceding sections as carotid intima-media thickness in-part indexes vascular atherosclerotic processes, and has been demonstrated to predict vascular events, it may be an important end-organ measure that enhances risk prediction in young adults. Nevertheless, there is some controversy as to the use of carotid intima media thickness in risk prediction. Indeed, there may be insufficient data supporting the

possibility that carotid intima-media thickness improves cardiovascular disease prediction beyond prediction by conventional risk factors (Folsom et al 2008; Lau et al 2008; Lorenz et al 2010; Simon et al 2010; Den Ruijter et al 2012; Inaba et al 2012; Robertson et al 2012; Spence et al 2012; van den Oord et al 2013). As much of the positive data on successfully detecting risk with carotid intima-media thickness may have been attributed to the measurements being performed in discrete areas of more advanced plaque formation, in more recent years the focus has shifted away from the use of intima-media thickness toward the detection of obvious plaque on carotid imaging. In this regard, meta-analyses and large studies have demonstrated that carotid plaque may be more predictive of cardiovascular events than intima-media thickness (Johnsen et al 2009; Inaba et al 2012; Spence et al 2012; Yoon et al 2017; Jeevarethinam et al 2018; Mitchell et al 2018; Sillesen et al 2018). There are indeed several reasons why carotid intima-media thickness may not be adequately predictive of atherosclerotic disease. What are these reasons?

Pathological intimal thickening, which may result in an increase in intima-media thickness, represents the earliest manifestation of progressive atherosclerosis. It is rich in proteoglycans and lipids and lacks smooth muscle cells and collagen. In contrast, medial thickening, which occurs as a consequence of adaptive hypertrophy, and as such is not a true representation of an atherosclerotic lesion, may also cause increases in intima-media thickness. Carotid intima-media thickness may therefore represent adaptive changes to biomechanical parameters with aging and may not be an accurate indicator of atherosclerotic changes. An increased intima-media thickness may also represent "fatty streaks" which consist of foamy macrophages, but these lesions have been shown to regress rather than progress to plaque formation (Wannarong et al 2013). Hence, there has been considerable uncertainty as to whether intima-media thickness or plaque best predict cardiovascular events. This may be particularly important in groups of African ancestry. Indeed, in a multi-ethnic study conducted in the United Kingdom, whilst carotid intima-media thickness was greater in a group of black African ancestry than in a comparable group of European descent, carotid plaque was less prevalent in black individuals as compared to with white individuals (Mackinnon et al 2010). This dissociation between intima-media thickness and plaque seen in black individuals suggests that an increase intima-media thickness in groups of black African ancestry may not directly relate to an increased atherosclerosis. Despite the evidence to support a role of plaque assessment over that of intima-media thickness in risk prediction, there are also arguments to suggest that intima-media thickness may complement the use of plaque. What are these arguments and

is there a possibility that this may be particularly important in risk prediction in young adults?

Although several studies suggest that carotid plaque predicts events beyond intima-media thickness (Johnsen et al 2009; Inaba et al 2012; Spence et al 2012; Yoon et al 2017; Jeevarethinam et al 2018; Mitchell et al 2018; Sillesen et al 2018) there are also studies that suggest that carotid intima-media thickness is superior to plaque at predicting events (Geeta et al 2015; Ren et al 2015; Barakoti, 2018). In this regard, as highlighted in aforementioned discussion, atherosclerosis can result in events attributed to either predominantly thrombotic or atherosclerotic (large plaque protruding into the lumen) arterial occlusion. In this regard, the presence of carotid plaque may be more likely in events caused by more extensive atherosclerosis (atherosclerotic occlusion). Alternatively, the extent of atherosclerosis and hence the chances of obvious plaque being detected may be less in those with largely thrombotic occlusion. Thus, more subtle atherosclerotic indexes, such as intima-media thickness may be better associated with arterial events where events are dominantly thrombotic in nature. In this regard, chronologically premature arterial events may be accounted for more by thrombotic than atherosclerotic occlusion (Collet et al 2006; Speidl et al 2007; Pineda et al 2009; Milgrom et al 2018). Whether carotid plaque is sufficiently sensitive to predict events in younger individuals is therefore unknown. In contrast, irrespective of the pathophysiological processes accounting for intima-media thickness, this may be a sensitive index of risk factor effects on early atheroma. However, whether the presence of plaque is equally as closely associated with arterial events over the younger adult age range, or where events may be attributed to more thrombotic than atherosclerotic occlusion, is unknown. As longitudinal studies cannot be readily conducted to assess events over a young adult age range, to further evaluate the role of carotid intima-media thickness versus plaque in the development of arterial events over this age range, *in the present thesis I therefore compared intima-media thickness and plaque in cases versus controls at different ages across the full adult lifespan. To evaluate whether age determines the extent to which lower limb thrombotic as opposed to atherosclerotic occlusion occurs, histological examination of amputated lower limb arteries was evaluated in those with CLI*. These data have been accepted for publication and is currently in press in the journal *Angiology* (Kolkenbeck-Ruh et al 2019) and are described in chapter 4.

1.4. Problem statement

Arterial vascular events frequently occur at a young age in African populations (stroke and CLI) and this has a major impact on the lives of the affected individuals, and immediate families and on the economy of any country. Whilst the detection and treatment of conventional risk factors is a major goal in preventing such events, the presence of risk factors alone may be markedly limited in risk assessment in the young. Thus, enhancing risk prediction beyond conventional risk factors is required to risk predict in young adults. In this regard, the role of low cost measures (ECG-LVH and eGFR) performed as part of a routine clinical work-up are advocated by guideline committees in South Africa. Although there is substantial evidence to support the use of these measures for enhancing risk prediction members of our group have demonstrated a poor performance for LVH detection using ECG criteria in African populations (Maunganidze et al 2014) with better approaches still only achieving a low sensitivity for LVH detection (Maunganidze et al 2014, Robinson et al 2016). In addition, LVH may not be a common finding in those with vascular events that occur at a young age (Naran et al, unpublished findings). Moreover, the utility of current formulae for estimating GFR in African populations may be remarkably limited. Although eGFR as determined using several of these formulae is independently associated with alternative end organ changes (Booysen et al 2016), whether CKD characterises those with events at a young age where insufficient time may have elapsed for risk factors to have produced CKD and where the extent of cardiovascular damage overall may be less than at an older age is unknown. If currently recommended low-cost end organ measures cannot be employed to predict risk at a young age, the question arises as to whether there are appropriate higher-cost end organ measures that may predict risk at a young age in Africa. As vascular events in Africa that occur at a young age (stroke and CLI) are likely to be driven to a large extent by atherosclerotic changes, one possible approach to assessing risk is the use of carotid intima-media thickness assessments. However, several questions arise in this regard. First, is carotid intima-media thickness sufficiently sensitive to detect vascular events at a young age when these events may be driven by less advanced atheroma? Second, will carotid intima-media thickness detect vascular events at a young age when it may index changes which reflect compensatory medial hypertrophy or fatty streaks which regress with time rather than clinically relevant atherosclerosis? Third, does carotid intima-media thickness index the relevant aspects of hypertensive pulsatile load that may drive events at a young age? This question is critical as hypertension may be a primary risk factor for events in groups of African ancestry. In this regard, previous studies suggest

that intima-media thickness may be driven by wave reflection and not that component of pulsatile load determined by arteriosclerotic abnormalities known to predict events. As intima-media thickness may to some extent reflect non-pathological changes rather than clinically relevant atherosclerotic changes, the question arises as to whether carotid plaque should be the preferred vascular change when risk assessing in the young to predict premature arterial events. Even though current evidence suggests that intima-media thickness may not add much to risk assessment beyond plaque, as highlighted there is some evidence to support this notion. In this regard, in the presence of less extensive atheroma, such as could occur in thrombotic occlusion which may occur more frequently in the young, plaque may not be sufficiently sensitive to detect risk. As a consequence of the aforementioned questions in the present thesis I examined the following hypotheses.

1.5. Hypotheses

In the present thesis, I therefore hypothesised that:

1. The low-cost end organ assessments, creatinine concentrations and eGFR as determined from standard formulae and the presence of CKD are independently associated with arterial vascular events (stroke and CLI) in groups of African descent not only at an older adult age but also in those with chronologically premature events. These independent relations are no worse than a higher cost direct measure of vascular pathology, carotid intima-media thickness.
2. The higher cost end organ measure, carotid intima-media thickness is independently associated with arterial vascular events (stroke and CLI) in groups of African ancestry not only at an older adult age but also in those with chronologically premature events.
3. In relations with blood pressure (the principle risk factors driving events in groups of Africa ancestry) at a community level, that carotid intima -media thickness is independently associated with pulsatile load driven by arteriosclerotic vascular changes (forward wave pressures), while cardiac end organ measures are more closely associated with pulsatile load determined by wave reflection.
4. When predicting risk for premature arterial events that because events at a younger age are often thrombotic in nature with less extensive atheroma mediating the arterial occlusion that earlier carotid atherosclerotic changes as indexed by carotid intima-media thickness may complement plaque in associations with events.

1.6 Aims

The aims of the present thesis are therefore as follows:

1. To assess the extent to which common carotid intima media thickness increases in vascular events (stroke and critical limb ischaemia) across the full adult age range, including at a young adult age and whether creatinine concentrations, eGFR or CKD are as well associated with vascular events as compared to carotid intima media thickness across the adult lifespan. These data are described in chapter 2 of the present thesis and have been published in the *Journal of Hypertension* (Kolkenbeck-Ruh et al 2019).
2. To compare the relative contribution of forward wave (arteriosclerotic changes) and backward wave pressures beyond steady-state pressures to variations in vascular and cardiac end organ measures across the adult lifespan in a large community sample. These data are described in chapter 3 of the present thesis and have been published in the *American Journal of Hypertension* (Kolkenbeck-Ruh et al 2019).
3. To assess the extent to which carotid plaque is better associated with premature vascular events than carotid intima media thickness across the adult lifespan or whether intima-media thickness complements plaque in this regard. To determine whether the ability of intima-media thickness to complement plaque in associations with vascular events can be explained by the degree of atherosclerotic (more advanced atheroma better detected using plaque) versus thrombotic (less advanced atheroma better detected using intima-media thickness) occlusion determined from histological assessment. These data are described in chapter 4 of the present thesis and is currently in press in the journal *Angiology* (Kolkenbeck-Ruh et al 2019).

CHAPTER 2

Carotid Intima-Media Thickness, but not Chronic Kidney Disease Independently Associates with Noncardiac Arterial Vascular Events in South Africa.

The data in this chapter has been published in the *Journal of Hypertension*:

Kolkenbeck-Ruh A., Woodiwiss A.J., Naran R., Sadiq S., Robinson C., Motau T.H., Monareng T., Mabena P., Manyatsi N., Gazwa P.Z., Abdool-Carrim T., Majane O.H.I., Veller M., Modi G., Norton G.R. (2019). Carotid intima-media thickness, but not chronic kidney disease independently associates with noncardiac arterial vascular events in South Africa. *Journal of Hypertension*. 37(4), 795-804. doi: 10.1097/HJH.0000000000001921.

2.1. Abstract

Background: Although chronic kidney disease (CKD) as determined from estimated glomerular filtration rate (eGFR) is recommended for risk prediction by current hypertension guidelines, the equations to derive eGFR may not perform well in black Africans. We compared whether across the adult lifespan, eGFR or CKD are as closely associated with non-cardiac arterial vascular events, as carotid intima-media thickness (IMT), in Africa.

Methods: In 1152 black South Africans (480 with non-cardiac arterial events, [294 with critical lower limb ischemia-CLI, 186 with stroke] of which 37 % were premature) and 672 age, sex and ethnicity-matched controls from a randomly selected community sample), we assessed relations between eGFR, CKD or carotid IMT (B-mode ultrasound) and arterial events.

Results: From 20 years until old age, with or without adjustments, IMT was increased in those with as compared to without events ($p<0.01$ at each decade of age). However, at any decade of age across the adult lifespan neither creatinine concentrations, nor eGFR were altered in those with arterial events ($p>0.28$). Whilst IMT was strongly and independently associated with the odds of an event (OR per 1 SD [0.171 mm] effect=2.19, CI=1.75 to 2.78, $p<0.0001$), neither creatinine concentrations ($p=0.89$), MDRD-derived ($p=0.07$), nor CKD-EPI-derived (OR per 1 SD [22.5 ml/min/1.73m^2] effect=1.06, CI=0.89 to 1.27, $p=0.51$) eGFR were independently associated with the odds of an event. Whilst many with premature events had an increased IMT (63 %), few with either premature events (8 %) or with events at an older age (21 %) had CKD and CKD had a poor performance (0.539±0.011) and low sensitivity (16 %) for event detection.

Conclusions: In black South Africans, despite carotid IMT strongly associating with non-cardiac arterial vascular events (stroke and CLI) consistently across the adult lifespan, few with events have CKD and CKD fails to associate with events.

2.2. Introduction

The kidney is a well-recognised target for damage by several cardiovascular risk factors including hypertension. Risk factor-related renal injury results in a decrease in glomerular filtration rate (GFR) (the best overall measure of kidney function) and kidney dysfunction contributes to vascular changes through several mechanisms (Chang and Kramer, 2011; Sud and Naimark, 2016; Hirakawa et al 2017). Importantly, the identification of chronic kidney disease (CKD) from a reduced GFR estimated from serum creatinine concentrations (eGFR) enhances the prediction of arterial vascular events beyond conventional risk factors (Mahmoodi et al 2012; Matsushita et al 2015). Consequently, CKD identified from a reduced eGFR is included in hypertension guidelines for risk prediction (Mancia et al 2013). In comparison to alternative measures of end-organ changes, eGFR is a simpler, cheaper and more cost-effective approach to risk prediction. Hence, hypertension guidelines in middle-income countries in Africa, give a higher priority to creatinine and eGFR assessments for risk prediction than more costly end-organ measures (Seedat and Rayner, 2012). However, the role of CKD in enhancing risk prediction in Africa, is uncertain.

Several formulae have been developed for assessing eGFR (Michels et al 2010; Lamb and Stevens, 2014), with a more systematic approach than that used to obtain previous equations resulting in the derivation of the recent Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Levey et al 2009). The CKD-EPI equation has been demonstrated to perform well with minimum bias across a range of GFR values in many ethnic groups (Stevens et al 2011). In addition, the CKD-EPI equation more accurately categorizes individuals regarding clinical risk (Matsushita et al 2010; White et al 2010; Matsushita et al 2012; Wang et al 2014; Parsh et al 2015) than the Modification of Diet in Renal Disease (MDRD) study equation, the equation predating the CKD-EPI equation. However, because the relationship between creatinine and GFR varies between ethnicities, neither the MDRD, nor the CKD-EPI equations perform as well in African as they do in Caucasian populations (Earley et al 2012; Lamb and Stevens, 2014), and the use of an ethnicity coefficient may not improve on performance in some African populations (Eastwood et al 2010; Stevens et al 2011; Van Deventer et al 2011). Moreover, arterial events in Africa are often premature, and hence occur at an age prior to when CKD more often occurs. Although in black South Africans eGFR derived from the CKD-EPI and MDRD equations associate with several alternative target organ measures, with more consistent associations noted for CKD-EPI-derived eGFR (Booysen et al 2016), whether

estimates of glomerular dysfunction and CKD are independently associated with cardiovascular events across the adult lifespan in Africa, is unknown. Hence, in the present study we aimed to determine the independent association of creatinine concentrations, eGFR or CKD with arterial events that frequently occur across the adult lifespan in Africa (critical limb ischaemia and stroke) and to compare the associations with that of carotid intima-media thickness (IMT), a well-accepted index of arterial end-organ changes.

2.3. Methods

2.3.1. Study groups

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M11-08-29, M14-04-29, M16-04-11, M02-04-72, M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. 1152 black South Africans participated in the present study. 480 consecutive black South Africans with stroke (n=186) or critical limb ischaemia (CLI) (n=294) were recruited from the Charlotte Maxeke Johannesburg Academic Hospital, South Africa. Patients with myocardial infarction (MI) were not included as MI most frequently occurs in older age groups in South Africans of African ancestry and the event may cause reductions in eGFR. The presence of CLI was defined as the occurrence of ischaemic leg pain at rest for more than two weeks, or the existence of ulcers or gangrene attributable to occlusive arterial disease. Patients with a new stroke, defined as a focal neurological deficit of vascular origin were evaluated. Participants with meningitis based on cerebrospinal fluid examination, or the presence of intracranial malignancy or intracranial mass lesions on imaging, or a vasculitis were excluded. Strokes were classified according to the Trial of Org 10172 in acute stroke treatment (TOAST) classification. Data obtained in patients with arterial vascular events were compared with data acquired over the same time period in 672 age- and sex-matched randomly recruited participants older than 16 years of black African descent living in the South West Township (SOWETO) of Johannesburg, South Africa, using the population census figures of 2001 (Booysen et al 2016). In this regard, black African patients attending the Charlotte Maxeke-Johannesburg Academic Hospital are of the same socioeconomic class as those living in the SOWETO community. Of the patients with arterial vascular events and the controls, 448 and 406 respectively had high quality carotid images available for the assessment of IMT.

2.3.2. Clinical, demographic and anthropometric measurements

A questionnaire was administered to obtain demographic information including each participant's medical history, the use of medication and tobacco and alcohol use. Clinical information was also extracted from the hospital records and confirmed by the attending physician. Clinical data included the presence and duration of risk factors (hypertension, and diabetes mellitus and the therapy thereof). Height and weight were measured using standard approaches and in all participants obesity was defined as a body mass index (BMI) $\geq$30 kg/m^2. Blood tests were performed including a fasting lipid profile and glucose concentration. Participants were considered to have diabetes mellitus if they had a fasting plasma glucose concentration $\geq$7mmol/l, or in whom glucose-lowering agents were prescribed. Brachial blood pressure (BP) was measured according to guidelines and taken as the mean of five measurements. Participants with a BP$\geq$140/90 mm Hg or those receiving antihypertensive medication were considered to have hypertension.

2.3.3. Estimated GFR

Serum creatinine concentrations were measured from blood samples obtained before endovascular procedures or surgery and in clinically well hydrated patients, using the Advia Chemistry systems (Siemens) with calibration traceable to isotope dilution mass spectrometry (IDMS). The 4-variable MDRD and CKD-EPI equations were employed to estimate GFR. The ethnicity factor as recommended in African Americans when calculating the MDRD and CKD-EPI eGFR was not applied in the present study as the use results in overestimation of kidney function in black Africans (Van Deventer et al 2011; Earley et al 2012).

2.3.4. Carotid intima-media thickness

Carotid IMT was determined using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington) employing a linear array 7.5 MHz probe as previously described (Booysen et al 2016). Images of at least 1cm length of the far wall of the distal portion of the right common carotid artery from an optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualized simultaneously) at least 1 cm proximal to the flow

divider were obtained (Figure 2.1). Carotid IMT measurements (Figure 2.2) were determined using semi-automated border-detection and quality control software.

2.3.5. Statistical analysis

As the present study compared cases and controls, the sample size required to show differences in eGFR with 80 % power was calculated using sample size calculation for case-control studies (Charan and Biswas, 2013). In order to calculate the minimum sample size required, I examined previous population-based studies that included individuals of black African ancestry and compared eGFR between two groups. Because there are no publications comparing eGFR in those with stroke and CLI versus controls, I examined meta-analyses of population studies where the impact of the risk factors, hypertension (Mahmoodi et al, 2012) and diabetes mellitus (Fox et al, 2012), common risk factors in the current study, was examined. In this regard, from 6 studies which included individuals of African ancestry and compared eGFR in hypertensive and normotensive individuals (Mahmoodi et al (2012), the mean SD was 16.9 (ml/min/1.73m^2) and the mean difference in eGFR between the two groups was 4.75 (ml/min/1.73m^2). Using the formula from Charan and Biswas (2013), the minimum sample size required to identify statistically significant differences (α=0.05) in eGFR with 80 % power between cases and controls was 99 in each group. In addition, from 5 studies which included individuals of African ancestry and compared eGFR between diabetic and non-diabetic individuals (Fox et al, 2012), the mean SD was 20.57 (ml/min/1.73m^2) and the mean difference of eGFR between the two groups was 5.5 (ml/min/1.73m^2). From the latter data using the formula by Charan and Biswas (2013)**,** the minimum sample size required to identify statistically significant differences in eGFR with 80 % power between cases and controls was 110 in each group. Although the current study employed considerably larger study samples (n=480 cases and n=672 controls) to show differences, prior to initiating the study we did not know how many patients we would be able to recruit at a younger age and hence considerably more participants were studied than that required to show differences in eGFR.

For database management and statistical analysis, SAS software, version 9.4 (SAS Institute Inc., Cary, NC) was employed. Continuous variables were expressed as mean (SD), or median (interquartile range) when non-normally distributed. Dichotomous variables were expressed as proportions or percentages. Age-related thresholds for IMT were derived from age-specific 75th and 95th percentiles of normotensive, non-diabetic participants from the community sample (Table 2.1). CKD was identified not only from an

eGFR<60 ml/min/1.73m^2, the presently accepted threshold, but also from age-specific thresholds derived from the 5th percentiles of normotensive, non-diabetic participants from the community sample (Table 2.2). Multiple logistic regression analysis was performed to determine the independent relations between eGFR or carotid IMT and vascular events. Adjustments included in multivariate models were those associated with vascular events. Odds ratios (OR) were compared using z statistics. Performance of IMT or eGFR for the detection of non-cardiac arterial vascular events was determined from the area under the receiver operator characteristic curve (AUC) and sensitivity and specificity for event detection using standard approaches.

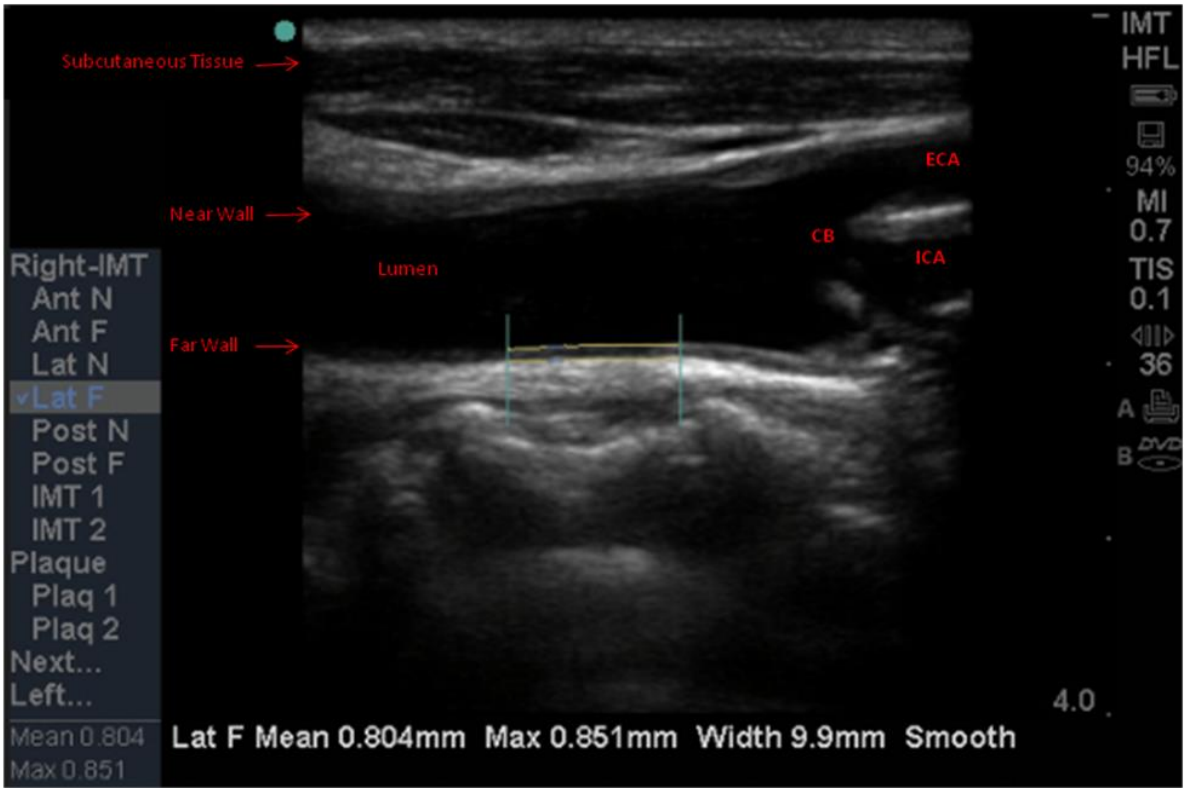


Figure 2.1. Ultrasonography of a 68 year old with critical limb ischaemia, showing the right lateral wall of the common carotid intima-media thickness (indicated by the double yellow line), 1 cm from the carotid bifurcation (CB) forming the right external (ECA) and internal (ICA) carotid arteries.

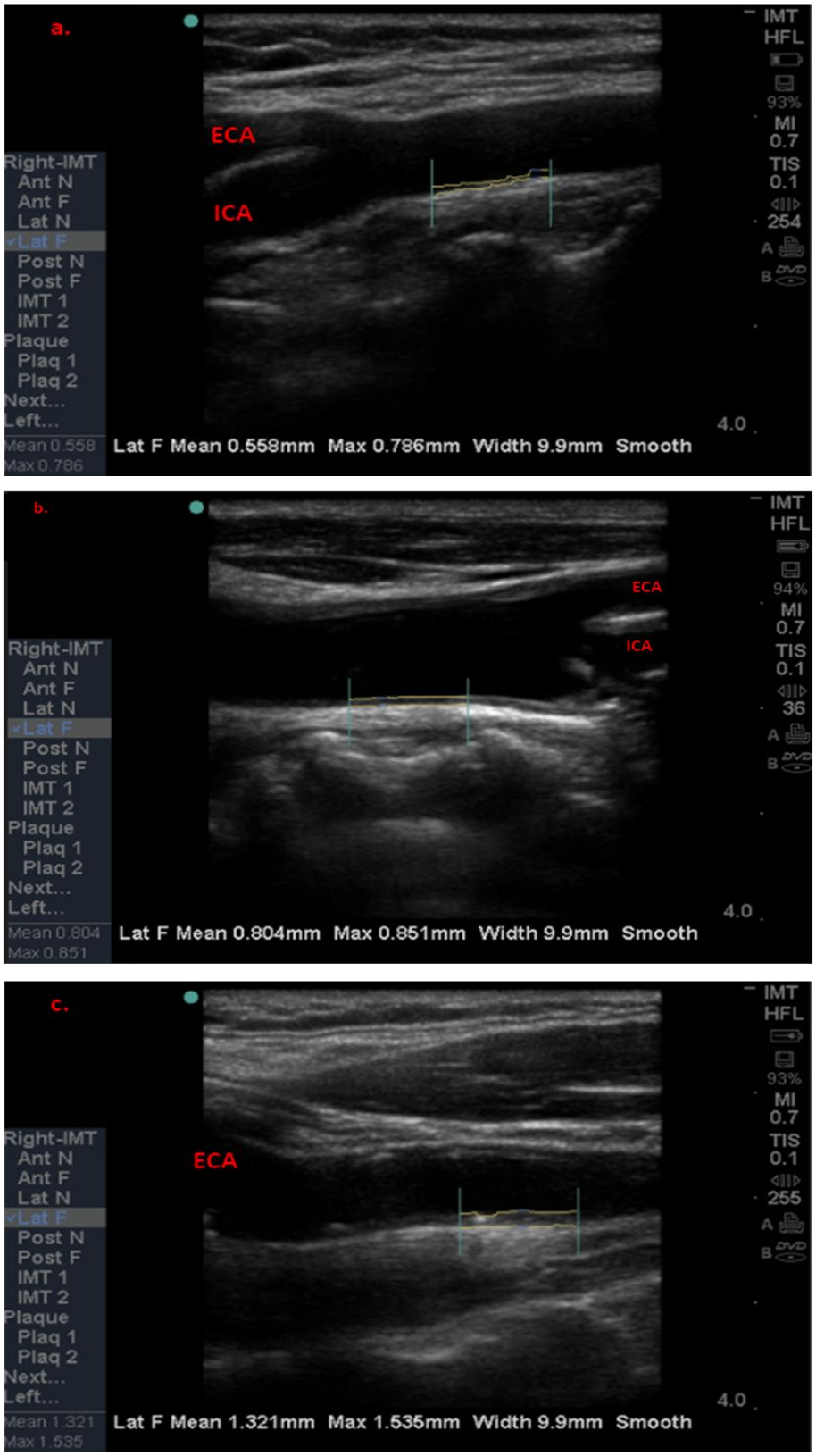


Figure 2.2. Ultrasonography showing three different carotid intima-media thickness measurements from three patients with critical limb ischaemia. a, IMT=0.558 mm; b, IMT=0.804; c, IMT=1.321 mm. ICA, internal carotid artery; ECA, external carotid artery.

Table 2.1. 75th and 95th percentiles of carotid intima-media thickness across the adult lifespan in normotensive, non-diabetic persons from a randomly selected community sample of African ancestry (n=422).

	n=			75th percentiles			95th percentiles		
Age category	All	Women	Men	All	Women	Men	All	Women	Men
< 20 years	43	25	18	0.564	0.551	0.578	0.595	0.594	0.632
20 - 29.9 years	136	81	28	0.558	0.551	0.581	0.629	0.601	0.633
30 - 39.9 years	81	53	28	0.580	0.579	0.582	0.655	0.640	0.741
40 - 49.9 years	68	55	13	0.663	0.658	0.684	0.761	0.761	0.788
50 -59.9 years	56	38	18	0.702	0.702	0.708	0.759	0.759	0.768
60 - 69.9 years	23	15	8	0.713	0.712	0.715	0.846	0.846	0.801
≥70 years	15	9	6	0.816	0.797	0.933	0.941	0.917	0.941

Table 2.2. 95th or 5th percentiles of serum creatinine concentrations or estimated glomerular filtration rate (eGFR) across the adult lifespan in normotensive, non-diabetic persons from a randomly selected community sample of African ancestry (n=762).

		Creatinine	MDRD-eGFR	CKD-EPI-eGFR
Age category	n=	95th percentile	5th percentile	5th percentile
< 20 years	87	94.0	76.6	82.9
20 - 29.9 years	257	94.0	73.7	80.5
30 - 39.9 years	145	96.0	71.2	77.5
40 - 40.9 years	120	98.0	67.5	72.2
50 - 59.9 years	88	102.0	60.8	62.4
60 - 69.9 years	38	104.0	53.3	52.6
≥70 years	27	120.0	53.2	49.8

eGFR, estimated glomerular filtration rate; MDRD: modification of diet and renal disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.

2.4. Results

2.4.1 Participant characteristics

The participant characteristics are given in Table 2.3. Differences in the characteristics between cases and controls were similar in those with versus those without high quality carotid imaging (Table 2.4). Of the patients admitted with stroke, 11.3 % were haemorrhagic, 17.2 % had a cardio-embolic cause (as assessed from clinical features and echocardiography); 16.7 % were classified as small artery occlusion (confirmed with intracranial imaging); 5.9 % with large artery occlusion (atherosclerotic) (confirmed with CT angiography); 41.4 % were indeterminate in origin and 7.5 % were from an alternative aetiology. 57.5 % of stroke and 23.5 % of those with CLI were chronologically premature (<55 years of age in women and 50 years of age in men).

Patients with arterial events had a greater prevalence of hypertension, diabetes mellitus and more patients smoked. HDL cholesterol concentrations were lower in patients with arterial events. Although LDL cholesterol concentrations were also lower in cases, 89.8 % of these patients were receiving lipid-lowering therapy at the time of performing fasting blood analysis. Body mass index was also lower in cases as compared to controls in both younger and older age groups. 41.8 % of patients had at least one of the modifiable conventional risk factors hypertension, diabetes mellitus or smoking, 36.2 % had two of these risk factors and 5.4 % had three of these risk factors.

Table 2.3. Participant characteristics.

	Controls (n=672)	Cases (n=480)
Strokes/CLI (n=)	-	186/294
Age (years)	54.5±15.2	56.2±14.4
Sex (% male)	55.2	60.6
Body mass index (kg/m^2)	27.7±5.3	27.8±8.2
% Hypertensive	61.6	65.8
% Diabetes mellitus	12.6	28.7*
% Regular smoking	22.5	40.4*
% Regular alcohol intake	26.2	50.0*
SBP/DBP (mm Hg)	136±22/87±12	134±22/80±13*
Total cholesterol (mmol/l)	4.80 (4.20 to 5.30)	3.85 (3.20 to 4.60)*
LDL cholesterol (mmol/l)	2.72 (2.30 to 3.20)	2.26 (1.62 to 2.86)*
HDL cholesterol (mmol/l)	1.36 (1.10 to 1.50)	1.02 (0.83 to 1.27)*
Glycated haemoglobin (%)	5.85 (5.60 to 6.20)	6.10 (5.70 to 7.70)*
Serum creatinine (µmol/L)	76.0 (65.5 to 90.0)	77.0 (65.0 to 94.0)
MDRD-eGFR (ml/min/1.73m^2)	88.9±25.1	91.3±36.7
CKD-EPI-eGFR (ml/min/1.73m^2)	87.4±20.7	85.4±24.7
Average carotid IMT (mm)	0.686±0.145 (n=406)	0.800±0.176* (n=448)

Data shown are proportions, mean±SD or median and interquartile range. CLI, critical limb ischaemia; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate; MDRD: modification of diet and renal disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; IMT, intima-media thickness. *$p<0.0001$ vs controls.

Table 2.4. Characteristics of participants with and without high quality carotid imaging.

	With		Without	
	Controls (n=406)	Cases (n=448)	Controls (n=206)	Cases (n=32)
Strokes/CLI (n)	-	164/284	-	22/10
Age (years)	54.3±15.3	56.6±14.4*	54.9±15.1	50.9±14.0
Sex (% male)	53.0	60.5*	58.9	62.5
Body mass index (kg/m^2)	27.5±5.3	27.7±8.1	28.0±5.2	29.1±9.9
% Hypertensive	58.1	66.1*	66.9	46.9*
% Diabetes mellitus	11.6	28.8*	14.3	28.1*
% Regular smokers	21.4	36.8*	24.1	25.0
% Regular alcohol intake	26.1	31.0*	26.3	25.0
Systolic blood pressure (mm Hg)	133±20	134±22	140±24	135±24
Diastolic blood pressure (mm Hg)	85±12	80±13*	89±13	81±13*
Total cholesterol (mmol/l)	4.8 (4.3-5.2)	3.8 (3.2-4.6)*	4.8 (4.0-5.6)	4.1 (3.3-4.5)*
LDL cholesterol (mmol/l)	2.7 (2.3-3.2)	2.2 (1.6-2.8)*	2.7 (2.2-3.5)	2.5 (1.9-3.1)
HDL cholesterol (mmol/l)	1.4 (1.2-1.5)	1.0 (0.8-1.3)*	1.3 (1.1-1.5)	0.9 (0.7-1.1)*
Glycated haemoglobin (%)	5.9 (5.6-6.2)	6.2 (5.7-7.8)*	5.8 (5.6-6.2)	6.1 (5.9-.6.3)
Serum creatinine (µmol/L)	74 (63-87)	77 (64-92)	80 (69-94)	84 (70-107)
MDRD-eGFR (ml/min/1.73m^2)	91.3±26.9	91.9±37.1	85.3±21.7	82.3±29.7
CKD-EPI=eGFR (ml/min/1.73m^2)	89.1±21.0	85.7±24.6*	84.7±19.9	81.5±25.7

CLI, critical limb ischaemia; LDL, low density lipoprotein; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate; MDRD: modification of diet and renal disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration. *p<0.05 vs controls.

2.4.2 Age-related changes in IMT and eGFR

Figure 2.3 shows the unadjusted and multivariate-adjusted age-related changes in IMT and eGFR (CKD-EPI equation) in the community sample and in patients experiencing arterial vascular events. Across the adult lifespan, age was associated with an increase in IMT and a decrease in eGFR in those with and without events. At all ages, IMT was increased in those with as opposed to without events. In contrast, CKD-EPI-derived eGFR was similar across all ages between those with and without events. The same results were noted when eGFR was determined from the MDRD equation (data not shown).

2.4.3 Independent associations of IMT, creatinine or eGFR as continuous traits with vascular events

Figure 2.4 shows the unadjusted and conventional risk factor-independent relations between IMT, creatinine concentrations or eGFR values and the risk of an arterial event (upper panels) and the performance (AUC) for events detection (lower panel) of IMT and eGFR. Whilst IMT showed strong unadjusted or independent relations with events and a strong performance for event detection, neither creatinine concentrations nor eGFR were associated with events either before or after adjustments and showed a poor performance for event detection (Figure 2.4). The relations between IMT and events, and the lack of relationship between creatinine concentrations, CKD-EPI derived or MDRD-derived eGFR and arterial events occurred for stroke or CLI considered separately; for haemorrhagic, small vessel and large vessel (atherosclerotic) strokes considered together, but separate from other causes of strokes; for cardio-embolic strokes considered separately from other causes of strokes; for premature events (<55 and 50 years of age for women and men respectively) or events in older age groups considered separately; and in hypertensives or normotensives (data not shown) considered separately (Table 2.5).

Table 2.5. Multivariate adjusted relations between carotid intima-media thickness (IMT), creatinine concentrations or estimated glomerular filtration rate (eGFR) and subgroups of non-cardiac arterial vascular events (stroke and critical limb ischaemia) in Africa.

Event vs	One SD effect	Odds ratios (95 % CI)	p-value
Stroke			
IMT	0.160 mm	2.33 (1.75 to 3.14)	<0.0001
Creatinine concentrations	52.6 µmol/l	1.17 (0.96 to 1.52)	=0.21
CKD-EPI eGFR	21.6 mls/min/1.73m^2	0.82 (0.63 to 1.07)	=0.14
MDRD eGFR	25.7 mls/min/1.73m^2	0.87 (0.68 to 1.10)	=0.26
Critical limb ischaemia			
IMT	0.169 mm	2.08 (1.63 to 2.68)	<0.0001
Creatinine concentrations	37.6 µmol/l	0.86 (0.68 to 1.03)	=0.15
CKD-EPI eGFR	22.0 mls/min/1.73m^2	1.19 (0.97 to 1.46)	=0.11
MDRD eGFR	31.0 mls/min/1.73m^2	1.18 (0.98 to 1.41)	=0.08
Haemorrhagic, small vessel and large vessel strokes (n=63)			
IMT	0.152 mm	2.25 (1.56 to 3.29)	<0.0001
Creatinine concentrations	56.2 µmol/l	1.15 (0.94 to 1.49)	=.020
CKD-EPI eGFR	20.9 mls/min/1.73m^2	0.78 (0.57 to 1.08)	=0.13
MDRD eGFR	25.1 mls/min/1.73m^2	0.78 (0.56 to 1.06)	=0.12
Cardio-embolic strokes (n=32)			
IMT	0.150 mm	1.76 (1.14 to 2.75)	=0.01
Creatinine concentrations	37.1 µmol/l	1.28 (0.72 to 1.67)	=0.15
CKD-EPI eGFR	20.8 mls/min/1.73m^2	0.63 (0.35 to 1.18)	=0.14
MDRD eGFR	25.0 mls/min/1.73m^2	0.66 (0.34 to 1.19)	=0.19
Premature events			
IMT	0.140 mm	1.79 (1.28 to 2.58)	=0.001
Creatinine concentrations	57.6 µmol/l	1.35 (0.93 to 2.81)	=0.37
CKD-EPI eGFR	20.4 mls/min/1.73m^2	0.97 (0.74 to 1.26)	=0.81
MDRD eGFR	29.6 mls/min/1.73m^2	1.10 (0.86 to 1.38)	=0.43
Events at an older age			
IMT	0.163 mm	2.12 (1.65 to 2.79)	<0.0001
Creatinine concentrations	42.6 µmol/l	0.85 (0.65 to 1.06)	=0.21
CKD-EPI eGFR	20.1 mls/min/1.73m^2	1.09 (0.89 to 1.33)	=0.41
MDRD eGFR	29.8 mls/min/1.73m^2	1.17 (0.96 to 1.42)	=0.13
	Hypertensives		
IMT	0.166 mm	1.80 (1.39 to 2.36)	<0.0001
Creatinine concentrations	59.5 µmol/l	1.00 (0.83 to 1.21)	=0.98
CKD-EPI eGFR	21.9 mls/min/1.73m^2	1.09 (0.89 to 1.35)	=0.40
MDRD eGFR	30.7 mls/min/1.73m^2	1.15 (0.95 to 1.38)	=0.15

IMT, carotid intima-media thickness; eGFR, estimated glomerular filtration rate; MDRD: modification of diet and renal disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration. Adjustments are for age, sex, hypertension, diabetes mellitus, smoking and HDL cholesterol concentrations.

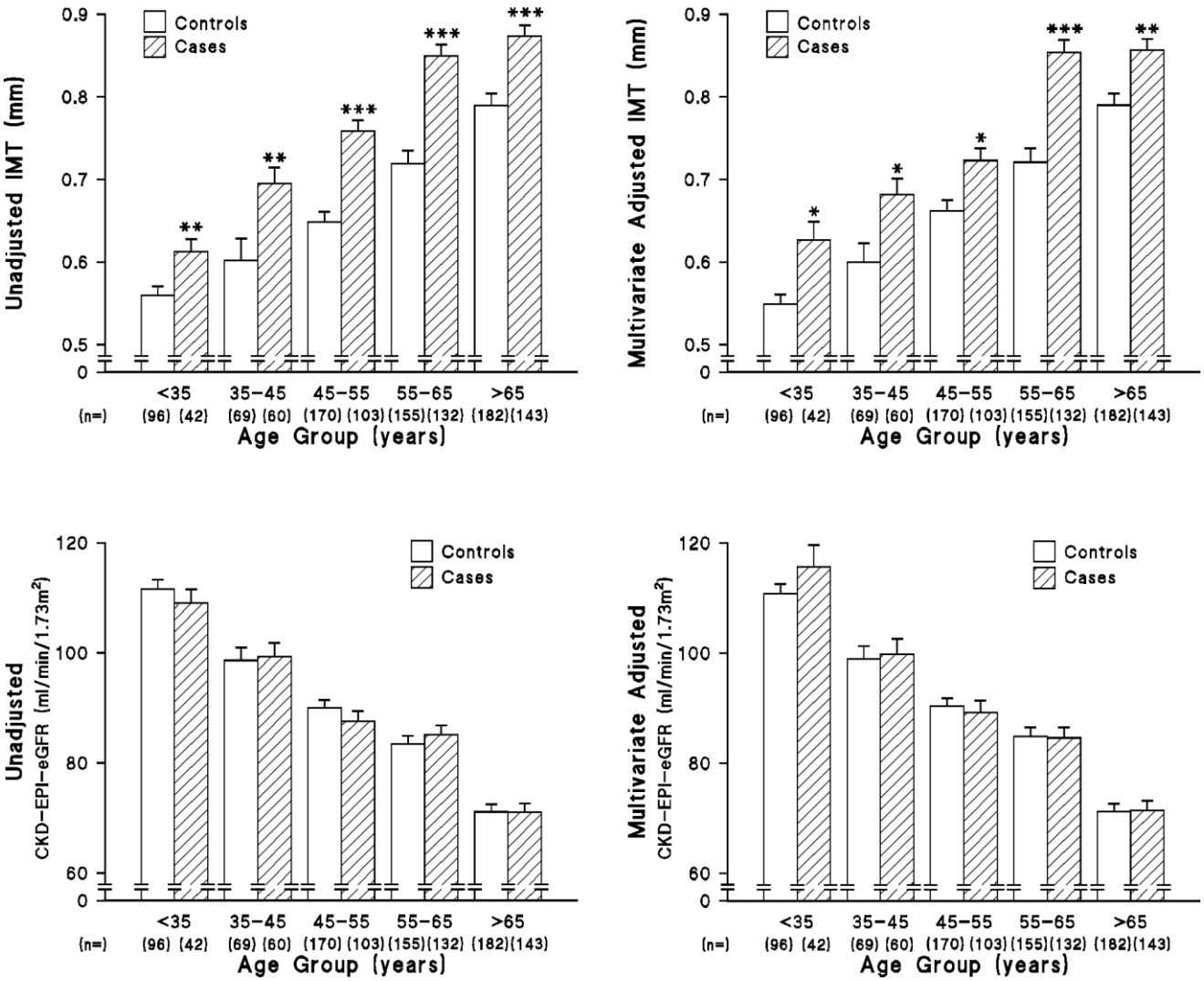


Figure 2.3. Unadjusted and multivariate-adjusted carotid intima-media thickness (IMT) and estimated glomerular filtration rate (eGFR derived from the CKD-EPI equation) across the adult lifespan in cases with non-cardiac arterial vascular events (stroke and critical limb ischaemia) and age, sex-and ethnicity-matched randomly selected community sample in Africa. Data shown are multivariate adjusted means and SEM. Adjustments are for sex, hypertension, diabetes mellitus, smoking and HDL cholesterol concentrations. *p<0.01, **p<0.001, ***p<0.0001 vs controls. IMT: carotid intima-media thickness; eGFR, estimated glomerular filtration rate; MDRD: modification of diet and renal disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.

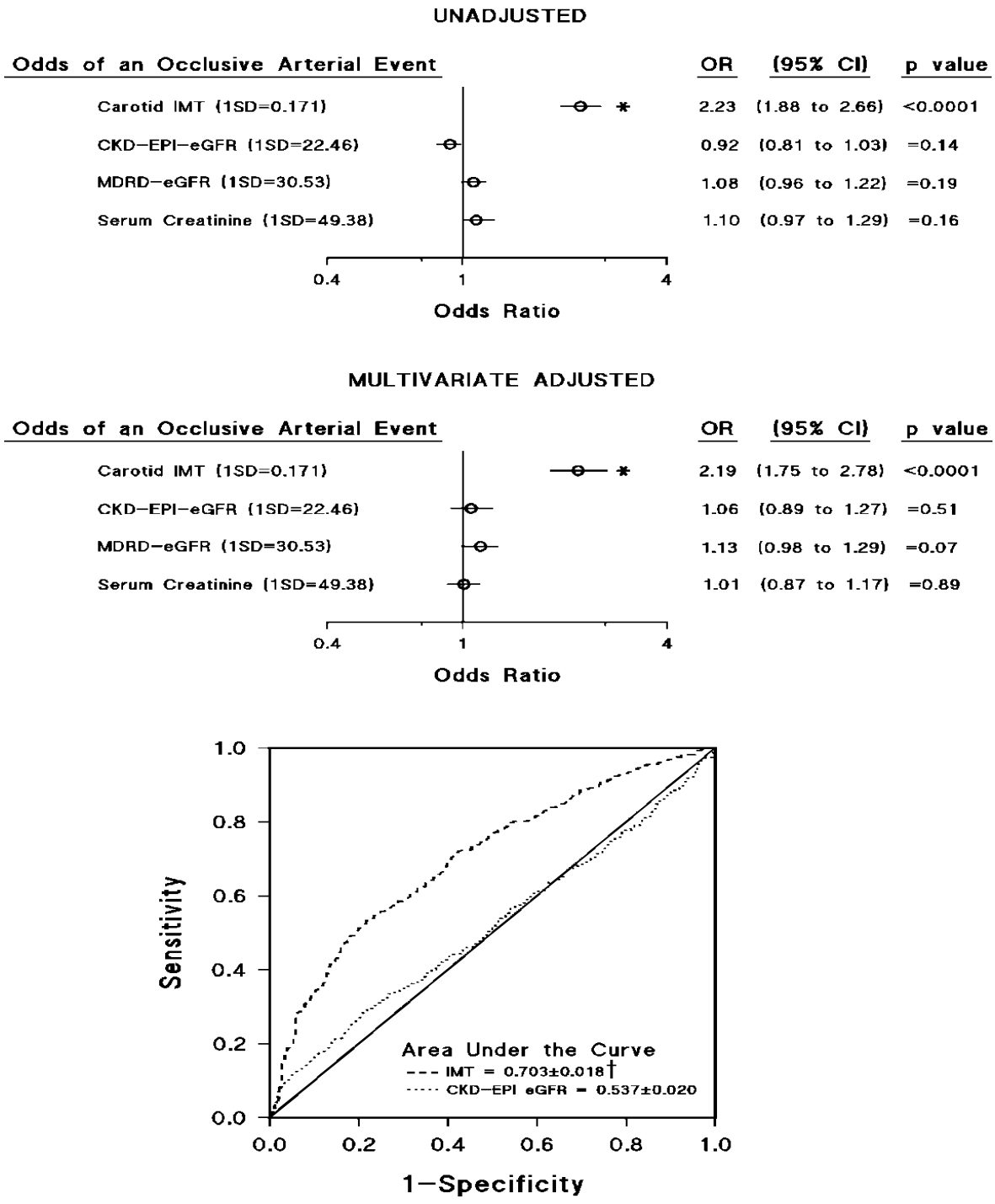


Figure 2.4. Unadjusted and multivariate adjusted relations between a one standard deviation (SD) change in carotid intima-media thickness (IMT), creatinine concentrations or estimated glomerular filtration rate (eGFR derived from the CKD-EPI equation) and non-cardiac arterial vascular events (stroke and critical limb ischaemia) in Africa (upper panel) and the performance (AUC) for event detection of IMT and eGFR (lower panel). Adjustments are for age, sex, hypertension, diabetes mellitus, smoking and HDL cholesterol concentrations. AUC, area under the receiver operator characteristic curves. One SD effects for IMT are in mm, for creatinine are in µmol/l and for eGFR are in mls/min/1.73m^2. *p<0.0001 versus OR for relations between creatinine or eGFR and events; †p<0.0001 versus AUC for eGFR.

2.4.4. Independent associations of values above or below thresholds of IMT or eGFR with vascular events

Figure 2.5 shows the proportion of cases and controls with either increases in IMT or the presence of CKD (eGFR<60 ml/min/1.73m^2 [CKD-EPI equation])(upper panels) and the performance (AUC) of increases in IMT or the presence of CKD for event detection (lower panel). Table 2.6 shows the unadjusted or multivariate adjusted relations between IMT values>0.9 mm or IMT values above age-specific thresholds, stage 2 and 3 CKD, or CKD defined according to eGFR values below age-specific thresholds and vascular events. Importantly, few patients with arterial events had CKD defined according to either CKD-EPI or MDRD equations. This was particularly noticeable in those with premature vascular events (<55 and 50 years of age for women and men respectively) (8 %) (Figure 2.4). An increased IMT based on values above either an absolute threshold of 0.9 mm or age-specific thresholds was independently associated with arterial events and showed a strong performance (Figure 2.5) and a reasonable sensitivity (Table 2.7) for event detection. Although in unadjusted models stage 2 and 3 CKD was associated with events (Table 2.6), with adjustments for conventional risk factors neither stage 2 and 3 CKD, nor CKD defined according to age-specific thresholds were associated with arterial events (Table 2.6) and stage 2 and 3 CKD showed a poor performance (Figure 2.5) and a very low sensitivity (Table 2.7) for event detection.

Table 2.6. Unadjusted and multivariate adjusted associations between increases in carotid intima-media thickness (IMT) or chronic kidney disease (CKD) and non-cardiac arterial vascular events.

Event vs	% cases / % controls with ↑IMT or CKD	Unadjusted Odds ratios* (95 % CI)	p-value	Multivariate adjusted Odds ratios* (95 % CI)	p-value
IMT>0.9 mm	24.3/5.9	5.12 (3.21 to 8.15)	<0.0001	4.31 (2.56 to 7.27)	<0.0001
IMT> age-specific thresholds (75th percentile)	67.6/36.9	3.57 (2.69 to 4.73)	<0.0001	3.06 (2.22 to 4.22)	<0.0001
IMT> age-specific thresholds (95th percentile)	42.9/12.6	5.22 (3.69 to 7.39)	<0.0001	4.24 (2.89 to 6.23)	<0.0001
CKD (-EPI) stages 2 and 3	16.3/10.0	1.58 (1.09 to 2.30)	<0.05	1.48 (0.95 to 2.30)	=0.08
CKD (-EPI) (<age-specific 5th percentile)	14.4/8.9	1.32 (0.88 to 1.97)	=0.18	1.50 (0.95 to 2.36)	=0.08
CKD (-MDRD) (<age-specific 5th percentile)	14.4/8.5	1.39 (0.92 to 2.09)	=0.12	1.39 (0.89 to 2.18)	=0.15
Creatinine (>age-specific 95th percentile)	14.8/10.0	1.32 (0.89 to 1.93)	=0.16	1.20 (0.77 to 1.86)	=0.42

IMT: carotid intima-media thickness; eGFR, estimated glomerular filtration rate; MDRD: modification of diet and renal disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration. *Adjustments are for age, sex, hypertension, diabetes mellitus, smoking and HDL cholesterol concentrations.

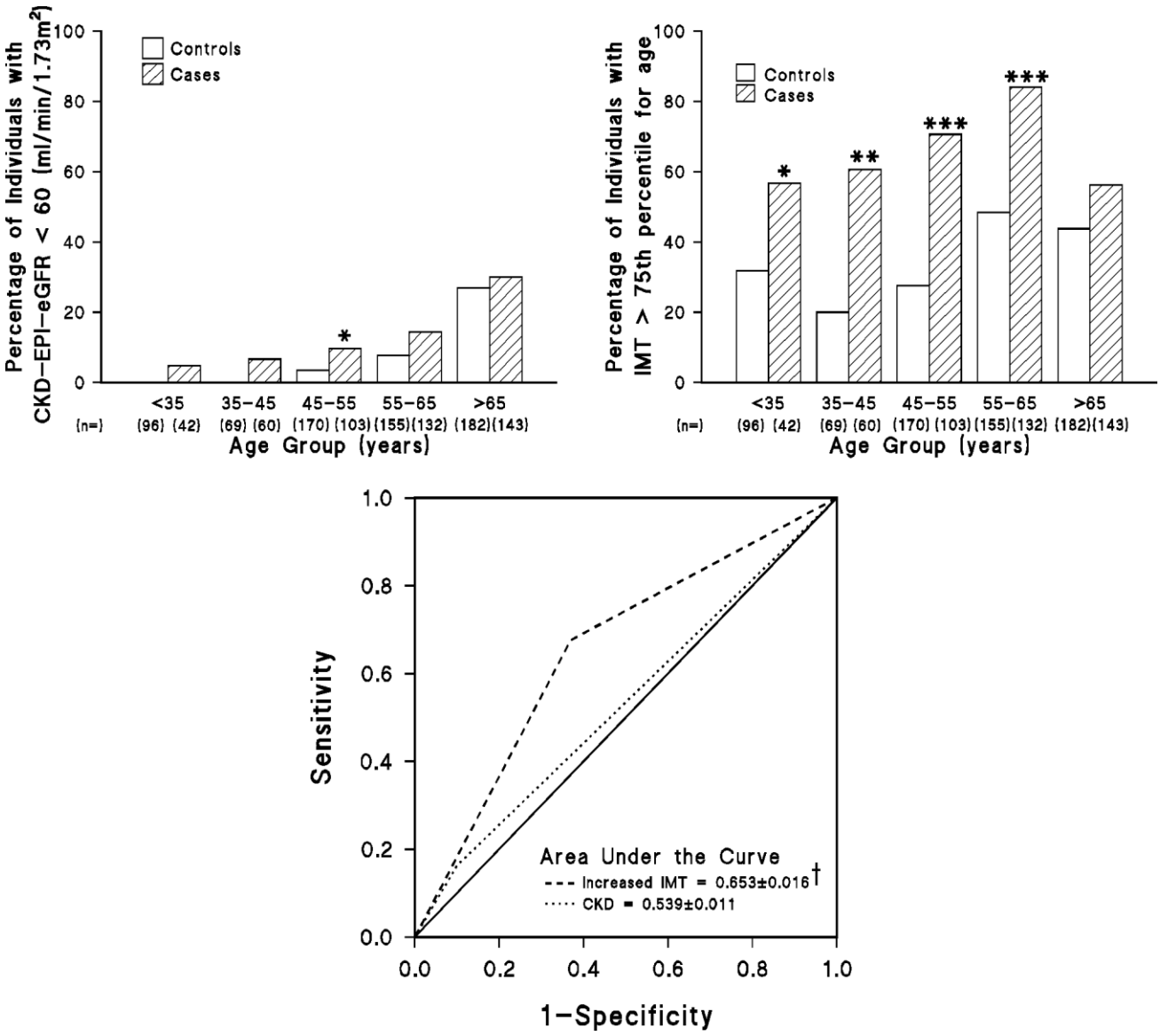


Figure 2.5. Proportion of cases with non-cardiac arterial vascular events (stroke and critical limb ischaemia) and age, sex-and ethnicity-matched randomly selected community sample in Africa with increased carotid intima-media thickness (IMT) or decreased estimated glomerular filtration rate (eGFR derived from the CKD-EPI equation) <60 ml/min/1.73m^2 (chronic kidney disease [CKD]) across the adult lifespan (upper panels) and the performance (AUC) for event detection of an increased IMT or CKD (lower panel). Data shown are unadjusted data. AUC, area under the receiver operator characteristic curves. *p<0.01, **p<0.001, ***p<0.0001 vs controls; †p<0.0001 versus AUC for CKD.

Table 2.7. Sensitivity or specificity for the detection of non-cardiac arterial vascular events (stroke or critical limb ischaemia) with an increased carotid intima-media thickness (IMT) or the presence of chronic kidney disease (CKD) in the study sample.

Vascular event vs	Sensitivity (%)	Specificity (%)
Increased IMT*	67.6	63.1
CKD	16.3	90.0

IMT: carotid intima-media thickness; CKD: Chronic Kidney Disease. *Based on age-specific 75th percentiles.

2.5. Discussion

The main findings of the present study are as follows: In a large case-control study conducted in a group of African ancestry, despite carotid IMT being uniformly greater across the adult lifespan in those with non-cardiac arterial vascular events (stroke and CLI) as compared to controls, creatinine-based indexes of glomerular function or after adjustments the presence of CKD based on these estimates were no different between those with and without events. Although, beyond all risk factors, IMT was independently associated with arterial events, neither creatinine concentrations, nor eGFR estimated using the CKD-EPI or the MDRD equations were associated with events either before or after multivariate adjustments. Importantly, few cases (16.3 %), particularly those with premature events (8 %), had CKD and assuming that similar eGFR values existed prior to as at the time of the event, eGFR showed a low sensitivity and poor performance for event detection. In sensitivity analyses, these relations were consistent for both stroke and CLI considered separately; after excluding strokes that were not necessarily haemorrhagic, small vessel or large vessel strokes (where the cause may not have been through conventional risk factors); for premature events and events occurring at an older age considered separately; as well as in hypertensives and normotensives considered separately.

Although controls were carefully age- sex- and ethnicity-matched participants from a randomly selected community sample and the sample size was reasonably large, as with any case-control analysis, the results of the present study may be subject to selection bias or residual confounding and consequently false negative or positive findings. However, the very low prevalence of CKD as defined by eGFR values <60 ml/min/1.73m^2 in those with premature events (8 %) and thus the low sensitivity of CKD for event detection markedly limits the value of CKD for detecting risk for a significant proportion of patients with events. Thus, even if residual confounding or a selection bias had limited associations between eGFR and events, too few patients experiencing non-cardiac arterial vascular events would have been identified as being at risk prior to the event, to place a high value on employing CKD identification as a sensitive marker for risk prediction. Against a notion that in the present study an inability of CKD to associate with non-cardiac arterial vascular events represents false negative findings is that in similar participants, an increased IMT was strongly and consistently associated with vascular events across the adult lifespan. Indeed, because conventional risk factors account for all end-organ changes, population stratification and an inability to adjust for residual confounding effects are likely to have

generated a bias against a role for all indices of end-organ changes, rather than a bias against a role for one (eGFR), but not another (IMT) end-organ measure.

Stroke may be caused by several pathophysiological mechanisms, and a significant proportion of patients in the present study had a stroke that may not have been caused by risk factors that affect glomerular function. Indeed, 17.2 % were cardio-embolic, where the cardiac pathology may have been other than coronary artery or hypertensive heart disease, 41.4 % were indeterminate in origin and 7.5 % were from an alternative aetiology. However, in the 11.3 % that were haemorrhagic (without congenital aneurysms on imaging); 16.7 % classified as small artery occlusion (confirmed with intracranial imaging); and 5.9 % with large artery occlusion (atherosclerotic) (confirmed with CT angiography) similar findings were noted as with all patients with stroke or those with critical limb ischaemia. Consequently, it is unlikely that the inclusion of patients with cardio-embolic or indeterminate causes (not caused by conventional risk factors) influenced the interpretation of the present results.

The present study suggests that in the majority of patients with non-cardiac-related arterial vascular events in South Africa and hence possibly other African regions, the event precedes the development of CKD. These data are contrary to alternative populations (Matsushita et al 2010; White et al 2010; Mahmoodi et al 2012; Matsushita et al 2012; Wang et al 2014; Matsushita et al 2015; Parsh et al 2015), where CKD may be useful in predicting a significant number of arterial events. Hence, generalised screening of either hypertensives or normotensives for CKD using creatinine-based estimations of GFR are unlikely to be of use in predicting the majority of non-cardiac-related arterial vascular events in South African and possibly other African populations. These findings oppose the proposal by hypertension guidelines in middle-income countries in Africa (Seedat and Rayner, 2012). In this regard, because of the low cost of eGFR assessments, these guidelines assign a greater priority to eGFR than more costly ultrasound-based assessments, such as carotid IMT, in detecting end-organ changes.

Several explanations are thought to account for the ability of CKD to predict events caused by vascular abnormalities beyond conventional risk factors (Chang and Kramer, 2011; Sud and Naimark, 2016; Hirakawa et al 2017). Importantly, the kidney is a well-recognized target organ for conventional risk factors including hypertension. Consequently, decreases in GFR are thought to provide an index of the accrual of the overall adverse effects of uncontrolled conventional risk factors, such as hypertension or diabetes mellitus, over time. In this regard, in Africa both CLI and stroke occur across the adult lifespan starting at an age as young as 20 years. It may therefore be argued that in younger

individuals there is insufficient time for risk factors such as hypertension to cause the development of CKD based on current definitions. In these circumstances, it is possible that a predisposition to pro-thrombotic conditions rather than vascular pathology is a more important cause of premature events (Collet et al 2006; Speidl et al 2007; Pineda et al 2008; Pineda et al 2009; Milgrom et al 2018). However, in the present study a strong, independent relationship between IMT and events was noted across the adult lifespan. Hence, vascular pathology is likely to have been a major cause of events across the adult lifespan. Moreover, risk factors are likely to have been present for sufficiently long, even in those with events at a younger age, to have caused carotid vascular changes. In addition, the lack of association of creatinine-based assessments of CKD and events was noted in both the young and the elderly and we assume that in the elderly, risk factors would have prevailed for a significant time-period. Moreover, the presence of CKD in the present study was defined not only according to standard approaches (at least <60 ml/min/1.73m^2), but also according to age-specific thresholds. Hence, inadequate definitions of CKD in the young cannot account for the lack of association of CKD with arterial events.

An alternative explanation for the lack of relationship between creatinine concentrations, eGFR or CKD and arterial events in the present study is that creatinine concentrations and eGFR are a poor index of glomerular function in Africa. Indeed, although the CKD-EPI equation has been demonstrated to perform well with minimum bias across a range of GFR values in many ethnic groups (Mancia et al 2013), the relationship between creatinine and GFR varies between ethnicities (Eastwood et al 2010; Mancia et al 2013). Thus, neither the MDRD, nor the CKD-EPI equations perform as well in African as they do in Caucasian populations (Michels et al 2010; Earley et al 2012). Moreover, the use of an ethnicity coefficient may not improve on performance in some African populations (Levey et al 2009; Eastwood et al 2010; Van Deventer et al 2011). However, prior studies (Dessein et al 2015; Booysen et al 2016) conducted in black African patients with rheumatoid arthritis and in the general population have demonstrated distinct independent associations between eGFR determined using CKD-EPI or MDRD equations and several alternative end-organ measures, including indexes of atheroma (plaque) (Dessein et al 2015). Hence, eGFR, determined using either MDRD or CKD-EPI equations may indeed be considered to be an appropriate end-organ measure in Africa. However, the present study suggests that the relationship between eGFR or CKD and non-cardiac arterial vascular events in Africa is remarkably limited.

A third possible explanation for the lack of relationship between creatinine concentrations, eGFR or CKD and arterial events in the present study is that eGFR and

CKD mainly predict cardiac rather than cerebral or peripheral vascular events. Cardiac events were not evaluated in the present study as they do not occur across the adult lifespan in groups of African ancestry in South Africa and they cause reductions in eGFR. In this regard, 50 % of events predicted by CKD are coronary events and a substantial portion of events predicted by CKD are caused by heart failure (Sud and Naimark, 2016). A dominant effect of CKD on cardiac as opposed to alternative cardiovascular events may occur through the cardio-renal syndrome. Indeed, eGFR is strongly and independently associated with left ventricular mass beyond all haemodynamic factors in groups of African ancestry in Africa (Maunganidze et al 2013). Whilst eGFR associates with left ventricular mass beyond all risk factors in African populations (Maunganidze et al 2013), the relationship between eGFR and carotid IMT is not independent of age (Booysen et al 2016). Thus, the adverse effects of CKD in Africa may be specific to cardiac rather than peripheral or cerebral vascular arterial events.

A fourth potential explanation for the lack of independent association between estimates of glomerular function or the presence of CKD and non-cardiac arterial vascular events in Africa is that the major risk factors that determine arterial events are those that do not affect renal function. In this regard, dyslipidaemia may be a major determinant of atherosclerosis, but have little impact on renal function. Indeed, in the present study although we could not determine the relationship between LDL cholesterol concentrations and occlusive arterial events (lipid lowering therapy was initiated on admission to hospital), a strong relationship between reduced HDL cholesterol concentrations and events was noted. However, in the present study, 68.1 % of those with arterial events had either hypertension or diabetes mellitus and these risk factors are well established as determinants of both CKD and occlusive arterial events. Moreover, at least for stroke, hypertension is well-recognised as the major risk factor in Africa.

An important limitation of the present study is that few possible confounding factors were determined and hence in statistical analysis adjustments for these unmeasured confounders could not be performed. Hence, with adjustments for these unmeasured confounders, differences in eGFR between cases and controls may have been noted. This limitation would nevertheless not have influenced the remarkably low number of cases with CKD, rendering identification of CKD a measure with a negligible sensitivity (16.3 %) for predicting events. An additional important limitation of the present study is that it was conducted at a single site (Johannesburg, South Africa) where extensive special investigations are available to ensure an appropriate diagnosis of the event. Although black Africans living in Johannesburg are largely representative of most chiefdoms in South

Africa, whether the results of the present study can be extrapolated to alternative black African populations in Africa is uncertain. However, in this regard, black African populations living in South Africa are thought to originate from the Bantu expansion from West Africa, and hence at least from a genetic perspective are thought to be similar. Nevertheless, further studies are warranted to explore whether the presence of CKD is as sensitive an index of non-cardiac arterial vascular events as vascular indexes of end-organ changes in other regions of Africa.

In conclusion, in the present study we show that in groups of African ancestry in South Africa, despite a consistent independent relationship between carotid IMT and non-cardiac-related arterial vascular events (stroke and CLI) across the adult lifespan, neither creatinine concentrations, eGFR calculated from either the MDRD or CKD-EPI equations, nor the presence of CKD associate with these events. Importantly, few patients in this study with non-cardiac arterial vascular events had eGFR values below currently accepted CKD thresholds and consequently the presence of CKD offered a negligible sensitivity for event detection. Thus, current creatinine-based assessments of glomerular function and the identification of CKD based on these thresholds is unlikely to significantly add to the prediction of the majority of non-cardiac arterial vascular events in South Africa. Whether current creatinine-based assessments of glomerular function enhance the ability to predict cardiac pathology in Africa or whether there is a necessity for identifying alternative equations that more accurately assess eGFR in Africa, requires further study.

CHAPTER 3

Organ-Specific, Age-Dependent Associations of Steady-State Pressures and Pulsatile Pressure Wave Components with End-Organ Measures.

The data in this chapter has been published in the *American Journal of Hypertension:*

Kolkenbeck-Ruh A., Motau T.H., Naran R., Libhaber C.D., Sareli P., Norton G.R., Woodiwiss A.J. (2019). Organ-specific, age-dependent associations of steady-state pressures and pulsatile pressure wave components with end-organ measures. *American Journal of Hypertension*. 32(3), 272-281. doi: 10.1093/ajh/hpy171.

3.1. Abstract

Background: The contribution of steady-state pressures and the forward (Pf) and backward (reflected) (Pb) wave pressure components of pulse pressure (PP) to risk prediction have produced contrasting results. We hypothesized that the independent contribution of steady-state pressures (mean arterial pressure [MAP]), Pf and Pb to cardiovascular damage is organ-specific and age-dependent.

Methods: In 1384 black South Africans from a community sample, we identified independent relations between MAP, Pf or Pb (applanation tonometry and SphygmoCor software) and either left ventricular mass index (LVMI) (n=997)(echocardiography), carotid intima media thickness (IMT)(n=804) (B-mode ultrasound), or aortic pulse wave velocity (PWV)(n=1217).

Results: Independent of risk factors, relations between Pf and IMT were noted in those over 50 years of age ($p<0.02$), whilst in those less than 50 years of age, MAP ($p<0.005$) was independently associated with IMT. Pb failed to show independent relations with IMT at any age ($p>0.37$) In contrast, independent relations between Pb and LVMI were noted in those less than ($p<0.0001$), and greater than ($p<0.02$) 50 years of age, whilst MAP was not independently associated with LVMI at any age ($p>0.07$) and Pf tended to show significant relations only in the elderly ($p=0.05$). Moreover, whilst MAP ($p<0.005$) and Pb ($p<0.01$) showed independent relations with PWV at any age, Pf failed to show independent relations ($p>0.10$).

Conclusion: Independent of confounders, steady-state and aortic forward and backward wave pressures show associations with end organ measures that are organ specific and age-dependent.

3.2. Introduction

The adverse haemodynamic effects of blood pressure (BP) are mediated by both a steady-state as well as a pulsatile component of BP. Several aortic changes contribute to pulsatile hemodynamic effects beyond steady-state pressures (mean arterial pressure [MAP]), and consequently determine cardiovascular damage (Vlachopoulos[a] et al 2010; Vlachopoulos[b] et al 2010; Wang et al 2010; Chirinos et al 2012; Weber et al 2012; Ben-Shlomo et al 2014; Zamaniet al 2014; Booysen et al 2015; Cooper et al 2015; Sibiya et al 2015; Zamani et al 2015). In this regard, an increased aortic stiffness and changes in aortic diameter enhance aortic characteristic impedance and thus forward wave pressures (Pf). Moreover, an increase in the magnitude of aortic backward (reflected) wave pressures (Pb) or the speed of wave reflection augment pulse pressure (PP). Understanding the relative roles of MAP, Pf and Pb in mediating cardiovascular events may provide a guide to possible novel therapeutic targets. Importantly, there are presently no therapeutic approaches that modify the structural aortic changes responsible for Pf (Agabiti-Rosei et al 2007). Furthermore, there is no evidence to show that BP reducing agents attenuate steady-state pressure-independent, age-related increases in Pb (Namasivayam et al 2009; Hodson et al 2017). Presently however, there is conflicting evidence regarding the relative roles of MAP, Pf and Pb in mediating cardiovascular damage.

Meta-analyses, including an individual patient meta-analysis have demonstrated the ability of increases in aortic stiffness, a fundamental determinant of Pf, to predict events beyond brachial BP (Vlachopoulos[a] et al 2010; Ben-Shlomo et al 2014). Moreover, in the Framingham Heart Study, an increased aortic stiffness (Mitchell et al 2010) and an enhanced Pf, but not Pb, predicted events (Cooper et al 2015) and associated with left ventricular mass (LVM) (Kaess et al 2016). In contrast however, in the Multiethnic Study in Atherosclerosis (MESA) a more important role of Pb as compared to Pf in mediating cardiovascular events (Chirinos et al 2012; Zamani et al 2014) and LVM (Zamani et al 2015) was noted. Although it is likely that the relative contribution of Pf and Pb may be different in different communities, events in the Framingham Heart Study (Cooper et al 2015) were not the same as those in the MESA (Chirinos et al 2012; Zamani et al 2014). It is therefore also possible that the contribution of MAP, Pf and Pb may differ depending on the organ that is affected. As Pb increases across the adult lifespan, whilst Pf increases over 50 years of age (Namasivayam et al 2009; Hodson et al 2017), it is also possible that MAP, Pb and Pf effects on different end organs are age-dependent. Consequently, in the present study we aimed to assess the relative contribution of MAP, Pf and Pb to variations in

several end organ measures across the adult lifespan in a large community-based sample.

3.3. Methods

3.3.1. Study groups

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. The present study design has previously been described (Woodiwiss et al 2009; Norton et al 2012). In the present study 1384 participants from randomly recruited (from the population census figures of 2001) families of black African descent (Nguni and Sotho chiefdoms) from the South West Township (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years of age, were evaluated. In sub-studies, 997 participants had echocardiographic measurements for the assessment of left ventricular mass (LVM), 804 had carotid intima-media thickness (IMT) measurements, and 1217 aortic pulse wave velocity (PWV) measurements.

3.3.2. Clinical, demographic, and anthropometric measurements

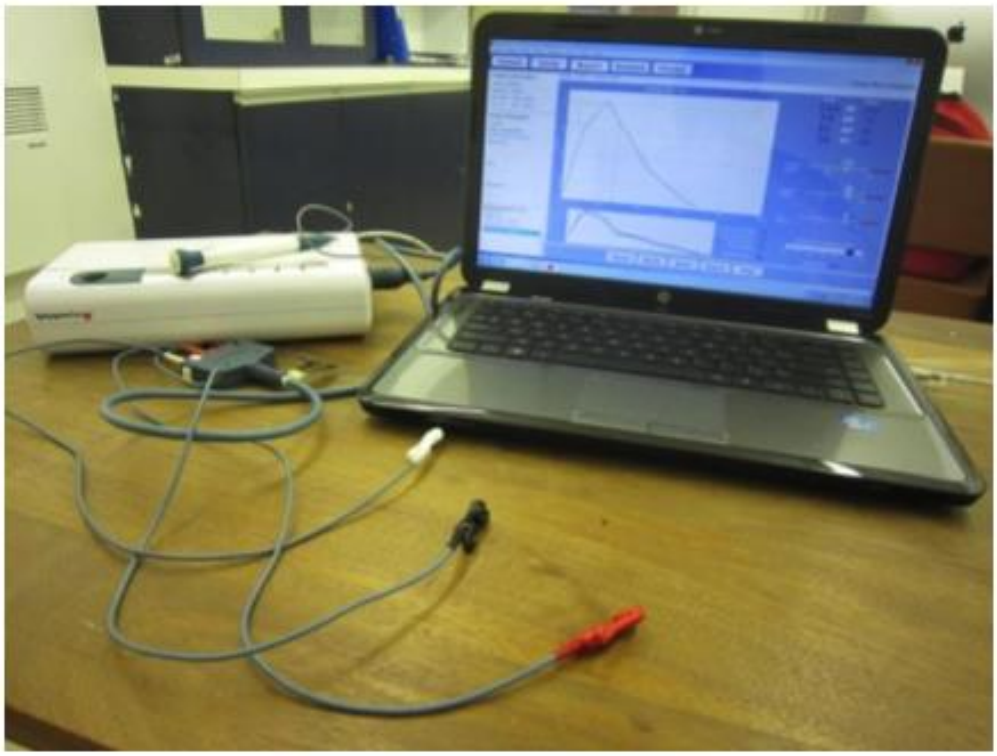
A questionnaire was administered to obtain demographic and clinical data.[17,18] Height and weight were measured using standard approaches and participants were considered to be overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Laboratory blood tests of renal function, liver function, blood glucose, hematological parameters, and percentage glycated hemoglobin (HbA_{1c}) were performed. Diabetes mellitus (DM) was defined as the use of insulin or oral glucose lowering agents or an HbA_{1c} value greater than 6.5 %. Uncontrolled glucose concentrations were considered to be present with an HbA1c above 6.1 %. High quality office brachial BP measurements were obtained in the seated position and after 5 minutes of rest, by a trained nurse-technician using a standard mercury sphygmomanometer according to guidelines (Woodiwiss et al 2009). The mean of 5 measurements obtained at least 30 seconds apart was taken as office BP. Hypertension was defined as a mean office BP≥140/90 mm Hg or the use of antihypertensive medication.

3.3.3. Pulse wave analysis

Central aortic haemodynamics were estimated using pulse wave and wave separation analysis as previously described (Norton et al 2012; Booysen et al 2015; Sibiya et al 2015; Hodson et al 2018) (Figure 3.1 and 3.2). After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm) pulse were recorded by applanation tonometry during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software. Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5 % or the amplitude of the pulse wave signal was less than 80 mV were discarded. Central aortic PP (PPc) was determined as the difference between aortic systolic (SBP) and diastolic (DBP) BP and aortic augmented pressure (Pa) as the difference between PPc – (height of the first systolic shoulder-diastolic BP). Augmentation index (AIx) was determined as Pa/PPc expressed as a percentage. Aortic forward (Pf) and backward wave (Pb) pressures were determined from wave separation analysis of the SphygmoCor-derived aortic pressure waveform using a 'triangular flow wave' (also from SphygmoCor software) (Booysen et al 2015; Sibiya et al 2015), and reflected wave magnitude (RM), as Pb/Pf, a Pf-independent index of the magnitude of wave reflection (Figure 3.2).

Aortic pulse wave velocity (PWV) was determined from sequential waveform measurements at carotid and femoral sites using applanation tonometry and SphygmoCor software. The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point. Pulse transit time was obtained from the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. An increased PWV was defined as a value> 10 m/sec as described by Van Bortel et al (2012) (Figure 3.3).

A



B



Figure 3.1. A SphygmoCor device coupled with an applanation tonometer (panel A) which was used to determine aortic haemodynamics. Panel B illustrates the applanation tonometer used to detect the pressure waveforms.

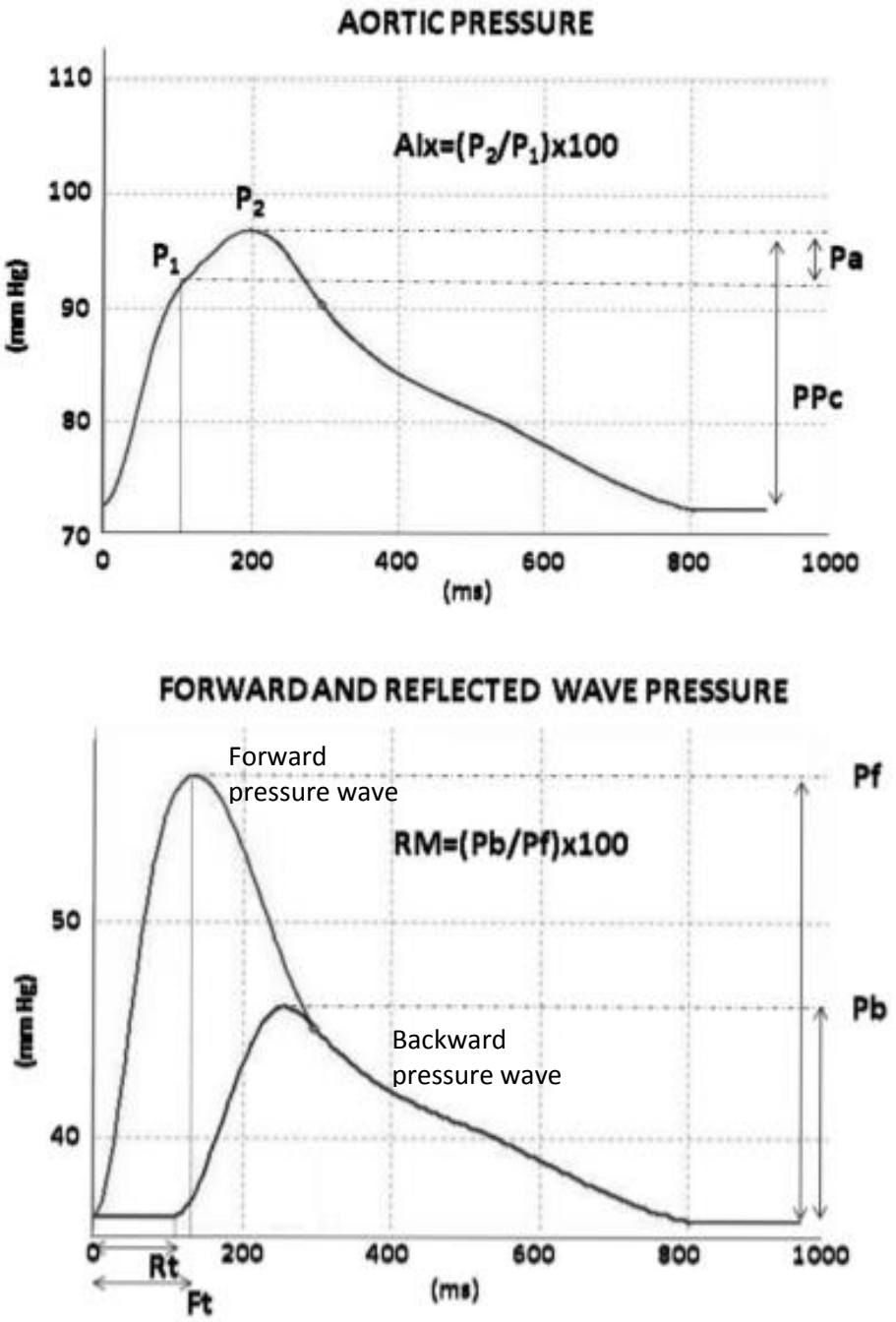


Figure 3.2. Derivation of aortic functional parameters from the aortic pressure waveform using SphygmoCor software. The aortic pressure wave was separated into the forward and backward waves assuming the aortic flow wave to have a triangular shape. Pa, augmented pressure; PPc, central aortic pulse pressure; AIx, augmentation index; Pf, forward wave pressure peak; Pb, backward (reflected) wave pressure peak; RM, reflection magnitude; Ft, time to peak forward wave; Rt, reflected wave time; P1, first systolic peak; P2, second systolic peak.

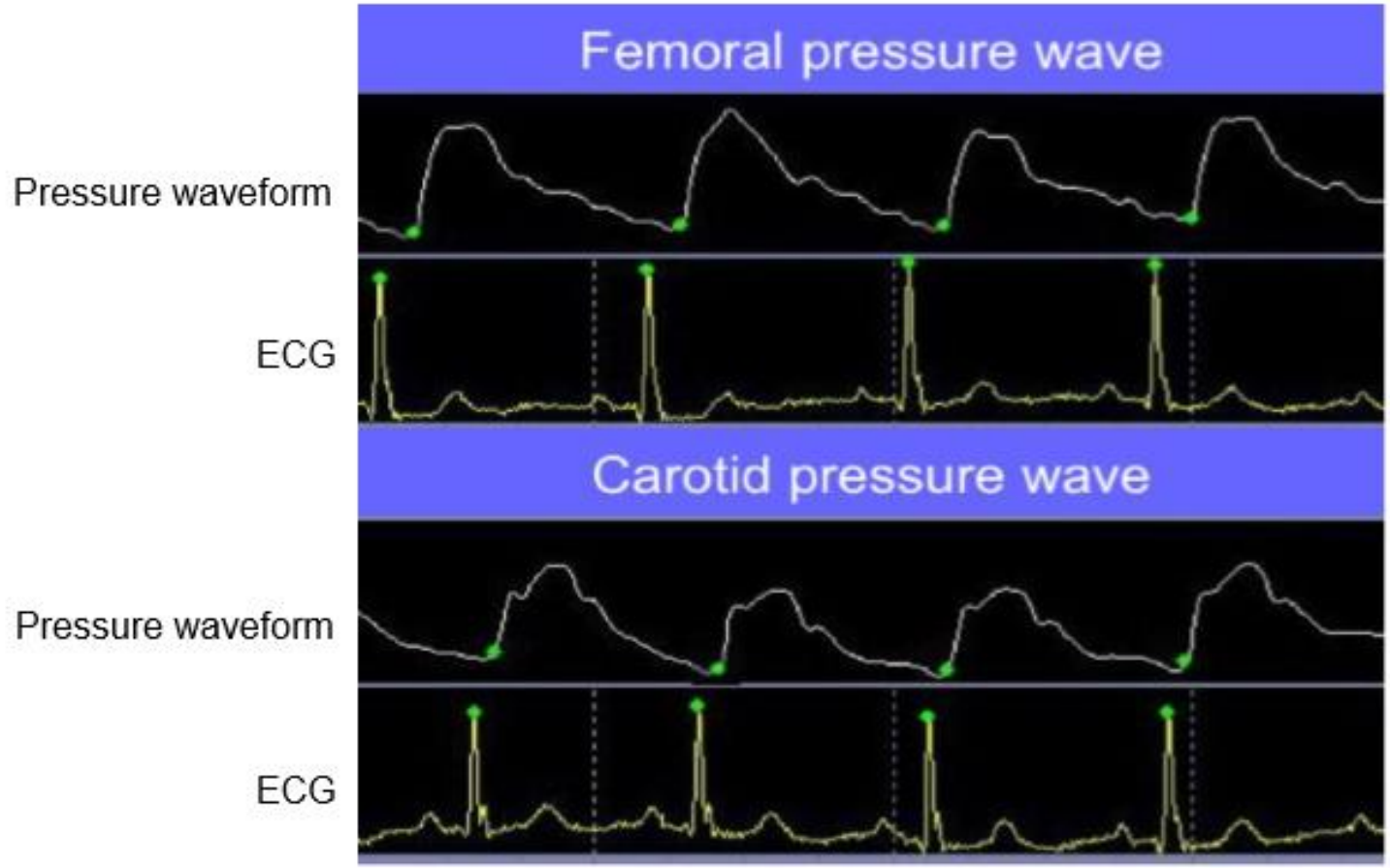


Figure 3.3. Aortic pulse wave velocity was determined from sequential pressure waveform measurements at carotid and femoral sites. The R-wave in a simultaneously recorded electrocardiogram (ECG) was used as a fiducial point to determine the time delay in the waves between the carotid and femoral sites (green dots on both ECG and pressure waveform).

3.3.4. Carotid IMT and LVM

Carotid intima-media thickness was determined using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington) as previously described (Norton et al 2012) employing a linear array 7.5 MHz probe as recommended (Touboul et al 2012). Images of at least 1 cm length of the far wall of the distal portion of the right common carotid artery from an optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualized simultaneously) at least 1 cm proximal to the flow divider were obtained. Carotid IMT measurements were determined using semi-automated border-detection and quality control software. An increased IMT was identified from age-specific thresholds (75^{th} percentiles) for IMT determined from normotensive, non-diabetic individuals from the community sample.

Echocardiographic measurements were recorded and analysed off-line by experienced investigators (AJW and CDL) who were unaware of the clinical data of the participants and whom had a low degree of inter- and intra-observer variability (Woodiwiss et al 2009). Left ventricular mass index (LVMI) was determined from transthoracic two-dimensional targeted M-mode echocardiography obtained in the long axis parasternal view. Variables were analysed according to the American Society of Echocardiography convention (Sahn et al 1978). Left ventricular mass was determined using a standard formula (Devereux et al 1986) and indexed (LVMI) to $height^{1.7}$ (Chirinos et al 2010). Left ventricular hypertrophy (LVH) was identified as an LVMI-$ht^{1.7}$>80 $g/m^{1.7}$ for men and >60 $g/m^{1.7}$ for women (Chirinos et al 2010).

3.3.5. Statistical analysis

As the present study assessed correlations between aortic haemodynamics and several end organ measures, the sample size required to show significant (α=0.05) correlations with 80 % power was calculated using the sample size guideline for correlation analysis (Bujang and Baharum, 2016). In order to calculate the minimum sample size required, I examined a previous population based study, which included individuals of African ancestry and assessed relationships between central aortic haemodynamics and left ventricular function (Kaess et al, 2016). In this study, the authors reported that the correlation coefficient of the relationship between backward wave pressure and left ventricular mass index was 0.1 and that this correlation was significant ($p<0.0001$). Using

the sample size guideline for correlation analysis (Bujang and Baharum, 2016), the minimum sample size required to identify statistically significant (α=0.05) correlations between aortic haemodynamics and end organ measures with 80 % power was 782. For the current study, the sample sizes for each of the end organ measures were above the minimum requirement for correlation analysis (IMT, n=804; LVM, n=997; PWV, n=1217).

For database management and statistical analysis, SAS software, version 9.4 (SAS Institute Inc., Cary, NC) was employed. Continuous variables are expressed as mean (±SD). Dichotomous variables are expressed as percentages. Multiple linear and logistic regression analysis was performed to determine the independent relations between aortic function and end-organ measures. Adjustments included in multivariate models were those factors associated with aortic function or end-organ measures as well as for steady-state pressures. Through inertial effects and Newton's 3rd Law of Motion, Pf determines Pb (Nichols et al 2011). More recently, it has also been suggested that through re-reflection of backward waves, Pb also determines Pf (Phan et al 2016). Hence, to assess the effects of Pb and Pf independent of each other in sensitivity analyses we evaluated relations between these two variables and end-organ measures in the same multivariate models.

3.4. Results

3.4.1. Participant characteristics

The participant characteristics are given in Table 3.1. A striking prevalence of participants were obese, or had diabetes mellitus or hypertension. A high proportion of hypertensives had uncontrolled hypertension despite a significant proportion of these individuals receiving antihypertensive medication. Average HbA1c values (8.32±2.41 %) in those with diabetes mellitus showed poor blood glucose control. A high proportion of participants had LVH or an increased IMT.

3.4.2. Relationships between indexes of aortic function

All indexes of aortic function were strongly correlated with MAP and with each other (Table 3.2).

3.4.3. Independent associations between steady-state, forward or backward wave pressures and LVM.

Independent of several confounders and when Pf and Pb were considered in separate regression models (Table 3.3 and Figure 3.4), Pb, but not MAP was independently associated with LVMI or LVH in those younger and older than 50 years of age. In contrast to Pb, which was independently associated with LVMI across the adult lifespan, there was only a trend for Pf to independently associate with LVMI in those older than 50 years of age (Table 3.3 and Figure 3.4). The inclusion of Pf and Pb in the same regression models nevertheless eliminated the relationship between Pb and LVMI or LVH in those older than 50 years of age (Table 3.4).

3.4.4. Independent associations between steady-state, forward and backward wave pressures and carotid IMT.

Independent of several confounders and when considered in separate (Table 3.5, Figures 3.5) or the same (Table 3.6) regression models, MAP and Pf, but not Pb (or RM)(data not shown) were independently associated with IMT. In addition when considered in separate (Table 3.5, Figures 3.5) or the same (Table 3.6) regression models either an independent association or a trend for an independent association between MAP or Pf and IMT values above age-specific thresholds was noted. Importantly however, whilst MAP was independently associated with IMT in those younger than 50 years of age, Pf was independently associated with IMT in those over 50 years of age (Tables 3.5 and 3.6, Figure 3.5).

3.4.5. Independent associations between steady-state, forward or backward wave pressures and aortic PWV.

Independent of several confounders and when considered in separate (Table 3.7, Figure 3.6) or the same (Table 3.8) regression models, MAP and Pb, but not Pf were independently associated with aortic PWV. In addition when considered in separate (Table 3.7, Figure 3.6) or the same (Table 3.8) regression models an independent association between MAP or Pb, but not Pf and increased aortic PWV values was noted in those over 50 years of age. Only 3 persons younger than 50 years of age had a PWV > threshold.

3.4.6. Relations between aortic pulse wave velocity, or brachial or aortic PP and end-organ measures.

Whilst aortic PWV was independently associated with LVMI in the younger, but not the older age groups, PWV was not independently associated with IMT at any age (Table 3.9). In regression models with MAP, both brachial and aortic PP were independently associated with LVMI in both younger and older age groups (Table 3.9). However, in keeping with the impact of Pf on IMT in older age groups, brachial and aortic PP were independently associated with IMT in older, but not younger age groups (Table 3.9). Brachial and aortic PP were independently associated with PWV at any age (Table 3.9).

3.4.7. Differential relations between aortic reflected wave magnitude and augmentation index and end-organ measures

In keeping with relationships between Pb and LVMI beyond Pf (Table 3.3), RM was robustly and independently associated with LVMI in those <50 and with PWV >50 years of age (Table 3.9). However, AIx showed only weak independent relationships with LVMI and only modest independent relations with PWV (Table 3.9).

Table 3.1. Participant characteristics.

	All	With LVMI	With IMT
n=	1384	997	804
Age (years)	45.2±18.4	46.0±18.2	46.7±18.2
Sex (% male)	33.5	33.0	32.3
Body mass index (kg/m^2)	29.7±8.0	29.8±7.7	30.0±8.0
% Overweight/obese	23.1/44.2	23.7/45.5	22.5/46.3
% Hypertensive	46.5	47.8	47.0
% Uncontrolled hypertension	35.5	35.0	33.2
% Treated for hypertension	25.1	27.3	27.5
% Diabetes mellitus	13.7	13.5	13.3
% Regular smokers	15.0	15.5	15.2
% Regular alcohol intake	20.9	20.3	21.0
Systolic blood pressure (mm Hg)	128±22	128±22	127±21
Diastolic blood pressure (mm Hg)	83±13	83±13	82±12
Mean arterial pressure (mm Hg)	100±16	100±16	98±15
Pulse pressure (mm Hg)	45±16	45±16	44±15
Aortic pulse pressure (mm Hg)	35±15	36±14	25±14
Forward wave pressure (mm Hg)	24.4±8.8	24.6±9.0	24.3±9.0
Backward wave pressure (mm Hg)	17.3±7.9	17.3±7.7	16.9±7.5
Aortic augmentation index	27.0±12.5	27.0±12.4	26.8±12.5
Reflected wave magnitude	0.71±0.21	0.71±0.21	0.70±0.21
Pulse wave velocity (m/sec)	6.28±2.6	6.11±2.65	6.01±2.50
	n=1217	n=881	n=727
Increase pulse wave velocity (%)	8.1	8.1	7.2
Left ventricular mass index (g/m$^{1.7}$)	-	66.1±23.3	-
Left ventricular hypertrophy (%)	-	45.8	-
Carotid intima-media thickness (mm)	-	-	0.65±0.14
Increase carotid intima-media thickness (%)	-	-	35.8

Table 3.2. Correlation matrix showing bivariate relationships between indexes of aortic function.

	Pf	Pb	MAP	PWV
Pf	-			
Pb	0.704	-		
MAP	0.462	0.607	-	
PWV	0.380	0.509	0.433	-

Numbers shown are correlation coefficients. Pf, forward wave pressure' Pb, backward wave pressure; MAP, mean arterial pressure; PWV, carotid-femoral pulse wave velocity. All correlations are $p<0.0001$.

Table 3.3. Relative contribution (standardized β-coefficient) in multivariate models of risk factors to variations in left ventricular mass indexed to height$^{1.7}$ or the presence of LV hypertrophy (LVH) at different age groups in a community sample.

	<50 years (n=566)				**≥50 years (n=431)**			
Models with →	Pb		Pf		Pb		Pf	
	β-coeff±SEM	p-value	β-coeff±SEM	p-value	β-coeff±SEM	p-value	β-coeff±SEM	p-value
LVMI vs								
Age	-0.046±0.049	=0.35	0.004±0.048	=0.94	0.085±0.050	=0.92	0.099±0.050	<0.05
Male gender	0.089±0.044	<0.05	0.068±0.044	=0.18	0.180±0.058	<0.001	0.176±0.053	<0.001
MAP	0.045±0.048	=0.35	0.188±0.045	=0.01	0.095±0.054	=0.08	0.126±0.050	<0.02
Pb	0.187±0.047	<0.0001	-	-	0.145±0.057	<0.02	-	-
Pf	-	-	0.035±0.041	=0.39	-	-	0.105±0.053	=0.05
Body weight	0.335±0.046	<0.0001	0.326±0.046	<0.0001	0.213±0.049	<0.0001	0.259±0.049	<0.0001
DM or HbA1c>6.1 %	0.008±0.042	=0.85	-0.015±0.042	=0.72	0.099±0.047	<0.05	0.092±0.048	=0.06
LVH vs	(207 out of 566 with LVH)				(250 out of 431 with LVH)			
Age	0.037±0.047	=0.43	0.071±0.046	=0.13	0.074±0.050	=0.14	0.093±0.049	=0.06
Female gender	0.306±0.043	<0.0001	0.321±0.043	<0.0001	0.216±0.052	<0.0001	0.218±0.053	<0.0001
MAP	0.002±0.046	=0.97	0.059±0.043	=0.17	0.020±0.053	=0.71	0.057±0.050	=0.25
Pb	0.132±0.046	<0.005	-	-	0.143±0.057	<0.02	-	-
Pf	-	-	0.018±0.039	=0.64	-	-	0.088±0.052	=0.09
Body weight	0.213±0.044	<0.0001	0.206±0.045	<0.0001	0.248±0.049	<0.0001	0.235±0.049	<0.0001
DM or HbA1c>6.1 %	0.004±0.040	=0.93	-0.002±0.040	=0.96	-0.010±0.047	=0.83	-0.016±0.048	=0.73

Standardized β-coefficients are for multivariate adjusted models. See figure 1 for partial r values and table 1 for additional abbreviations. Additional adjustors include treatment for hypertension, regular smoking, regular alcohol intake and total and HDL cholesterol concentrations.

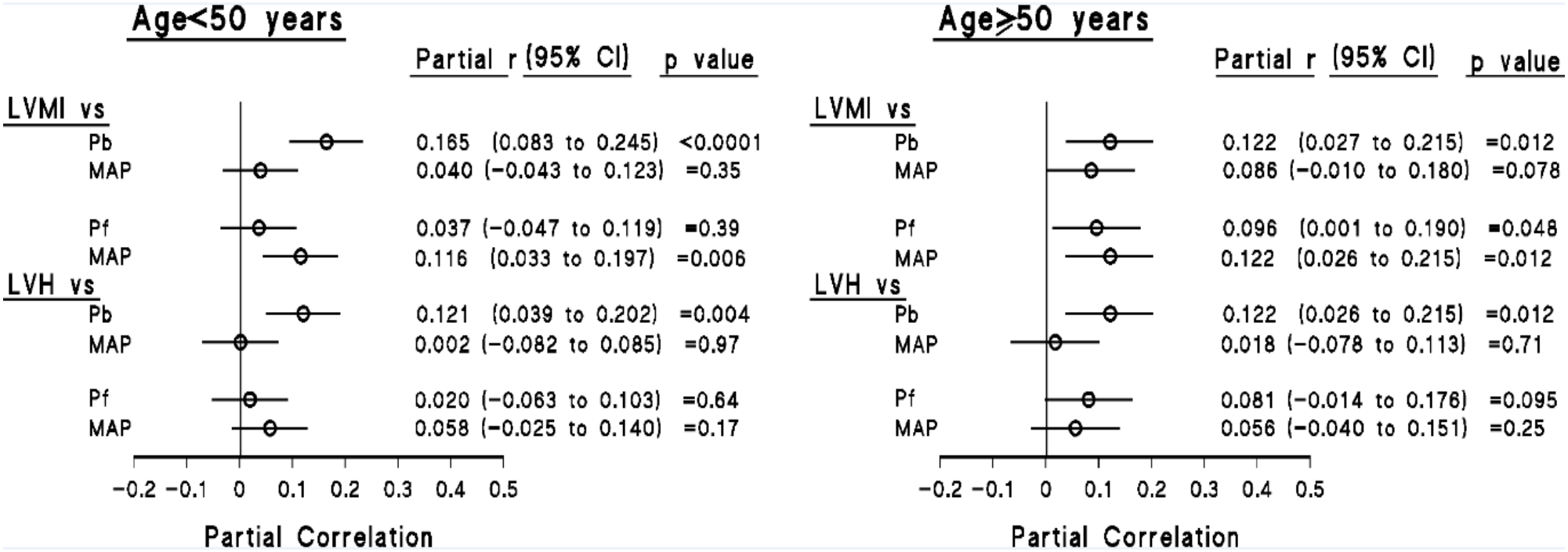


Figure 3.4. Multivariate adjusted relationships between steady-state (mean arterial pressure [MAP]) or aortic forward (Pf) or backward (Pb) wave pressures and left ventricular mass index (LVMI) or LV hypertrophy (LVH) at different ages in a community-based sample. The figure shows partial correlations and 95 % confidence intervals for the relations between LVMI or LVH and MAP, Pf or Pb. The relations are with MAP and Pf or MAP and Pb in the same regression models. Additional adjustments are for age, sex, treatment for hypertension, body weight, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.1 % and total and HDL cholesterol concentrations.

Table 3.4. Relative contribution (standardized β-coefficient) in multivariate adjusted models of backward (Pb) and forward (Pf) wave pressures (in the same regression models) to variations in left ventricular mass indexed to height$^{1.7}$ or the presence of LV hypertrophy (LVH) at different age groups in a community sample.

	<50 years (n=566)		**≥50 years (n=431)**	
	β-coefficient±SEM	p-value	β-coefficient±SEM	p-value
LVMI vs				
Age	0.064±0.051	=0.21	0.081±0.051	=0.11
Male gender	0.093±0.044	<0.05	0.176±0.053	<0.002
Pb	0.228±0.056	<0.0001	0.117±0.068	=0.085
MAP	0.047±0.048	=0.33	0.090±0.055	=0.10
Pf	-0.067±0.047	=0.16	0.048±0.068	=0.44
Body weight	0.339±0.046	<0.0001	0.268±0.050	<0.0001
DM or HbA1c>6.1 %	-0.009±0.042	=0.83	0.095±0.048	<0.05
LVH vs	(207 out of 566 with LVH)		(250 out of 431 with LVH)	
Age	0.022±0.049	=0.66	0.072±0.050	=0.16
Female gender	0.302±0.043	<0.0001	0.218±0.053	<0.0001
Pb	0.167±0.054	<0.005	0.128±0.067	=0.057
MAP	0.003±0.046	=0.95	0.017±0.054	=0.76
Pf	-0.057±0.046	=0.22	0.025±0.062	=0.68
Body weight	0.216±0.044	<0.0001	0.246±0.049	<0.0001
DM or HbA1c>6.1 %	0.003±0.040	=0.94	-0.013±0.047	=0.78

Standardized β-coefficients are for multivariate adjusted models. See table 1 for additional abbreviations. Additional adjustors include treatment for hypertension, regular smoking, regular alcohol intake and total and HDL cholesterol concentrations.

Table 3.5. Relative contribution (standardized β-coefficient) in multivariate models of risk factors to variations in carotid intima-media thickness (IMT) or the presence of an increased IMT at different age groups in a community sample.

	<50 years (n=435)				**≥50 years (n=369)**			
Models with→	Pb		Pf		Pb		Pf	
	β-coeff±SEM	p-value	β-coeff±SEM	p-value	β-coeff±SEM	p-value	β-coeff±SEM	p-value
IMT vs								
Age	0.421±0.052	<0.0001	0.437±0.051	<0.0001	0.331±0.054	<0.0001	0.312±0.053	<0.0001
Male gender	0.038±0.049	=0.44	0.030±0.043	=0.53	0.119±0.062	=0.06	0.106±0.062	=0.09
MAP	0.140±0.049	<0.005	0.146±0.046	<0.002	0.065±0.059	=0.27	0.054±0.053	=0.32
Pb	0.043±0.048	=0.37	-	-	0.072±0.062	=0.25	-	-
Pf	-	-	0.051±0.041	=0.22	-	-	0.133±0.056	<0.02
Body mass index	0.063±0.052	=0.23	0.062±0.052	=0.24	0.050±0.059	=0.40	0.035±0.059	=0.56
DM or HbA1c>6.1 %	0.094±0.043	<0.05	0.093±0.042	<0.05	0.029±0.052	=0.58	0.017±0.052	=0.74
Increased IMT vs	(127 out of 435 with ↑IMT)				(161 out of 369 with ↑IMT)			
Age	0.018±0.062	=0.77	0.009±0.061	=0.88	0.019±0.058	=0.75	-0.035±0.058	=0.54
Male gender	0.026±0.058	=0.65	0.020±0.057	=0.72	0.018±0.068	=0.79	0.006±0.068	=0.94
MAP	0.131±0.059	<0.05	0.120±0.055	<0.05	0.036±0.065	=0.57	0.030±0.058	=0.60
Pb	-0.033±0.057	=0.56	-	-	0.083±0.068	=0.22	-	-
Pf	-	-	-0.014±0.049	=0.77	-	-	0.137±0.061	<0.05
Body mass index	0.120±0.062	=0.06	0.121±0.062	=0.05	0.074±0.065	=0.25	0.057±0.0564	=0.38
DM or HbA1c>6.1 %	0.046±0.050	=0.36	0.047±0.050	=0.35	0.008±0.057	=0.88	0.003±0.057	=0.96

Standardized β-coefficients are for multivariate adjusted models. See figure 2 for partial r values and table 1 for additional abbreviations. Additional adjustors include treatment for hypertension, regular smoking, regular alcohol intake, and total and HDL cholesterol concentrations.

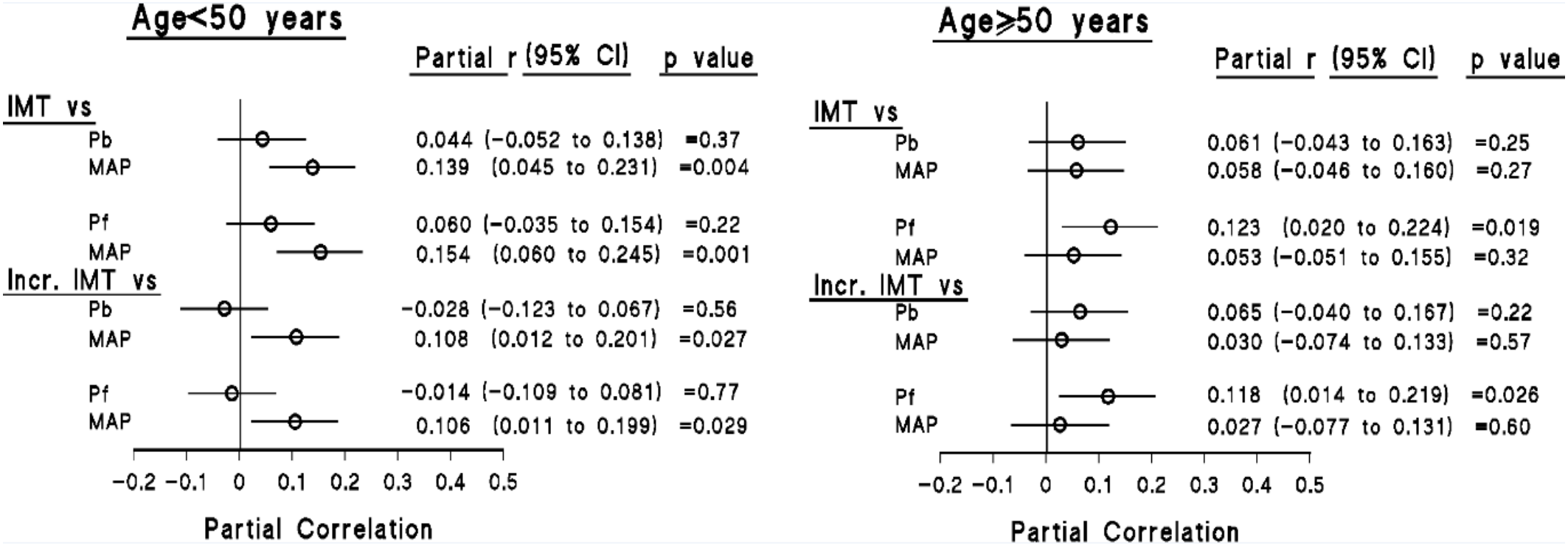


Figure 3.5. Multivariate adjusted relationships between steady-state (mean arterial pressure [MAP]) or aortic forward (Pf) or backward (Pb) wave pressures and carotid intima media thickness (IMT) or an increased IMT at different ages in a community-based sample. The figure shows partial correlations and 95 % confidence intervals for the relations between IMT or increased IMT and MAP, Pf or Pb. Relations are with MAP and Pf or MAP and Pb in the same regression models. Additional adjustments are for age, sex, treatment for hypertension, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.1 % and total and HDL cholesterol concentrations.

Table 3.6. Relative contribution (standardized β-coefficient) in multivariate adjusted models of backward (Pb) and forward (Pf) wave pressures (in the same regression models) to variations in carotid intima-media thickness (IMT) or the presence of an increased IMT at different age groups in a community sample.

	<50 years (n=435)		≥50 years (n=369)	
	β-coefficient±SEM	p-value	β-coefficient±SEM	p-value
IMT vs				
Age	0.432±0.053	<0.0001	0.314±0.054	<0.0001
Male gender	0.033±0.049	=0.50	0.105±0.062	=0.09
Pb	0.018±0.055	=0.75	-0.015±0.075	=0.84
MAP	0.141±0.049	<0.005	0.059±0.059	=0.32
Pf	0.044±0.048	=0.36	0.140±0.068	<0.05
Body mass index	0.062±0.052	=0.24	0.033±0.060	=0.58
DM or HbA1c>6.1 %	0.094±0.043	<0.05	0.017±0.052	=0.74
Increased IMT vs	(127 out of 435 with ↑IMT)		(161 out of 369 with ↑IMT)	
Age	0.018±0.064	=0.78	-0.036±0.059	=0.54
Male gender	-0.026±0.058	=0.66	0.006±0.066	=0.93
Pb	-0.033±0.066	=0.62	0.004±0.080	=0.96
MAP	0.131±0.059	<0.05	0.029±0.065	=0.66
Pf	-0.0004±0.057	=0.99	0.135±0.072	=0.06
Body mass index	0.120±0.062	=0.06	0.057±0.065	=0.38
DM or HbA1c>6.1 %	0.046±0.050	=0.36	-0.003±0.057	=0.96

Standardized β-coefficients are for multivariate adjusted models. See table 1 for additional abbreviations. Additional adjustors include treatment for hypertension, regular smoking, regular alcohol intake, and total and HDL cholesterol concentrations.

Table 3.7. Relative contribution (standardized β-coefficient) in multivariate models of risk factors to variations in aortic pulse wave velocity (PWV) or the presence of an increased PWV at different age groups in a community sample.

	<50 years (n=706)				≥50 years (n=511)			
Models with→	Pb		Pf		Pb		Pf	
	β-coeff±SEM	p-value	β-coeff±SEM	p-value	β-coeff±SEM	p-value	β-coeff±SEM	p-value
PWV vs								
Age	0.196±0.043	<0.0001	0.233±0.042	<0.0001	0.298±0.043	<0.0001	0.357±0.042	<0.0001
Male gender	0.049±0.043	=0.25	0.021±0.042	=0.61	0.143±0.048	<0.005	0.122±0.049	<0.02
MAP	0.214±0.043	<0.0001	0.258±0.039	<0.0001	0.130±0.047	<0.01	0.223±0.044	<0.0001
Pb	0.128±0.043	<0.005	-	-	0.230±0.054	<0.0001	-	-
Pf	-	-	0.057±0.035	=0.11	-	-	0.066±0.047	=0.16
Body mass index	0.114±0.043	<0.01	0.103±0.044	<0.02	-0.044±0.045	=0.33	-0.054±0.046	=0.25
DM or HbA1c>6.1 %	-0.023±0.035	=0.52	-0.028±0.035	=0.42	0.166±0.039	<0.0001	0.178±0.040	<0.0001
Increased PWV vs	(3 out of 706 with ↑PWV)				(96 out of 511 with ↑PWV)			
Age	-	-	-	-	0.265±0.045	<0.0001	0.314±0.044	<0.0001
Male gender	-	-	-	-	0.091±0.051	=0.07	0.066±0.051	=0.20
MAP	-	-	-	-	0.099±0.049	<0.05	0.176±0.046	<0.0001
Pb	-	-	-	-	0.246±0.057	<0.0001	-	-
Pf	-	-	-	-	-	-	0.115±0.049	<0.02
Body mass index	-	-	-	-	-0.041±0.047	=0.39	-0.055±0.048	=0.26
DM or HbA1c>6.1 %	-	-	-	-	0.090±0.041	<0.05	0.098±0.041	<0.02

Standardized β-coefficients are for multivariate adjusted models. See figure 3 for partial r values and table 1 for additional abbreviations. Additional adjustors include treatment for hypertension, regular smoking, regular alcohol intake, heart rate and total and HDL cholesterol concentrations.

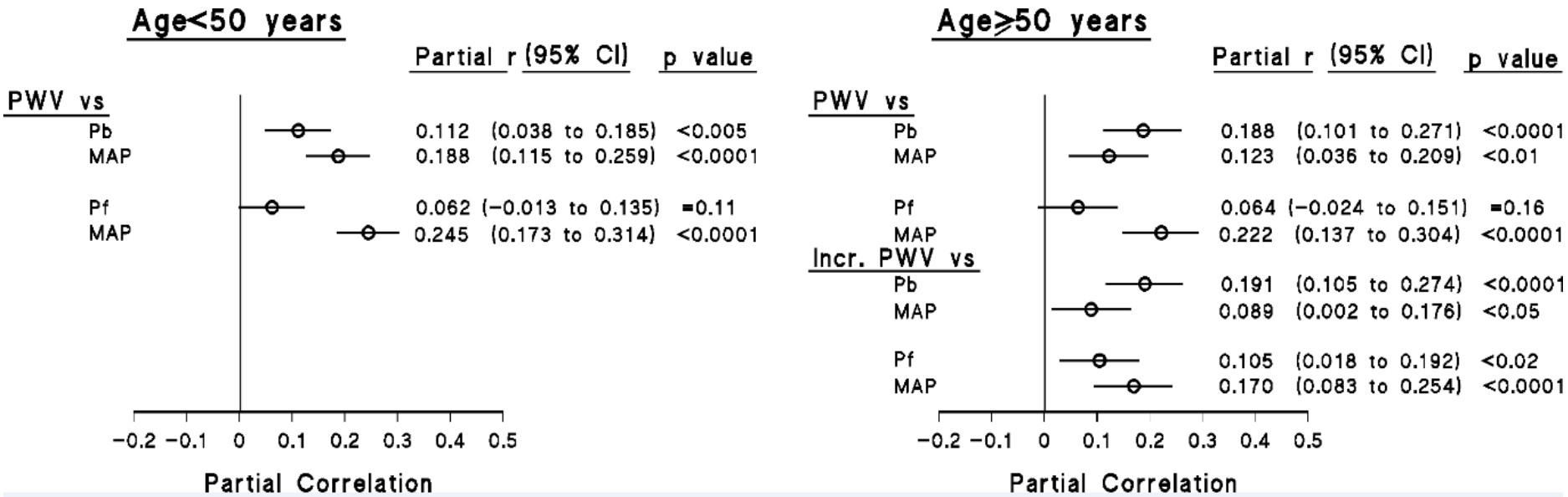


Figure 3.6. Multivariate adjusted relationships between steady-state (mean arterial pressure [MAP]) or aortic forward (Pf) or backward (Pb) wave pressures and aortic pulse wave velocity (PWV) or an increased PWV at different ages in a community-based sample. The figure shows partial correlations and 95 % confidence intervals for the relations between PWV or increased PWV and MAP, Pf or Pb. Relations are with MAP and Pf or MAP and Pb in the same regression models. Additional adjustments are for age, sex, heart rate, treatment for hypertension, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.1 % and total and HDL cholesterol concentrations.

Table 3.8. Relative contribution (standardized β-coefficient) in multivariate adjusted models of backward (Pb) and forward (Pf) wave pressures (in the same regression models) to variations in aortic pulse wave velocity (PWV) or the presence of an increased PWV at different age groups in a community sample.

	<50 years (n=706)		≥50 years (n=511)	
	β-coefficient±SEM	p-value	β-coefficient±SEM	p-value
PWV vs				
Age	0.188±0.045	<0.0001	0.302±0.043	<0.0001
Male gender	0.054±0.044	=0.21	0.159±0.049	<0.002
Pb	0.152±0.060	<0.02	0.324±0.073	<0.0001
MAP	0.211±0.043	<0.0001	0.137±0.047	<0.005
Pf	-0.028±0.049	=0.57	-0.119±0.062	=0.06
Body mass index	0.117±0.044	<0.01	-0.033±0.046	=0.47
DM or HbA1c>6.1 %	-0.022±0.035	=0.53	0.169±0.039	<0.0001
Increased PWV vs	(3 out of 706 with ↑PWV)		(96 out of 511 with ↑PWV)	
Age	-	-	0.266±0.045	<0.0001
Male gender	-	-	0.097±0.051	=0.06
Pb	-	-	0.282±0.077	<0.0005
MAP	-	-	0.101±0.049	<0.05
Pf	-	-	-0.046±0.065	=0.48
Body mass index	-	-	-0.037±0.048	=0.44
DM or HbA1c>6.1 %	-	-	0.091±0.041	<0.05

Standardized β-coefficients are for multivariate adjusted models. See table 1 for additional abbreviations. Additional adjustors include treatment for hypertension, regular smoking, regular alcohol intake, heart rate and total and HDL cholesterol concentrations.

Table 3.9. Multivariate adjusted (including for steady-state pressures) relationships between aortic pulse wave velocity (PWV), brachial or aortic pulse pressure (PP) or aortic augmentation index (AIx) or reflected wave magnitude (RM) and end-organ measures.

	<50 years of age			≥50 years of age		
	n=	Partial r (95 % CI)	p-value	n=	Partial r (95 % CI)	p-value
PWV vs						
LVMI	501	0.094 (0.006 to 0.181)	<0.05	380	0.020 (-0.082 to 0.122)	=0.70
Carotid IMT	397	0.045 (-0.055 to 0.144)	=0.38	330	0.068 (-0.042 to 0.177)	=0.22
Brachial PP vs						
LVMI	566	0.110 (0.027 to 0.191)	<0.01		0.153 (0.058 to 0.245)	<0.005
Carotid IMT	435	0.042 (-0.053 to 0.137)	=0.38		0.115 (0.012 to 0.216)	<0.05
Aortic PWV	706	0.094 (0.020 to 0.167)	<0.02		0.121 (0.034 to 0.207)	<0.01
Aortic PP vs						
LVMI	566	0.137 (0.054 to 0.218)	=0.001		0.143 (0.048 to 0.235)	<0.005
Carotid IMT	435	0.038 (-0.057 to 0.133)	=0.43		0.105 (0.001 to 0.206)	<0.05
Aortic PWV	706	0.113 (0.039 to 0.186)	<0.005		0.127 (0.040 to 0.212)	<0.005
Aortic augmentation index (AIx) vs						
LVMI	566	0.086 (0.003 to 0.168)	<0.05	431	-0.021 (-0.117 to 0.075)	=0.67
Carotid IMT	435	0.032 (-0.063 to 0.127)	=0.51	369	0.021 (-0.083 to 0.124)	=0.70
Aortic PWV	706	0.027 (-0.047 to 0.101)	=0.48	511	0.111 (0.023 to 0.196)	<0.02
Reflected wave magnitude (RM=Pb/Pf) vs						
LVMI	566	0.148 (0.066 to 0.228)	<0.0005	431	0.026 (-0.070 to 0.121)	=0.59
Carotid IMT	435	(-0.145 to 0.045) =0.30	=0.30	369	-0.011 (-0.115 to 0.092)	=0.83
Aortic PWV	706	(-0.012 to 0.136)	=0.10	511	0.162 (0.075 to 0.246)	<0.0005

Partial r values are for multivariate adjusted models. LVMI, left ventricular mass index; IMT, intima-media thickness. Adjustments are for age, mean arterial pressure, heart rate (PWV and AIx), sex, uncontrolled hypertension, body weight (LVMI), body mass index (IMT), regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.1 %, and total and HDL cholesterol concentrations.

3.5. Discussion

The main findings of the present study are as follows: In a large, randomly recruited, community-based sample, independent of risk factors including age, steady-state pressures (MAP), and forward and backward wave pressures showed associations with indexes of cardiac and vascular end-organ changes that were organ and age-specific. In this regard, Pb, but not MAP or Pf showed robust independent relations with LVMI and LVH in those younger than 50 years of age, and Pb and MAP with PWV in those at any age. In contrast, MAP (in those younger than 50 years of age) and Pf (in those older than 50 years of age), but not Pb showed independent relations with carotid IMT.

There is presently ongoing debate as to the relative role of steady-state pressures and aortic Pf or Pb, the component waveforms of aortic PP, in risk prediction. In this regard, in the Framingham Heart Study, beyond steady-state pressures an enhanced Pf, but not an increased Pb, predicted events (Cooper et al 2015) and associated with LVM (Kaess et al 2016). In keeping with the Framingham Heart Study, where most events were vascular in origin (Cooper et al 2015), in the present study we show that whilst beyond steady-state pressures and additional confounders Pb was not associated with carotid IMT at any age, Pf was independently associated with IMT over the age of 50 years. In contrast to the Framingham Heart Study, in the MESA a more important role of Pb as compared to Pf in predicting events (Chirinos et al 2012; Zamani et al 2014) and associating with LVM (Zamani et al 2015) was noted. In keeping with the dominant effects of Pb on LVM and events in the MESA, in the present study we noted, independent of steady-state pressures, a more important role for Pb as compared to Pf in associations with both LVMI and PWV.

Although prior large studies have evaluated the role of Pf and Pb in correlations with LVMI (Zamani et al 2015; Kaess et al 2016), no large studies have compared the relative impact of MAP, Pf and Pb in associations with several end-organ measures. In this regard, the present study provides clear evidence for organ-specific associations of MAP, Pf and Pb with end-organ changes in the same individuals of a community sample, effects that are age-specific. In this regard, the present results suggest that in community samples where Pb contributes substantially to variations in aortic PP as in the present sample (Sibiya et al 2015), that IMT indexes largely Pf effects. In contrast, in the same community LVMI or aortic PWV index MAP and Pb effects. Consequently, consideration should be given to the possibility of enhancing risk reduction beyond decreases in steady-state pressures and arteriolar resistance, by targeting both proximal aortic stiffness and hence impedance (and thus Pf) in those over 50 years of age, and across the adult lifespan, by reducing those

determinants of Pb that are independent of arteriolar function. Presently however, no convincing therapeutic approaches have yet emerged as being able to reduce aortic aortic stiffness and hence impedance beyond distending pressure effects. Moreover, no vasodilators have been demonstrated to decrease age-dependent increases in Pb beyond arteriolar function.

In a prior study, we reported on the brachial PP or SBP-independent associations of Pf and Pb with end-organ measures, and either failed to show or showed only modest age-independent relations between Pf or Pb and LVMI, IMT or PWV (Sibiya et al 2015). However, that prior study (Sibiya et al 2015) was conducted in a smaller study sample and was specifically designed to determine whether Pf or Pb explained brachial PP-independent relations between aortic PP and end-organ measures. In contrast, the present study was conducted in a larger study sample with sufficient power to show age-independent relations and in the present analysis we did not adjust for brachial PP, which reflects at least some of the impact of Pf and Pb. The present study therefore provides insights into the extent of the age and steady-state pressure independent relations between Pf or Pb and end-organ measures irrespective or brachial BP in a large community–based study sample.

The limited independent association between Pf and LVMI in the present study, despite providing clear evidence of differential adverse effects on end-organs, does not exclude a possibility that Pf contributes to adverse cardiac effects. Indeed, although in the Framingham Heart Study aortic PWV was not independently associated with LVMI, Pf in that study was (Kaess et al 2016). Moreover, in the present community-based sample, aortic PWV, but not Pf was independently associated with LVMI in women, but not in men (Bello et al 2017). Importantly, these studies together suggest that the impact of aortic PWV and Pf are independent of each other (Kaess et al 2016; Bello et al 2017). In this regard, aortic stiffness may mediate increases in LVM through several possible mechanisms, including through direct coupling of the left ventricle to the proximal aorta (Bell et al 2015), where proximal aortic stiffness may load the left ventricle through effects that could be independent of aortic Pf and hence PP.

Importantly, through inertial effects and Newton's 3rd Law of Motion Pf determines Pb. In addition, it is now also recognised that through re-reflection of Pb, Pb determines Pf (Phan et al 2016). Consequently, although this approach is likely to over adjust, in sensitivity analyses we assessed the relative contribution of Pf and Pb in relations with end-organ measures with Pf and Pb in the same regression models. In this regard, whilst Pb was independently associated with LVMI and LVH beyond Pf in the younger age group, and with PWV across all age groups, Pf was independently associated with IMT beyond Pb in

the older age group. Consequently, the relations between Pb or Pf and end-organ measures reported on in the present study do not necessarily reflect the impact of Pf on Pb or Pb on Pf.

There are several limitations to the present study that warrant consideration. First, the present study was cross-sectional in design and hence no conclusions regarding causality can be drawn. As the present study was not longitudinal in design, the apparent age-dependence of end-organ changes on MAP, Pf and Pb require confirmation in longitudinal analysis. However, this would require follow-up of participants across most of the adult lifespan. Second, the use of the 'triangulation method' of aortic wave separation to derive aortic forward and backward wave pressures has been questioned (Kips et al 2009). However, the same limitations to the use of the 'triangulation method' of aortic wave separation apply to associations between Pf or Pb and either LVMI or IMT, and differential associations were noted. Thus, irrespective of the limitations of deriving forward and backward wave pressures, in the present study, the conclusion that Pf and Pb show differential associations with LVMI versus IMT still hold. Third, in the present study, calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries (Picone et al 2015). Hence, aortic pressures may have been underestimated using the current approach. However, both forward and backward wave pressures would have been similarly affected by this calibration error. Fourth, the value of carotid IMT as a vascular marker of damage that predicts events has been questioned. However, being a community-based study few participants under 50 years of age had carotid plaque and hence we were not statistically powered to assess relations between MAP, Pf or Pb and atherosclerotic plaque over the full adult age range. Fifth, more women than men volunteered for the study and thus we were not able to perform sex-specific analyses in different age categories. Hence, the present findings may be specific for women. Last, the present study was conducted in one ethnic group. Our findings may therefore not be translatable to other ethnic groups.

In conclusion, in a large, community-based study, whilst steady-state pressures (MAP) and aortic Pf show independent relations with carotid IMT in those younger and older than 50 years of age respectively, Pb and steady-state pressures (for PWV) showed independent relations with LVMI and aortic PWV. These data suggest that in community samples with high reflected waves (Hodson et al 2017), steady-state pressures, Pf and Pb produce organ-specific and age-dependent effects on end organ measures. Whether these organ-specific effects translate into differential effects of MAP, Pf and Pb on cardiovascular events at different ages requires further study.

CHAPTER 4

Complementary Impact of Carotid Intima Media Thickness with Plaque in Associations with Non-Cardiac Arterial Vascular Events.

The data in this chapter have been accepted for publication and is currently in press in the journal, *Angiology:*

Kolkenbeck-Ruh A., Woodiwiss A.J., Monareng T., Sadiq E., Mabena P., Robinson C., Motau T.H, Stevens B., Manyatsi N., Tiedt S., Dembskey R., Abdool-Carrim T., Veller M., Cassimjee I., Modi G., Hale M., Norton G.R. (2019). Complementary impact of carotid intima media thickness with plaque in associations with non-cardiac arterial vascular events. *Angiology*: In press.

4.1. Abstract

Background: The ability of carotid intima-media thickness (IMT) to predict risk beyond plaque is controversial. We aimed to determine whether relationships with events for IMT complement the impact of plaque over a young age range depending on the extent of thrombotic versus atherosclerotic disease.

Methods: In 1157 participants (473 with critical limb ischemia [CLI] or stroke; 684 community participants), we assessed relations between carotid IMT or plaque (B-mode ultrasound) and arterial events. The extent of atherosclerotic versus thrombotic occlusion (histology) was determined in 54 patients with CLI requiring amputations.

Results: Thrombotic occlusion in CLI was associated with a younger age ($p<0.0001$) and less carotid plaque ($p=0.02$). Consequently, independent relations between plaque and CLI were noted over an older (>50 years) ($p<0.005$ to <0.0001), but not younger ($p>0.38$) age range, whilst independent relations between plaque and stroke ($p<0.005$ to <0.0001) and between IMT and CLI ($p<0.0001$) were noted over a younger age range. Although in the performance for event detection (area under the receiver operator characteristic curve), an IMT>threshold failed to add to plaque alone in those older than 50 years of age (0.680 ± 0.020 vs 0.664 ± 0.017, $p=0.27$), IMT improved the performance for combined stroke and CLI detection when added to plaque in those younger than 50 years of age (0.719 ± 0.023 vs 0.631 ± 0.026, $p<0.0001$).

Conclusions: Because over a younger age range the high prevalence of thrombotic occlusion in CLI is associated with less carotid plaque, IMT adds information to plaque in a complementary fashion in associations with arterial vascular events.

4.2. Introduction

Carotid intima-media thickness (IMT) is a well-described index of subclinical atherosclerosis that is readily acquired using B-mode ultrasound. Although IMT predicts atherosclerotic cardiovascular events (Den Ruijter et al 2012; Centurión, 2016), there is nevertheless considerable uncertainty as to the role of IMT in risk prediction beyond the identification of atherosclerotic plaque. Marked dissociations that exist in carotid IMT and plaque between ethnic groups (Mackinnon et al 2010), suggests that IMT and plaque index different pathophysiological changes. In this regard, IMT may reflect non-atherosclerotic arterial wall changes such as compensatory medial hypertrophy in response to a high wall stress (Touboul et al 2012), or early fatty streaks which regress rather than progress to atherosclerotic plaque (Insull, 2009; Wannarong et al 2013). Importantly, meta-analyses and large studies have demonstrated that carotid plaque may be more predictive of cardiovascular events than IMT (Johnsen et al 2009; Inaba et al 2012; Spence et al 2012; Yoon et al 2017; Jeevarethinam et al 2018; Mitchell et al 2018; Sillesen et al 2018). However, some studies suggest that IMT is superior to plaque at predicting events (Geeta et al 2015; Ren et al 2015; Barakoti, 2018). Thus, a better understanding of the role of IMT versus plaque in risk determination is required.

Atherosclerosis can result in events attributed to either predominantly thrombotic or atherosclerotic (large plaque protruding into the lumen) arterial occlusion. In this regard, the presence of carotid plaque may be more likely in events caused by more extensive atherosclerosis (atherosclerotic occlusion). Alternatively, the extent of atherosclerosis and hence the chances of obvious plaque being detected may be less in those with largely thrombotic occlusion. Thus, more subtle atherosclerotic indexes, such as IMT may be better associated with arterial events where events are dominantly thrombotic in nature. In this regard, chronologically premature arterial events may be accounted for more by thrombotic than atherosclerotic occlusion (Collet et al 2006; Speidl et al 2007; Pineda et al 2009; Milgrom et al 2018). Whether carotid plaque is sufficiently sensitive to predict events in younger individuals is therefore unknown. In contrast, irrespective of the pathophysiological processes accounting for IMT, IMT may be a sensitive index of risk factor effects on early atheroma. Indeed, strong independent associations between IMT and arterial events are noted across the adult lifespan, including at a chronologically premature age (Kolkenbeck-Ruh et al 2019). However, whether the presence of plaque is equally as closely associated with arterial events over the younger adult age range, or where events may be attributed to more thrombotic than atherosclerotic occlusion, is unknown. As

longitudinal studies cannot be readily conducted to assess events over a young adult age range, to further evaluate the role of carotid IMT versus plaque in the development of arterial events over this age range, we therefore compared IMT and plaque in cases versus controls at different ages across the full adult lifespan in a country where these events commonly occur over a young adult age (Kolkenbeck-Ruh et al 2019). We assessed relationships with non-cardiac arterial vascular events (stroke and critical limb ischemia [CLI]) as coronary artery disease is infrequent at a young age in the ethnic group in the country where the study was performed (Kolkenbeck-Ruh et al 2019). To evaluate whether age determines the extent to which lower limb thrombotic as opposed to atherosclerotic occlusion occurs, histological examination of amputated lower limb arteries was evaluated in those with CLI.

4.3. Methods

4.3.1 Study groups.

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M17-04-01, M16-04-11, M14-04-29, M12-04-108, M11-08-29, M07-04-69, and M02-04-72). Participants gave informed, written consent. Consecutive patients of black African ancestry (n=473) with stroke (n=164) or CLI (n=309) were recruited from the Charlotte Maxeke Johannesburg Academic Hospital, South Africa. The presence of CLI was defined as the occurrence of ischemic leg pain at rest for more than two weeks, or the existence of ulcers or gangrene attributable to occlusive arterial disease. Patients with a new stroke, defined as a focal neurological deficit of vascular origin were evaluated. Participants with meningitis based on cerebrospinal fluid examination, or the presence of intracranial malignancy or intracranial mass lesions on imaging, and vasculitis were excluded. Strokes were classified according to the Trial of Org 10172 in acute stroke treatment (TOAST) classification. Data obtained in patients with vascular events were compared with data acquired over the same time-period in 684 age- and sex-matched randomly recruited participants older than 16 years of black African descent living in the South West Township (SOWETO) of Johannesburg, South Africa, using the population census figures of 2001. In this regard, black African patients attending the Charlotte Maxeke-Johannesburg Academic Hospital are of the same socioeconomic class as those living in the SOWETO community.

4.3.2. Clinical, demographic, anthropometric measurements.

A questionnaire was administered to obtain demographic information including each participant's medical history, the use of medication and tobacco and alcohol use. Clinical information was also extracted from the hospital records and confirmed by the attending physician. Clinical data included the presence of risk factors (hypertension, and diabetes mellitus and the therapy thereof). Height and weight were measured using standard approaches and in all participants obesity was defined as a body mass index (BMI) $\geq$30 kg/m^2. Blood tests were performed including a fasting lipid profile. Participants were considered to have diabetes mellitus if they had a fasting plasma glucose concentration $\geq$7mmol/l, or in whom glucose-lowering agents were prescribed. Brachial blood pressure (BP) was measured according to guidelines and taken as the mean of five measurements. Participants with a BP$\geq$140/90 mm Hg or those receiving antihypertensive medication were considered to have hypertension.

4.3.3. Carotid intima-media thickness and plaque.

Carotid IMT was determined using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington) employing a linear array 7.5 MHz probe as recommended (Touboul et al 2012). As described in Chapter 2, images of at least 1 cm length of the far wall of the distal portion of both the right and left common carotid arteries from an optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualized simultaneously) at least 1 cm proximal to the flow divider, were obtained (Figure 2.1). Carotid IMT measurements (Figure 2.2) were determined using semi-automated border-detection and quality control software. At least 3 measurements were obtained from both the right and left sides and the mean of data from both sides employed for analysis. Carotid plaque was determined from both longitudinal and cross-sectional images (Figure 4.1a) in and around the carotid bifurcation (Touboul et al 2012; Mitchell et al 2018). Carotid plaque was defined according to the Mannheim consensus (Touboul et al 2012) as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50 % of the surrounding IMT value or demonstrates a thickness >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface (Figure 4.1b).

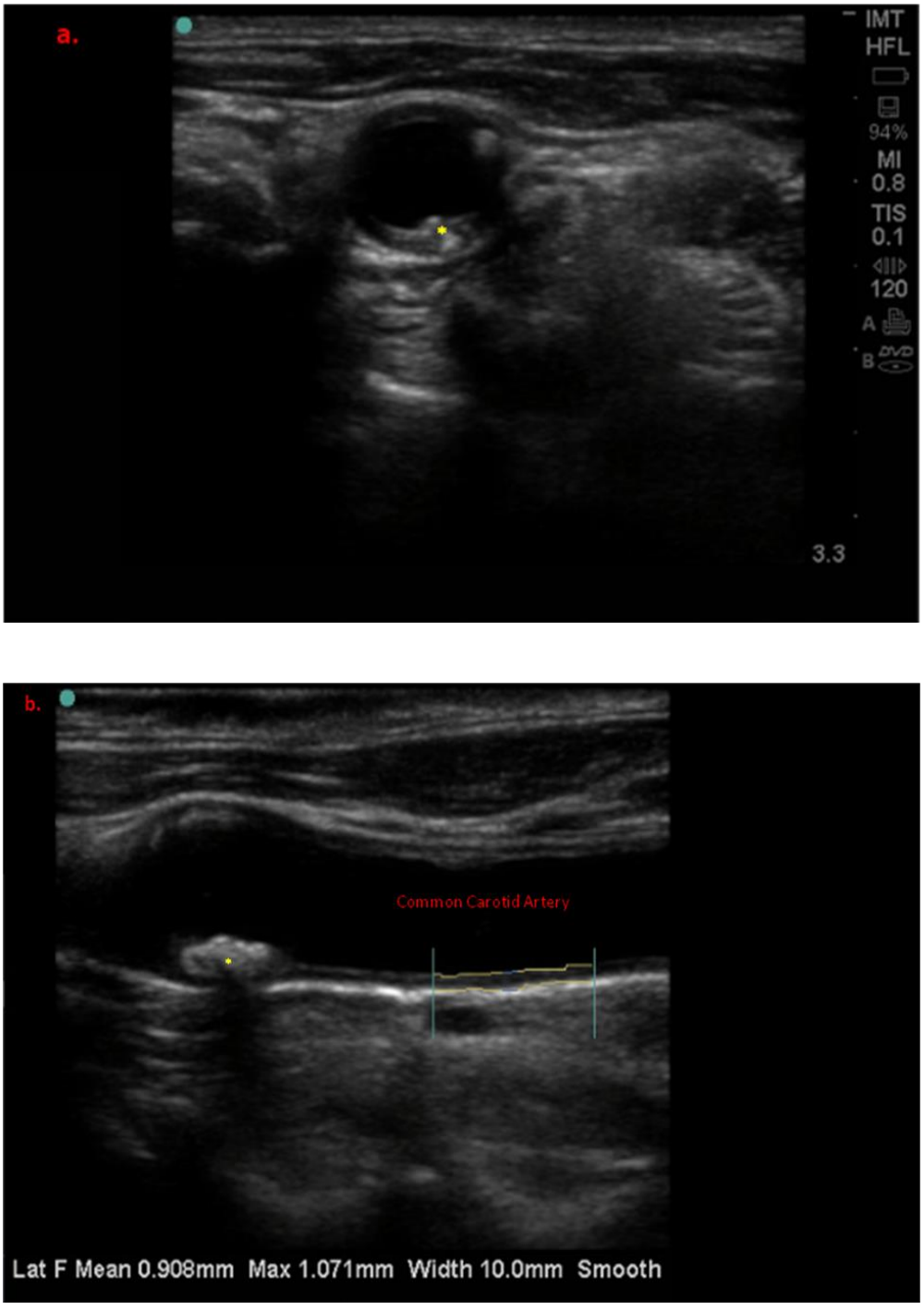


Figure 4.1. Ultrasonography showing the cross sectional (a) and longitudinal sections (b) in and around the carotid bifurcation for the identification of the presence of plaque (indicated by the yellow *).

4.3.4. Histological assessment.

Histological assessment of amputated limbs was performed in 54 patients with CLI requiring amputation. The amputated lower limb was fixed in 10 % formalin immediately after surgery. Standard dissection of the arterial tree throughout the lower limb from the point of occlusion, diagnosed from clinical and CT-angiogram assessment, to the dorsalis pedis artery was performed. Thereafter, the femoral, popliteal, posterior and anterior tibial, fibular, dorsalis pedis and planter arteries were dissected, where they were sectioned transversally at 50 mm intervals from proximal to distal. After routine processing and paraffin embedding, 5 µm sections were obtained and stained with hemotoxylin and eosin. All specimens were examined microscopically for the presence of atheroma and thrombi using the American Heart Association classification of histological atherosclerosis (Stary, 2000) and classified as normal (no pathology) (Figure 4.2), thrombotic (Figure 4.3a), atherosclerotic (Figure 4.3b) or mixed occlusions (Figure 4.3c). Histological assessments were confirmed by an experienced anatomical pathologist (MH). The stenosis of the lower limb arteries from both atherosclerotic plaques and thrombi was graded based on the luminal narrowing of the lower limb arteries as grade, grade 1 (0-25 % stenosis), grade 2 (26-50 % stenosis), grade 3 (51-75 % stenosis) and grade 4 (76-100 % stenosis) (Porwal et al 2016) (Figure 4.4).

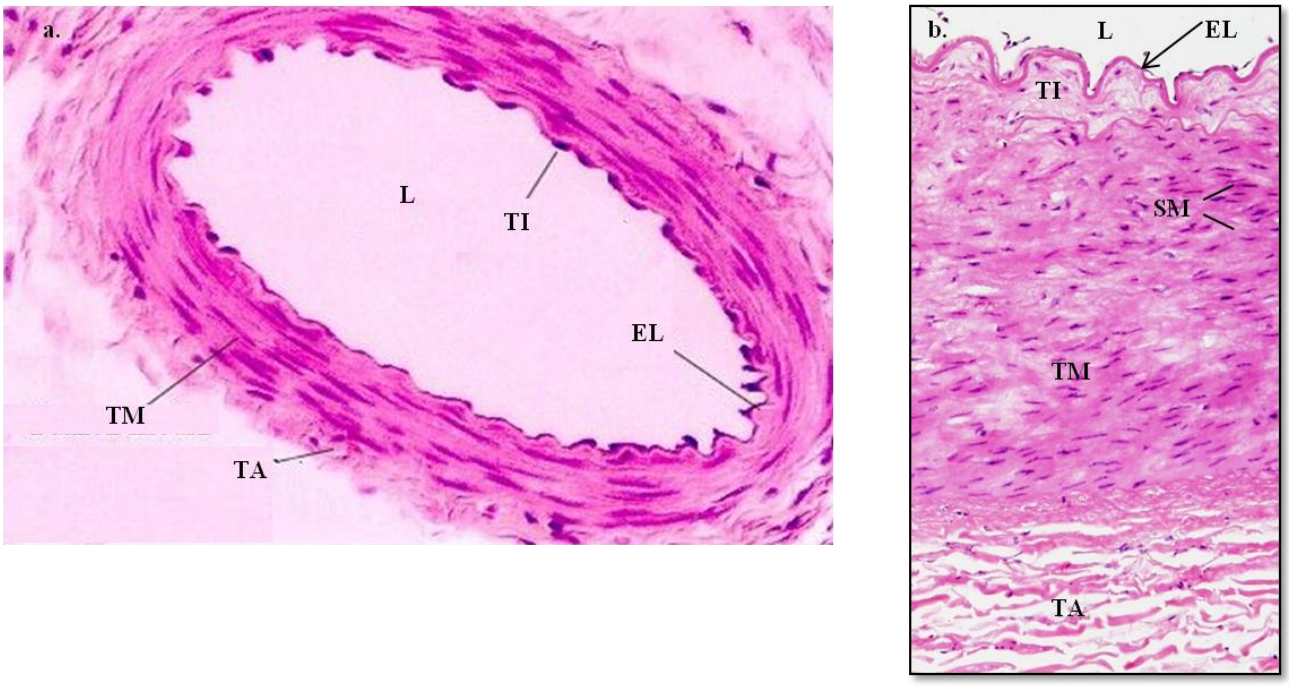


Figure 4.2. Hematoxylin Eosin staining of a normal muscular artery showing the 3 tunica layers. (a) Showing the cross sectional image (b) Showing the 3 tunica layers under a higher magnification. Adapted from Barakoti et al 2018. EL, elastic lamina; L, lumen; SM, smooth muscle cells; TA, tunica adventitia; TI, tunica intima; TM, tunica media.

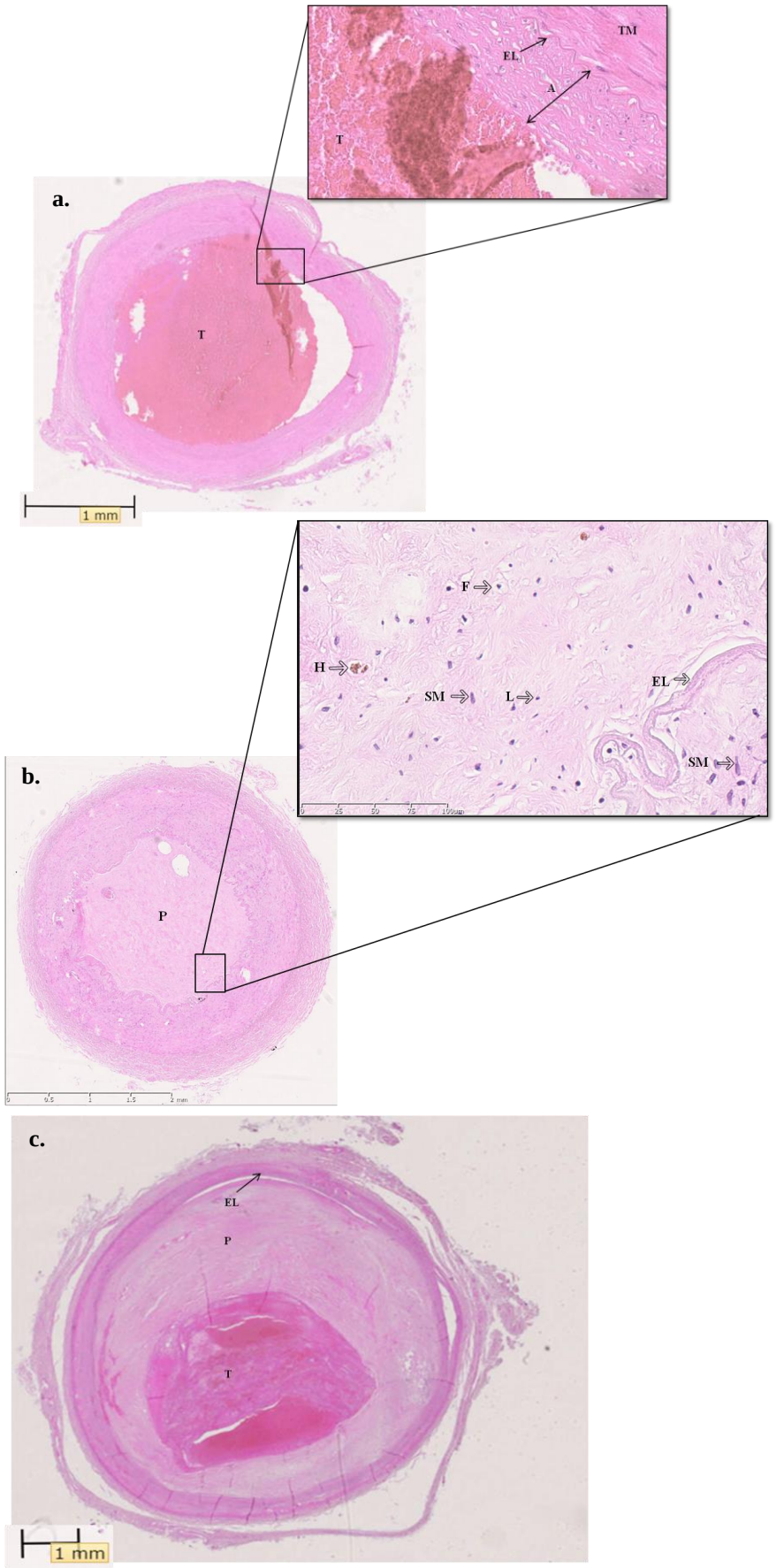


Figure 4.3. Hematoxylin Eosin staining showing thrombotic (a), atherosclerotic (b) and mixed (c) occlusions in the popliteal (a,c) and posterior tibial arteries from different amputated limbs of patients with critical limb ischaemia. A, atheroma; EL, elastic lamina F, foam cells; H, hemosiderin; L, lymphocytes; P, plaque; SM, smooth muscle cells; T, thrombus; TM, tunica media.

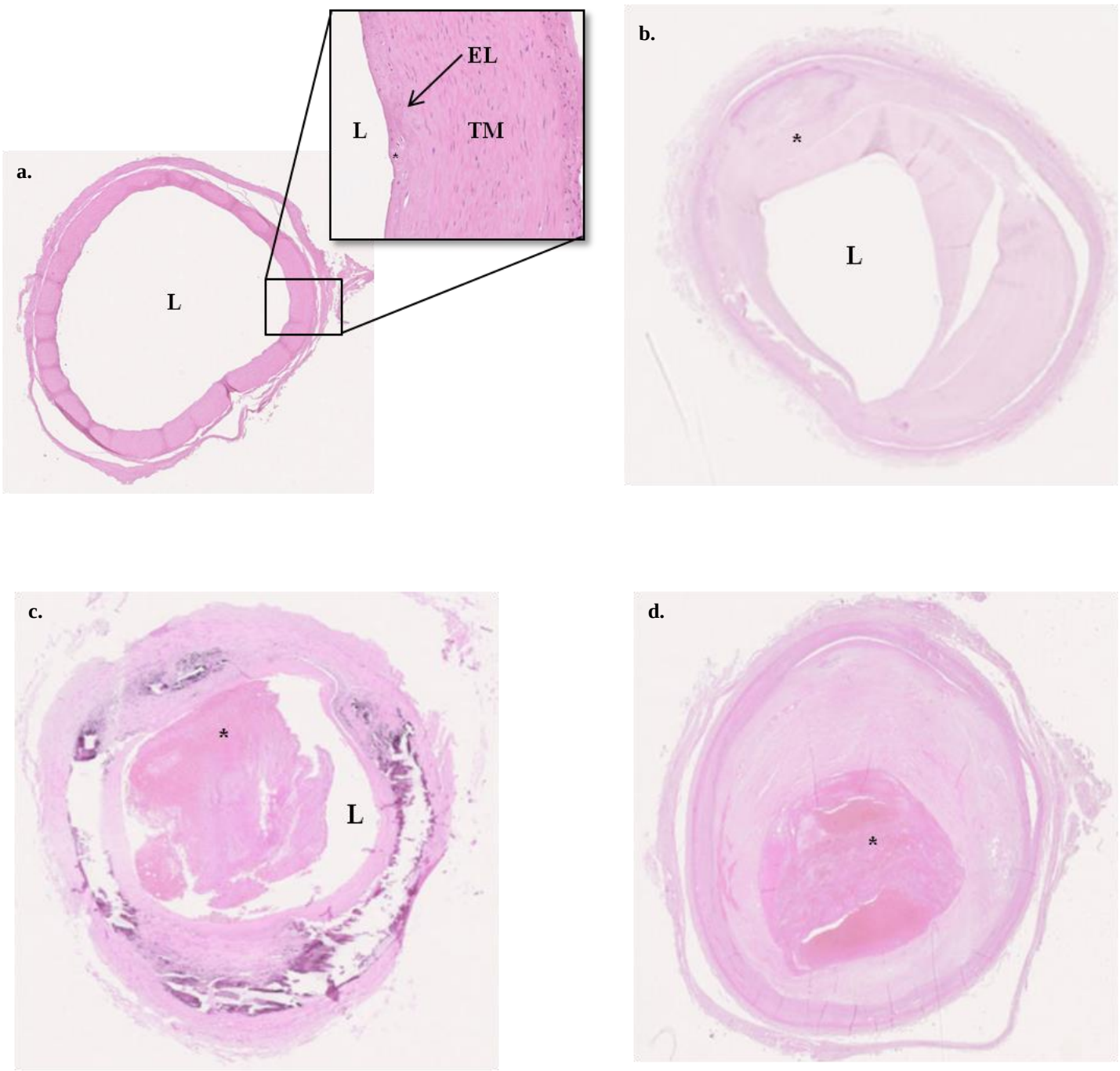


Figure 4.4. Degree of luminal narrowing from either thrombotic or atherosclerotic occlusions (indicated by the *). (a) grade 1 (0-25 %); (b) grade 2 (26-50 %); (c) grade 3 (51-75 %); grade 4 (76-100 %). EL, elastic lamina; L, lumen; TM, tunica media.

4.3.5. Statistical analysis.

As the present study compared cases and controls, the sample size required to show differences in IMT with 80 % power was calculated using sample size calculation for case-control studies (Charan and Biswas, 2013). In order to calculate the minimum sample size required, I examined a previous population-based study that included individuals of black African ancestry and compared carotid IMT between individuals who had a stroke compared to healthy individuals that had not had a stroke (Chambless et al, 2000). In this study the difference in carotid IMT between the cases and controls was 0.13 (mm) and the mean SD was 0.75 (mm). Using the formula from Charan and Biswas (2013), the minimum sample size required to identify statistically significant differences (α=0.05) in carotid IMT with 80 % power between cases and controls was 259 in each group. Although the current study employed considerably larger study samples (n=473 cases and n=479 controls) to show differences, prior to initiating the study we did not know how many patients we would be able to recruit at a younger age and hence considerably more participants were studied than that required to show differences in carotid IMT.

For database management and statistical analysis, SAS software, version 9.4 (SAS Institute Inc., Cary, NC) was employed. Continuous variables were expressed as mean (SD), or median (interquartile range) when non-normally distributed. Dichotomous variables were expressed as proportions or percentages. An increased IMT was considered as either >0.80 mm or >age-related thresholds for IMT derived from age-specific 95th percentiles of normotensive, non-diabetic participants from the community sample as previously described (Kolkenbeck-Ruh et al 2019). Multiple logistic regression analysis was performed to determine the independent relations between carotid IMT or plaque and vascular events. Adjustments included in multivariate models were those associated with vascular events. Odds ratios (OR) were compared using z statistics. Performance of IMT or plaque for the detection of non-cardiac arterial vascular events was determined from the area under the receiver operator characteristic curve (AUC) and sensitivity and specificity for event detection using standard approaches.

4.4. Results

4.4.1. Participant characteristics.

The participant characteristics are given in Table 4.1. Of the patients admitted with stroke, 11.0 % had hemorrhagic stroke, 18.9 % had a cardio-embolic cause (as assessed from clinical features and echocardiography); 17.7 % were classified as small artery occlusion (confirmed with intracranial imaging); 5.5 % with large artery occlusion (atherosclerotic) (confirmed with CT angiography); 39.0 % were indeterminate in origin and 7.9 % were from an alternative etiology.

Patients with arterial events had a greater prevalence of hypertension, diabetes mellitus and more patients with CLI smoked. HDL cholesterol concentrations were lower in patients with arterial events. Although LDL cholesterol concentrations were also lower in cases, 81.3 % of these patients were receiving lipid-lowering therapy at the time of performing fasting blood analysis. Body mass index was also lower in cases with CLI as compared to controls. 43.7 % of patients had only one of the modifiable conventional risk factors hypertension, diabetes mellitus or smoking, 37.8 % had two of these risk factors and 5.7 % had three of these risk factors. The characteristics of the patients with CLI requiring amputation and whom had histological assessment of the amputated limb (Table 4.2) were similar to those of the whole group except (Table 4.1) they tended to have a lower BMI, BP and HDL cholesterol concentrations.

Table 4.1. Participant characteristics.

	Controls (n=254)	CLI (n=309)	Controls (n=430)	Stroke (n=164)
Age (years)	58.6±11.3	60.3±12.5	49.5±13.4	49.8±14.8
Sex (% male)	63.4	71.2	42.3	41.5
Body mass index (kg/m^2)	27.8±6.3	26.3±6.0*	29.3±7.4	29.9±8.2
% Hypertensive	56.7	65.4*	47.4	66.9**
% Diabetes mellitus	12.6	34.0**	9.8	18.9*
% Regular smoking	21.3	46.9	18.4	24.6
SBP/DBP (mm Hg)	135±19/86±10	138±22/81±12**	130±19/85±12	129±20/80±14**
Total cholesterol (mmol/l)	4.93±0.98	3.86±1.21**	4.77±1.00	4.16±1.03**
LDL cholesterol (mmol/l)	2.84±0.73	2.10±0.98**	2.75±0.77	2.56±0.88*
HDL cholesterol (mmol/l)	1.37±0.46	1.08±0.48**	1.39±0.40	1.09±0.37**
Glycated aemoglobin (%)	6.31±1.65	7.21±2.43**	7.12±3.06	5.91±1.09**
% Carotid plaque	7.9	34.6**	4.2	42.1**
Average carotid IMT (mm)	0.711±0.138	0.808±0.185**	0.662±0.124	0.765±0.181**

Data shown are proportions, mean±SD. CLI, critical limb ischaemia; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; IMT, intima-media thickness. *$p<0.05$, **$p<0.0001$ vs controls.

Table 4.2. Characteristics of participants with critical limb ischemia with histological assessment of arterial tree of amputated limbs (n=54).

Age (years)	62.0±14.6
Sex (% male)	74.1
Body mass index (kg/m^2)	24.4±4.7*
% Hypertensive	57.4
% Diabetes mellitus	25.9
% Regular smoking	35.2
SBP/DBP (mm Hg)	127±21/77**±12*
Total cholesterol (mmol/l)	3.67±1.35
LDL cholesterol (mmol/l)	1.90±0.88
HDL cholesterol (mmol/l)	0.83±0.42**
Glycated aemoglobin (%)	7.26±2.69

Data shown are proportions, mean±SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein. *p<0.05; **p<0.005 vs. all critical limb ischemia.

4.4.2. Thrombotic versus atherosclerotic occlusion in CLI.

The proportion of the lumen occupied by atheromatous plaque at several arterial points below the level of the occlusion was markedly less in participants with thrombotic as compared to atherosclerotic or mixed occlusions (Table 4.3). As compared to thrombotic occlusions, atherosclerotic and mixed occlusions were strongly associated with age with those with thrombotic occlusions being younger (Table 4.3). As compared to those with thrombotic occlusions, the proportion of those with atherosclerotic and mixed occlusions with carotid plaque was greater (Table 4.3). However, age-adjusted carotid IMT values did not differ between those with thrombotic as compared to atherosclerotic and mixed occlusions (Table 4.3).

4.4.3. Plaque is not associated with CLI over a younger age range.

Whilst as compared to age- and sex-matched controls a high proportion of participants with stroke had carotid plaque even over a younger age range, participants with CLI only demonstrated a marked excess in carotid plaque over a much older age range (Figure 4.5, upper panel). Thus, carotid plaque was independently associated with stroke, but not CLI over a younger age range (Figure 4.6).

4.4.4. Carotid IMT independently associates with vascular events beyond plaque.

At several ages in those with stroke or CLI, IMT was independently associated with arterial events (Figure 4.7) and these relationships were also independent of carotid plaque (Figure 4.1, lower panel). However, in contrast, to the lack of independent relationship between carotid plaque and CLI over this age range (Figure 4.9, upper panel), beyond conventional risk factors, increases in IMT were most marked in younger participants with CLI (Figure 4.9, lower panel). Consequently, as compared to controls a higher prevalence of those with an increased IMT was noted in those with CLI (>95th percentile for age or >0.80 mm) (Figure 4.8), and an independent association between an increased IMT and CLI (Figures 4.6 and 4.9) was noted over a younger age range. Carotid IMT and plaque were not associated with stroke subtypes (TOAST classification) (Table 4.4).

Table 4.3. Characteristics of participants with critical limb ischemia with thrombotic versus mixed or atherosclerotic occlusion of the lower limb.

	Thrombotic (n=14)	Mixed or atherosclerotic (n=40)	p value
Age (years)	47.6±12.9	67.1±11.5	<0.0001
Carotid plaque (%)	21.4	57.5	=0.02
Age-adjusted IMT (mm)	0.846±0.039	0.845±0.021	=0.69
% Mixed	-	20	-
76-100% Occlusion (%)	92.9	87.5	=0.19
Level of occlusion			
Femoral (%, n=)	7.1 (1)	5.0 (2)	=0.76
Popliteal (%, n=)	50.0 (7)	45.0 (18)	=0.75
Posterior tibial (%, n=)	35.7 (5)	42.5 (17)	=0.66
Anterior tibial (%, n=)	7.1 (1)	7.5 (3)	=0.97
% Luminal atheroma in arterial tree below level of occlusion			
Popliteal (%)	10.0	57.7	=0.01
Posterior tibial (%)	28.6	64.9	=0.02
Anterior tibial (%)	20.0	50.0	=0.09
Fibular (%)	33.3	37.5	=0.85
Dorsalis pedis (%)	40.0	50.0	=0.69

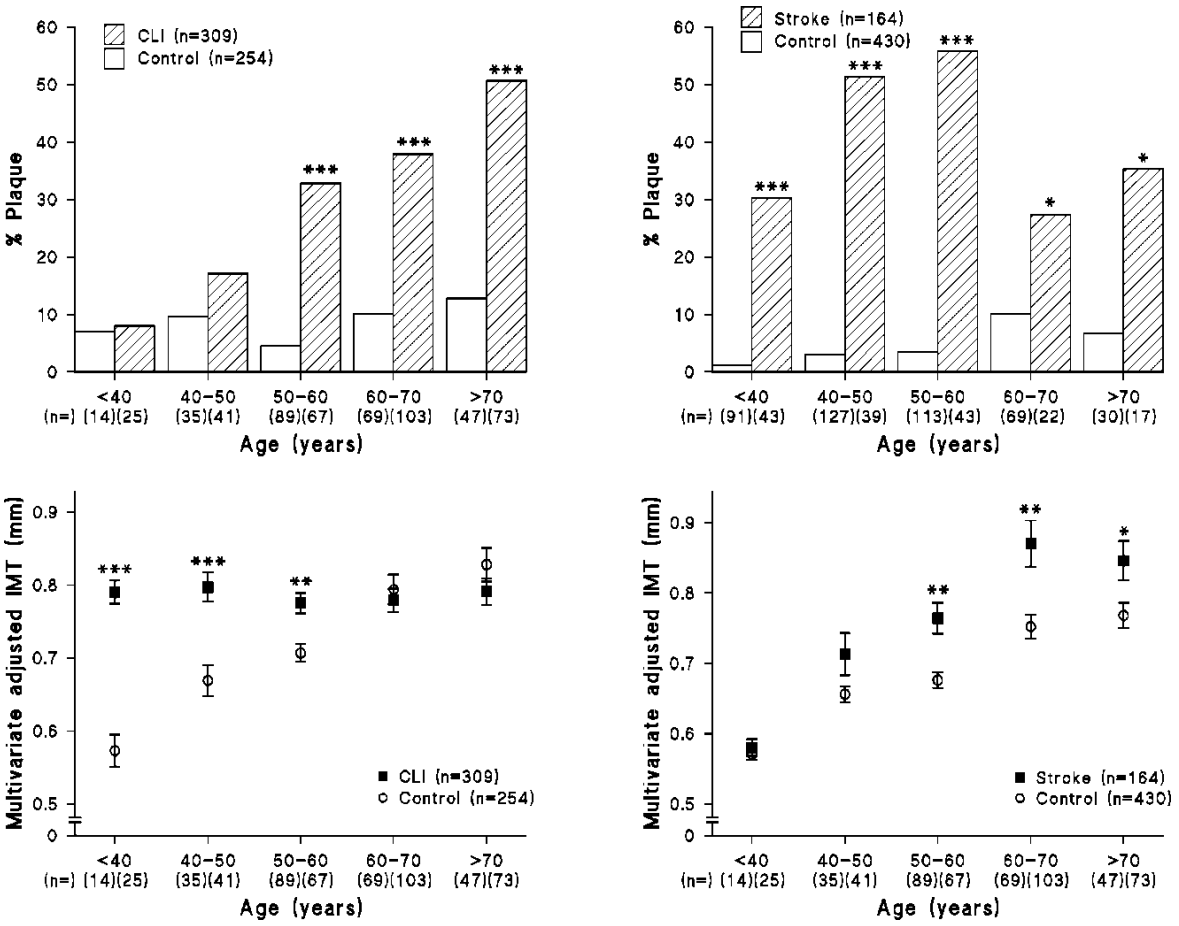


Figure 4.5. Prevalence of carotid plaque (upper panel) and multivariate-adjusted carotid intima-media thickness (IMT)(lower panel) values beyond plaque across the adult lifespan in cases with non-cardiac arterial vascular events (stroke and critical limb ischemia [CLI]) and age, sex-and ethnicity-matched randomly selected controls from a community sample in Africa. Data shown in lower panel are multivariate adjusted means and SEM. Adjustments are for carotid plaque, hypertension, diabetes mellitus, and smoking. *p<0.05, **p<0.005, ***p<0.0001 vs controls.

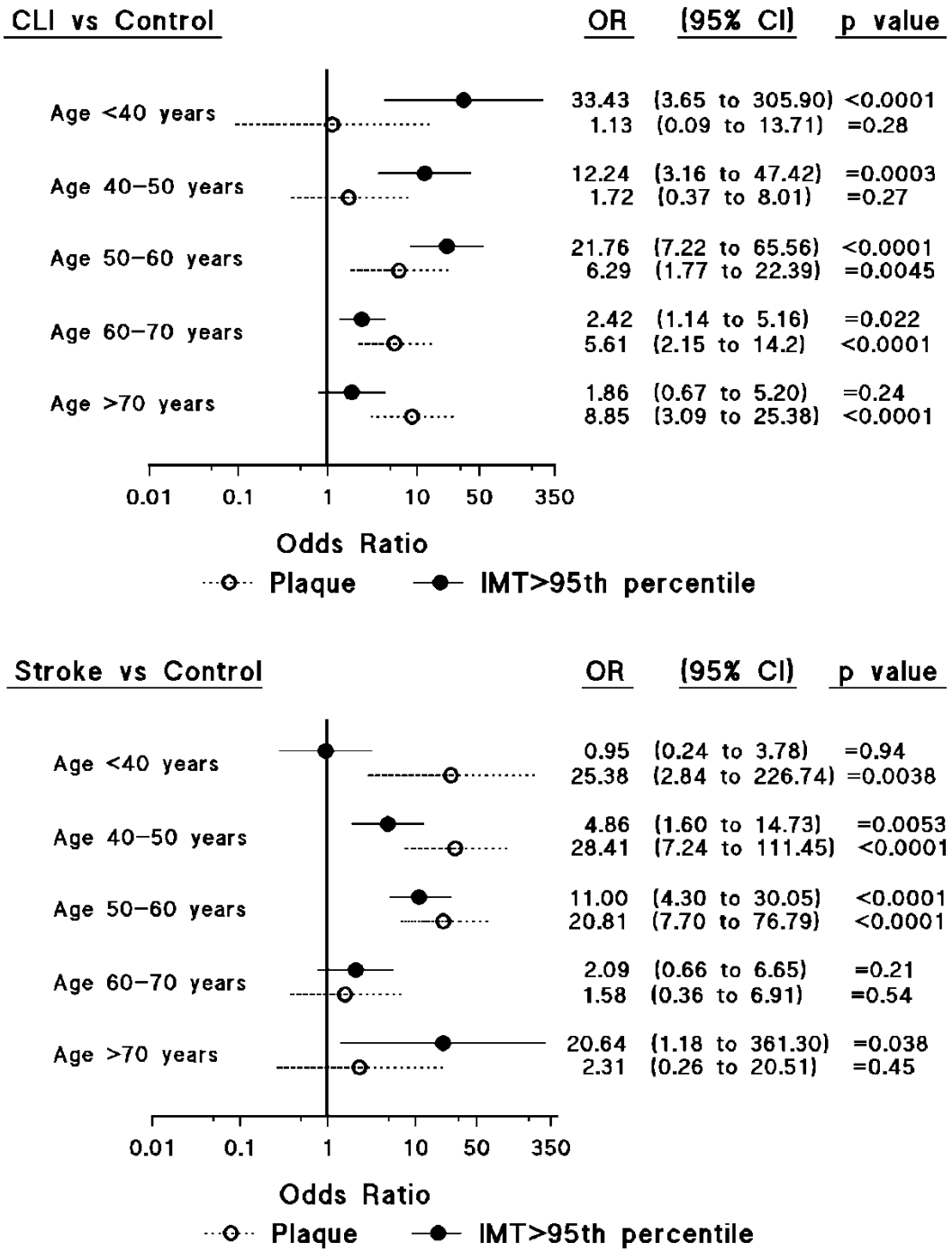


Figure 4.6. Multivariate adjusted odds (Odds ratio [OR] and 95 % confidence interval [CI]) of a non-cardiac arterial vascular event (stroke or critical limb ischemia) across the adult lifespan with the presence of carotid plaque (open circles) or an increased carotid intima-media thickness (IMT) (closed circles) in Africa. Adjustments are for hypertension, diabetes mellitus, and smoking. An increased IMT was defined as values above the 95th percentile for age in the community.

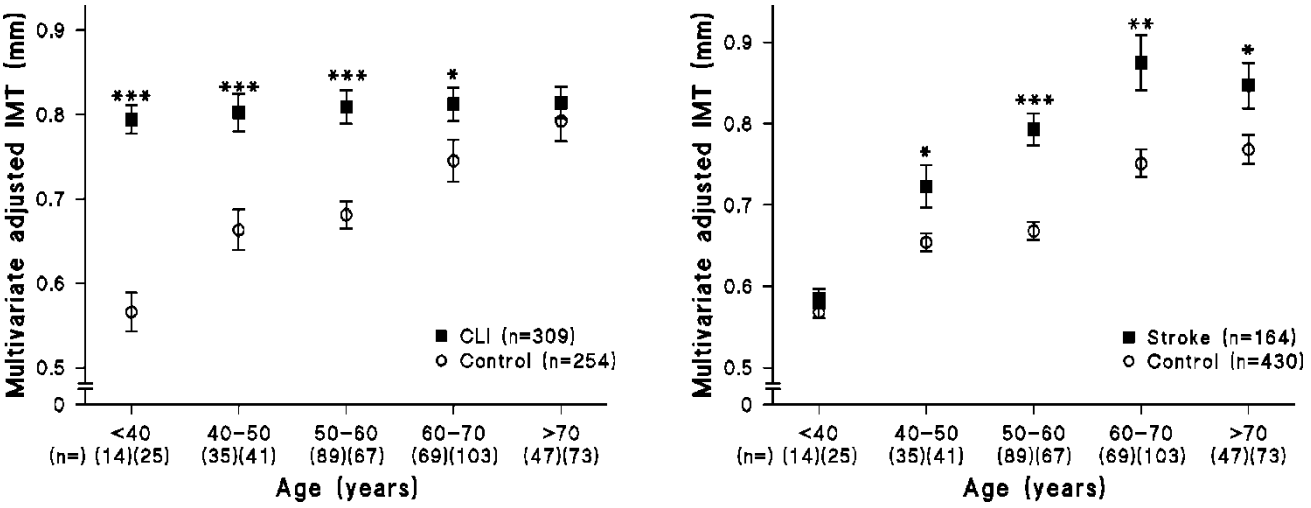


Figure 4.7. Multivariate-adjusted carotid intima-media thickness (IMT) across the adult lifespan in cases with non-cardiac arterial vascular events (stroke and critical limb ischemia [CLI]) and age, sex-and ethnicity-matched randomly selected controls from a community sample in Africa. Data shown are multivariate adjusted means and SEM. Adjustments are for sex, hypertension, diabetes mellitus, and smoking. *p<0.05, **p<0.005, ***p<0.0001 vs controls.

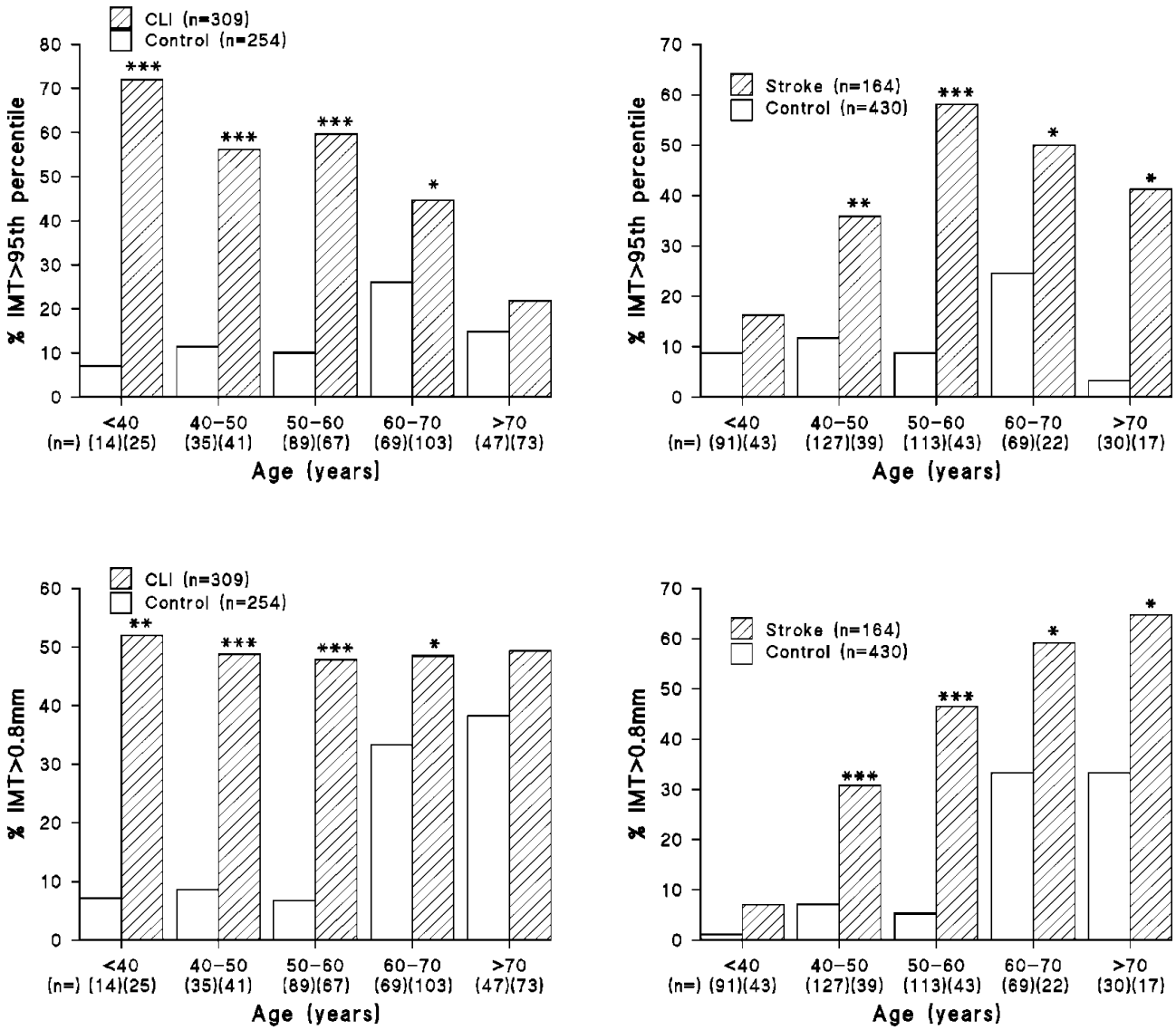


Figure 4.8. Prevalence of an increased carotid intima-media thickness (IMT) across the adult lifespan in cases with non-cardiac arterial vascular events (stroke and critical limb ischemia [CLI]) and age, sex-and ethnicity-matched randomly selected controls from a community sample in Africa. Upper panels show prevalence rates for thresholds >95 % confidence intervals for age and lower panels for thresholds >0.80 mm at any age. *p<0.05, **p<0.001, ***p<0.0001 vs controls.

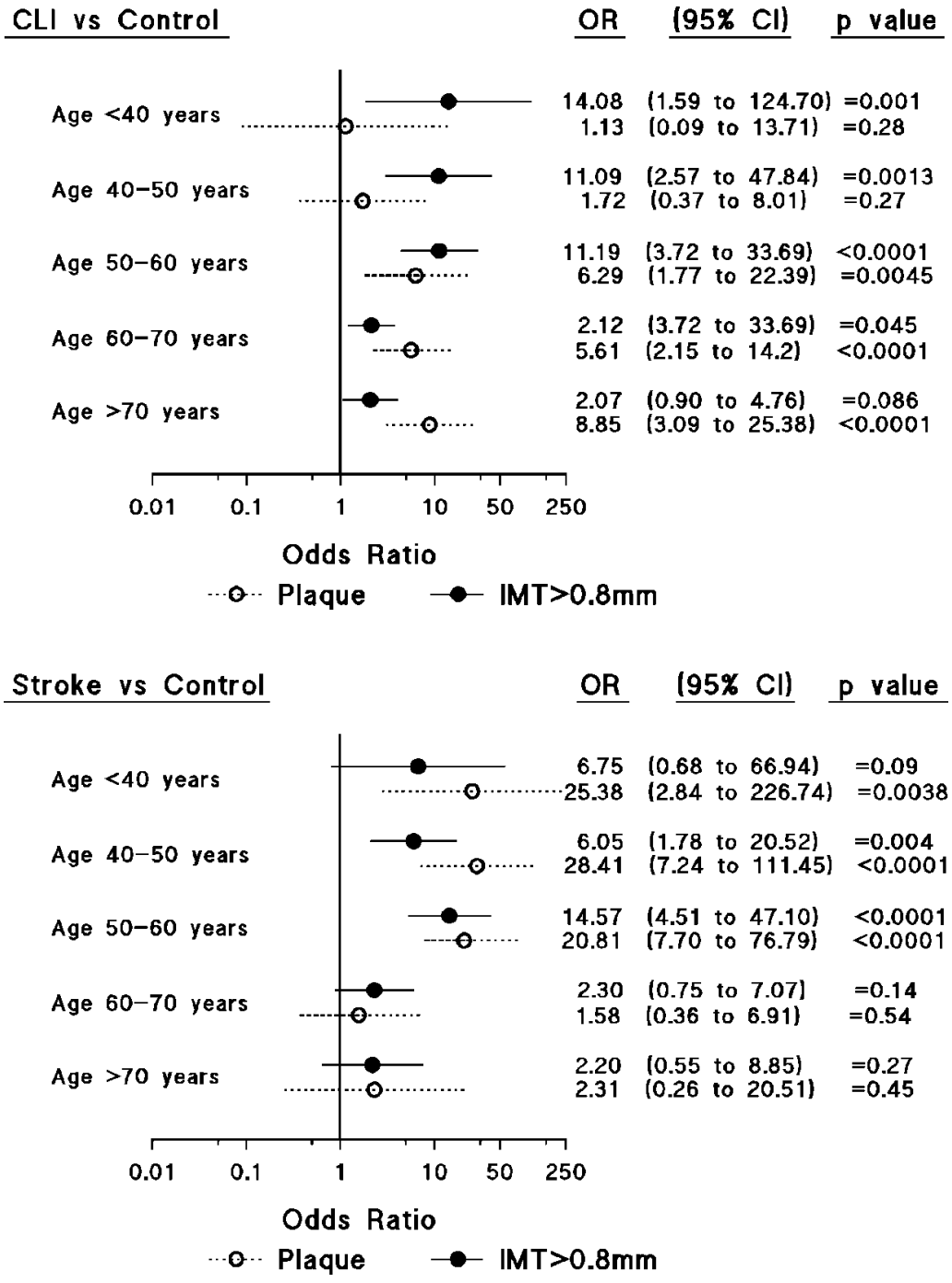


Figure 4.9. Multivariate adjusted odds (Odds ratio [OR] and 95 % confidence interval [CI]) of a non-cardiac arterial vascular event (stroke or critical limb ischemia) across the adult lifespan with the presence of carotid plaque (open circles) or an increased carotid intima-media thickness (IMT) (closed circles) in Africa. Adjustments are for hypertension, diabetes mellitus, and smoking. An increased IMT was defined as values above 0.80 mm.

Table 4.4. Age adjusted carotid intima-media thickness (IMT) and plaque in different stroke subtypes based on the TOAST classification.

	IMT (mm)	Plaque (%)
Hemorrhagic (n=18)	0.796±0.034	44.4
Atherosclerotic (n=9)	0.750±0.048	33.3
Small vessel (n=29)	0.773±0.027	55.2
Cardio-embolic (n=31)	0.750±0.026	48.4
Other determinants (n=13)	0.717±0.043	23.1
Indeterminate (n=64)	0.772±0.018	37.5

4.4.5. Carotid IMT complements the performance of carotid plaque for event detection.

Although IMT as a continuous trait showed either a stronger (younger age group) or similar (older age group) performance (AUC) for event detection as carotid plaque (Table 4.5.), when using either age-specific 95th percentiles as thresholds or thresholds of 0.8 mm, carotid plaque showed a similar performance for event detection as IMT (Table 4.5). However, considering either the presence of carotid plaque or an IMT>95th percentile or >0.80 mm as pathological, IMT improved the ability (performance {AUC] and sensitivity) to detect arterial events as compared to plaque or IMT alone in those <50 years of age (Figure 4.10, Table 4.5). In contrast, the addition of IMT to plaque failed to improve on the performance for arterial event detection in those older than 50 years of age (Figure 4.10, Table 4.5).

Table 4.5. Performance (area under the receiver operator characteristic curve [AUC]), sensitivity and specificity for the detection of arterial vascular events (stroke or critical limb ischemia) with carotid plaque and intima-media thickness (IMT) over different age ranges.

Event→	≤50 years of age			≥50 years of age		
	AUC±SEM	Sensitivity	Specificity	AUC±SEM	Sensitivity	Specificity
Carotid index						
Carotid index	0.631±0.026	28.4	97.8	0.664±0.017	71.2	91.6
IMT (continuous variable)	0.736±0.028	-	-	0.643±0.024	-	-
IMT>0.80 mm	0.642±0.020	32.4	96.0	0.621±0.021	49.9	74.3
IMT> 95th age percentile	0.658±0.023	41.9	89.6	0.646±0.019	44.6	84.7
Plaque or IMT>0.80 mm	0.711±0.022*	48.0	94.1	0.656±0.021	59.4	71.8
Plaque or IMT>95th age percentile	0.719±0.023†	55.4	88.3	0.680±0.020	55.4	80.7

All AUC values are significant at $p<0.0001$; *$p<0.0001$ versus plaque alone or IMT>0.80 mm alone; $^{\dagger}p<0.0001$ versus plaque alone or IMT>95th percentile alone.

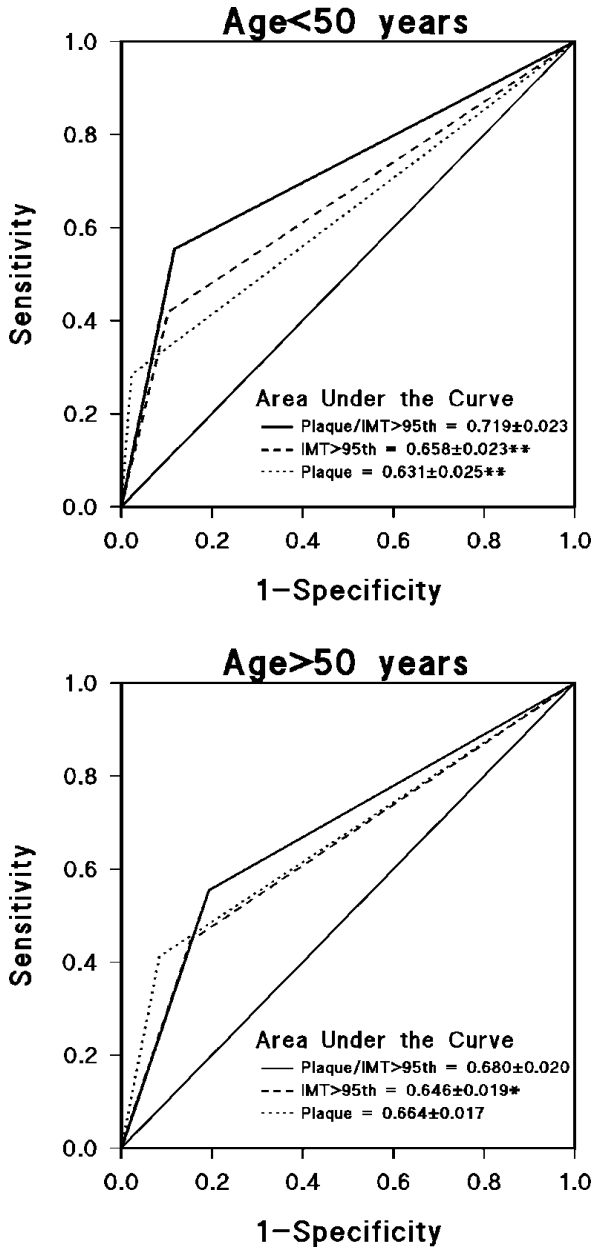


Figure 4.10. Impact of the addition of an increased carotid intima-media thickness (IMT) and carotid plaque to receiver operator characteristic (ROC) curves for the detection of critical limb ischemia or stroke at an age less than (upper panel) or more than (lower panel) 50 years of age . An increased IMT was defined as values above the 95th percentile for age in the community. Curves are for the presence of plaque or an increased IMT considered separately or for either the presence of plaque or an increased IMT considered together. *p<0.005; **p<0.0001 versus the presence of plaque or an increases IMT considered together.

4.5. Discussion

The main findings of the present study are as follows: In a large case-control analysis involving arterial events which frequently occur across the adult lifespan in some developing countries (stroke and CLI), we noted that carotid IMT complements plaque in independent associations with events over a younger age range (<50 years). In this regard, a high prevalence of histological thrombotic as opposed to atherosclerotic occlusion was noted over a younger age range in those with CLI and thrombotic occlusion was associated with less carotid plaque. Consequently, plaque was not independently associated with CLI over a younger age range, whilst over the same age range, IMT was strongly and independently associated with CLI. Thus, by enhancing the sensitivity for event detection, IMT above thresholds added to plaque in the performance (AUC) for event detection in those <50 years of age. In contrast, IMT added little to plaque in the performance for event detection over an older age range.

As carotid IMT indexes not only pathological intimal changes, but also compensatory medial hypertrophy or hyperplasia and fatty streaks that may regress with time (Insull, 2009; Touboul et al 2012; Wannarong et al 2013), the role of IMT as an index of risk-related atherosclerotic disease is uncertain. Although IMT may predict risk beyond plaque (Geeta et al 2015; Ren et al 2015; Barakoti, 2018), there is little understanding of why or when this may occur. One possibility is that IMT is a measure of earlier pathology than plaque and hence may be a more sensitive index for detecting risk. However, almost without exception all studies evaluating the role of carotid IMT and plaque in risk prediction have assessed the impact on events at a middle-age or in the elderly (Johnsen et al 2009; Inaba et al 2012; Spence et al 2012; Geeta et al 2015; Ren et al 2015; Yoon et al 2017; Barakoti et al 2018; Jeevarethinam et al 2018; Mitchell et al 2018; Sillesen et al 2018), with little consideration given to the fact that events at a younger age may reflect earlier atherosclerotic changes. To the best of our knowledge, the present study therefore, although cross-sectional in design, provides the first evidence, across the full adult age range, to explore the possibility that IMT rather than plaque may best index the development of arterial events when events reflect earlier atherosclerotic changes. Indeed, in patients with CLI, thrombotic occlusion was far more prevalent in those at a younger than older age, and those with thrombotic occlusion had less carotid plaque. Consequently, plaque failed to associate with CLI over the younger adult age range, whilst IMT showed strong independent associations with CLI in this age range. Thus, across the full adult age

range, both IMT and plaque provide complementary information in associations with events.

There are limited studies assessing changes in carotid IMT and the extent of carotid plaque in those with events that occur over a younger adult age range (chronologically premature vascular events). In this regard to the best of our knowledge only one study has been conducted in a large cohort of young stroke (Saeed et al 2014), and none in large cohorts of alternative arterial vascular events such as MI or CLI. Consistent with the high prevalence of carotid plaque noted in young stroke (Saeed et al 2014) we show that plaque is strongly and independently associated with stroke over a young age range. In contrast however, we also show that plaque is not independently associated with CLI over a young age range and that a dissociation between IMT and plaque in relationships with events is noted. Indeed, whilst plaque was strongly associated with stroke at a younger age, the strength of relations between IMT and stroke over this age range was less impressive. In contrast, whilst IMT was strongly associated with CLI at a younger age, plaque showed no independent relations with CLI over this age range.

The ability of IMT to complement plaque in CLI detection in the present study does not suggest that IMT is able to show these effects through pathological atherosclerotic changes in the intima. In this regard, IMT may still reflect compensatory medial changes or non-pathological fatty streaks (Insull, 2009; Touboul et al 2012; Wannarong et al 2013). These effects may nevertheless effectively index an accrual of the adverse impact (compensatory or pathological) of uncontrolled risk factors over time. In contrast however, the lack of association of carotid plaque with CLI over a younger age range, suggests that carotid plaque is inadequate for event detection over this age range and hence that alternative end-organ measures indexing less advanced atherosclerotic or other vascular effects are required to predict risk. Although the present study was not longitudinal in design, it does nevertheless show strong independent relations between IMT and CLI over a younger adult age range and hence may possibly provide such an early measure.

Importantly, stroke may reflect not only large artery atherosclerotic occlusion, but also small artery disease, cardio-embolic disease (Ohira et al 2011), or alternative causes where the pathophysiological mechanisms are not necessarily through atherosclerosis. Although it may be argued that carotid plaque is more likely to occur with atherosclerotic stroke, in keeping with previous studies (Saeed et al 2014), in the present study we failed to show similar relations. In the present study although carotid plaque failed to show strong independent relations with CLI over a younger age range, carotid plaque was strongly related to stroke over this age range. Consequently, in contrast to CLI, it is unlikely that

over the younger age range, stroke reflects earlier atherosclerotic changes than over the older age range.

Although in the present study carotid IMT was strongly associated with CLI over a younger age range, carotid IMT was not independently associated with CLI over an older age range. In addition, although carotid plaque was strongly associated with stroke over a younger age range, plaque was not independently associated with stroke over an older age range. In this regard, the lack of independent impact of IMT on CLI or plaque on stroke over an older age range could be accounted for by the limitations of the study design. In this regard, the incorporation of controls from the community sample with high IMT values or the presence of plaque and hence at a high risk of events would bias against showing independent relations with events. Nevertheless, IMT and plaque were independently associated with stroke and CLI respectively over an older age range, and hence it is possible that IMT and plaque have a limited role to play in risk prediction for CLI and stroke respectively over this age range. This may be related to a differential impact of risk factors on IMT and plaque at different ages, a hypothesis that requires further study.

In the present study, IMT considered as a continuous trait showed a stronger performance for the detection of events than plaque over a younger age range. Thus, the possibility that in developing nations at least, IMT may be better than plaque at detecting risk should be entertained. However, thresholds for IMT are necessary and in this regard, the performance of IMT using either age-specific thresholds above the 95^{th} percentile or a value of 0.80 mm showed a similar performance for events detection as compared to plaque. Thus IMT should not be employed for risk detection in preference to plaque. Rather these assessments should be employed to complement each other.

There are several limitations of the present study that require consideration. First, case-control analysis is limited by the use of non-random selection of control samples with a small sample size, resulting in population stratification. To limit population stratification, in the present study however we employed a large study sample and age-, sex- and ethnically-matched subjects from the same socio-economic background of a large, randomly selected community sample as control subjects. In this regard, the consistency of the case-control findings across several categories of age suggests a high level of reproducibility and hence a limited degree of population stratification. Second, the present study was not longitudinal and hence whether the presence of increases in IMT or plaque at the time of the event are also noted well before the development of events, is uncertain. However, atherosclerosis is a structural change that requires extensive time to develop and hence the presence of an increased IMT or plaque at the time of the event suggests that

these structural changes have been present for some time preceding the event. Importantly however, the lack of plaque at the time of the development of CLI is indicative of a lack of plaque preceding the event and hence there is no question that plaque is a poor end-organ measure for the prediction of CLI at a younger age. Third, to limit population stratification, and to ensure that a significant number of events were captured at a younger adult age, only one ethnic group was studied and MI was excluded as it is an infrequent occurrence at a younger age in African populations. Hence, further studies in different populations and with the inclusion of MI are required to evaluate whether the present findings are unique to developing populations and translatable to MI as well.

In conclusion, in the present large case-control analysis conducted across the adult age range in a population of African descent, we show that carotid IMT measurements complement carotid plaque is associations with non-cardiac, arterial vascular events. In this regard, we show that because over a younger age range CLI has a high prevalence of dominantly thrombotic, rather than atherosclerotic occlusion, where carotid plaque occurs less frequently, that CLI over a younger age range is not associated with the presence of carotid plaque. However, IMT is strongly associated with CLI over this age range and hence IMT adds to plaque to enhance the performance and sensitivity for event detection. These data suggest that both carotid plaque and IMT should be employed in risk detection in a complementary manner with neither one used in preference over the other.

CHAPTER 5

Contextual Narrative and Conclusion

5.1. Introduction

As highlighted in the introduction to the present thesis, Sub-Saharan Africa is presently facing a health-related epidemiological transition with a shift from infectious diseases and diseases of poverty to non-communicable diseases (Boutayeb and Boutayeb, 2005; Bradshaw et al 2010; Dhalal et al 2011; Kapiga, 2011). In this regard, cardiovascular disease is the most common non-communicable disease accounting for death in Africa. Whilst the burden of cardiovascular disease in developed countries is on the middle aged to elderly, as also highlighted in the introduction to the present thesis several types of arterial vascular events in Africa including stroke and the end stage of peripheral arterial disease, critical limb ischaemia (CLI), occur not only in the elderly, but also at a young adult age, ranging from late teens to middle age.

Events at a younger adult age have a devastating impact on disability adjusted life years. In this regard, if surviving these cardiovascular events, the individual loses the most productive years of their life, is often unable to reach their full potential in the work and family environment, often becomes a burden to families and friends, is frequently unable to support their families limiting the capacity to provide for or educate their children often driving families below the breadline, and cannot contribute to the economy of a country, often placing a burden on the healthcare system and hence on a countries economy. Identifying those at risk of events at an early adult age and preventing such events is therefore a primary goal in Africa.

There is no question that because of a perception that cardiovascular events are largely restricted to the elderly, risk prediction of events at a young adult age is limited by many factors. In this regard, because young adults seek medical care less frequently than older adults, and because comprehensive risk assessments are performed less often in younger adults even if they seek medical care, younger adults are seldom assessed for risk factors. Even if younger adults are assessed for risk factors, the presence of the risk factor is often perceived as being an early change with insufficient time to produce adverse effects. Because age is the strongest risk factor for cardiovascular disease the overall clinical risk score often assigned to those with risk factors at a young age is relatively low and there is then a risk that behavioural interventions will be employed well before drug therapy and maintained for far too long before drug therapy is instituted or doses of drugs are increased. There is therefore no question that more effective approaches to risk prediction and thus prevention in the young in Africa are required. In this regard, a well recognised approach to enhancing risk prediction is through the assessment of preclinical

end-organ changes. There has nevertheless always been significant argument as to the cost-effectiveness of the measurement of end organ changes in developing countries. Indeed, few countries in sub-Saharan Africa recommend comprehensive end-organ assessments as part of risk prediction at any age. Some South African guidelines suggest only limited routine end organ assessments with the determination of electrocardiographic (ECG) left ventricular hypertrophy and estimated glomerular filtration rate (eGFR) being the only assessments that are recommended (Seedat and Rayner, 2012). In the present thesis, I explored the possibility that eGFR is indeed useful for enhancing the ability to detect an arterial vascular event at a young age in Africa. I compared this with the ability of a well-recognised vascular marker for predicting events and that is carotid intima media thickness (IMT). I further identified the key factors that account for variations in IMT with a focus on the role of the most important risk factor in Africa and that is hypertension and subsequently whether IMT is indeed useful beyond more accepted measures of atherosclerosis (plaque) for risk prediction. In chapters 2 - 4 of the present thesis I have described and discussed my findings in detail. In the present chapter, I will nonetheless discuss the importance of these findings from a more general context.

5.2. The role of risk prediction of premature events using end organ measures with little additional cost involved.

Unlike economically developed countries, in low and middle-income countries considerable thought must be given to providing healthcare with maximal benefits at minimal cost. Although the detection of electrocardiographic (ECG) left ventricular hypertrophy (LVH) and the assessment of eGFR cost little and are generally considered to be part of routine clinical assessment, particularly in those with hypertension, whether they are effective at risk prediction across the full adult age range, including over a younger adult age range is unknown. In this regard, although ECG LVH was not explored as a possible risk factor over a young adult age range in the present thesis, previous studies from our group have demonstrated a limited ability of either Cornell or Sokolow-Lyon criteria to identify LVH in communities of African descent in South Africa (Maunganidze et al 2013). Indeed, our group have demonstrated that obesity markedly limits the performance or sensitivity for ECG LVH detection largely because of a marked effect of obesity on ECG voltages (Maunganidze et al 2013). Although our group have subsequently demonstrated that a combination of standard risk factors together with ECG voltages (Maunganidze et al 2013) or an approach which accounts for the impact of obesity on ECG voltages (Robinson

et al 2016) can improve on the performance and sensitivity of ECG criteria for LVH detection, data from our group suggest that even echocardiographic LVH is not associated with critical limb ischaemia (Brand et al 2012) or stroke (Naran et al, unpublished data) in South Africa. As these are the two arterial vascular events that occur at a chronologically premature age in South Africa, although further work is required to test this hypothesis, it is unlikely that even echocardiographic LVH will be a useful risk predictor for premature events in South Africa. Although there are several ECG changes that have been suggested as being able to predict risk beyond LVH (strain patterns, Q-T interval, etc), whether they are useful in predicting specifically arterial vascular events other than myocardial infarction and whether risk prediction is as effective using these measures at a younger as opposed to older adult age range is unknown. Hence, pending further studies to address these questions, there is nevertheless little chance that ECG criteria will be of value in predicting chronologically premature stroke or CLI. Although ECG evaluation is critical for identifying arrhythmias, and hence should form part of the initial assessment of young adults with risk factors, the utility of ECG LVH criteria in detecting those at risk for an event has to be questioned. What is the value of eGFR in risk assessment in young adults?

In the present thesis I explored the ability of eGFR to associate with arterial vascular events across the adult lifespan in a group of African ancestry (Kolkenbeck-Ruh et al, 2019). In this regard, the role of eGFR in risk prediction in groups of African ancestry had previously been questioned. Indeed, the relationship between creatinine and GFR varies between ethnicities. Hence neither the MDRD, nor the CKD-EPI equations for estimating GFR perform as well in African as they do in Caucasian populations (Earley et al 2012; Lamb and Stevens, 2014), and the use of an ethnicity coefficient does not improve on performance in some African populations (Eastwood et al 2010; Stevens et al 2011; Van Deventer et al 2011). Despite these concerns, previous studies from our group conducted in black South Africans demonstrated that eGFR derived from the CKD-EPI and MDRD equations were well associated with several alternative target organ measures, with more consistent associations noted for CKD-EPI-derived eGFR (Booysen et al 2016). Hence, at that time (Booysen et al 2016) our group had provided evidence to indicate that estimates of glomerular dysfunction and chronic kidney disease (CKD) may indeed be independently associated with cardiovascular events across the adult lifespan in Africa. However, at that time (Booysen et al 2016) relationships between eGFR or CKD and arterial vascular events across the adult lifespan had not been studied. In the present thesis I therefore performed such an evaluation. What were the findings and their clinical implication?

As described in chapter 2 and published in the *Journal of Hypertension* (Kolkenbeck-Ruh et al, 2019), I demonstrated in a large case-control study conducted in a group of African ancestry, that despite carotid IMT being uniformly greater across the adult lifespan in those with non-cardiac arterial vascular events (stroke and CLI) as compared to controls, creatinine-based indexes of glomerular function or after adjustments the presence of CKD based on these estimates were no different between those with and without events. Few cases (16.3 %), particularly those with premature events (8 %), had CKD and assuming that similar eGFR values existed prior to as at the time of the event, eGFR showed a low sensitivity and poor performance for event detection. These data therefore provided important evidence that the use of eGFR is markedly insensitive for the detection of risk for an event. Thus, the utility of these measures for enhancing risk prediction of those with an event at a younger age is highly limited. In this regard, too few patients experiencing non-cardiac arterial vascular events will be identified as being at risk prior to the event, to place a high value on employing CKD identification as a sensitive marker for risk prediction. Thus, in the majority of patients with non-cardiac-related arterial vascular events in South Africa and hence possibly other African regions, the event precedes the development of CKD. Hence, generalised screening for CKD using creatinine-based estimations of GFR are unlikely to be of use in predicting the majority of non-cardiac-related arterial vascular events in South African and possibly other African populations. These findings oppose the proposal by hypertension guidelines in middle-income countries in Africa (Seedat and Rayner, 2012). In this regard, because of the low cost of eGFR assessments, these guidelines assign a greater priority to eGFR than more costly ultrasound-based assessments, such as carotid IMT, in detecting end-organ changes.

It is important not to discount the utility of eGFR assessments for risk prediction of events other than stroke and CLI. As highlighted in chapter 2, eGFR and CKD may mainly predict cardiac rather than cerebral or peripheral vascular events. Cardiac events were not evaluated in the present study as they do not occur across the adult lifespan in groups of African ancestry in South Africa. In this regard, 50 % of events predicted by CKD are coronary events and a substantial portion of events predicted by CKD are caused by heart failure (Sud and Naimark, 2016). A dominant effect of CKD on cardiac as opposed to alternative cardiovascular events may occur through the cardio-renal syndrome. Indeed, eGFR is strongly and independently associated with left ventricular mass beyond all haemodynamic factors in groups of African ancestry in Africa (Maunganidze et al 2013). Whilst eGFR associates with left ventricular mass beyond all risk factors in African populations (Maunganidze et al 2013), the relationship between eGFR and carotid IMT is

not independent of age (Booysen et al 2016). Thus, the adverse effects of CKD in Africa may be specific to cardiac rather than peripheral or cerebral vascular arterial events. In addition, given the high prevalence of risk factors (hypertension and diabetes mellitus)-related end stage renal failure in Africa, there is still an important need to assess eGFR when risk predicting in Africa. The results of the present thesis should therefore be seen as highlighting a need for additional risk evaluations beyond eGFR rather than evidence to suggest that eGFR should not be assessed in young patients at risk of premature events.

5.3. Carotid IMT may be a useful index of risk for premature events, but what is carotid IMT an index of?

As low cost measurements for predicting risk beyond conventional risk factors are likely to be ineffective at predicting non-cardiac arterial vascular events in Africa, consideration must be given to alternative measures that may enhance risk prediction. There are several additional measures of end organ changes that have been developed including the assessment of carotid IMT, the detection of the presence of plaque in large arteries, the evaluation of calcification in coronary vessels, measures of large artery stiffness, and more sensitive indexes of renal dysfunction including urinary micro-albumin evaluations, all of which have been demonstrated to add to risk prediction (Stein et al 2008; Matsushita et al 2010; White et al 2010; Chang and Kramer, 2011; Mahmoodi et al 2012; Matsushita et al 2012; Wang et al 2014; Matsushita et al 2015; Parsh et al 2015; Sud and Naimark, 2016;. Several of these indexes are too expensive for routine assessment in low to middle income countries including coronary calcification scores which require technology that is too expensive to purchase and maintain, or the measure of urinary micro-albumin concentrations which is not just device driven but carries a significant cost per assay. The fact that the common chronologically premature events in sub-Saharan Africa are vascular in origin suggests that vascular indexes are most likely to effectively predict such events. In this regard, as described in chapter 2 and published in the *Journal of Hypertension* (Kolkenbeck-Ruh et al 2019), I showed that carotid IMT indeed independently and consistently associates with non-cardiac arterial vascular events over a younger adult age range. As carotid IMT is presently an easy to acquire and reliable measure using semi-automated software, and the cost of portable devices with high resolution software has considerably decreased over the past decade, the possibility that young individuals with risk factors developing at an early age could be screened using IMT to complement risk prediction and subsequently managed, should be given due consideration. There are

nevertheless several considerations that must be addressed before advocating such an approach.

One important consideration is whether carotid IMT does indeed index an accrual of the long term adverse effects of the most important risk factors responsible for premature arterial vascular events. In this regard an ongoing debate is whether end organ damage in hypertension, the risk factor that carries the most population attributable risk for cardiovascular events in Africa, is caused mostly by the adverse effects of steady-state pressures or pulsatile pressures and if pulsatile pressure is important, which determinant of pulsatile pressure is responsible for these effects. With respect to pulsatile pressure effects, the key ongoing question is whether these effects are driven by forward wave or backward wave pressure components and their determinants. As arteriosclerotic changes drive forward wave pressures and arteriosclerosis is a key pathophysiological alteration demonstrated to predict largely vascular events (CHD and stroke) (Ben-Shlomo et al 2014; Vlachopoulos[a] et al 2010), it is essential that the end organ measure employed to predict events in the young reflect any arteriosclerotic alterations that contribute to the events. Thus, in the present thesis I explored whether in African populations, vascular damage, as indexed by carotid IMT is determined by steady-state or pulsatile pressures and if pulsatile pressures are involved which pulsatile pressure waves are important? What were the findings and implications thereof.

In chapter 3 of the present thesis and published in the *American Journal of Hypertension* (Kolkenbeck-Ruh et al 2019) I showed that in a large community sample of African ancestry living in South Africa, that whilst backward wave pressures are primary determinants of left ventricular mass and arterial stiffness, that mean arterial pressures and the forward pressure wave component of pulse pressure are independent determinants of carotid IMT. Importantly, in addition to demonstrating that both steady-state and pulsatile components of blood pressure are independently associated with carotid IMT, I also showed that beyond age, alternative risk factors, including smoking, diabetes mellitus, and circulating concentrations of lipids were not independently associated with IMT beyond age (Chapter 3 and Kolkenbeck-Ruh et al 2019). Consequently, the results of the present thesis provide strong evidence that IMT does indeed index the adverse effects of the principle risk factor for cardiovascular disease in Africa (hypertension), that this effect is noted across the adult lifespan, but that IMT is not an adequate index of alternative risk factors including diabetes mellitus, smoking or dyslipidaemia. These findings therefore not only provide more clarity on what aspects of blood pressure are important in mediating arterial vascular changes, but also provide further evidence to support the view extensively

discussed in the introductory chapter that IMT may not be an appropriate index of atherosclerotic effects, and hence an adequate index of the adverse vascular effects of the major risk factors smoking, diabetes mellitus or dyslipidaemia. In the following section I will therefore first address the issue of those aspects of blood pressure that could account for premature vascular changes and hence premature vascular events, Consequently, the issue of whether alternative indexes of atherosclerotic changes other than IMT are better at predicting premature events will be discussed.

5.4. Which aspects of blood pressure account for IMT changes and hence possibly events in young adulthood?

As indicated in the aforementioned discussion, carotid IMT was strongly associated with mean arterial and not pulsatile pressure in early adulthood (up until 50 years of age) (chapter 3, Kolkenbeck-Ruh et al 2019). In contrast, forward wave pressures were independently associated with IMT after 50 years of age when these pressures normally begin to increase in most persons (chapter 3, Kolkenbeck-Ruh et al 2019). As IMT is independently associated with arterial events across the adult lifespan (chapter 2 and Kolkenbeck-Ruh et al 2019), these data suggest that one need only consider managing steady-state pressures to prevent premature arterial events in Africa and that little attention needs to be given to the pulsatile pressure component of BP to prevent premature events. This has important implications as current antihypertensive therapy is indeed designed to decrease steady state pressures and not the determinants of the pulsatile components of blood pressure that are independent of steady-state pressures. Indeed, as highlighted in the introductory chapter, antihypertensive therapy has never been demonstrated to decrease the determinants of forward wave pressures beyond steady-state effects. Thus, at first glance, there appears to be a ready solution to addressing IMT changes in early adulthood and thus possibly events in the early years of life. However, further work by our group (Motau et al, unpublished) which still requires wave separation analysis using actual aortic flow waves to confirm the findings and calculations of characteristic impedance to support the findings suggest that the premature arterial events described in the present thesis are accounted for by increases in forward wave pressures without increases in pulse pressure. Although further work is required to assess whether these changes are accounted for by alterations to the impedance to flow beyond steady-state pressures and whether these effects are attributed to arteriosclerotic aortic stiffness or diameter changes is still underway, it is possible that conventional blood pressure measurements, which do not assess forward wave

pressures or aortic stiffness or diameter are insufficient to account for IMT changes and hence possibly arterial vascular events at a chronologically premature age. Therefore, carotid IMT may not only act as an index of the accrual of the adverse effects of blood pressure, but may also index the adverse effects of increases in aortic stiffness and impedance beyond blood pressure effects. In this regard, increases in aortic stiffness may produce adverse effects on vascular structure through a direct impact on endothelial function (Wang et al 2010; Chirinos et al 2012; Weber et al 2012; BenShlomo et al 2014; Zamaniet al 2014; Booysen et al 2015; Cooper et al 2015; Sibiya et al 2015; Zamani et al 2015) and a reduction in the normal impedance mismatch that exists between the aorta and more distal vessels, a mismatch that normally reduces load on distal vessels (Agabiti-Rosei et al 2007; Vlachopoulos[a] et al 2010; Vlachopoulos[b] et al 2010).

5.5 Does carotid IMT index premature arterial vascular events beyond more effective indexes of atherosclerosis?

To recapitulate, simple and cheap measures of end organ changes currently recommended by guidelines for risk prediction in Africa are either insensitive (eGFR) or likely inaccurate (ECG LVH) in detecting those at risk for the major chronologically premature arterial vascular events that occur in Africa. However, carotid IMT is strongly and independently associated with these events over the full adult age range. In this regard, IMT not only has an advantage in that it strongly indexes the adverse effects of the principle risk factor for cardiovascular disease in Africa, namely hypertension, but possibly also the adverse effects of arteriosclerotic changes beyond blood pressure. However, as indicated in the aforementioned discussion IMT is a poor index of the adverse effects of alternative risk factors including diabetes mellitus, smoking and dyslipidaemia. Hence, consideration should be given to whether alternative end organ measures, which more effectively index the adverse effects of all risk factors on atherosclerotic vascular changes, and hence are better risk predictors over the young adult age range than IMT. In this regard, the presence of carotid plaque provides a better ability to index atherosclerotic changes than IMT. Nevertheless, whether the presence of carotid plaque, which is an index of more advanced atherosclerosis than IMT is sufficiently sensitive to detect events at a younger age is unknown. Thus, in the present thesis I also hypothesized that because at a younger age, occlusive arterial events are often more thrombotic than atherosclerotic in nature that carotid plaque may not always be a better index than IMT of premature events. What was I able to show and what are the implications of these findings?

In chapter 4 of the present thesis, and accepted for publication in the journal *Angiology* (Kolkenbeck-Ruh et al 2019), using histological assessments of the large arteries of amputated limbs from patients with critical limb ischaemia, I explored whether critical limb ischaemia may occur over a younger age range in association with less advanced atherosclerotic changes. In this regard, I demonstrated that a significant proportion of those with critical limb ischaemia at a young age develop dominantly pathological thrombotic as opposed to atherosclerotic occlusion. In contrast, over an older age range, the arterial occlusion is dominantly atherosclerotic in nature. Importantly, those with pathological thrombotic occlusion had significantly less carotid plaque. Thus, in a large case-control analysis, over the young adult age range, whilst carotid plaque was independently associated with stroke, it was not independently associated with critical limb ischaemia. Carotid plaque only independently associated with critical limb ischaemia over an older adult age. As a consequence, when considering stroke and critical limb ischaemia together, over the younger adult age range either an increased IMT or the presence of carotid plaque (either/or) showed a greater sensitivity and performance for event detection than an increased IMT or the presence of plaque considered separately. These findings suggest that IMT measurements are indeed a necessary vascular measurement to enhance risk prediction over a younger adult age range, but that these should be performed together with plaque assessments.

The study described in chapter 4 although focussed on evaluating the relative role of carotid plaque versus IMT in risk evaluation across a younger adult age range provides additional important insights. This study provides one explanation as to why although carotid plaque surpasses IMT in risk evaluation that it does not replace IMT. Indeed, IMT provides a more sensitive index when the extent of atherosclerosis responsible for the event at hand is in the earlier phase of development and thrombotic changes are largely responsible for the occlusion. This also raises the question as to why in some patients thrombosis is so extensive causing an early event. Nevertheless, this was not a goal of the studies in the present thesis, and hence much more work is required to evaluate this question with the possibility that many of these patients having underlying thrombophilia, being considered.

5.6. Clinical Applications

As highlighted in the previous section and in the introduction to the present thesis, the burden of stroke and critical limb ischaemia in South Africa is not only on the elderly,

but also on those at a young adult age, ranging from late teens to middle age. Identifying individuals likely to have these events at a young adult age and intervening to prevent chronologically premature events is therefore an important consideration. In this regard, at present approaches adapted from the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) guidelines for the identification and management of hypertension (Mancia et al 2013) are primarily used for assessing and managing cardiovascular risk in South Africa (Seedat et al 2014). Importantly, current guidelines recommend that once the diagnosis of hypertension is established, patients with blood pressure ≥ 160/100 mmHg should commence drug therapy in conjunction with lifestyle modification, while patients with stage 1 hypertension (the majority of those with events at a young adult age) should receive lifestyle modification (Seedat et al 2014). However, drug therapy should be implemented irrespective of hypertension status if they are stratified as high risk according to the following criteria: three or more major risk factors, the presence of diabetes mellitus, evidence of target-organ damage or the development of a complication of hypertension (Seedat et al 2014). As indicated in several sections throughout the present thesis at a young adult age those who develop events seldom have more than two major risk factors and although a substantial number have diabetes mellitus, the majority do not. Thus, at a young adult age there is a high degree of reliance on identifying end-organ changes to stratify as high risk. As in-part demonstrated in the prese thesis (for eGFR), whilst low cost end organ assessments (eGFR and ECG criteria for LVH) provide little useful information to predict the risk of stroke or CLI at a young adult age, the presence of normal values is misleading when risk predicting and may lead to relatively low risk scores being assigned and drug therapy being withheld for inappropriate periods. In contrast, as also demonstrated in the present thesis, carotid IMT when used in conjunction with an assessment of the presence of carotid plaque provides a potentially sensitive end organ measure that may herald the development of either stroke or CLI at a young adult age. The presence of increases in carotid IMT or plaque may therefore result in more timely use of blood pressure lowering or lipid-lowering therapy or aspirin and associated agents (Klug et al 2012, Seedat et al 2014, Zanchetti et al 202) and more careful follow-up of those who develop risk factors at an early age. Nevertheless, in the present health-care systems in Africa whether there is the capacity to provide services for the assessment of carotid IMT or plaque in those who develop risk factors at a young adult age is uncertain.

5.7. Limitations

Throughout the present thesis I have highlighted the potential limitations of the studies conducted. However, there are some limitations that are worthy of further emphasis. First, the studies conducted in chapters 2 - 4, were not longitudinal in design. Therefore, the apparent age dependence of end organ changes on mean arterial pressure, forward and backward wave pressures (chapter 3), chronic kidney disease (chapter 2) and carotid intima-media thickness and plaque (chapter 4), require confirmation in longitudinal analysis that would require follow-up of participants and patients across most of their adult lifespan. Second, in chapters 2 and 4, case-control analysis was necessary as longitudinal studies to identify premature vascular events are not feasible. Hence, there is a possibility that false positive or negative findings may have occurred. However, this mainly occurs when controls show population stratification which is limited by the use of large study samples and an ability to show consistent findings. To limit population stratification, I employed a large study sample and age-, sex-, and ethnically matched subjects from the same socio-economic background of a large randomly selected Johannesburg community sample as control subjects. In this regard, the consistency of the case-control findings (chapters 2 and 4) across several categories of age suggests a high level of reproducibility and hence a limited degree of population stratification. Third, I was unable to perform histology on arteries of patients with stroke. However, carotid plaque was associated with stroke across most of the adult age range. Fourth, I only reported on the presence of plaque and did not evaluate plaque quantity (total plaque area mm^2) or quality (greyscale analysis) as described by Mitchell et al (2018). Nevertheless, few with critical limb ischemia at a young age had plaque and almost no control subjects over this age range had plaque. Fifth, I did not attempt to identify the presence of plaque in the femoral artery using ultrasound approaches. In this regard, in the absence of carotid plaque at a young adult age in those with CLI, femoral plaque may have been more extensive. However, histological thrombotic occlusions in the lower limb are by definition associated with a less extensive degree of atheroma than atherosclerotic occlusions and hence the presence of even femoral artery plaque may have been difficult to detect. Nevertheless, future studies should assess the relationships between femoral plaque and critical limb ischaemia, particularly in younger individuals. Sixth, in the present thesis I did not perform cost-effective analysis as many of the variables (such as the cost to perform the assessments in various countries) could not be estimated to perform this analysis, and hence that future studies require this analysis once these variables are determined. Seventh, the studies described in the present thesis were

conducted on one ethic group at a single site within South Africa, Johannesburg. Although black Africans living in Johannesburg are largely representative of most chiefdoms in South Africa, future studies in different ethnic groups and at different sites are warranted to explore whether the presence of chronic kidney disease and carotid plaque and or carotid intima-media thickness are sensitive indices of chronologically premature non-cardiac arterial events (stroke and critical limb ischaemia).

5.8. Conclusions

In conclusion, in the present thesis I provide evidence to suggest possible approaches to enhancing risk prediction for arterial events over a young adult age range (20-50 years of age) in groups of black African ancestry. In this regard, I show that whilst eGFR is too insensitive to predict risk for an early event, IMT, which is determined by the most important risk factor for events at this age (hypertension and associated arteriosclerotic pulsatile load), is strongly associated with events at a young adult age. I also show that because of the high prevalence of thrombotic occlusion at a younger age in those with lower limb occlusion where less extensive carotid atheroma is noted, IMT complements the assessment of carotid plaque to enhance risk prediction over this age range.

APPENDICIES

Appendix A

Ethical clearance certificates

R14/49 Miss Andrea Kolkenbeck-Ruh et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160411

NAME: (Principal Investigator)	Miss Andrea Kolkenbeck-Ruh et al
DEPARTMENT:	Surgery Charlotte Maxeke Johannesburg Academic Hospital Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Vascular Pathology in Human Immunodeficiency Virus (HIV)-Infected versus Non HIV-Infected Patients Presenting with Critical Lower Limb Ischaemia
DATE CONSIDERED:	06/05/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Gavin Norton and Prof Angela Woodiwiss
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/10/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated,from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review in April and will therefore be due in the month April each year.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Prof Angela Woodiwiss and Prof Gavin Norton

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170401

NAME: (Principal Investigator)	Prof Angela Woodiwiss and Prof Gavin Norton
DEPARTMENT:	School of Physiology
PROJECT TITLE:	Gene Candidates as Determinants of Blood Pressure and Intermediary Phenotypes in the Pathogenesis of Hypertension in Black South Africans
DATE CONSIDERED:	26/04/2002 (Initial Approval)
DECISION:	Approved unconditionally
CONDITIONS:	Renewal for 5 Years Valid for the Period 01 April 2017 - 30 April 2022 Previously M1204108, M070469 and M020472
SUPERVISOR:	Prof Angela Woodiwiss
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/04/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in April and will therefore be due in the month of April each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Dr Eitzaz Sadiq and Dr Harold Majane et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140429

NAME: (Principal Investigator) Dr Eitzaz Sadiq and Dr Harold Majane et al

DEPARTMENT: Neurosciences
Charlotte Maxeke Johannesburg Academic Hospital

PROJECT TITLE: Risk Predictors for Stroke in Human Immundeficiency Virus Infected South Africans

DATE CONSIDERED: 25/04/2014

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR:

APPROVED BY: _____
Professor P Cleaton-Jones, Co-Chairperson, HREC (Medical)

DATE OF APPROVAL: 06/06/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

_____ _____
Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B

Turn-it-in report

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ORIGINALITY REPORT

12%	2%	10%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	PanVascular Medicine, 2015. Publication	4%
2	Andrea Kolkenbeck-Ruh, Tshegofatso H Motau, Ravi Naran, Carlos D Libhaber, Pinhas Sareli, Gavin R Norton, Angela J Woodiwiss. "Organ-Specific, Age-Dependent Associations of Steady-State Pressures and Pulsatile Pressure Wave Components with End-Organ Measures", American Journal of Hypertension, 2018 Publication	2%
3	Christopher J McAloon, Luke M Boylan, Thomas Hamborg, Nigel Stallard, Faizel Osman, Phang B Lim, Sajad A Hayat. "The changing face of cardiovascular disease 2000–2012: An analysis of the world health organisation global health estimates data", International Journal of Cardiology, 2016 Publication	2%
4	Nitheesha Ganta, Shahabuddin Soherwadi,	1%

	Kakra Hughes, Richard F. Gillum. "Burden of Peripheral Artery Disease in Sub-Saharan Africa and the Caribbean 1990 to 2015", Vascular and Endovascular Surgery, 2018 Publication	
5	Anahita Dua, Cheong J. Lee. "Epidemiology of Peripheral Arterial Disease and Critical Limb Ischemia", Techniques in Vascular and Interventional Radiology, 2016 Publication	1%
6	clinicalgate.com Internet Source	1%
7	www.uct.ac.za Internet Source	1%
8	www.nature.com Internet Source	1%
9	F. Gerry R. Fowkes, Victor Aboyans, Freya J. I. Fowkes, Mary M. McDermott, Uchechukwu K. A. Sampson, Michael H. Criqui. "Peripheral artery disease: epidemiology and global perspectives", Nature Reviews Cardiology, 2016 Publication	1%

Exclude quotes	On	Exclude matches	< 1%

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