Arteriosclerotic Aortic Dysfunction in Groups of African Ancestry in Africa

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Abstract

Arterial events in developing countries frequently occur over a young adult age, starting as early as the third decade of life. Atherosclerosis (atheroma formation) is the vascular alteration that ultimately occludes arteries causing coronary artery disease (CAD), stroke or peripheral arterial disease (PAD). In contrast, arteriosclerosis is a process that is thought to in-part be caused by atherosclerosis, but which may also occur independently thereof, and contribute toward the development of vascular damage that leads to occlusion or rupture of the artery. Little is known about the pathogenesis of premature arterial events (events occurring in men < 50 years of age or women < 55 years of age). As arteriosclerosis, which enhances pulse pressure (PP) and hence blood pressure (BP) by increasing aortic stiffness, is presently perceived as largely a disorder of ageing, conventional thought is that it is unlikely to contribute. Arteriosclerosis may even occur at a young age, but the impact may not be detected from BP measures. In the present thesis I therefore evaluated the contribution across the adult age range of arteriosclerotic aortic dysfunction to arterial disease in groups of African ancestry living in South Africa.

Through the impact of conventional risk factors on arteries, several arteriosclerotic arterial functional changes contribute to cardiovascular events. It is nevertheless uncertain whether these effects are accurately reflected by changes in PP in either central (PPc) or peripheral arteries. I therefore, aimed to determine the extent to which relations between modifiable risk factors and arterial function translate into increases in PP in 1232 black South Africans from the South Western Township (SOWETO) of Johannesburg. With adjustments for confounders, diabetes mellitus was associated with an increased carotid-femoral pulse wave velocity (PWV) and forward travelling wave pressures (Pf) but neither brachial PP, PPc, nor backward travelling pressure waves (Pb). Independent of confounders, uncontrolled hypertension was associated with an increased Pf, but not with changes in brachial PP, PPc or Pb. Thus, neither brachial nor central arterial PP are adequate indexes of relations between modifiable conventional risk factors and risk-related arteriosclerotic aortic functional changes.

As the age at which arteriosclerosis begins to contribute to events is uncertain, I subsequently determined, across the full adult lifespan, the extent to which arteriosclerosis-related changes in arterial function occur in those with precipitous arterial events (stroke and critical limb ischaemia [CLI]). Despite similar brachial PP and BP levels, as compared to 726 age, sex and ethnicity-matched randomly selected controls, over 10-year increments in age from 20 to 60years, multivariate-adjusted aortic PWV, characteristic impedance (Zc), Pf, and

early systolic pulse pressure amplification (PPamp) were consistently and markedly altered in 356 participants with either CLI or stroke (35.4% premature). Of the patients with stroke, 10.7% were haemorrhagic in origin, 22.1% had a cardio-embolic cause; 17.6% were classified as small artery occlusion and 2.5% as large artery occlusion (atherosclerotic), 31.9% were indeterminate in origin and 15.2% were from alternative aetiology. Aortic stiffness accounted for Zc increases (no differences in aortic diameter), and Pf was accounted for by changes in Zc and not aortic flow or wave re-reflection. Compared to age- and sex-matched controls, independent of risk factors, PWV and Zc were more strongly associated with premature events than events in older persons and Pf and early systolic PPamp were at least as strongly associated with premature events as events in older persons. Despite similar BP levels, arteriosclerosis-related changes in arterial function are consistently associated with arterial events beyond risk factors from as early as 20 years of age.

The extent to which arteriosclerotic aortic dysfunction mediates arterial damage through effects beyond not only peripheral, but also central arterial BP, is uncertain. I therefore aimed to determine whether the impact of aortic stiffness on atherosclerotic or small vessel end-organ damage beyond brachial BP depends in part on stiffness-induced increases in central arterial pressures produced by an enhanced Zc. In 1021 participants, 287 with stroke or CLI, and 734 from a community sample with atherosclerotic or small vessel end-organ measures although Zc and carotid-femoral PWV were correlated, these relations were not independent of confounders. Both Zc and hence central arterial pressures generated by the product of Zc and aortic flow (Q) (P_{QxZc}), as well as PWV were independently associated with carotid intima-media thickness, estimated glomerular filtration rate (eGFR), endothelial activation markers (V-CAM-1) and events. With further adjustments for brachial PP or systolic BP (SBP), PWV and P_{QxZc} were both associated with eGFR, V-CAM-1 and events. Relationships between PWV and eGFR, V-CAM-1 or events were independent of P_{QxZc} and relationships between P_{QxZc} and eGFR, V-CAM-1 or events were independent of PWV. Thus, beyond brachial BP, the impact of aortic stiffness on arterial damage involves effects that are both dependent (proximal aortic Zc and hence P_{QxZc}) and independent (PWV indexes stiffness across the full length of the aorta) of central arterial pulsatile load. Hence, PWV and brachial PP may be insufficient to account for all of the damage mediated by increases in aortic stiffness.

In conclusion, I provide evidence published in the high impact journals *Art Thromb Vasc Biol (ATVB)*, *J Hypertension* and *Am J Hypertens* to advance our understanding of arteriosclerosis as a possible cause of arterial disease. In this regard, I show that in contrast

to what has previously been thought, that arteriosclerotic aortic dysfunction is as strongly associated with arterial events over a younger as over an older adult age. Moreover, I demonstrate that these effects go undetected because the impact on arterial function produced by risk factors is largely on central arterial forward wave pressures with little impact on brachial or peak central arterial pressures. Further, I show that the impact of arteriosclerosis on arterial damage may occur through both forward wave pressure (P_{QxZc}) effects and pressure-independent effects (as indexed by aortic PWV).

Declaration

I Tshegofatso Harold Motau declare that the work contained 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 for any degree or examination in this university, or any other university.

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Tshegofatso Harold Motau

Signed on……20th……day of ……April……. 2021

I certify that the studies contained in this thesis have the approval of the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg. The ethics clearance numbers are M160411, M170401 (M1204108, M0700469, M020472, M140429).

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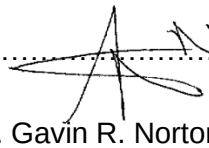
Tshegofatso Harold Motau

Signed on……20th……day of …April……. 2021



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Prof. Gavin R. Norton (Supervisor) Prof. Angela J. Woodiwiss (Supervisor)

Dedication

In loving memory of my father Stephen Thabo Motau (1948-2000), affectionately known as Graven, and mother Pulane Eusybia Musi (1948-2017). Their memory continues to inspire me to pursue my goals amidst adversity. Also, to my wife Dr Loyiso F. Mpuntsha-Motau, your support, encouragement and unconditional love carried me throughout this journey.

Publications and Presentations

Publications

1) **Motau, T.H**., Norton, G.R., Sadiq, E., Manyatsi, N., Kolkenbeck-Ruh, A., Robinson, C., Tade, G., Mabena, P., Monareng, T., Naran, R., Peters, F., Peterson, V., Abdool-Carrim, T., Veller, M., Majane, O.H.I., Sareli, P., Cassimjee, I., Modi, G., Woodiwiss, A.J. (2020). Marked Arterial Functional Changes in Patients with Arterial Vascular Events Across the Early Adult Lifespan. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 40(6), 1574-1586. doi:10.1161/ATVBAHA.119.313734.

2) **Motau, T. H**., Norton, G. R., Sareli, P., and Woodiwiss, A. J. (2018). Aortic Pulse Pressure Does Not Adequately Index Cardiovascular Risk Factor-Related Changes in Aortic Stiffness and Forward Wave Pressure. *American Journal of Hypertension*, 31(9), 981-987. doi:10.1093/ajh/hpy061.

3) **Motau, T.H.**, Norton, G.R., Mmopi, K.N., Bello, H., Peterson, V.R., Libhaber, C., Sadiq, E., Naran, R., Da Silva Fernandes, D., Masiu, M., Mthembu, N., Gomes, M., Monareng, T., Abdool-Carrim, T., Veller, M., Cassimjee, I., Peters, F., Modi, G., Sareli, P., Woodiwiss, A.J. (2021). Relations of aortic stiffness with arterial damage beyond brachial pressure are both dependent and independent of central arterial pulsatile load. *Journal of Hypertension,* 39(4):718-728.

4) 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. (*****equal contribution). *Although this work was not included in the thesis, I made major contributions toward it and this paper generated the results which prompted the work described in the present thesis.*

Oral Presentations

1) **45th Conference of Physiology Society of Southern Africa (PSSA)**, University of Pretoria, Waterkloof, Pretoria, South Africa, 27-31 August 2017. Lack of Association of Current Thresholds of Carotid-Femoral Pulse Wave Velocity and Occlusive Arterial Disease in Young South Africans. Podium presentation.

2) **Stroke and Hypertension Society (SAHS) Congress** (held in Stellenbosch, Protea Hotel, 3-5 August 2018): Aortic Pulse Pressure Does Not Adequately Index Cardiovascular Risk Factor-Related Changes in Aortic Stiffness and Forward Wave Pressure. Oral poster presentation.

3) **Stroke and Hypertension Society (SAHS) Congress** (held in Stellenbosch, Protea Hotel, 3-5 August 2018): Arteriosclerosis-Related Aortic Functional Changes Characterise Arterial Events Across the Early Adult Lifespan. Podium presentation, Top-6 Abstracts (Silver Award).

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List of Abbreviations and Acronyms

A

AGE	Advanced glycation end-product
AJW	An experienced observer
AUC	Area under the curve

B

β-coeff	Beta-coefficient
BMI	Body mass index
BP	Blood pressure

C

CAD	Coronary artery disease
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	CKD-Epidemiology Collaboration
CLI	Critical limb ischaemia
cm	centimetre
CPGRU	Cardiovascular Pathophysiology and Genetics Research Unit
CT	Computed tomography
CVD	Cardiovascular disease

D

DBP	Diastolic blood pressure
DM	Diabetes mellitus

dyne.s.cm^2	dyne-second per square centimetre

E

ECM	Extracellular matrix
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
ESH	European Society of Hypertension

G

GTF	Generalised transfer function

H

HbA1c	glycosylated haemoglobin
HDL	High-density lipoprotein
HIV	Human immuno-deficiency virus
HR	Heart rate

I

I-CAM-1	Intercellular adhesion molecule 1
IDMS	Isotope dilution mass spectrometry
IHD	Ischaemic heart disease
IMT	Intima-media thickness
ISH	Isolated systolic hypertension

L

LDL	Low-density lipoprotein
LV	Left ventricle

LVMI	Left ventricular mass index
LVOT	Left ventricular outflow tract
M	
m/sec	meter per second
MAP	Mean arterial pressure
MAP	Mean arterial pressure
MESA	Multi-ethnic Study in Atherosclerosis
MI	Myocardial infarction
ml/min	millilitre per minute
mm	millimetre
mm Hg	millimetres of mercury
mmol/l	millimole per litre
MMPs	Matrix metalloproteases
ms	milliseconds
N	
n	number (sample size)
NRF	National Research Foundation
O	
OR	Odds ratio
P	
P1	First systolic peak or inflection point
P2	Second systolic peak

p value	Probability value
Pa	Augmentation pressure
PAD	Peripheral arterial disease
Pb	Backward wave pressure
PD	Peripheral waveform length averaged over 10 seconds
Pf	Forward wave pressure
Pi	Incident wave pressure
PP	Pulse pressure
PPamp	Pulse pressure amplification
PPb	Brachial pulse pressure
PPc	Central aortic pulse pressure
PQxZc	Product of Q and Zc
PSSA	Physiology Society of Southern Africa
PWV	Pulse wave velocity

Q

Q	Flow
QRS	Q-wave, R-wave and S-wave complex

R

r	Correlation coefficient
RAGE	Receptor for AGE
RAP	Right atrial pressure
ROC	Receiver operator characteristic

S

SAHS	Stroke and Hypertension Society
SASS	South African Stroke Society
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
SHEP	Systolic Hypertension in the Elderly Program
SOWETO	South Western Townships
STOP	Swedish Trial in Old Patients with Hypertension
SV	Stroke volume
SVR	Systemic vascular resistance

T

TAC	Total arterial compliance
TGF-β	Transforming growth factor-beta
TOAST	Trial of Org 10172 in Acute Stroke Treatment
t_{Q95}	the point at which flow achieves 95% of its peak

V

V-CAM-1	Vascular cell adhesion molecule 1
VSMC	Vascular smooth muscle cells

Z

Zc	Characteristic impedance

Preface

Cardiovascular disease is presently the dominant cause of death and disability in the world today. Although in the 20th century, communicable diseases were the overwhelmingly important cause of hospitalisations and death in developing countries, in the 21st century non-communicable diseases are rapidly achieving equivalent importance. Of the non-communicable diseases, cardiovascular disease is the major cause of morbidity and mortality in developing countries and several arterial events are frequently reported to occur on average 10-20 years earlier than in other populations. However, there is little understanding of the pathophysiological mechanisms responsible for premature arterial events. As premature events have a major impact on disability-adjusted life years and ultimately on the lives of individuals, family members, extended families and the economy of a country, a better understanding of the mechanisms responsible for premature arterial events is required.

A well-accepted pathophysiological mechanism responsible for the arterial disease is arteriosclerosis (hardening of the arteries), as opposed to atherosclerosis (which represents the arterial change responsible for occlusion). However, it is only in the past 2-3 decades that a better understanding of the potential mechanisms through which arteriosclerosis produces its adverse effects has begun to emerge. Although arteriosclerosis has for almost a century been acknowledged as producing arterial damage through increases in pulse pressure and hence systolic blood pressure (BP), there is increasing evidence that the adverse effects of arteriosclerosis go well beyond BP as measured at the periphery. However, whether the adverse impact of arteriosclerosis is beyond even BP determined in central arteries has been a matter of considerable debate over the past decade. As arteriosclerosis is thought to be an ageing effect, the possibility that arteriosclerosis mediates premature events, rather than just events in the elderly, has also never been considered. However, several lines of evidence suggest that arteriosclerosis and the adverse effects thereof may not simply be an ageing effect. The uncertainty as to whether arteriosclerosis mediates adverse effects beyond both peripheral and central arterial BP, and the potential of arteriosclerosis playing an important role at even an early adult age, prompted me to further explore these questions as the topic of my PhD thesis.

The present thesis comprises of a series of semi-independent data chapters (Chapters 2 to 4), each having its own introduction, methods, results and discussion sections. In the introduction to this thesis (Chapter 1), I critically review the available evidence on the role of arteriosclerosis and argue in favour of conducting the studies that have been included in the

present thesis. The conclusions chapter of the thesis (Chapter 5) consolidates the findings of the data presented, underscoring the novelty of and giving perspective to these findings based on what is presently known and understood in the field. In support of the present thesis, the data presented in Chapter 2 has been published in the *American Journal of Hypertension* (Motau *et al*., 2018), the data presented in Chapter 2 in *Arteriosclerosis Thrombosis and Vascular Biology* (*ATVB*) (Motau *et al*., 2020), and the data presented in Chapter 4 are in-press in the *Journal of Hypertension* (Motau *et al*., 2020).

Chapter 1 Introduction

Arteriosclerotic-Related Aortic Haemodynamic Changes

1.0 Introduction

Cardiovascular disease (CVD), being responsible for over 17.3 million deaths per year globally, is the most common cause of death worldwide (Roth *et al.*, 2015). Low to middle-income countries including South Africa (which is an upper-middle-income country) carry 80% of the burden of cardiovascular deaths (Bowry *et al.*, 2015). Importantly, the economic burden of CVD translates into an estimated reduction in gross domestic product by up to 7% in low to middle-income countries (Bowry *et al.*, 2015). This is therefore seen as a critical obstacle to the growth of such countries contributing to an inability to compete on the world stage. Arterial disease is a major cause of CVD and includes diseases of several arterial beds. In this regard, arterial disease may affect the coronary arteries leading to ischaemic heart disease (IHD) or coronary artery disease (CAD) and myocardial infarction (MI); the cerebral arteries leading to ischaemic or haemorrhagic stroke (cerebrovascular disease);or the vasculature of the lower (and occasionally the upper) limb resulting in peripheral arterial disease (PAD) and ultimately critical limb ischaemia (CLI). Although several alternative arterial changes contribute to mortality and morbidity including aneurysms of the aorta or elsewhere, which may ultimately lead to dissection or rupture; IHD, stroke and PAD account for most major cardiovascular events caused by arterial pathology, Arterial disease is the major cause of CVD in developed countries (WHO, 2011), and as shall be discussed in subsequent sections, the arterial disease is increasing in prevalence in low or middle-income countries, including in African countries. A marked cause for concern is that for stroke and CLI, these events often occur in younger age groups in developing African countries.

1.1 Cardiovascular disease in sub-Saharan Africa

Low and middle-income countries are presently in an epidemiological transition where although diseases of poverty and infectious (communicable) diseases are relatively more common in comparison to CVD, non-communicable diseases including CVD now add to the healthcare burden (Moran *et al.*, 2013). Hence, in South Africa, the mortality from communicable diseases, in particular, human immunodeficiency virus (HIV) infections and tuberculosis are compounded by deaths attributed to CVD (Boutayeb and Boutayeb, 2005; Dalal *et al.*, 2011; Kapiga, 2011). Indeed, in the year 2000, the South African National Burden of Disease Study estimated CAD and stroke to be the second and third most common causes of death in South Africa after HIV infections (Bryer *et al.*, 2010). Importantly, the mortality rate of stroke is more than 2-fold higher in black women (160 per 100 000), in comparison to white men (72 per 100 000) (Bryer *et al.*, 2010). Although the increasing burden of CVD in developing countries may in-part be attributed to infectious causes,

including the impact of HIV infections (Chetty, 2001; Brand *et al.*, 2012; Brand *et al.*, 2014), or rheumatic heart disease (Carapetis, 2007; Mayosi *et al.*, 2006; Engel *et al.*, 2015), the increasing burden of CVD in developing countries is most likely attributed to increased risk factors caused by urbanisation and lifestyle changes. In this regard, hypertension is that risk factor that appears to account for most of the increase in CVD in many developing countries in Africa and in particular in South Africa. Indeed, based on the South African Demographic and Health Survey, the prevalence of hypertension is 30.4% in the adult population (age > 15 years old) in South Africa (Kandala *et al.*, 2013; Seedat *et al.*, 2014), with older adults (age ≥ 50 years old) disproportionately affected at a rate of 77.3% (Peltzer and Phaswana-Mafuya, 2013). Although, the incidence of hypertension increases with advancing age, in high-income countries, the greatest absolute burden of hypertension is in old age groups (age ≥ 60 years), whereas in low- and middle-income countries the major absolute burden is in the middle aged groups (40-59 years) (Mills *et al.*, 2016). Moreover, in Africans (Nigerians) the risk of cardiovascular complications for a given blood pressure level is higher than in Caucasians (Flemish) (Odili *et al.*, 2017). Of particular concern in South Africa, the incidence of arterial events such as stroke and PAD is increasing in younger individuals within the 35 to 54-year age group (Bryer *et al.*, 2010; Brand *et al.*, 2012) an observation which applies to arterial events in many African countries. In this context, stroke aetiology, is based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification which denotes five subtypes of ischemic stroke including: large-artery atherosclerosis (LAA), cardioembolic, small-vessel occlusion, stroke of other determined aetiology, and stroke of undetermined aetiology (Adams *et al.*, 1993). So, what is the evidence to suggest that there is cause for concern regarding the age at which arterial events occur in Africa?

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

Age is the strongest risk factor for arterial events with events usually occurring in those older than 60 years of age (Johansson *et al.,* 2010; McAloon *et al.,*2016). Indeed, nearly 20% of adults aged 70 years and older have PAD and most of those with PAD are older than 65 years of age (Dua and Lee, 2016). Nevertheless, globally, CVD attributable deaths account for at least 11% of premature deaths in individuals as young as 30-34 years of age (Roth *et al.*, 2015). Importantly, populations living in Africa or who originate from Africa differ in the age distribution of arterial events. In this regard, in sub-Saharan Africa stroke frequently occurs in younger individuals (less than 65 years of age) (Connor *et al.,*2007; Sarfo *et al.,*2015). The lower age of stroke in Africa is consistent across African countries with the average age varying from 59 years to 64 years (Sarfo *et al.*, 2015; Nkusi *et al.*, 2017; Keates *et al.*, 2017; 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 South Africa, the average age of hospitalisations for stroke in referral hospitals is 45-55 years(Connor *et al.*, 2009; Kolkenbeck-Ruh *et al.*, 2019b; Kolkenbeck-Ruh *et al.*, 2020), with events occurring in those as young as 18 years of age. Thus, in some African countries half of all strokes may be considered as chronologically premature (<50 years of age for men and 55 years of age for women). Concerning PAD, in contrast to the occurrence being largely in the very elderly in other ethnic groups, the mean age of PAD in African Americans is 56 years (Collins *et al.*, 2017). In this regard, similar low mean ages for PAD are reported in Africa. Indeed, in Nigeria, the prevalence of PAD in those less than 65 years of age has been reported as being 40-52.5% (Oyelade *et al.*, 2012; Ogbera *et al.*, 2015). In South Africa, the average age of hospitalisations for CLI in referral hospitals is 45-55 years (Brand *et al.*, 2012; Kolkenbeck-Ruh *et al.*, 2019b; Kolkenbeck-Ruh *et al.*, 2020) with events occurring in those also as young as 18 years of age. Thus, some arterial events, particularly stroke and CLI, the result of PAD, frequently occur over a young adult age range in Africa. In contrast, IHD has never been reported to occur commonly at particularly young adult age in African populations.

1.1.2 Impact of cardiovascular events at a younger adult age

As some cardiovascular events, including stroke and PAD or CLI, occur in populations of African origins and in African countries 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). Similarly, the survival rate of young individuals with PAD is higher compared to older individuals. However, the adverse impact of stroke or PAD at a young as compared to older age on disability-adjusted life years is far greater. Stroke is well-recognised 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 stroke eventually return to their jobs after the stroke, whilst 70 % of patients remain disabled and unemployable (Reshi *et al.*, 2017). Patients who survive PAD similarly face considerable life-changing adaptations particularly when lower limb amputation has occurred (Koureas *et al.*, 2017). Pain and impaired mobility are major features of PAD (Aquarius *et al.*, 2006; Regensteiner *et al.*, 2008; Koureas *et al.*, 2017; Wu *et al.*, 2017). Even in cases where amputation does not occur, mobility becomes increasingly restricted leading to a need for help with daily activities, a decrease in productively, and ultimately, an inability to work (Koureas *et al.*, 2017; Wu *et al.*, 2017). In addition, a restricted

ability to participate in social activities in those with PAD, results in isolation and psychosocial issues including depression (Aquarius *et al.*, 2006; Koureas *et al.*, 2017). The occurrence of a premature arterial event (below age 50 years) therefore results in considerable loss of potential and productivity with notable effects on personal well-being, wealth and the wealth of the country (Nakibuuka *et al.*, 2015). 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 arterial events in the young affects not only the individual but is a major threat to immediate dependents such as children or spouses and the economy due to loss of effectiveness and productivity at working age (Smajlović, 2015) and the dependence on the state for survival. There is therefore a need to better understand the pathophysiological mechanisms that may contribute to premature events and hence to identify better approaches to both detecting those at risk for these events and perhaps to prevent these events.

1.1.3 Premature arterial events in the absence of ageing: Could arteriosclerosis play a role?

As age is by far the strongest risk factor for arterial events, an elementary question that remains unanswered is why arterial vascular events occur at a young adult age? Of critical importance is that the fundamental arterial changes that contribute to these events are two pathophysiological processes in the arterial wall, one of which is ***atherosclerosis*** and the second of which is ***arteriosclerosis***. In this regard, atherosclerosis (atheroma formation) is the vascular alteration that ultimately occludes arteries causing CAD, stroke or PAD. In contrast, arteriosclerosis is a process that is thought to in-part be caused by atherosclerosis, but which may also occur independently thereof (see below), and contribute toward the development of vascular damage (atherosclerosis or alternative changes) that leads to occlusion or rupture of the artery (see below). Previous studies from our group have demonstrated that atherosclerotic changes in the carotid artery are striking in those with events at young adult age in Africa (Kolkenbeck-Ruh *et al.*, 2019b; Kolkenbeck-Ruh *et al.*, 2020), What has not been adequately reported on is whether arteriosclerotic changes contribute to the development of events at a young adult age. In this regard, as will be discussed, arteriosclerosis is thought to occur largely as a consequence of an ageing process that is exacerbated by the presence of risk factors. Moreover, conventional thought is that arteriosclerosis mediates adverse effects through an impact of increases in blood pressure (BP) readily detectable at the brachial artery as increased pulse pressure (PP) and hence systolic BP (SBP). As increases in PP and SBP at the brachial artery only begin to increase from an older adult age (50 years of age onward), traditional thought holds that

arteriosclerosis cannot contribute to arterial events at a younger adult age. Hence current thinking is that premature arterial event although mediated by atherosclerotic changes, are unlikely to be attributed to arteriosclerosis. However, as will be discussed, there is evidence to suggest that arteriosclerosis may contribute to events well beyond brachial BP (including PP and SBP) and this prompted me to evaluate the relative role of arteriosclerotic arterial functional changes in those with arterial events at a younger as opposed to an older age. Hence, in the present Chapter I will discuss the mechanisms responsible for *arteriosclerosis* and the role of arteriosclerosis as a possible cause of arterial vascular events. In so doing I will highlight the missing evidence that prompted me to perform the studies described in the present thesis.

1.2 Mechanisms of arteriosclerosis and subtypes

The term "*arteriosclerosis*" refers to "*hardening of arteries*" due to a loss of elasticity and thickening of the wall of elastic arteries produced by ageing, sustained conventional cardiovascular risk factors such as hypertension and diabetes mellitus, or secondary to connective tissue disorders (Kumar *et al.*, 2013; Meyer, 2001). The essential change that occurs in elastic arteries which "*harden*" is an increased stiffness of the wall of the vessel. What are the arterial changes responsible for arteriosclerosis and hence arterial stiffness and how do these changes occur?

1.2.1 Cellular changes and mechanisms responsible for arteriosclerosis

Large *elastic* arteries in addition to the three layers found in all large arteries (tunica intima, tunica media and tunica adventitia) also comprise of internal and external elastic laminae in the intima-medial and media-adventitial boundaries, respectively (Chirinos, 2016). Collagen and elastin form part of the extracellular matrix (ECM) of the vessel wall, and in so doing provide structural integrity and elasticity (Zieman *et al.*, 2005). The tunica media has elastin fibres that are organised as an interconnecting, fenestrated network (Wagenseil and Mecham, 2012). These layers of elastin fibres are associated with a layer of circumferential vascular smooth muscle cells (VSMC's) and collagen fibres, forming a lamellar composite unit (Wagenseil and Mecham, 2012). However, the deposition of elastin in large arteries is limited to the foetal development period and during early infancy (Chirinos, 2016). Thereafter, the expression of elastin is switched-off in early childhood (Chirinos, 2016). In this regard, the number of lamellar units established during early development is directly related to the tension in the arterial wall with more elastin lamellar resulting in a greater elasticity and a reduced wall stiffness and hence tension (Wagenseil and Mecham, 2012). This fixed deposition of elastin fibres implies both an intrinsic wall tension from childhood

and that elastin fibre damage is essentially irreparable, leading to irreversible increases in arterial stiffness (Chirinos, 2016).

How does progressive elastin degradation occur with advancing age? The two mechanisms that have been hypothesised involve mechanical stress and elastase-mediated proteolysis, with collagen deposits occurring predominantly in the tunica media at the sites of elastin fragmentation (Chirinos, 2016). Both collagen and elastin are tightly regulated by catabolic matrix metalloproteases (MMPs) (Zieman *et al.*, 2005). Since the aorta is highly pulsatile, cyclic stress, especially in hypertensive individuals, stimulates VSMCs (and inflammatory cells) to release collagenases (MMP-1, MMP-8, MMP-13) and elastases (MMP-7 and serine proteases) (Jacob, 2003). Although there are tissue inhibitors of MMPs that counter this enzymatic response in controlling vascular remodelling, damaged elastin fibres are not replaced, whilst more collagen is deposited (Wagenseil and Mecham, 2012). As a result, with vascular tissue injury there is generally an increased collagen-to-elastin ratio, and hence an increased tensile vascular strength and stiffness (Wagenseil and Mecham, 2012). Importantly several additional processes contribute to the increased stiffness in elastic arteries.

The accumulation of Advanced Glycation End-products (AGEs) is thought to play a major role in the development of arteriosclerotic changes in elastic arteries. In this regard, AGEs are a heterogeneous group of molecules produced by glycation in cells or long-lived extracellular proteins (Chaudhuri *et al.*, 2018). The process of protein glycation is a collection of non-enzymatic sequential reactions referred to as the Maillard reaction, present in all tissues and fluids where the significant concentration of glucose, fructose, or more reactive dicarbonyls react with proteins (Sell *et al.*, 1992; Chaudhuri *et al.*, 2018). In a glucose-mediated glycosylation reaction, the initial by-product formed is fructosyl-lysine, an Amadori adduct of glucose to lysine (Ansari *et al.*, 2011; Rahbar *et al.*, 1969). For instance, the glycosylated haemoglobin adduct, HbA1c, is one such product and this has revolutionised the clinical monitoring of patients with diabetes mellitus (Rahbar *et al.*, 1969). In arterial tissue, AGEs form irreversible cross-links between collagen and elastin molecules (Lee and Cerami, 1992; Bailey, 2001), generating stiffer collagen that is resistant to hydrolysis, and reducing the elastic matrix of the arterial wall (Winlove *et al.*, 1996; Konova *et al.*, 2004; Zieman *et al.*, 2005). Receptor for AGE (RAGE), a multiligand receptor of the immunoglobulin superfamily, can trigger an inflammatory response, oxidant radical formation, proinflammatory cytokines, growth factors, and vascular adhesion molecules (Yan *et al.*, 1994; Throckmorton *et al.*, 1995) that mediate vascular stiffness via the enzymatic action of MMPs (Kuzuya *et al.*, 2001). In addition, the interaction of RAGE receptor with AGE

ligands can stimulate a profibrotic response, independently through the Transforming Growth Factor-Beta (TGF-β) pathway (Li *et al.*, 2003; Zieman *et al.*, 2005). Although several alterations in ECM molecules lead to an increased arterial stiffness, particularly in hypertensives and in those with uncontrolled diabetes mellitus where AGEs are formed in excess, an increased stiffness of elastic arteries may also occur through cellular effects. How may this occur?

Vascular smooth muscle cells may play a major role in mediating increases in arterial stiffness through cell signalling and increased vascular smooth muscle tone (Bassiouny *et al.*, 1994; Benetos *et al.*, 1993; Gillessen *et al.*, 1995; McEniery *et al.*, 2006). The process of elastin fragmentation and collagen accumulation may activate signalling pathways that promote VSMC osteogenic differentiation and vascular calcification (Duca *et al.*, 2016). Moreover, there is a growing consensus that increased vascular stiffness with ageing is also attributable to the intrinsic mechanical property changes in VSMCs with ageing(Qiu *et al.*, 2010; Lacolley *et al.*, 2017). In this regard, intrinsic vascular smooth muscle stiffness with ageing has been demonstrated to occur by an increase in the expression of cytoskeletal proteins (particularly actin filaments), smooth muscle α-actin, as well as increased β1-integrin adhesion molecules between VSMCs and the ECM of the aorta (Qiu *et al.*, 2010). The integrins provide a mechanical connection between VSMCs cytoskeleton and the ECM (Schwartz and Ginsberg, 2002; Delon and Brown, 2007). Indeed, changes in the contractile state of VSMC are associated with parallel changes in the focal adhesion points (facilitated by integrin adhesion molecules) between the ECM fibronectin, and VSMC cytoskeletal proteins (Qiu *et al.*, 2010). As a result, integrin-ECM adhesions increase cytoskeleton remodelling, thus conferring mechanical resistance of VSMCs (Qiu *et al.*, 2010). In addition, the abundance of smooth muscle α-actin in “aged vessels” transfers significant force to the ECM in high-pressure states, thus contributing to ECM remodelling (Humphrey *et al.*, 2014; Qiu *et al.*, 2010). Furthermore, the increased inclusion of α-actin within the contractile filament results in cytoskeletal fibres that contract more forcefully (Humphrey *et al.*, 2014).

1.2.2 The traditional classification of arteriosclerosis

The traditional classification of arteriosclerosis includes mainly three vascular lesions: atherosclerosis, Mönckeberg’s medial calcific sclerosis, and arteriolosclerosis (Fishbein and Fishbein, 2015; Kumar *et al.*, 2013). Atherosclerotic arteriosclerosis affects large elastic and medium muscular arteries and forms characteristic raised atheromatous lesions in the tunica intima (Kumar *et al.*, 2013). Best described by the vascular response-to-injury hypothesis model, atherosclerosis is a chronic inflammatory response of the arterial wall to endothelial

injury and involves the interaction of mainly oxidised-Low Density Lipoproteins (ox-LDL), monocyte-derived macrophages, T lymphocytes and cellular constituents of the arterial wall, forming characteristic raised atheromatous lesions (containing cholesterol and necrotic debris-containing lipid cores) in the tunica intima (Kumar *et al.*, 2013). Whilst the atherosclerotic changes associated with atherosclerotic arteriosclerosis are the processes that ultimately lead to occlusion of the artery and hence to arterial events, Mönckeberg's arteriosclerosis is calcific lesions that involve the tunica media and do not encroach on the lumen (Kumar *et al.*, 2013). These calcific lesions maybe idiopathic or associated with diabetes mellitus, renal failure or both, and are typically seen in individuals above the age of 50 years. Overall, arterial stiffening due to age appears to be diffuse, predominantly medial and associated with dilated arteries (Nichols *et al.*, 2011). Although atherosclerosis is considered to play little role in contributing to the arteriosclerotic changes in Mönckeberg's arteriosclerosis, as will be discussed in subsequent sections, arteriosclerosis leads to atherosclerosis and hence the distinction may be difficult. In contrast to other forms of arteriosclerosis, arteriolosclerosis is a thickening of the wall of arterioles and hence contributes to small vessel and not large vessel disease. The single layer of smooth muscle cells in arterioles is deposited by intimal fibromuscular tissue or "hyaline", a pathology usually associated with hypertension or diabetes mellitus (Fishbein and Fishbein, 2015).

Despite the aforementioned pathological classification by earlier proponents and Kumar *et al.* (2001), there has been some controversy on the terminology and the mechanisms linking arterial stiffness and atherosclerosis (Fishbein and Fishbein, 2015). In this regard, the Rotterdam Study has shown the association between arterial stiffness and atherosclerosis at various sites in the vascular tree (Van Popele *et al.*, 2001), whilst earlier studies have suggested that aortic stiffening can occur in the absence of atherosclerosis (Avolio *et al.*, 1985; Vaitkevicius *et al.*, 1993). Moreover, a positive but weak correlation has been shown between a verified degree of aortic and systemic atherosclerosis (based on a pathological atherosclerotic index), and aortic stiffening (Sawabe *et al.*, 2005). Nevertheless, atherosclerosis does not necessarily occur as a result of ageing and may begin in childhood. Indeed, the majority of children show some degree of aortic fatty streaks by 3 years of age, which increase after 8 years of age and develop into atherosclerotic plaques in the coronary arteries during adolescence (Urbina *et al.*, 2009). In addition, progressive and linear increases in carotid intima-media thickness, an index of atherosclerosis, occurs with increasing age starting from an early adult age, while arteriosclerotic aortic functional changes generally only start occurring from midlife and increase at an exponential rate thereafter(Nichols *et al.*, 2011). Therefore, although atherosclerosis contributes to arterial stiffening and calcification, arteriosclerosis is considered as an independent pathogenetic

process that may or may not be accompanied by atherosclerosis. However, as will be discussed, arteriosclerotic changes are likely to be major causes of arterial events and hence are considered as key role players in the development of atherosclerosis. Thus, just as atherosclerosis is sometimes considered to be a central determinant of the development of arteriosclerosis, so arteriosclerosis is considered as a critical cause of atherosclerosis. How then can arteriosclerosis cause arterial events and what is the evidence to support this effect?

1.3 Arteriosclerosis and cardiovascular disease: A role for blood pressure effects

The presence of arteriosclerosis in those with CVD has been well-recognised for at least the past 60-70 years. However, the role of arteriosclerosis as a potential causative factor has traditionally been attributed to effects produced by increases in blood pressure (BP), specifically systolic BP (SBP) or pulse pressure (PP) as detected at the brachial pulse. As PP as measured at the brachial pulse only begins to increase in the general population from around 50-60 years of age (Franklin and Wong, 2016) (due to increases in SBP [across the adult lifespan], and decreases in DBP from around 50-60 years of age [Franklin and Wong, 2016; Staessen *et al.*, 1990], this effect has largely been attributed to an impact on the very elderly with little importance attached to effects in the young to middle-aged. However, as will be discussed in subsequent sections, over the last three decades there has been an exponential increase in scientific literature identifying a potential role for aortic stiffness induced functional changes in the development of CVD that is beyond that detected at the brachial pulse. Most importantly, the effects of aortic stiffness have been noted in the middle-aged or even at a younger age, well before the impact of arteriosclerosis is thought to produce its adverse effects. In the following sections, I will therefore discuss the importance of elastic arteries in maintaining a normal cardiovascular function, the impact of a loss of elasticity of these arteries on a normal function and the evidence to support a role for arteriosclerotic arterial dysfunction in mediating CVD. In so doing I will highlight the evidence to suggest that brachial PP is limited in the ability to index the adverse effects of arteriosclerosis and hence lead the reader through a series of arguments that led me to perform the work conducted in the present thesis.

1.3.1 The importance of central arteries (aorta) as elastic organs

Arterial stiffness describes the capacity of an artery to expand and subsequently return to normal volumes in response to pressure changes (Cecelja and Chowienczyk, 2012). A low arterial stiffness occurs when the vessel is compliant or distensible and hence has a high capacity to expand in response to pressure changes. In contrast, a high arterial stiffness

occurs when the vessel has a low degree of distensibility or compliance and hence has a low capacity to expand in response to pressure changes. In this regard, in keeping with its very high elastin-to-collagen ratio, the most distensible artery in the body is the aorta and the importance of aortic distensibility is often thought to be best described by the “Windkessel” model of arterial function (Nichols *et al.*, 2011; Laurent *et al.*, 2006).

Since the first direct BP measurement by the philosopher, Stephen Hales in 1733 (Westerhof *et al.*, 2009) in a horse, an interest in the pulsatile nature of arterial pressures has existed. Hales noticed that the height of the blood column in the glass pipe inserted into an artery, fluctuated and these fluctuations were synchronous with the heartbeat (Tsyrlin *et al.*, 2016). These fluctuations suggested the generation of pressure waves that were intermittent and this sparked interest in the factors that determine the extent of the pressure change during each contraction of the heart and how these pressure changes came to produce the peripheral pulse. In understanding BP propagation proximally from the heart to distal arteries, the mechanism was compared to a fire-hose system referred to as a Windkessel – a German word literally meaning “air chamber” (Salvi, 2012; Laurent *et al.*, 2006). Briefly, a Windkessel was coupled to an intermittent pump employed to provide water to fight fires, which in its own right would generate intermittent flow if the pump was simply connected to a hose. This of course would be a hopelessly ineffective manner of delivering water to fight fires. Indeed, steady rather than intermittent flow was required to act as an effective delivery system. To generate steady flow from a pump that was intermittent two approaches could be employed. Either use high resistance hosepipes (e.g. dampening of pulsatility occurs as one goes down the arterial system), which of course, according to the equation Flow (F)=P (Pressure)/R (Resistance), was not an option as this would limit flow. Thus, in order to provide a steady flow of water without having to use high resistance hosepipes that limit flow, during the ejection phase of the pump in the Windkessel system, most of the water was stored in a closed tank (Windkessel) with compressed air, whilst some of the water was pumped out in a continuous stream (Salvi, 2012). The air acted as a cushion and hence allowed for dampening of the system thus reducing the pulsatility of flow. According to the Windkessel model as applied to the cardiovascular system, an analogy popularised by Otto Frank (Westerhof *et al.*, 2015), the role of large elastic proximal arteries (i.e. the aorta and its main branches) include a cushioning function, which dampens high-pressure pulsatility generated by intermittent ventricular ejection, and a conduit function, whilst muscular distal arteries and arterioles contribute to the distribution of blood (Safar *et al.*, 2003). This “Windkessel effect”, was attributed to the properties of a compliant arterial system with the capacitance to accommodate an increase in blood volume and pressure generated by ejection of blood from the left ventricle (LV) (Dart and Kingwell, 2001).Thus,

during systole, about 50% of the stroke volume and 10% of the energy accumulates in the aorta as it expands during ejection (London and Pannier, 2010). During diastole, the stored energy in the aorta then generates elastic recoil which creates the force to produce flow during this phase of the cardiac cycle, thus ensuring continuous blood flow even in the diastolic period (London and Pannier, 2010). The capacitance component of the Windkessel model corresponds to compliance (C), which can be described as C = Δ volume/ Δ pressure (Dart and Kingwell, 2001) or approximated as C = stroke volume (SV)/PP (Chemla *et al.*, 1998; Stergiopulos *et al.*, 1999). As such, according to the Windkessel model, when arteriosclerosis reduces aortic elasticity and hence decreases arterial compliance (C) (or increases stiffness), it reduces the cushioning effect of the aorta and increases pulsatility with a subsequent widening of PP and consequent increases in SBP (Alfie *et al.*, 1999).

The function of the aorta as a "Windkessel" has considerably expanded over the years to now incorporate several additional concepts. In this regard, as liberally stated in basic texts, there is an erroneous assumption that large vessels, such as the aorta, offer little resistance to flow. The assumption is that the only way that a stiff aorta could generate a higher pressure was through a reduced cushioning effect and hence compliance. Unlike in smaller vessels, where resistance to flow is a determinant of pressure (P=F x R), it is commonly thought that large vessels cannot produce the same effect. The inability of large vessels such as the aorta to generate resistance to flow nevertheless only applies to steady flow in the arterial system and has little relevance to pulsatile flow. Indeed, pulsatile flow rate in the aorta is so marked during ejection that even small changes in aortic diameter or stiffness may have striking effects on the resistance to flow. Resistance to flow in a pulsatile system is called impedance. In essence, the cushioning effect of the aorta prevents impedance to flow. In contrast, arteriosclerotic-mediated increases in aortic stiffness determine an increased characteristic impedance to flow (impedance to flow in a pulsatile system in the absence of wave reflection), abbreviated as Zc (Nichols *et al.*, 2011). The pressure generated during ventricular ejection follows the usual formula P=F x R but is this instance PP=Aortic flow (abbreviated as Q) x Zc (Nichols *et al.*, 2011). Thus, in addition to a cushioning effect of the aorta or the lack thereof influencing PP, the Windkessel model also incorporates the notion that increases in Zc in the proximal aorta are what determines arteriosclerotic effects on PP and hence SBP. Importantly, SV/PP or C discussed in the aforementioned paragraph is determined not only by proximal aortic stiffness but also by the compliance of the arterial system further downstream including much smaller vessels whose function is not strongly influenced by arteriosclerosis and which are often modified by vasoconstrictor effects. Thus, C is better termed total arterial compliance (TAC) and although influenced by arteriosclerotic aortic dysfunction, TAC is also strongly determined by vascular tone of arteries downstream

from the aorta. Importantly, a major limitation cited with the Windkessel model, is that a distinction between total compliance provided by large arteries and that purely attributable to small peripheral arteries cannot be clearly drawn, as through increases in stiffness large arteries can impose resistance whilst smaller vessels can also provide compliance albeit limited (Chirinos and Segers, 2010). Consequently, contemporary views incorporate the notion that systemic blood flow is driven by several vascular factors including TAC and proximal aortic Zc, both of which are determined by aortic stiffness. However, of the two Zc is the only one of these mechanical factors that is determined by central arterial properties alone.

1.3.2 Impact of increases in aortic stiffness on blood pressure and cardiovascular damage

Through the mechanisms discussed in the aforementioned section, arteriosclerotic-induced increases in aortic stiffness produce marked increases in proximal aortic Zc, TAC and hence PP and SBP and when very extensive, decreases in DBP (through a reduced blood flow during diastole as a consequence of reduced elastic recoil of the aorta) (Sethi *et al.*, 2014). Thus, increases in aortic stiffness enhance pulsatile load on the cardiovascular system. The importance of the pulsatile (as opposed to the steady) component of BP as a cause of cardiovascular damage has been underscored by several important findings. Indeed, independent of steady component pressures (MAP), PP has been demonstrated to be a strong predictor of CAD (Franklin *et al.*, 1997; Madhavan *et al.*, 1994; Alderman *et al.*, 1998; Benetos *et al.*, 1997; Benetos *et al.*, 1998; Franklin *et al.*, 1999), stroke (Darne *et al.*, 1989) and cardiovascular events in both hypertensive patients and in the general population. In addition, isolated systolic hypertension (ISH), which is associated with a widened PP, and often a lower than normal DBP, is independently associated with several end-organ measures and events including LV mass (Palmieri *et al.*, 2006; Pini *et al.*, 2008), carotid plaque (Pini *et al.*, 2008), estimated glomerular filtration rate (eGFR) (Townsend *et al.*, 2010), microalbuminuria (Hashimoto and Ito, 2011), stroke, MI, and heart failure (Mitchell *et al.*, 2010a), coronary artery events (Mitchell *et al.*, 2016), chronic kidney disease (CKD), peripheral arterial disease (Mao *et al.*, 2017) as well as cardiovascular mortality (Chirinos *et al.*, 2005; Pini *et al.*, 2008; Jankowski *et al.*, 2008; Roman *et al.*, 2007; Lin *et al.*, 2016; Safar *et al.*, 2002).

The highest level of evidence in support of an independent role of pulsatile as opposed to steady pressure load as a cause of cardiovascular disease comes from intervention data targeting BP in patients with ISH. In this regard, in a large randomised, double-blind,

placebo-controlled trial, the Systolic Hypertension in the Elderly Program (SHEP) involving elderly patients (n=4,736; age ≥ 60 years) with ISH (SBP ≥ 160 mm Hg and DBP < 95 mmHg) and thus a widened PP, treating increases in BP produced a reduction in strokes by 36%, MI's and CAD by 27%, all CVD by 32% and total mortality by 13% (Perry *et al.*, 2000). Other intervention studies included in a meta-analysis of the ISH trials (Staessen *et al.*, 2000), including the Systolic Hypertension in Europe (Syst-Eur) (Staessen *et al.*, 1997) and Syst-China (Wang *et al.*, 2000) trials showed similar benefits from treatment of ISH with calcium channel blocker-based antihypertensive therapeutic regimen. The SHEP trial involved a two-step randomised treatment which included chlorthalidone (diuretic), followed by atenolol (beta-blocker) or reserpine (Perry *et al.*, 2000). Important to note in the latter study, is that the stroke incidence was markedly reduced in patients that reached the treatment goal of a 20 mm Hg reduction from baseline to < 160 mm Hg in comparison to patients not reaching the targeted treatment goal (Perry *et al.*, 2000). In the Syst-Eur study, initial treatment with a calcium channel blocker (nitrendipine), before the addition of an angiotensin-converting enzyme inhibitor (ACE-I), enalapril, and/or a diuretic, (hydrochlorothiazide) showed a significant reduction of 41% in stroke and a reduction of 26% in combined coronary events (Staessen *et al.*, 1997). Elderly Chinese patients with ISH enrolled in the Syst-China study showed an improved prognosis particularly in those with pre-existing diabetes (Wang *et al.*, 2000). The Swedish Trial in Old Patients with Hypertension (STOP), involving much older patients (70-84 years of age), with SBP between 180 and 230 mm Hg, who are most likely to have striking increases in central arterial stiffness, achieved robust reductions in cardiovascular morbidity and mortality subsequent to treatment with β-blockers and a diuretic (Dahlöf *et al.*, 1991). Although there is no question as to the importance of pulsatile load generated by arteriosclerotic-induced increases in aortic stiffness as a cause of marked cardiovascular damage, the past two decades has heralded ongoing debate as to the relative role of various determinants of a pulsatile load as causes of cardiovascular damage. Although the Windkessel model provides important insights into the cause of increases in pulsatile load, it is limited in some respects. What then is the contemporary view of the factors that determine central arterial PP and which subsequently drive increases in SBP?

1.3.2.1 Determinants of pulsatile load

As indicated in Figure 1.1, the aortic (central arterial) pulse is generated by a composite of several waveforms, with the two dominant waveforms being the incident or forward travelling pressure wave, the peak of which is labelled as Pf and a reflected or backward travelling pressure wave, the peak of which is labelled as Pb in the figure (Hughes *et al.*, 2013;

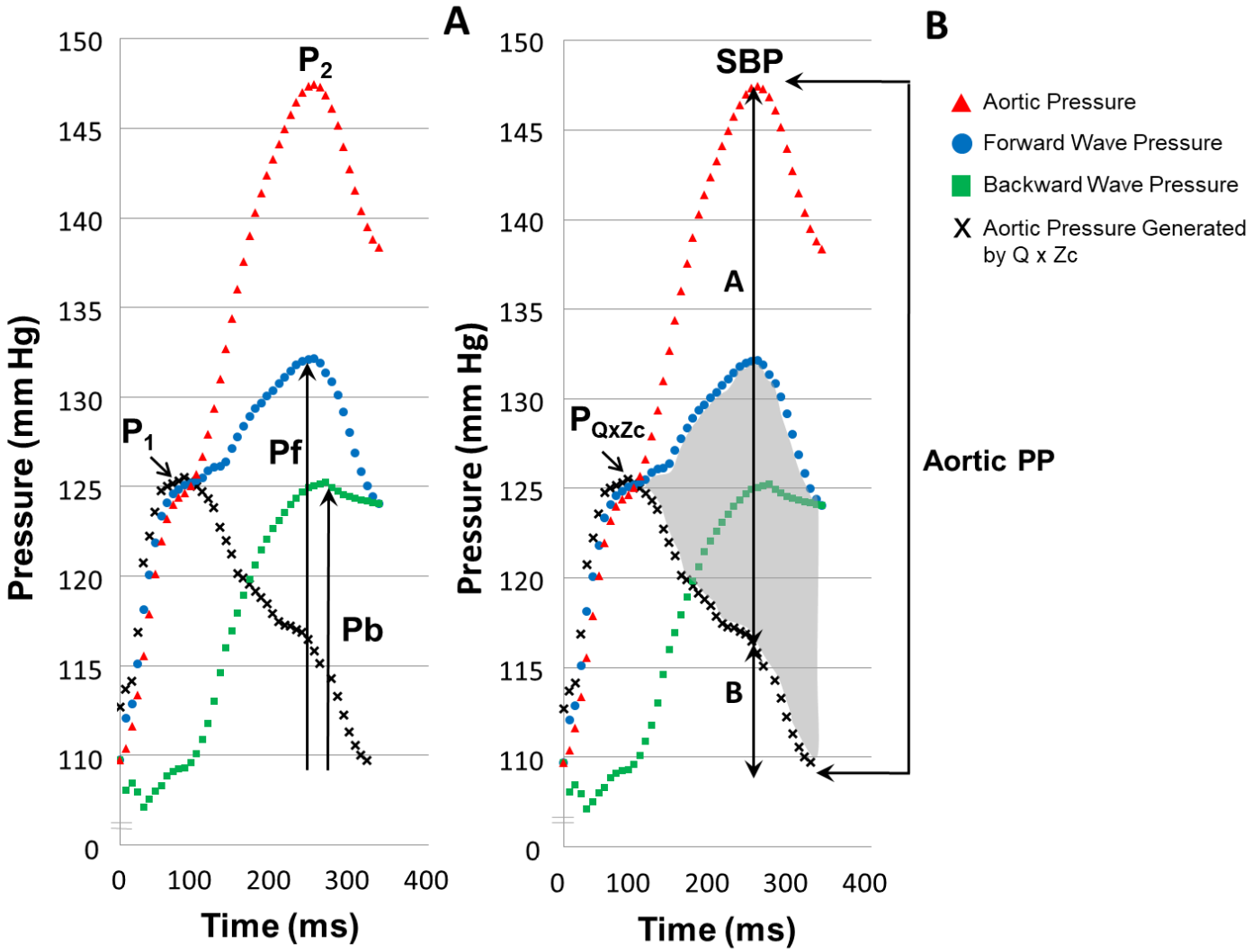

Figure 1.1. Wave components of aortic pulse pressure (PP) showing the forward and backward wave pressures (panel A) superimposed on the aortic pressure wave and the pressure waves generated by the product of flow (Q) and characteristic impedance (Zc) and wave re-reflection (panel B). The shaded area in panel B shows that component of the forward pressure wave that represents wave re-reflection ("rectified waves" reflecting off structures in the proximal aorta). Pb, peak backward wave pressure; Pf, peak forward wave pressure; P1, first systolic shoulder; P2, second systolic shoulder; P_{QxZc}, peak pressure generated by the product of aortic flow (Q) and characteristic impedance (Zc); SBP, systolic blood pressure; A, reflected + re-reflected wave pressures; B, pressure generated by the product of Q and Zc at peak PP.

Hashimoto and Ito, 2009; Murgo *et al.*, 1980; Nichols *et al.*, 2011). As alluded to in the aforementioned sections, the incident pressure wave is produced by LV ejection into the aorta which generates aortic flow (Q). This wave is therefore in part determined by stroke volume (SV) and hence several cardiac effects (contractility, Frank-Starling mechanisms mediated by alterations in preload and diastolic function) as well as afterload to the LV. Although peak aortic flow is determined mainly by the impact of SV, it may also be influenced by several additional factors including ejection duration (an increased ejection duration may be associated with a lower Q although SV is unchanged). In this regard, as SV is the product of velocity time integral (area under the aortic flow velocity curve) and aortic root area, if ejection duration is increased then although the peak of aortic flow (Q) may be reduced, the velocity time integral (area) and hence SV may remains unchanged (Nichols *et al.*, 2011). As indicated in the previous discussion, Q interacts with proximal aortic Zc which is in turn determined by the extent of aortic stiffness (arteriosclerotic aortic effects) and the diameter of the aorta (a decreased diameter generates a higher resistance or impedance and hence Zc). Thus a large component of forwarding wave pressures is generated by the pressure produced by the product of Q and Zc (P_{QxZc} in Figure 1.1) (Nichols *et al.*, 2011); Torjesen *et al.*, 2014) and this, in turn, is the major pressure wave produced centrally that increases when arteriosclerotic aortic dysfunction occurs. What of the role of reflected waves?

During ventricular ejection, the forward travelling pressure wave, as with any oscillating wave (such as a sound wave) is transmitted throughout the systemic arterial tree. When oscillating pressure waves reach areas of bifurcation or significant tapering, the impedance mismatch (e.g. a change from a very compliant aorta to the less compliant peripheral artery) results in wave reflection and the oscillating reflected pressure wave travels back up the arterial tree to the proximal aorta. Several points of impedance mismatch occur along the arterial tree resulting in the generation of multiple oscillating reflected waves. These oscillating reflected pressure waves travel sufficiently rapidly that that meet with the oscillating forward travelling pressure wave at various points along the arterial tree. As with any oscillating wave (sound waves being a key example), the forward and reflected waves are often timed so that at points where antinodes (points along oscillating waves that undergo maximum velocity and displacement in the y-direction) overlap, the summation of the two waves will occur (Nichols *et al.*, 2011). Although innumerable reflected pressure waves are generated at multiple points in the arterial tree, many will return to the aorta and summate together to generate what appears to be a single reflected wave (backward wave) in the proximal aorta the peak of which is labelled Pb in Figure 1.1.

Importantly, at a peripheral level, as reflected waves do not have far to travel before encountering forward waves, reflected wave antinodes will occur close to forward wave antinodes, thus producing maximal summation and generating a pressure wave which appears as a single pulsatile wave. In contrast, closer to the proximal aorta reflected waves will have had further to travel before encountering forward waves. Thus, the chances of reflected wave antinodes being synchronised with forwarding wave antinodes will decrease and significantly less summation will occur generating a pressure wave which appears as two pulsatile waves with a first and second systolic shoulder (Figure 1.1). In children and adolescents, the reflected wave travels sufficiently slowly that it only summates with the forward wave in diastole, an effect that enhances diastolic BP and thus coronary perfusion pressures, thus contributing to increases in coronary flow during exercise. As the reflected wave occurs in diastole at this age it does not contribute to central arterial PP and SBP. However, from early adulthood, reflected waves arrive sufficiently early in the central aorta that summation with the forward wave markedly augments central arterial PP and SBP (Figure 1.1). Thus, it is now recognised that wave reflection is a major determinant of central arterial PP and SBP. As peripherally, reflected wave antinodes closely correspond with forwarding wave antinodes, one would expect that the impact of wave reflection that occurs centrally is closely indexed by BP at the brachial pulse. However, as will be discussed, this is indeed not the case. What are the determinants of wave reflection?

Through Newton's Laws of Motion (inertial effects and for every action, there is an equal and opposite reaction), increases in forwarding wave pressures usually result in increases in backwards-wave pressures. However, increases in wave reflection are also attributed to alterations in the vasculature, with increases in arteriolar tone and more proximal arterial vessel tone producing marked effects on wave reflection. Moreover, mainly through harmonic effects on oscillating waves, decreases in heart rate increase the amplitude of the reflected wave by reducing the wave frequency (Xiao *et al.*, 2018). Increases in aortic stiffness have frequently been cited as a major cause of increases in central arterial PP because of an enhanced speed of wave reflection (a stiffer aorta will convey pressure waves more rapidly). The argument is that this will cause an earlier return of reflected waves, and thus a greater pressure augmentation in central arteries. However, wave reflection *per se* is a major determinant of pressure augmentation and there is presently no evidence to support the notion that pressure augmentation mediates cardiovascular damage or events beyond the magnitude of wave reflection.

More recently, the role of wave re-reflection as a determinant of forward travelling pressures waves has been highlighted (Phan *et al.*, 2016). In this regard, the peak of the forward

travelling pressure wave shows an excess of pressure beyond that identified from the product of Q and Zc (Figure 1.1) (Phan *et al.*, 2016). This excess pressure is theoretically generated by re-reflected or “rectified waves” reflecting off structures in the proximal aorta and this pressure adds to the forward travelling pressure wave. Importantly, the amplitude of re-reflected waves will be determined by the extent of wave reflection, which as indicated in the previous paragraph, involves changes in distal arterial structure and function rather than in the aorta *per se*. Thus, a portion of the forward travelling pressure wave is generated by factors that have little to do with either Q or Zc. Consequently, to accurately assess the impact of aortic stiffness on central arterial pulsatile load, some of the excess pressure generated by wave re-reflection needs to be eliminated from the overall forward wave effects and the impact of the pressure generated by just Zc x Q at peak forward wave pressures determined (Figure 1.1.). Importantly, although it is well-accepted that pulsatile load is determined by several pressure waves, there are marked limitations in the ability of the brachial pulse to detect these effects. What are these limitations?

1.3.2.2 Are the determinants of pulsatile load adequately detected at the brachial pulse?

Under physiological conditions, mean arterial pressure (MAP) and DBP remain relatively constant along the arterial tree, whilst PP and thus SBP, progressively increase from the aorta to peripheral vessels. Pulse pressure in peripheral arteries including the brachial artery may be as much as 20-50% greater than central aortic PP and hence SBP may be up to 40 mm Hg higher in the brachial artery than in the aorta (Kroeker and Wood, 1955; Pauca *et al.*, 2001; Ohte *et al.*, 2007). The increase in PP from the aorta to peripheral arteries is referred to as PP amplification (Pauca *et al.*, 1992; Nichols *et al.*, 2011). These differences in central arterial and brachial PP render the brachial pulse limited in the ability to accurately index the adverse effects of central arterial pressures (PP and SBP). What are the mechanisms that explain PP amplification? In this regard, there are two factors which account for PP amplification and changes in PP amplification.

The progressive increase in PP and SBP from central to peripheral arteries is in part driven by a gradient in arterial stiffness and diameter between the proximal aorta and the peripheral arteries, an effect that generates an impedance mismatch between these vessels (Smulyan and Safar, 1997; Benetos *et al.*, 2010; O’Rourke and Nichols, 2005; Hashimoto and Ito, 2010; McEniery *et al.*, 2014).In this regard, the progression from central to peripheral arteries is associated with a smaller diameter and stiffer vessels, both of which will increase the impedance to flow. As impedance is increased, although this will have no effect on MAP,

which is largely determined by the diameter of arterioles, the pressure generated by pulsatile flow will increase and PP will be enhanced. Arteriosclerotic changes largely influence aortic as compared to more peripheral vessel stiffness and hence impedance to flow. Thus, as aortic stiffness increases in response to risk factors and age, the increase in PP that occurs centrally may not be produced to the same extent peripherally. Thus, central aortic PP will increase more than peripheral PP and PP amplification will decrease. As the impedance to flow in the proximal aorta is lowest at a younger age when elastin fibres are largely intact, PP amplification is most marked over this age. Thus, arteriosclerotic effects on aortic function in the young may translate into marked increases in central arterial pulsatile load with little impact on brachial BP. What is the alternative mechanism responsible for decreases in PP amplification?

The second reason why through PP amplification, brachial PP and SBP do not adequately index changes in central arterial PP and SBP is through the impact of reflection phenomena. As indicated in the aforementioned discussion, peripherally, reflected waves have a much greater impact on PP and SBP than centrally and hence one may reason that peripheral BP should closely index changes in wave reflection. However, it is now recognised that variations in central arterial backward wave pressures are determined largely by variations in wave reflection from the lower part of the body (Morgan *et al.*). In contrast, the impact of wave reflection on the peak pressure noted in the brachial pulse (first systolic shoulder) is derived from waves generated in the upper limb which contribute little to variations in central arterial backward wave pressures. As variations in wave reflection centrally are more closely related to central arterial than peripheral PP and SBP, an assumption is that variations in wave reflection from the lower part of the body differ markedly from those generated in the upper limb. Although reflected waves generated from the lower part of the body are transmitted outward to form a second systolic shoulder on the peripheral pulse, unlike the central arterial pulse where these reflected waves augment PP producing a second systolic shoulder that is higher than the first, in the peripheral pulse they do not augment PP and thus produce a second systolic shoulder that is lower than the first (Figure 1.2). Thus, those increases in backwards-wave pressures that contribute to central arterial PP do not faithfully translate into increases in brachial PP and brachial PP changes and thus do not closely index reflected wave pressure-induced increases in central arterial PP and SBP. Consequently, increases in central arterial backward wave pressures result in an enhanced aortic PP with little changes in brachial PP. Thus, PP amplification is also decreased when central aortic backward wave pressures are increased. As backward wave pressures increase linearly starting from a young adult age, increases in wave reflection over this age range may translate into marked increases in central arterial pulsatile load with little impact

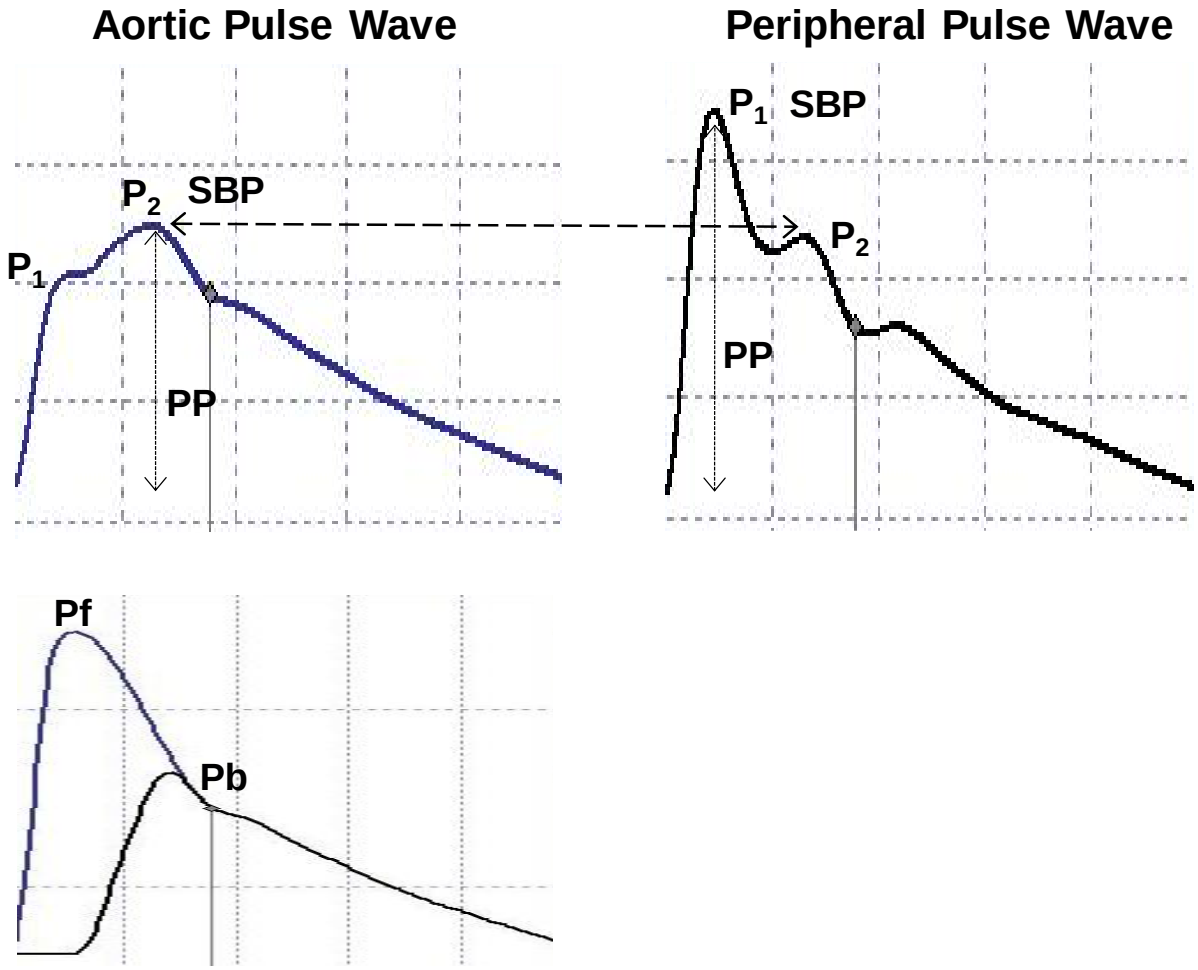

Figure 1.2. The aortic pulse wave compared to the peripheral pulse wave, showing pulse pressure (PP) amplification and the comparative first (P1) and second (P2) systolic shoulders. The wave components (forward [Pf] and backward wave pressures [Pb]) of the aortic pulse wave are shown in the lower-left panel. SBP, systolic blood pressure.

on brachial BP.

As a consequence of the understanding that reductions in PP amplification herald the adverse effects of increases in aortic stiffness and wave reflection, several studies have been performed to determine whether PP amplification acts as a risk marker. In this regard, even when PP measured in either the aorta or the peripheral arteries fails to predict cardiovascular events, PP amplification is able to do so (Benetos *et al.*, 2010; Bursztyn *et al.*, 2016; Regnault *et al.*, 2012; Safar *et al.*, 2002). Whether the effects noted in these studies (Benetos *et al.*, 2010; Bursztyn *et al.*, 2016; Regnault *et al.*, 2012; Safar *et al.*, 2002) could be attributed to increases in aortic stiffness or wave reflection is nevertheless uncertain. The question, however, is to what extent does arteriosclerosis-associated increases in aortic stiffness and hence, forward wave pressures account for increases in pulsatile load?

1.3.2.3 Contribution of forward wave pressures and the determinants thereof to pulsatile load

There is an ongoing debate as to the relative contribution of forward and backward wave pressures to variations in PP. Although in the same regression model backward wave pressures contribute markedly more to variations in central arterial PP than forward wave pressures (Booysen *et al.*, 2015), there has been a particular focus on age-related effects as these account for most of the PP changes in hypertension. In this regard, although some studies suggest that increases in backwards-wave pressures contribute more than forward wave pressures to age-related increases in PP and hence SBP (Cecelja *et al.*, 2009; Namasivayam *et al.*, 2009; Namasivayam *et al.*, 2016), this is not consistent with other studies, Indeed, several studies conducted in different population samples including the Framingham Heart Study sample and a community sample studied by our group in Africa, have provided clear evidence that forward travelling pressure waves are at least equally as important if not more important when the effect over the age of 50 years is considered (when most PP changes occur) (Mitchell *et al.*, 2010b). These data therefore raise the question of the extent to which arteriosclerotic-mediated aortic dysfunction contributes to this effect?

Importantly, the Framingham Heart Study attributed age-related increases in aortic PP and SBP to an enhanced Zc and not to increases in peak aortic flow (Q) (Mitchell *et al.*, 2010b). This effect was not associated with differences in aortic root diameter but was associated with age-related increases in carotid-femoral (aortic) pulse wave velocity (PWV), an index of stiffness across the full length of the aorta, rather than just in the proximal aorta (Mitchell *et al.*, 2010b). These effects are therefore likley to reflect an impact of ageing and risk factors

on arteriosclerosis. Additional studies support the view that age-related increases in PP are attributed to increases in forward travelling pressure waves through an enhanced Zc and not to Q (Mitchell, 2015; Segers *et al.*, 2007; Torjesen *et al.*, 2014). These data are further supported by findings in studies such as the Dallas Heart Study that hypertension-related increases PP and SBP are attributed to enhanced forward wave pressure through an impact on aortic stiffness and Zc (Goel *et al.*, 2017; Mitchell, 2015).Nevertheless, ISH has been reported to show an enhanced SV and hence increases in PP may also be attributed additionally to Q (McEniery *et al.*, 2005; Alfie *et al.*, 1999; Pasierski *et al.*, 1991). Moreover, in a recent paper accepted for publication in the journal *Hypertension* (Woodiwiss *et al.*, 2020), our group show that both Q and Zc are the principal determinants of age-related increases in PP and SBP in groups of African ancestry living in Africa. Thus, age-and hypertension-related increases in PP and SBP are indeed largely attributed to arteriosclerotic effects on aortic function, although in Africa and ISH in some populations an additional effect of Q contributes. Although Zc is a major determinant of age and hypertension-associated increases in PP, the role of changes in aortic root diameter on Zc have nevertheless been controversial. What is the evidence to support or refute a role for aortic root diameter?

The aorta is well recognised as dilating with age and is often noted on chest roentgenograms as “unfolding” of the aorta. It is argued that although an increased diameter reduces resistance to flow and hence should decrease Zc, increases in aortic diameter may also transfer the haemodynamic load to stiffer collagen fibres, thus resulting in an elevated Zc (O’Rourke and Nichols, 2005). However, using magnetic resonance imaging of the aorta, a reduced diameter at three aortic locations from the aortic root has been demonstrated to associate with an increased PP in older individuals above the age of 70 years (Torjesen *et al.*, 2014). Thus, the role of aortic diameter as a cause of increases in PP remains speculative and the fundamental mechanism responsible for increases in forward wave pressures and hence PP and SBP is therefore thought to be an increased Zc generated by an enhanced aortic stiffness. Irrespective of the relative impact of the determinants of pulsatile load on PP and SBP, a critical question is which of these factors produces cardiovascular damage? Is arteriosclerosis-associated aortic dysfunction a cause of cardiovascular damage and to what extent are these effects beyond brachial BP?

1.3.2.4 Contribution of arteriosclerosis-associated increases in pulsatile load to cardiovascular damage beyond brachial BP

Several studies have now produced data to show that a significant impact on end-organs of pulsatile load in central arteries is mediated by that wave component that is determined by an enhanced aortic stiffness (forward pressure wave) and that these effects are often beyond brachial BP. As will be discussed, however, there is similarly equally as important data to show largely backward wave effects, while forward wave effects were not detected, In this regard, in the Framingham Heart Study, a study largely conducted in groups of European ancestry, an enhanced forward but not backward wave pressure predicted cardiovascular events beyond brachial BP (Cooper *et al.*, 2015). Moreover, forward but not backward wave pressure was associated with left ventricular mass index (LVMI) in the Framingham sample (Kaess *et al.*, 2016). In contrast, in the Multi-ethnic Study in Atherosclerosis (MESA), backward wave pressures were noted to be more important than forward wave pressures in mediating cardiovascular events beyond brachial BP (Chirinos *et al.*, 2012; Zamani *et al.*, 2014). Furthermore, in the MESA, backward wave pressures were more important than forward wave pressures in associations with LVMI beyond brachial BP (Zamani *et al.*, 2015). Our group have similarly demonstrated a critical role for backward wave pressures as opposed to forward wave pressures in the development of several end-organ changes in a group of African descent beyond brachial BP (Booysen *et al.*, 2015; Sibiya *et al.*, 2015). Moreover, more recent evidence obtained by our group published in *Hypertension*, demonstrates that backward wave pressures rather than the pressure generated by the product of Q x Zc, Zc itself, or systemic vascular resistance (SVR) is the critical factor accounting for marked age-related BP effects on LVMI and diastolic function in a group of African ancestry and that these effects are beyond brachial BP(Bello *et al.*, 2020). However, also in recent work, we have demonstrated that while backward wave pressures contribute to several cardiac effects in groups of African descent, the major impact of forward wave pressures beyond brachial BP is on the vasculature and that these differential effects are age-dependent (Kolkenbeck-Ruh *et al.*, 2019a). Thus, the possibility that differential effects of forward and backward wave pressures may occur beyond brachial BP must be considered and that these effects are dependent on age, the end-organ effect identified, and the ethnic group involved also requires careful deliberation. Indeed, the events in the Framingham Heart Study, which were largely arterial in origin (a stroke or MI) were noted in those of European ancestry (Cooper *et al.*, 2015), while events in the MESA study were dominantly cardiac in origin (heart failure) and noted in a multi-ethnic sample (Chirinos *et al.*, 2012; Zamani *et al.*, 2014).

In summary, there is sound evidence to support a role for that pulsatile component of the central arterial pulse determined by arteriosclerotic effects (forward wave pressure) as a cause of cardiovascular damage beyond brachial BP. As PP amplification is greatest over a young adult age range, these data raise the question of how much arteriosclerotic damage is occurring in young adults as a result of risk factors, is not detected at the brachial pulse? In other words, are arteriosclerotic-induced increases in Zc and hence, forward wave pressures contributing to premature vascular events even when no detectable changes at the brachial artery are noted? In this regard, as premature arterial events are uncommon findings in high-income countries this question remains unanswered and in-part prompted me to explore this question in South Africa whereas previously indicated stroke and CLI are events that frequently occur over a young adult age range. Nevertheless, more recently the possibility that the adverse effects of arterial stiffness are mediated by effects beyond pulsatile load altogether (central or peripheral arteries) has also been considered. What is the evidence that the aortic stiffness determines cardiovascular damage beyond not only brachial BP, but also central arterial BP?

1.4 Aortic stiffness and cardiovascular damage beyond pulsatile load: Are these effects beyond even central arterial pulsatile load?

There is emerging evidence that abnormalities of aortic stiffness mediate cardiovascular damage beyond not only brachial BP but also central arterial BP. However, as will be discussed, whether these effects are entirely beyond central arterial pulsatility and thus not amenable to targeting by antihypertensive therapy, has not been fully resolved. What is the current evidence for an impact of aortic stiffness effects on cardiovascular damage beyond not only brachial BP but also central arterial BP?

1.4.1 Aortic stiffness as an independent determinant of cardiovascular damage: A possible effect in the young?

Most of the evidence to support a role for aortic stiffness as a possible cause of CVD beyond pulsatile loads has been derived from an indirect measure of arterial stiffness and that is carotid-femoral (aortic) pulse wave velocity (PWV). This current gold-standard assessment of aortic stiffness, evaluates the speed of pressure wave travel across the length of the aorta. According to the Moens-Korteweg equation [$PWV = \sqrt{(E \times h)/2r\rho}$], PWV is proportional to the square root of Young's elasticity modulus (E), given the constant ratio of the wall thickness, h, to vessel radius, r, and blood density, ρ (Gosling and Budge, 2003; Korteweg, 1878). The propagative model [$PWV\ (m/s) = D/\Delta t$] determines aortic stiffness by dividing the direct or indirect anatomical distance (D) between two arterial sites, femoral and common

carotid arteries, and the transit time (Δt) of the pulse between the two sites. Transit time is the difference in the period from the R-wave of the QRS complex generated simultaneously by an electrocardiogram, to the foot of the femoral versus carotid pressure waveforms (Laurent *et al.*, 2006; Van Bortel *et al.*, 2012; Nichols, 2005). What is the evidence that aortic PWV predicts events and is this independent of pulsatile load?

In meta-analyses of several major studies, aortic PWV has been shown to be an independent predictor (beyond brachial BP) of future cardiovascular events including MI, stroke, revascularisation and aortic syndromes, and all-cause mortality (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014). Importantly, these events are largely events considered arterial in nature. As a result of these data (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014), the European Society of Hypertension/European Society of Cardiology (ESH/ESC) guidelines for the management of arterial hypertension suggests the use of aortic PWV as a tool for assessment of subclinical target organ damage to enhance risk prediction (Mancia *et al.*, 2013; Van Bortel *et al.*, 2012). This measurement is in addition to brachial PP also employed as an index of arterial stiffness effects (Mitchell *et al.*, 2010a; Boutouyrie *et al.*, 2002; Laurent *et al.*, 2001; Blacher *et al.*, 1999; Mattace-Raso *et al.*, 2010) and hence the ability to predict cardiovascular events is seen as an impact that is beyond brachial BP (PP or SBP).Surprisingly, in a meta-analysis on aortic stiffness (PWV) and cardiovascular event prediction, the effect of PWV was noted over a younger adult age range (below 61 years of age) and even showed a trend for a greater effect [effect size: 2.04 (1.70-2.44)] over this age range than over an older age range [above 61 years of age; effect size: 1.43 (1.28-1.61)] (Ben-Shlomo *et al.*, 2014). Nevertheless, the number of events in the younger age group in the meta-analysis was too small(n=328) in comparison to the older age group (n=1,457) to draw meaningful conclusions as the original studies were performed in developed countries where arterial events seldom occur in the young. Thus, the role and frequency of arteriosclerotic causes of cardiovascular events within a younger adult age group is unknown. Importantly, the age threshold employed to identify “young” adults (Ben-Shlomo *et al.*, 2014) was such that most events could have occurred in those who were middle-aged, a finding that is not unexpected given the striking exponential increase in aortic stiffness from 50-60 years of age. As several major cardiovascular risk factors are independently associated with arteriosclerotic aortic functional changes (PWV) in younger individuals (Townsend *et al.*, 2015) the possibility that arteriosclerosis may contribute to events over a younger adult age range nevertheless requires consideration. Indeed, one study has reported on a high prevalence of arteriosclerotic vascular abnormalities (PWV) in stroke in the young (Saeed *et al.*, 2014), but failed to compare aortic functional changes with age-matched controls and did not report on all central arterial functional changes that

characterise arteriosclerotic effects. How then could PWV predict events beyond brachial PP and SBP?

One suggestion for the impact of aortic stiffness on cardiovascular disease beyond brachial BP is that aortic stiffness increases central arterial, but not necessarily brachial PP and SBP. In this regard, the argument is that because of the impedance mismatch between the aorta and peripheral vessels (see aforementioned discussion), aortic stiffness-induced increases in central arterial PP fail to faithfully translate into brachial BP effects, and hence can only be detected in central arteries. The question therefore arise as to whether central arterial BP predicts events beyond brachial BP? Indeed, reviews and meta-analysis show that central SBP or PP have better relations with cardiovascular target organ damage over brachial BP (Kollias *et al.*, 2016; Roman and Devereux, 2014). Moreover, in some studies central aortic as compared to brachial SBP and/or PP have been demonstrated to better predict cardiovascular outcomes (Lu *et al.*, 2001; Pini *et al.*, 2008; Roman *et al.*, 2007; Safar *et al.*, 2002; Vlachopoulos *et al.*, 2010; Williams *et al.*, 2006). However, in a meta-analysis, the ability of aortic PP or SBP to predict risk beyond peripheral PP or SBP failed to achieve significance (Vlachopoulos *et al.*, 2010). Moreover, the Framingham Heart Study failed to show an ability of aortic BP to predict events beyond brachial SBP despite an ability of PWV to predict events independent of brachial BP (Mitchell *et al.,* 2010b). Furthermore, in several other studies, whilst PP amplification better predicted cardiovascular mortality or events (Benetos *et al.,* 2010; Bursztyn *et al.,* 2016; Regnault *et al.,* 2012; Safar *et al.,* 2002), and heart disease (Salvi *et al.,* 2010) than brachial BP and added to brachial PP in risk prediction (Bursztyn *et al.,* 2016), these studies failed to show a better effect of central PP or SBP in risk predicting than brachial PP or SBP. Importantly, the extent to which aortic PWV predicts events beyond that component pulsatile pressure wave determined by aortic stiffness (forward travelling pressure wave) is unclear. Thus, the possibility still exists that an important effect of aortic stiffness beyond central arterial PP or SBP is mediated by that component of central pressures specifically determined by increases in aortic stiffness. Indeed, as indicated in Figure 1.1, the peak of the pressures generated by Zc (P_{QxZc}) precedes peak aortic PP and SBP, with the peak pressure being the summation of several pressure waves that are strongly influenced by wave reflection. As wave reflection varies according to heart rate and vascular tone, it is possible that the impact of forward wave pressures on peak aortic PP cannot be detected by snapshot measurements in a clinical setting as both heart rate and vascular tone show diurnal variations which differ markedly from individual to individual. Alternatively, in the generation of arterial damage, the impact of forward wave pressures may be independent of peak aortic PP and backward wave pressure effects. In this regard, peak aortic PP is produced by summation of forward wave

pressures with a multitude of backward wave pressures produced in the lower part of the body. In contrast, in distal arterial sites, PP is produced by summation of forward wave pressures with backward wave pressures produced only in that vascular bed. As shall be discussed, the forward travelling pressure wave may be particularly important in producing arterial damage in distal arterial sites when aortic stiffness enhances the transmission of pulsatile energy into these arterial sites by enhancing flow. Nevertheless, at present consideration must be given to the possibility that aortic stiffness mediates adverse effects beyond even peak central arterial BP. If this is so, then conventional thinking that events occur through arteriosclerotic changes only in the elderly or very elderly, requires reconsideration. How could central arterial stiffness mediate effects beyond both brachial and central arterial BP?

1.4.2 Mechanisms that may explain the impact of aortic stiffness on arterial events beyond central arterial pulsatile load

Several mechanisms may explain relations between central arterial stiffness and arterial events beyond even central arterial pulsatile load. One explanation is that as discussed in previous sections, the more elastic and distensible aorta is coupled with stiffer distal muscular arteries, creating an impedance gradient that increases progressively from the heart to periphery (Mitchell, 2018). This impedance gradient dampens the transmission of forward travelling waves into the distal arterial circulation and ultimately the microcirculation (Laurent *et al.*, 2006). This impedance gradient is particularly important for vascular beds that have pulsatile flow at a microcirculatory level including the brain and the kidneys (O'Rourke and Adji, 2010; Hashimoto and Ito, 2011; Mitchell, 2011; Woodard *et al.*, 2015; Webb *et al.*, 2012; Smulyan *et al.*, 2016; Cooper and Mitchell, 2016; Cooper *et al.*, 2016a; Cooper *et al.*, 2016b). In this regard, the high impedance of the more distal arteries prevents transmission of energy into the distal arteries and hence maintains pulsatile flow and hence pressure in these arteries at a minimum. However, with an increased aortic stiffness, the aortic characteristic impedance is enhanced, but the distal arterial impedance fails to increase to the same extent. The consequence is that the normal impedance mismatch that exists between the aorta and more distal vessels diminishes. Thus, pulsatile energy is transmitted into more distal arteries and pulsatile flow increases in distal arterial beds and at a microcirculatory level (O'Rourke *et al.*, 2010; Hashimoto and Ito, 2011; Mitchell *et al.*, 2011; Woodard *et al.*, 2015; Webb *et al.*, 2012; Cooper *et al.*, 2016b; Cooper and Mitchell, 2016; Smulyan *et al.*, 2016). The consequence is that irrespective of what aortic pulse pressure is, pulsatile flow and hence the pressure in more distal arteries is greater than normal with arterial damage being the effect. These effects have been suggested from

evidence obtained in several studies with the possibility of these effects noted for small vessel disease of the brain (Cooper *et al.*, 2016a; Mitchell *et al.*, 2011) and in the kidney (Hashimoto and Ito, 2011; Woodard *et al.*, 2015). Whether similar effects of a high pulsatile flow can explain atherosclerosis in more proximal arterial beds has not been identified. Coupled with an enhanced forward wave pressure generated by an aortic stiffness-induced increase in Zc, the augmented transmission of pulsatile energy would have more marked deleterious effects. Thus, increases in central arterial PP may indeed contribute to this effect, but not through an impact of peak PP, but rather through effects determined largely by forward wave pressures. Under physiological conditions, through autoregulatory effects an increased tone of resistance vessels in response to an increased PP, is predicted to be able to limit the transmission of pulsatility into the microcirculation of high-flow organs (Loutzenhiser *et al.*, 2006). If resistance vessels remodel in response to an elevated PP, then blood flow will further decrease (Mitchell, 2018; 2008). However, increased arterial stiffness and pulsatility are associated with microvascular lesions in high-flow organs, suggesting that the small resistance vessels in these organs may be damaged by the high pulsatile flow energy, blunting autoregulation of these vessels (Mitchell, 2018).

That an impedance mismatch may explain the impact of arteriosclerotic-induced aortic stiffness on cardiovascular events is an important conceptual issue concerning events at a younger age. In this regard, as indicated in the previous discussion, the aorta dilates with age and this in part compensates for age-related increases in aortic stiffness and hence Zc. If early aortic arteriosclerosis occurs, even minimal changes may produce a far greater effect on Zc at a younger age as compensatory aortic dilatation may not be present. Thus, even modest early arteriosclerosis in the young may have dramatic effects on the impedance mismatch. Consequently, the impact of several major cardiovascular risk factors on arteriosclerotic aortic functional changes in younger individuals (Townsend *et al.*, 2015) may have profound consequences for damage downstream in distal arteries. Although as indicated in aforementioned sections, one study has reported on a high prevalence of arteriosclerotic vascular abnormalities in stroke in the young (Saeed *et al.*, 2014), this study failed to compare aortic functional changes with age-matched controls and it is unclear whether the aortic functional changes also translated into parallel increases in central arterial pulsatile load (forward wave pressures as determined by Zc and peak aortic PP). Moreover, although major cardiovascular risk factors associate with arteriosclerotic aortic functional changes in younger individuals (Townsend *et al.*, 2015), it is unclear whether the impact is also on central arterial pulsatile load (forward wave pressures and hence peak aortic PP or forward wave pressures alone). In this regard, as argued above, a central question which remains unanswered is how does arterial stiffness increase without increases in central

arterial pressures? The possibility exists that aortic stiffness does increase the forward travelling pressure wave through effects on Zc, but that an ability to detect an impact on peak aortic PP through snapshot measurements in a clinical setting is difficult because backward wave pressure effects on peak aortic PP vary considerably throughout 24 hours. Thus, further studies are required to determine the central arterial pulsatile effects and the determinants thereof that occur with risk factor-related alterations in aortic stiffness and increases in stiffness in those with premature arterial events.

A second possibility that may explain an adverse effect of increases in aortic stiffness on arterial events beyond pulsatile load is through an impact on endothelial dysfunction, a well-recognised precursor of atherosclerosis. In this regard, several studies have demonstrated independent relations between aortic stiffness and endothelial dysfunction (Nigam *et al.*, 2003; Kobayashi *et al.*, 1996; Jadhav and Kadam, 2005; McEniery *et al.*, 2006; Wallace *et al.*, 2007; Soltész *et al.*, 2009; Kopeć *et al.*, 2009; Bruno *et al.*, 2012). Nevertheless, none of the above studies excluded an impact of pulsatile load in central arteries as an explanation for these relations. Thus, the effects of increases in aortic stiffness on endothelial function may simply reflect the effects of the aforementioned increases in central arterial PP. However, there are several lines of evidence from *ex vivo* studies that indicate that increases in arterial stiffness indeed decrease endothelial function independent of pressure effects. In this regard, even in the presence of exposure to comparable pulsatile pressures, cultured endothelial cells show marked abnormalities in function when they are plated on stiffer as opposed to less stiff conduits (Peng *et al.*, 2003). In addition, whilst collagen, which increases arterial stiffness has been demonstrated to directly activate endothelial cell adhesion molecules in the vascular wall, elastin, which improves aortic elasticity, enhances endothelial function (Orr *et al.*, 2009; González-Santiago *et al.*, 2002). Although *ex vivo* data support a view that endothelial dysfunction, which occurs at any age in the presence of risk factors, may be accentuated in the presence of increases in vascular stiffness without an impact of central pulsatile load, there is limited evidence for similar effects *in vivo*. Whether this mechanism applies *in vivo* therefore still requires further study. Moreover, how this effect could promote atherosclerotic effects in distal arteries largely unaffected by arteriosclerotic changes, is an unanswered question?

1.5 Could arteriosclerotic aortic dysfunction determine premature arterial events? Summary of the argument and the missing evidence

As highlighted in earlier sections of this chapter, the present thesis was conducted to better identify possible determinants of arterial events over a younger adult age, an age where

these events frequently occur in middle-income countries such as South Africa. As underscored in the present chapter, because PP and SBP, which are strongly determined by arteriosclerotic-induced increases in proximal aortic Zc only begin to increase at 50-60 years of age, a commonly held belief is that the impact of arteriosclerosis only begins to contribute to arterial events in late middle and old age. Several lines of evidence however suggest that arteriosclerotic aortic dysfunction may play a central role in the pathogenesis of events even over a younger adult age. First, as argued, brachial BP does not adequately detect abnormalities in central arterial pressure in the young and hence does not serve as an appropriate index of arteriosclerotic changes over the young adult age. Thus, marked arteriosclerotic changes may contribute to arterial events over a young age that would go undetected if only brachial BP were measured. Second, evidence has emerged over recent years to indicate that arteriosclerotic aortic dysfunction may mediate arterial damage through several mechanisms unrelated to peak pulsatile load even in central arteries. One particularly important mechanism that may play a role in the young is through an impedance mismatch between the aorta and more peripheral vessels (which increases the transmission of pulsatile energy). As over a young adult age, the aorta is not dilated, even modest arteriosclerotic changes in the aorta will have a marked effect on aortic impedance, thus decreasing the impedance mismatch and increasing the transmission of pulsatile energy into distal arteries. Third, there is evidence that several major cardiovascular risk factors associated with arteriosclerotic aortic functional changes (PWV) in younger individuals (Townsend *et al.*, 2015) effects that may have profound consequences for damage downstream in distal arteries. Fourth, one study has reported on a high prevalence of arteriosclerotic vascular abnormalities (PWV) in stroke in the young (Saeed *et al.*, 2014). However, there are several lines of evidence that are missing that would provide a more convincing argument for the role of arteriosclerotic aortic dysfunction in arterial events over a young adult age. What are these issues?

First, whether cardiovascular risk factors that associate with aortic functional changes (PWV) in younger individuals (Townsend *et al.*, 2015) are indeed also associated with increases in central arterial pulsatile load (the forward travelling pressure wave) despite a minimal impact on either brachial or peak central arterial PP, is unknown. This has important implications as if the effect is indeed on central arterial pulsatile load (forward wave pressures), this renders the adverse effect potentially susceptible to central arterial BP reduction with current antihypertensives and/or identifies a possible additional risk marker of the adverse effects of arteriosclerosis. Moreover, the study reporting on a high prevalence of arteriosclerotic vascular abnormalities (PWV) in stroke in the young (Saeed *et al.*, 2014) failed to compare these data with an age-matched control group across the adult lifespan; only assessed

arterial function in a single arterial event (stroke) and did not assess the extent to which these effects were associated with alterations in central arterial pulsatile load and the determinants thereof (Zc and forward wave pressures as well as peak aortic PP). Thus, whether arteriosclerotic changes do indeed possibly contribute to arterial events over a younger adult age and whether these events are potentially preventable by targeting central arterial BP with current antihypertensives, is unknown. Moreover, there are limited data to show whether PWV effects are indeed independent of the central arterial pulsatile effects determined by Zc, that is the pressure generated by the product of Q and Zc. In this regard, if forward wave pressure effects produced by the pressures generated by increases in Zc are indeed simply an alternative index to PWV of central arterial stiffness, then it is likely that targeting central arterial pulsatile load is of little consequence. However, if forward wave pressure effects produced by the pressures generated by increases in Zc associate with cardiovascular damage beyond PWV, then measuring these pressures may further improve risk prediction beyond even PWV, and may offer a guide to preventing the adverse effects of increases in central arterial stiffness by reducing central arterial pressures. The work described in the present thesis was therefore performed to in-part address these questions.

1.6 Aims

Thus, in the present thesis I aimed to determine the following:

1) Whether relations between modifiable conventional risk factors and indices of arteriosclerotic-induced increases in aortic stiffness (aortic PWV) and that component of central arterial pulsatile load determined by arteriosclerotic aortic stiffness (forward wave pressures) are adequately indexed by peak central arterial PP?
2) The extent to which across the full adult lifespan arteriosclerotic aortic dysfunction (PWV and Zc) and the effects on central arterial pulsatile load (forward wave pressures) associate with several arterial events (stroke and CLI) independent of brachial BP and peak central arterial PP in comparison to age-matched randomly selected controls in a middle-income country where events are often precipitous.
3) The extent to which the relationships between arteriosclerotic-induced increases in aortic stiffness and arterial end-organ measures and events are dependent or independent of alterations in that component of central arterial pulsatile load (forward wave pressures as indexed by the pressure generated by the product of Q and Zc) influenced by arteriosclerotic effects?

Chapter 2 Aortic Pulse Pressure does not Adequately Index Cardiovascular Risk Factor-Related Changes in Aortic Stiffness and Forward Wave Pressure

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2.0 Abstract

Background: Through the impact of conventional risk factors on arteries, several changes in aortic function contribute to cardiovascular events. It is nevertheless uncertain whether these effects are accurately reflected by changes in central aortic pulse pressure (PPc). We, therefore, aimed to determine the extent to which relations between modifiable risk factors and aortic function translate into increases in PPc.

Methods: In 1,232 black South Africans from the South Western Township (SOWETO) of Johannesburg, we determined risk factors and aortic function from carotid-femoral pulse wave velocity (PWV), aortic PPc, forward wave pressures (Pf), and reflected (backward) wave pressures (Pb) (applanation tonometry and SphygmoCor software).

Results: With adjustments for alternative risk factors and distending pressure (mean arterial pressure (MAP)), diabetes mellitus (treatment or HbA1c > 6.5%, n = 151) was associated with an increased PWV (7.10 ± 2.09 vs. 6.17 ± 2.00 m/s, P < 0.0001), and Pf (26 ± 8 vs. 24 ± 8 mm Hg, P < 0.005), but neither brachial PP (46 ± 14 vs. 45 ± 13, P = 0.19), PPc (36 ± 12 vs. 35 ± 11 mm Hg, P = 0.48), nor Pb (17 ± 6 vs. 17 ± 6 mm Hg, P = 0.83). Moreover, independent of alternative risk factors and MAP, uncontrolled hypertension (office blood pressure > 140/90 mm Hg, n = 433) was associated with an increased Pf (26 ± 12 vs. 24 ± 10 mm Hg, P < 0.01), but not with changes in brachial PP (45 ± 19 vs. 44 ± 17, P = 0.75), PPc (35 ± 16 vs. 35 ± 15 mm Hg, P = 0.93), or Pb (18 ± 8 vs. 17 ± 8 mm Hg, P = 0.46).

Conclusions: Neither brachial nor aortic PP are adequate indexes of relations between the modifiable conventional risk factors, uncontrolled hypertension or diabetes mellitus, and risk-related aortic functional changes.

2.1 Introduction

The adverse hemodynamic effects of blood pressure (BP) are mediated by both a steady as well as a pulsatile component of BP. Several aortic changes produced by modifiable conventional risk factors contribute to pulsatile hemodynamic effects beyond the steady component of BP, and consequently determine cardiovascular damage (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014; Wang *et al.*, 2010; Chirinos *et al.*, 2012; Weber *et al.*, 2012; Zamani *et al.*, 2014; Cooper *et al.*, 2015; Booysen *et al.*, 2015; Sibiya *et al.*, 2015). In this regard, an increased aortic stiffness enhances aortic characteristic impedance and thus forward wave pressures (Pf). Moreover, increases in the magnitude of aortic backward wave pressures (Pb) or the speed of wave reflection augment pulse pressure (PP). Although these aortic changes influence brachial PP, increases in aortic stiffness (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014), as well as Pf and Pb (Wang *et al.*, 2010; Chirinos *et al.*, 2012; Weber *et al.*, 2012; Zamani *et al.*, 2014; Cooper *et al.*, 2015; Booysen *et al.*, 2015; Sibiya *et al.*, 2015; Vlachopoulos *et al.*, 2010), predict events or associate with end organ changes independent of brachial PP. That these aortic functional changes predict events independent of brachial PP is attributed to amplification of PP from the aorta to the brachial artery, thus rendering brachial PP a poor index of aortic pressure pulsatility. However, there is also presently considerable uncertainty as to whether aortic rather than brachial PP adequately indexes aortic functional changes.

Although meta-analyses of a number of studies have demonstrated that aortic pulse wave velocity (PWV), an index of aortic stiffness, is a striking independent predictor of cardiovascular events (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014), meta-analyses of the impact of aortic PP on events has not produced similarly impressive effects (Vlachopoulos *et al.*, 2010). Furthermore, in the Framingham Heart Study, while aortic PWV (Mitchell *et al.*, 2010a) and consequent increases in Pf (Cooper *et al.*, 2015) were demonstrated to predict events, aortic PP failed to do so beyond brachial PP (Mitchell *et al.*, 2010a). An adverse effect of aortic stiffness on end-organs beyond central aortic PP (PPc) has, therefore, been postulated. In this regard, it is proposed that an increased aortic impedance enhances the transmission of energy into distal vessels (not necessarily through increases in aortic PP) by decreasing the impedance mismatch between these vessels (O'Rourke *et al.*, 2010; Hashimoto and Ito, 2011; Mitchell *et al.*, 2011; Webb *et al.*, 2012; Smulyan *et al.*, 2016). A limited ability of PPc to index risk-related changes in aortic PWV and Pf may be accounted for by the relatively greater contribution of Pb rather than Pf to variations in PPc (Booysen *et al.*, 2015). Presently there are no data from large studies to indicate whether independent associations between risk factors and aortic functional

changes beyond steady component pressures manifest with parallel increases in PPc. Hence, in the present study conducted in a large community-based sample, we aimed to determine whether relations between modifiable conventional risk factors and PWV and Pf independent of steady component pressures (mean arterial pressure [MAP]) are adequately indexed by PPc.

2.2 Methods

2.2.1 Study participants

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, 1,232 participants from randomly recruited (from the population census figures of 2001) families of black African descent (Nguni and Sotho chiefdoms) from the South Western Township (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years, were evaluated.

2.2.2 Clinical, demographic, and anthropometric measurements

A questionnaire was administered to obtain demographic and clinical data (Woodiwiss *et al.*, 2009; Norton *et al.*, 2012). Height and weight were measured using standard approaches and participants were considered to be overweight if their body mass index was ≥25 kg/m^2 and obese if their body mass index was ≥30 kg/m^2. Laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA1c) were performed. Diabetes mellitus was defined as the use of insulin or oral hypoglycemic agents or an HbA1c value greater than 6.5%. High-quality office brachial BP measurements were obtained according to guidelines in the seated position and after 5 minutes of rest, by a trained nurse-technician using a standard mercury sphygmomanometer (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. Uncontrolled hypertension was defined as office BP values >140 or 90 mm Hg systolic or diastolic BP, respectively.

2.2.3 Pulse wave analysis

Central aortic hemodynamics were estimated using pulse wave and wave separation analysis as previously described (Booysen *et al.*, 2015; Sibiya *et al.*, 2015; Woodiwiss *et al.*, 2009; Norton *et al.*, 2012). 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, TX) 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 generalised transfer function (GTF) 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 BP and diastolic BP. As SphygmoCor software employs a GTF to derive PPc (GTF-derived PPc), we also determined PPc from the second systolic shoulder of the radial pulse (P2-derived Pc) (Norton *et al.*, 2012). Aortic Pf and Pb 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).

Aortic 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.

2.2.4 Statistical analysis

For database management and statistical analysis, SAS software, version 9.4 (SAS Institute Inc., Cary, NC) was employed. Continuous variables were expressed as mean (SEM or SD). Dichotomous variables were expressed as proportions or percentages. Multiple regression analysis was performed to determine the independent relations between conventional risk factors and aortic function. Adjustments included in multivariate models were those associated with aortic function as well as for steady-state pressures or distending pressure effects on pulsatile hemodynamic parameters (MAP).

2.3 Results

2.3.1 Participant characteristics

The participant characteristics are given in (Table 2.1). A high proportion of participants were obese or had diabetes mellitus or hypertension. A significant percentage of hypertensives had uncontrolled hypertension despite a high proportion (around half) of these individuals receiving antihypertensive medication.

2.3.2 Risk factors independently associated with aortic function

In stepwise regression models, age, MAP, diabetes mellitus, and uncontrolled hypertension were independently associated with aortic Pf (Table 2.2). The use of HbA1c instead of a diagnosis of diabetes mellitus was not independently associated with Pf ($P = 0.28$). While diabetes mellitus (Table 2.2) or in separate regression models HbA1c ($P < 0.0001$) were independently associated with aortic PWV, with adjustments, including for MAP, uncontrolled hypertension was not (Table 2.2). A diagnosis of hypertension, irrespective of treatment, failed to independently associate with any aortic hemodynamic variable (data not shown). Importantly, only age and MAP were independently and directly associated with brachial and aortic PP and Pb (Tables 2.2 and 2.3). Neither a diagnosis of diabetes mellitus (Tables 2.2 and 2.3), nor HbA1c (brachial PP, $P = 0.54$; GTF-derived PPc, $P = 0.39$; P2-derived PPc, $P = 0.57$), were independently associated with either brachial or aortic PP. Neither body mass index, nor smoking were independently and directly associated with any hemodynamic variable (Tables 2.2 and 2.3).

2.3.3 Multivariate-adjusted changes in aortic function

Both diabetes mellitus (Figure 2.1) and uncontrolled hypertension (Figure 2.2) were independently associated with an increased multivariate-adjusted Pf. Moreover, diabetes mellitus (Figure 2.1), but not uncontrolled hypertension (Figure 2.2), was independently associated with PWV. Importantly, however, neither diabetes mellitus nor uncontrolled hypertension were independently associated with brachial and aortic PP, or Pb (Figures 2.1 and 2.2).

Table 2.1. Participant characteristics.

n	1,232
Age (years)	45 ± 19
Sex (% male)	35
Body mass index (kg/m2)	29.0 ± 7.4
% Overweight/obese	24/41
% Hypertensive	46
% Uncontrolled hypertension	35
% Treated for hypertension	25
% Diabetes mellitus	12
% Regular smokers	16
% Regular alcohol intake	22
Systolic blood pressure (mm Hg)	129 ± 22
Diastolic blood pressure (mm Hg)	84 ± 12
Total cholesterol (mmol/l)	4.63 ± 1.03
LDL cholesterol (mmol/l)	2.67 ± 0.88
HDL cholesterol (mmol/l)	1.41 ± 0.40
Brachial pulse pressure (mm Hg)	45 ± 16
Aortic SBP (mm Hg)	119 ± 22
GTF-derived PPc (mm Hg)	35 ± 15
P2-derived PPc (mm Hg)	36 ± 14
Forward wave pressures (mm Hg)	25 ± 9
Backward wave pressures (mm Hg)	17 ± 8
Aortic pulse wave velocity (m/s)	6.28 ± 2.60

Abbreviations: DBP, diastolic blood pressure; GTF, generalised transfer function; HDL, high-density lipoprotein; LDL, low-density lipoprotein; P_2, second systolic shoulder of the radial pulse; PPc, central aortic pulse pressure; SBP, systolic blood pressure.

Table 2.2. Independent relations between risk factors and aortic pulse wave velocity (PWV), forward wave pressures (Pf) or aortic backward wave pressures (Pb) in a community sample.

Risk factors vs→	PWV			Pf			Pb		
Risk factors	β-coeff.±SEM	partial r	p-value	β-coeff.±SEM	partial r	p-value	β-coeff.±SEM	partial r	p-value
Age	0.498±0.027	0.60	<0.0001	0.189±0.030	0.20	<0.0001	0.423±0.025	0.36	<0.0001
Male sex	0.051±0.026	0.04	=0.05	0.039±0.029	0.03	=0.17	0.047±0.024	0.03	=0.05
Mean arterial pressure	0.212±0.032	0.19	<0.0001	0.284±0.036	0.46	<0.0001	0.414±0.030	0.61	<0.0001
Uncontrolled hypertension	0.006±0.032	0.00	=0.85	0.126±0.036	0.09	<0.001	0.047±0.029	0.03	=0.11
Diabetes mellitus*	0.117±0.023	0.11	<0.0001	0.077±0.026	0.07	<0.005	0.005±0.021	0.00	=0.83
Smoking	-0.031±0.024	-0.03	=0.20	-0.022±0.026	-0.07	=0.42	0.023±0.023	0.02	=0.30
Alcohol	0.046±0.024	0.04	=0.05	-0.016±0.027	-0.01	=0.54	0.004±0.022	-0.00	=0.87
Body mass index	-0.073±0.027	-0.08	<0.01	-0.022±0.030	-0.02	=0.47	-0.093±0.025	-0.07	<0.0005

β-coeff., standardized β-coefficient. *diagnosed according to treatment or an HbA1c>6.5%.Heart rate was also included in models with PWV.

Table 2.3. Independent relations between risk factors and brachial or aortic pulse pressure (PP) in a community sample.

Risk factors vs→	Brachial PP			GTF-derived aortic PP			P_2-derived Aortic PP		
Risk factors	β-coeff.±SEM	partial r	p-value	β-coeff.±SEM	partial r	p-value	β-coeff.±SEM	partial r	p-value
Age	0.299±0.028	0.27	<0.0001	0.384±0.025	0.34	<0.0001	0.414±0.024	0.36	<0.0001
Male sex	0.018±0.027	0.01	=0.51	-0.023±0.024	-0.02	=0.37	-0.072±0.023	-0.06	<0.005
Mean arterial pressure	0.388±0.034	0.54	<0.0001	0.433±0.031	0.60	<0.0001	0.475±0.029	0.64	<0.0001
Uncontrolled hypertension	0.036±0.033	0.02	=0.28	0.024±0.030	0.02	=0.43	0.010±0.028	0.00	=0.72
Diabetes mellitus*	0.032±0.024	0.03	=0.19	0.016±0.022	0.01	=0.48	0.021±0.021	0.02	=0.32
Smoking	-0.018±0.026	-0.02	=0.48	0.014±0.023	0.01	=0.55	0.027±0.027	0.02	=0.22
Alcohol	-0.010±0.025	-0.01	=0.67	-0.016±0.022	-0.01	=0.49	-0.007±0.021	-0.00	=0.74
Body mass index	-0.038±0.028	-0.04	<0.02	-0.059±0.026	-0.04	<0.05	-0.085±0.024	-0.05	<0.001

β-coeff., standardized β-coefficient. *diagnosed according to treatment or an HbA1c>6.5.

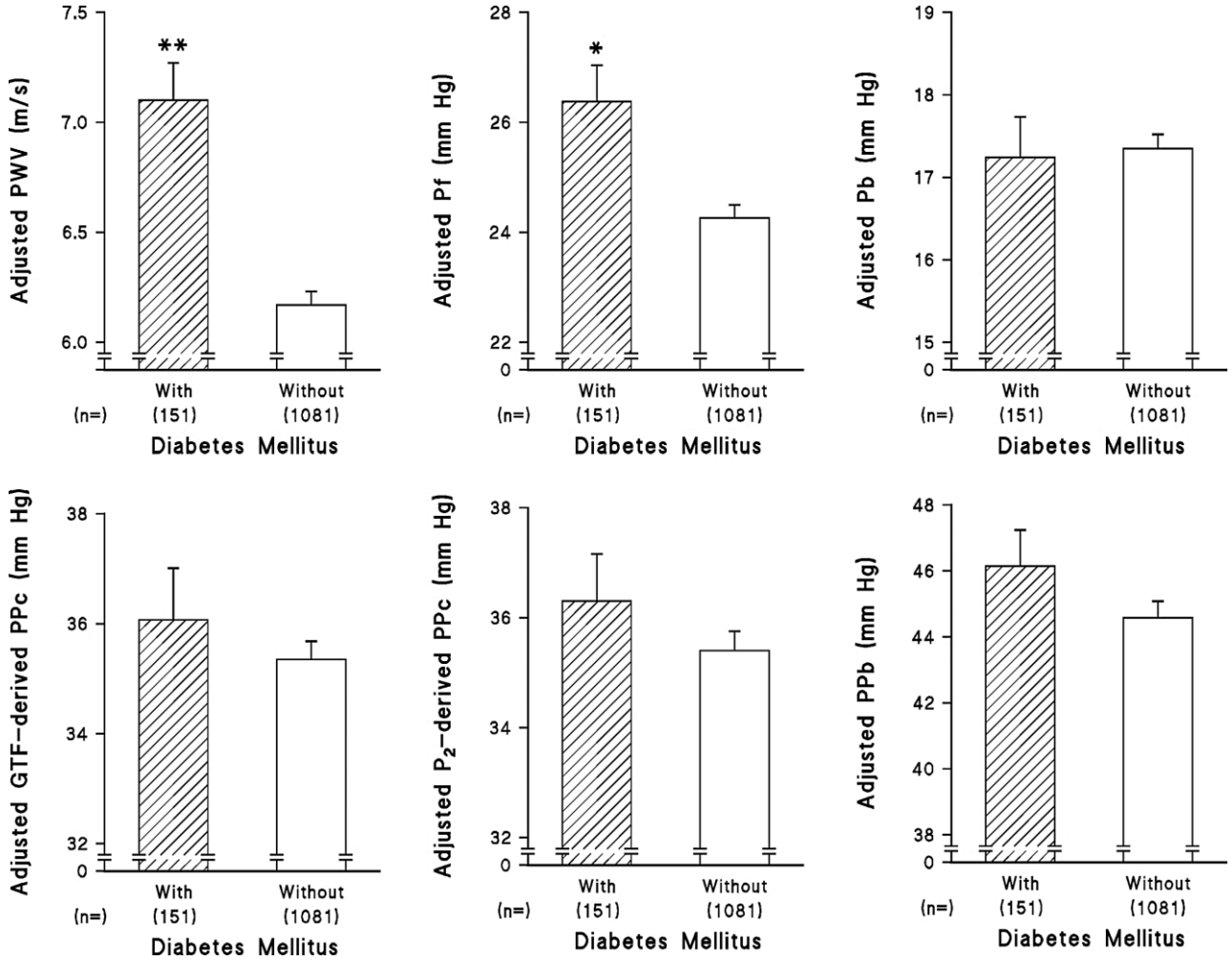

Figure 2.1. Multivariate-adjusted aortic and brachial hemodynamic parameters in patients with vs. without diabetes mellitus in a community-based sample. Adjustments are for age, sex, uncontrolled hypertension, mean arterial pressure, heart rate (for PWV only), body mass index, regular smoking, and regular alcohol intake. Abbreviations: Pb, backward wave pressures; Pf, forward wave pressures; PPb, brachial pulse pressure; PWV, aortic pulse wave velocity. GTF-derived PPc, central aortic pulse pressure derived from a generalised transfer function; P2-derived PPc, central aortic pulse pressure derived from the second systolic shoulder of the radial pulse. Data shown are multivariate-adjusted means and SEM. *P < 0.005, **P < 0.0001 vs. non-diabetics.

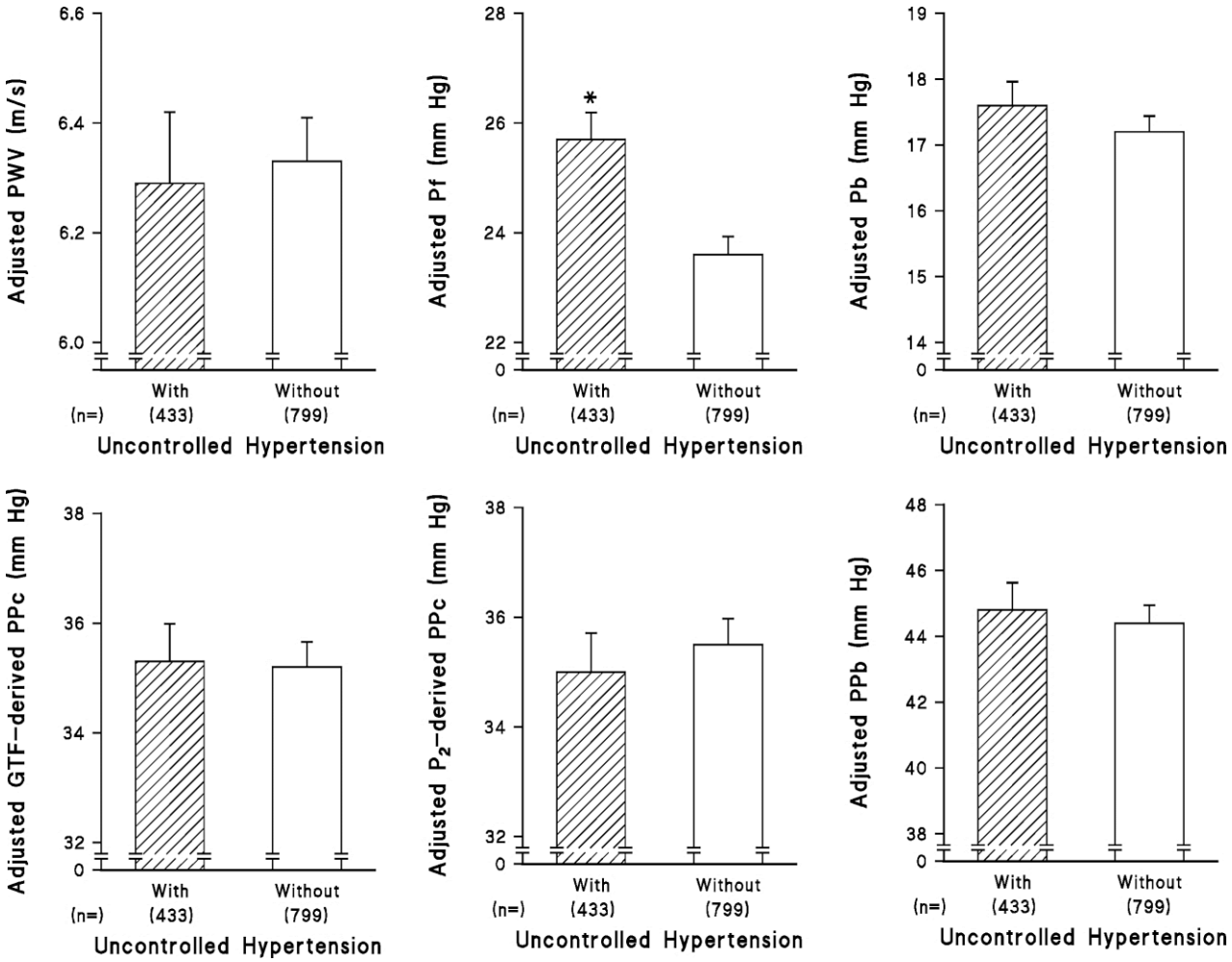

Figure 2.2. Multivariate-adjusted aortic and brachial hemodynamic parameters in patients with vs. without uncontrolled hypertension in a community-based sample. Adjustments are for age, sex, diabetes mellitus, HbA1c, mean arterial pressure, heart rate (for PWV only), body mass index, regular smoking, and regular alcohol intake. Abbreviations: Pb, backward wave pressures; Pf, forward wave pressures; PPb, brachial pulse pressure; PWV, aortic pulse wave velocity. GTF-derived PPc, central aortic pulse pressure derived from a generalised transfer function; P2-derived PPc, central aortic pulse pressure derived from the second systolic shoulder of the radial pulse. Data shown are multivariate-adjusted means and SEM. *P < 0.0005 vs. those with a controlled blood pressure.

2.4 Discussion

The main findings of the present study are as follows: In a large, representative, community-based sample with a high prevalence of modifiable conventional risk factors, independent of distending pressures (MAP) and additional confounders, both diabetes mellitus and uncontrolled hypertension were associated with aortic Pf, but not with either brachial or aortic PP. Importantly, neither diabetes mellitus nor uncontrolled hypertension were associated with aortic Pb.

It is well accepted that because of aortic-to-brachial PP amplification, brachial PP is an inadequate index of the adverse hemodynamic effects of changes in aortic function. More recently, however, the value of aortic as opposed to brachial PP, as an appropriate index of aortic dysfunction, has been questioned (Vlachopoulos *et al.*, 2010; Mitchell *et al.*, 2010a). In this regard, in the present study, we show that the 2 major modifiable risk factors for aortic dysfunction, uncontrolled hypertension and diabetes mellitus, despite being independently associated with aortic PWV (diabetes mellitus) and Pf (diabetes mellitus and uncontrolled hypertension), were not independently associated with aortic PP. These findings are in-part in agreement with studies that show that while aortic PWV (Mitchell *et al.*, 2010a), Pf (Cooper *et al.*, 2015), or PP amplification (Benetos *et al.*, 2010; Regnault *et al.*, 2012; Benetos *et al.*, 2012; Bursztyn *et al.*, 2016) may predict events, in these same studies (Benetos *et al.*, 2010; Regnault *et al.*, 2012; Benetos *et al.*, 2012; Bursztyn *et al.*, 2016; Cooper *et al.*, 2015) aortic PP failed to predict events beyond brachial PP. Hence, taken together, the results of the present and previous (Benetos *et al.*, 2010; Regnault *et al.*, 2012; Benetos *et al.*, 2012; Bursztyn *et al.*, 2016; Cooper *et al.*, 2015; Mitchell *et al.*, 2010a) studies suggest that risk-related changes in aortic function are poorly indexed by alterations in not only brachial PP, but aortic PP as well.

What is the possible explanation for the lack of impact of diabetes mellitus-induced increases in aortic PWV and risk factor-related increases in aortic Pf on aortic PP? In this regard, aortic PP is determined by forward and backward component waveforms. When the impact of these component waveforms on aortic PP is assessed in the same regression models in the present study sample, the aortic Pb has previously been noted to contribute substantially more than the Pf to variations in aortic PP (Booysen *et al.*, 2015). Moreover, aortic Pb have been demonstrated in the present study sample to largely account for brachial PP-independent associations between aortic PP and end organ measures (Booysen *et al.*, 2015; Sibiya *et al.*, 2015). Consequently, increases in aortic PP could better reflect the impact of an enhanced Pb effect, at least in the present sample. Thus, although

computational modeling suggests that most variations in aortic PP are determined by the effects of impedance and flow (Vennin *et al.*, 2017), changes which determine Pf, it is nevertheless possible that in the present sample aortic PP is less likely to accurately reflect Pf effects.

Characteristic impedance, a key determinant of Pf, is driven in-part by aortic stiffness which is indexed by aortic PWV. In the present study, although diabetes mellitus was independently associated with aortic PWV and hence Pf, uncontrolled hypertension was independently associated with Pf, but not aortic PWV. The question, therefore, arises as to whether increases in aortic stiffness account for relations between uncontrolled hypertension and Pf. Importantly, to eliminate the impact of steady and distending pressure effects on aortic stiffness, we adjusted for MAP. In associations between hypertension and aortic stiffness, this approach would in-part account for an uncontrolled BP and hence over-adjust for the impact of hypertension. Therefore, it is likely that in the present study, uncontrolled hypertension does indeed account for a high proportion of the variation in aortic PWV and that this in-part determines Pf, but that the impact was masked by over-adjustment in regression models. However, adjustments for MAP did not eliminate relations between uncontrolled hypertension and Pf. Consequently, at least some of the relationship between uncontrolled hypertension and aortic Pf could be accounted for by an impact of factors other than stiffness along the length of the aorta. In this regard, it is possible that mainly proximal aortic stiffness, not measured in the present study, better accounts for changes in Pf (Bell *et al.*, 2015). Alternatively, uncontrolled hypertension may contribute to alterations in aortic diameter and aortic diameter may influence characteristic impedance and hence Pf (Torjesen *et al.*, 2014). Further studies are, therefore, warranted to assess whether Pf effects produced by uncontrolled hypertension or diabetes mellitus are accounted for more by proximal aortic stiffness rather than stiffness over the full length of the aorta, or by alterations in aortic diameter.

The present study was conducted in one ethnic group. Importantly, in apparently healthy individuals of the present community sample, markedly higher aortic augmentation indexes have been noted as compared to alternative communities around the world (Chirinos *et al.*, 2011). Although aortic augmentation index is influenced by several variables, a strong determinant is aortic Pb. As variations in aortic PP in the present community sample are strongly governed by aortic Pb (Booysen *et al.*, 2015), the inability of aortic PP to index the impact of conventional risk factors on Pf or the determinants thereof may be a finding specific to the present community. In contrast, in alternative ethnic groups in which backward waves may not contribute as much to variations in aortic PP as in the present population,

aortic PP may adequately index the adverse effects of Pf or the determinants thereof. Further studies in alternative ethnic groups are, therefore, required to evaluate this hypothesis.

There are several alternative 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. Second, the use of the "triangulation method" of aortic wave separation to derive aortic Pf and Pb has been questioned (Kips *et al.*, 2009). However, relations between diabetes mellitus and aortic PWV failed to translate into similar relations between PWV and aortic PP. Thus, irrespective of the limitations of deriving Pf, in the present study, the conclusion that aortic PP does not adequately reflect aortic functional changes still holds. 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 Pf and Pb would have been similarly affected by this calibration error, and aortic Pf, but not Pb, were independently associated with uncontrolled hypertension and diabetes mellitus. Fourth, PWV measurements were obtained from pulse transit distance using subtracted distances (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), prior to the recommendation that a standardized approach of 80% of the direct carotid-femoral distance be used (Van Bortel *et al.*, 2012). Hence, our PWV values are marginally different from standard non-invasive measurements (Norman *et al.*, 2016). Fifth, SphygmoCor software employs an undisclosed GTF to derive aortic BP values. However, we also estimated aortic PP from the second systolic shoulder of the radial pulse (Norton *et al.*, 2012), which does not require the use of a GTF and using this approach we obtained essentially the same results as with GTF-derived aortic PP.

In conclusion, we show that in a large, community-based study with a high prevalence of hypertension and diabetes mellitus, that despite these risk factors being associated with several aortic functional changes, including aortic stiffness, and Pf independent of steady-state pressures and additional confounders, that similar relations with both brachial and aortic PP do not occur. Hence, these data support the view that neither brachial nor aortic PP adequately index aortic functional changes associated with cardiovascular risk.

Chapter 3 Marked Arterial Functional Changes in Patients with Arterial Vascular Events Across the Early Adult Lifespan

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3.0 Abstract

Objective: The age at which arteriosclerosis begins to contribute to events is uncertain. We determined, across the adult lifespan, the extent to which arteriosclerosis-related changes in arterial function occur in those with precipitous arterial events (stroke and critical limb ischemia [CLI]).

Approaches and results: In 1082 black South Africans (356 with either CLI [n=238] or stroke [n=118] [35.4% premature], and 726 age, sex and ethnicity-matched randomly selected controls), arterial function was evaluated from applanation tonometry and velocity and diameter measurements in the outflow tract. Compared to age and sex-matched controls, over 10-year increments in age from 20-60years, multivariate-adjusted (including steady-state pressures) aortic pulse wave velocity (PWV), characteristic impedance (Zc), forward wave pressures (Pf), and early systolic pulse pressure amplification (PPamp) were consistently altered in those with arterial events. Increases in Zc were accounted for by aortic stiffness (no differences in aortic diameter) and Pf by changes in Zc and not aortic flow or wave re-reflection. Multivariate-adjusted PWV (7.48±0.30 versus 5.82±0.15m/sec, $p<0.0001$), Zc ($p<0.0005$) and Pf ($p<0.0001$), were higher and early systolic PPamp lower ($p<0.0001$) in those with precipitous events than in controls. In comparison to age- and sex-matched controls, independent of risk factors, PWV and Zc ($p<0.005$ and <0.05) were more closely associated with premature events than events in older persons and Pf and early systolic PPamp were at least as closely associated with premature events as events in older persons.

Conclusions: Arteriosclerosis-related changes in arterial function are consistently associated with arterial events beyond risk factors from as early as 20 years of age.

3.1 Introduction

As opposed to atherosclerosis, arteriosclerosis is thought to largely reflect an ageing process that results in changes in the aorta that contribute to stroke, coronary artery disease and peripheral arterial disease, mainly in the elderly. In this regard, arteriosclerosis enhances aortic stiffness and increases characteristic impedance (Zc)(resistance to flow in a pulsatile system in the absence of wave reflection)(Nichols *et al.,* 2011).A greater aortic Zc amplifies aortic forward wave pressures (Pf) and therefore increases pulsatile load and decreases the impedance mismatch between the aorta and more distal vessels, allowing for a greater transmission of pulsatile energy into these vessels (O'Rourke *et al.,* 2010; Hashimoto and Ito, 2011; Mitchell *et al.,* 2011; Webb *et al.,* 2012; Smulyan *et al.,* 2016). An increased pulsatile load, or the transmission of pulsatile energy to either the cerebral, coronary or lower limb circulation, is thought to contribute to vascular damage and hence to stroke, coronary artery disease and peripheral arterial disease. The strong age-dependency of arteriosclerosis has rendered the view that it contributes to arterial events largely in the elderly. Although a number of major cardiovascular risk factors produce arteriosclerotic changes in younger individuals (Motau *et al.,* 2018; Townsend 2015), the extent to which arteriosclerosis contributes to arterial events over a younger age range is nevertheless uncertain.

Several age-related aortic hemodynamic changes (Namasivayam *et al.,* 2009; Mitchell *et al.,* 2010b; Namasivayam *et al.,* 2016; Hodson *et al.,* 2017; Hodson *et al.,* 2016), some of which are attributed to arteriosclerosis, contribute to vascular damage. In this regard, over a younger adult age there is a greater probability that the aortic hemodynamic alterations responsible for events are non-arteriosclerotic in nature. Indeed, aortic backward wave pressures (Pb) largely produced by wave reflection off arterial sites distal to the aorta, and which are therefore not caused by arteriosclerosis, increase with age across the full adult lifespan beginning in the late teenage years (Namasivayam *et al.,* 2009; Namasivayam *et al.,* 2016; Hodson *et al.,* 2017; Hodson *et al.,* 2016). In contrast, arteriosclerosis-related aortic changes (aortic stiffness and hence Pf) largely increase after the age of 50 years (Namasivayam *et al.,* 2009; Mitchell *et al.,* 2010b; Namasivayam *et al.,* 2016; Hodson *et al.,* 2017; Hodson *et al.,* 2016). Arteriosclerotic-induced increases in Pf therefore determine end organ effects mainly in those over 50 years of age(Kolkenbeck-Ruh *et al.*, 2019b). Consequently, although arteriosclerosis-related aortic functional changes predict arterial events (Vlachopoulos *et al.,* 2010b; Ben-Shlomo *et al.,* 2014; Cooper *et al.,* 2015), it is possible that non-arteriosclerosis-related aortic reflected wave changes, which also predict events (Wang *et al.,* 2010; Chirinos *et al.,* 2012; Weber *et al.,* 2012; Zamani *et al.,* 2014), are

the aortic functional changes responsible for chronologically premature events. However, aortic stiffness may predict events in younger as well as in older persons (Ben-Shlomo *et al.,* 2014), data nevertheless obtained in populations where few events are precipitous. As the role of arteriosclerotic changes in contributing to arterial events over a younger adult age range is unknown, in the present study we therefore aimed to determine the age at which arteriosclerotic-induced changes in arterial function begin to associate with arterial events and whether these changes are as marked at a younger as compared to an older adult age. The present study was conducted in a middle-income country where hospitalizations for events often occur in the 20-40 year age group (Connor *et al.,* 2009; Kolkenbeck-Ruh *et al.,* 2019b; Kolkenbeck-Ruh *et al.,* 2020). As capturing a significant number of precipitous events across the 20-40 year age group in longitudinal studies is difficult, we conducted a case-control analysis in a large study sample of hospitalized (case) and community-based (control) participants.

3.2 Materials and Methods

3.2.1 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=356) with stroke (n=118) or CLI (n=238) were recruited from the Charlotte Maxeke Johannesburg Academic Hospital, South Africa (Kolkenbeck-Ruh *et al.,* 2019b; Kolkenbeck-Ruh *et al.,* 2020). Inclusion was based on a diagnosis of either stroke or CLI and ethnic origins being of black African ancestry, and in order to avoid a selection bias, there were no exclusion criteria other than an inability to consent or to obtain consent from a family member in the case of a dysphasia. All patients had arterial function assessed from applanation tonometry (Norton *et al.,* 2012; Booysen *et al.,* 2015; Sibiya *et al.,* 2015) and 287 patients also had simultaneous high-quality velocity and diameter measurements in the outflow tract which allowed for more in-depth analysis (Mitchell *et al.,* 2010b; Segers *et al.,* 2017; Westerhof *et al.,* 1972; Murgo *et al.,* 1981). Patients with myocardial infarction (MI) were not included as MI most frequently occurs in older age groups in South Africans of African ancestry.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. Patients with

meningitis based on cerebrospinal fluid examination, or the presence of intracranial malignancy or intracranial mass lesions on imaging, and those with 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 vascular events were compared with data acquired over the same time-period in 726 age- and sex-matched randomly recruited participants older than 16 years of age of black African descent living in the South Western 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 ethnic origins and socioeconomic class as those living in the SOWETO community. All community participants had arterial function assessed from applanation tonometry and 390 also from simultaneous high-quality velocity and diameter measurements in the outflow tract (Mitchell *et al.,* 2010b; Segers *et al.,* 2017; Westerhof *et al.,* 1972; Murgo *et al.,* 1981).

3.2.2 Clinical, demographic, anthropometric and blood pressure measurements

A questionnaire was administered to obtain demographic and clinical information including each participant's medical history, the use of medication and tobacco and alcohol use. Clinical information, including brain imaging data, 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 obesity was defined as a body mass index (BMI) ≥30 kg/m^2. Blood tests were performed including a fasting glucose and lipid profile. Participants were considered to have diabetes mellitus if they had a fasting plasma glucose concentration ≥7 mmol/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≥140/90 mm Hg or those receiving antihypertensive medication were considered to have hypertension.

3.2.3 Central arterial hemodynamic assessments

Central arterial function was determined from pulse wave analysis (Namasivayam *et al.,* 2016; Hodson *et al.,* 2017; Norton *et al.,* 2012; Booysen *et al.,* 2015; Sibiya *et al.,* 2015) and from more in-depth analysis (Mitchell *et al.,* 2010b; Segers *et al.,* 2017; Westerhof *et al.,* 1972; Murgo *et al.,* 1981) conducted in 677 participants with additional aortic velocity and diameter assessments obtained in the outflow tract. 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 arterial waveform using a validated generalised 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 arterial PP (PPc) was determined as the difference between central arterial SBP and diastolic BP (DBP) and peak central arterial-to-brachial PP amplification as brachial PP/PPc. Whilst central arterial pressure waveforms were obtained, aortic velocity and diameter measurements were acquired by an experienced observer (AJW) in participants in the left lateral decubitus position using an Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.). Velocity waveforms were obtained in the 5-chamber view. Aortic diameter measurements were obtained just proximal to the aortic leaflets in the long axis parasternal view. The largest diameter recorded in early systole was employed to construct the aortic flow waveform (Mitchell *et al.,* 2010b). Carotid-femoral (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.

3.2.4 Aortic characteristic impedance and pressure wave components.

Aortic flow waveforms were generated from aortic velocity and diameter measurements. Taking care to avoid any overshoot of the image, the leading (outer) edge or the most dense, or brightest, portion of the spectral image of the velocity waveform was outlined using graphics software and using aortic diameter measurements employed to construct a flow waveform. Characteristic impedance (Zc) was calculated in the time domain as change in pressure/change in flow from the foot of the pulse wave up until 95% of peak flow (Mitchell *et al.,* 2010b; Segers *et al.,* 2017). Using Zc values and the flow and pressure waveforms, wave separation analysis was performed based on the following formulae: Forward wave pressure=(aortic PP + QxZc)/2 and Backward wave pressure=(aortic PP – QxZc)/2 (Cooper *et al.,* 2015; Westerhof *et al.,* 1972; Murgo *et al.,* 1981). An example of the waves generated from wave separation analysis is provided in Figure 3.1. Wave separation analysis was performed in the time domain base upon consensus that this analysis can be conducted

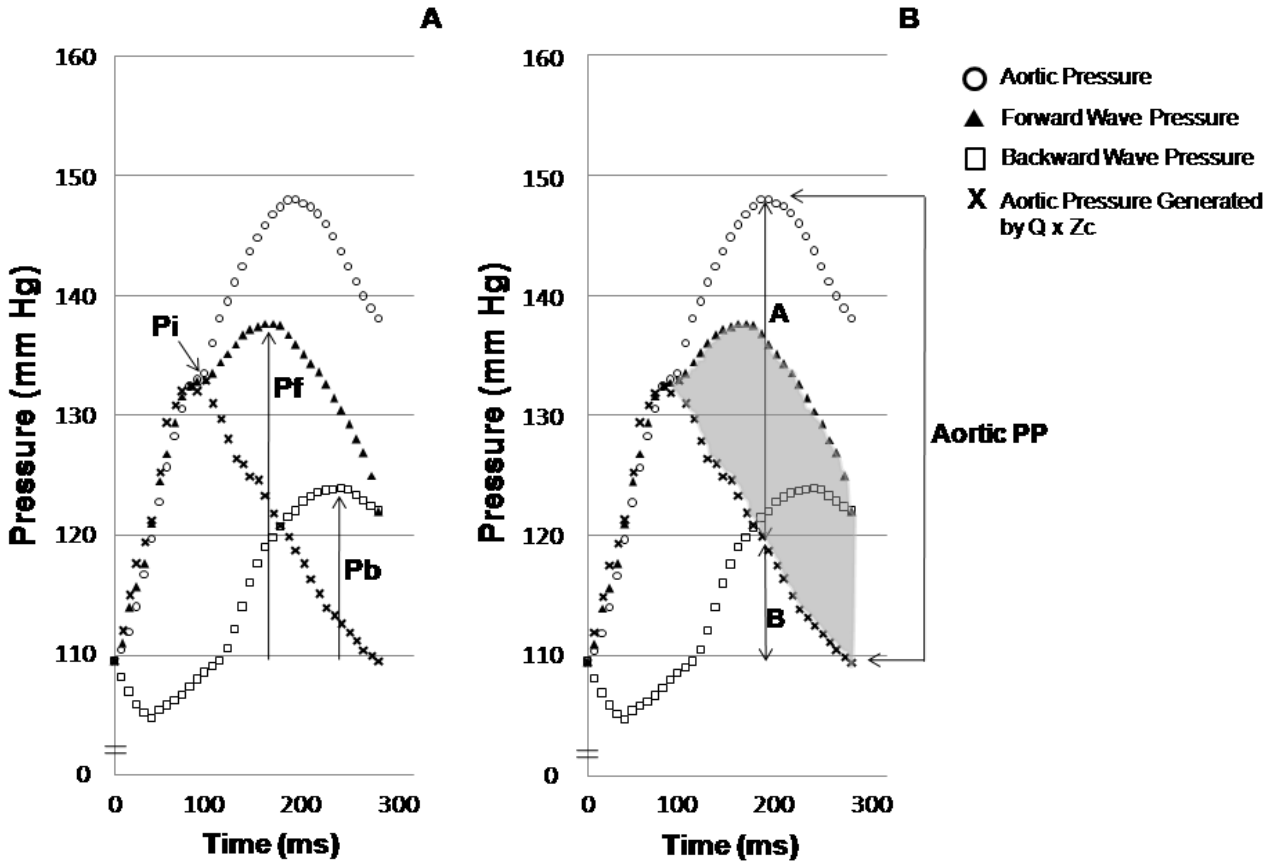

Figure 3.1. Wave components of aortic pulse pressure (PP) showing the forward and backward wave pressures (panel A) superimposed on the aortic pressure wave and the pressure waves generated by the product of flow (Q) and characteristic impedance (Zc) and wave re-reflection (panel B). The shaded area in panel B shows that component of the forward pressure wave that represents wave re-reflection. Waves are derived from wave separation analysis of original recordings as described in the text. Pb, peak backward wave pressure; Pf, peak forward wave pressure; A, reflected + re-reflected wave pressures; B, pressure generated by the product of aortic flow (Q) and characteristic impedance (Zc) at peak PP.

in either the time or the frequency domain (Segers *et al.,* 2017). To determine the contribution of the pressures generated by the product of aortic flow (Q) and Zc as opposed to wave re-reflection (Phan*et al.,* 2016) to Pf, the product of Q and Zc (P_{QxZc}) at peak aortic Pf was determined (Figure 3.1). To determine the contribution of wave re-reflection to aortic Pf (Phan *et al.,* 2016) the difference between Pf and P_{QxZc} at peak Pf was identified (Figure 3.1). As not all patients with events had high-quality velocity assessments in the left ventricular outflow tract, wave separation analysis was also conducted in both cases and controls using an assumed triangular flow wave and SphygmoCor software (Booysen *et al.,* 2015; Sibiya *et al.,* 2015). Where Pf and Pb were not available from wave separation analysis using an actual aortic flow wave, Pf and Pb were derived from data obtained assuming a triangular flow wave. To exclude the impact of alterations in central arterial reflection wave pressures on peak central arterial-to-brachial PP amplification, amplification was also determined from the ratio of brachial PP/the peak pressure generated by P_{QxZc} (early systolic PP amplification).

3.2.5 Statistical analysis

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 proportions or percentages. Age-related thresholds of central arterial function were derived from normotensive, non-diabetic participants from the community sample (n=648)(Table 3.1). As age-specific 75th percentiles did not differ substantially between men and women (Table 3.1), thresholds were identified from the age-specific 75th percentiles of both sexes considered together (Table 3.1). To compare conventional risk factors between cases and controls, comparisons of age and sex-adjusted data were made within each age category. Multiple logistic regression analysis was performed to determine the independent relations between central arterial function and vascular events. Multivariate-adjusted analysis of variance was employed to determine differences in arterial function between cases and controls. Adjustments were for age and sex, those factors associated with vascular events, and for distending pressure effects on pulsatile hemodynamic parameters (mean arterial pressure). Adjustments for age were within in each age category. Factors associated with events that were included in the models were hypertension, diabetes mellitus, smoking and HDL cholesterol concentrations. Odds ratios (OR) were compared using z statistics. The predictive accuracy of central arterial function in the detection of vascular events was determined from the area under (AUC) the receiver operator characteristic (ROC) curve. As PP amplification is inversely associated with cardiovascular damage, for comparative purposes with the impact of alternative

Table 3.1. 75th and 95th percentiles of aortic pulse wave velocity (PWV) across the adult lifespan in normotensive, non-diabetic persons from a randomly selected community sample of African ancestry (n=648).

	n=			75th percentiles			95th percentiles		
Age category	All	Women	Men	All	Women	Men	All	Women	Men
<20 years	83	47	36	4.99	4.81	5.21	6.04	6.04	6.05
20-29.9 years	230	145	85	5.40	5.40	5.40	6.20	6.06	6.43
30-39.9 years	126	84	42	5.90	5.90	5.92	7.18	6.90	7.59
40-49.9 years	98	74	24	6.16	6.16	6.21	7.79	7.79	8.11
50-59.9 years	63	42	21	7.07	7.07	6.94	9.64	9.07	9.64
60-69.9 years	30	21	9	8.05	7.96	8.05	9.77	9.62	9.77
≥70 years	18	8	10	9.20	8.05	11.51	13.20	9.69	13.20

parameters of aortic function on arterial events, PP amplification was expressed as a reciprocal value.

3.3 Results

3.3.1 Participant characteristics

The participant characteristics in broad age categories that define the presence of a premature versus late onset event are given in Table 3.2 and differences in the major conventional risk factors in more discrete age categories across the full adult age range in Table 3.3. Importantly, little difference was noted in the characteristics of participants with and without high-quality velocity measurements in the outflow tract and similar differences between cases and controls were noted in those with or without these measures (Table 3.4). 35.4% of the cases (21.3% stroke and 14.1% CLI) experienced premature arterial events. Irrespective of whether events were premature or occurred in older age groups, patients with vascular events had a greater prevalence of diabetes mellitus and more patients smoked (Table 3.2). A markedly higher proportion of cases with premature events as compared to controls had hypertension, but both cases and controls at an older age had a similarly high proportion with hypertension (Table 3.2). HDL cholesterol concentrations were lower in patients with vascular events (Table 3.2). Although LDL cholesterol concentrations were also lower in cases (Table 3.2), 86.6% of these patients were receiving lipid-lowering therapy at the time of obtaining fasting blood samples. Body mass index was also lower in cases as compared to controls in both younger and older age groups (Table 3.2). Of the patients with premature events 53.2% had one of the modifiable conventional risk factors, hypertension, diabetes mellitus or smoking, 34.9% had at least two of these risk factors and 3.2% had three of these risk factors.

3.3.2 Stroke subtypes

Of the patients admitted with stroke 10.7% were haemorrhagic in origin, 22.1% had a cardio-embolic cause (as assessed from clinical features and echocardiography); 17.6% were classified as small artery occlusion (confirmed with imaging) and 2.5% as large artery occlusion (atherosclerotic) (confirmed with CT angiography). Of the remaining strokes 67.7% were indeterminate in origin and 32.3% were from alternative aetiology. No differences in the proportion of patients classified according to the TOAST classification were noted in those with premature events as compared to at an older age (data not shown).

Table 3.2. Participant characteristics according to age categories that define the presence of premature or late onset events.

	Age<50 or 55 years[a]		Age≥50 or 55 years[a]	
	Controls (n=441)	Cases (n=126)	Controls (n=285)	Cases (n=230)
Strokes/CLI (n=)	-	76/50	-	42/188
Age (years)	39.9±9.8$^{\#\#}$	41.4±7.8††	63.9±8.3	64.9±8.9
Sex (% male)	46.5$^{\#\#}$	54.0†	61.1	69.1
Body mass index (kg/m^2)	29.0±7.6	27.3±7.3*	28.0±4.9	27.4±6.6
% Hypertensive	39.2$^{\#\#}$	80.9**	75.4	82.2
% Diabetes mellitus	3.9$^{\#\#}$	15.9**††	18.9	38.7**
% Regular smokers	22.2	35.7*	18.9	39.6**
% Regular alcohol intake	27.9	60.0**	26.7	50.3**
Systolic blood pressure (mm Hg)	126±17$^{\#\#}$	128±19††	142±22	138±22*
Diastolic blood pressure (mm Hg)	84±12$^{\#}$	79±14**	87±12	81±13**
Total cholesterol (mmol/l)	4.53±0.99$^{\#\#}$	3.94±1.07**	4.97±0.98	3.95±1.08**
LDL cholesterol (mmol/l)	2.58±0.80$^{\#\#}$	2.30±0.85*	2.89±0.86	2.22±0.94**
HDL cholesterol (mmol/l)	1.42±0.42$^{\#}$	1.06±0.39**	1.35±0.45	1.11±0.43**

[a]Refers to men < or ≥50 years of age or women < or ≥55 years of age. CLI, critical limb ischemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein. *p<0.05, **p<0.0005 vs controls. Adjustments are for age and sex within each age category. †p<0.01, ††p<0.0001 versus cases at an older age, $^{\#}$p<0.01, $^{\#\#}$p<0.0001 versus healthy controls at an older age.

Table 3.3. Comparison of major conventional risk factors across the full adult age range in participants with arterial events (cases=stroke and critical limb ischemia) versus controls.

	% Hypertensives	% DM	% Smokers	HDL cholesterol (mmol/l)
Age categories	Controls/cases	Controls/cases	Controls/cases	Controls/cases
1. <30 years	21.4/92.3***	1.0/15.4*	34.7/38.5	1.41±0.34/0.89±0.30***
2. 30 to 40 years	22.9/69.4***	2.4/12.8*	39.8/50.0	1.40±0.47/0.98±0.45***
3. 40 to 50 years	47.9/83.1***	2.6/16.9**	13.2/30.8*	1.43±0.46/1.12±0.36**
4. 50 to 60 years	64.5/85.2**	15.4/43.2***	17.2/44.4**	1.43±0.50/1.11±0.43***
5. 60 to 70 years	80.3/82.6	20.5/40.2**	15.0/46.7**	1.31±0.33/1.07±0.39***
6. ≥70 years	78.0/33.3	18.6/33.3	20.3/20.3	1.27±0.37/1.14±0.47*

DM, diabetes mellitus; HDL, high-density lipoprotein. *p<0.05, **p<0.005, ***p<0.0001 vs controls. Probability values are derived from comparisons of age and sex-adjusted data within each age category.

Table 3.4. Characteristics of participants with and without high-quality velocity measurements in the left ventricular outflow tract.

	Cases		Controls	
	With	Without	With	Without
Strokes/CLI (n=)	287	69	390	336
Age (years)	55±14	60±12**††	54±13	44±15††
Sex (% male)	61	72*†	57	47†
Body mass index (kg/m^2)	27.9±7.3	26.9±5.5*	28.4±6.3	28.8±7.1
% Hypertensive	82.5**	79.0**	56.5	49.0
% Diabetes mellitus	31.9**	27.0**	12.3	6.8†
% Regular smokers	38.5**	38.0**	22.6	17.7
% Regular alcohol intake	55.3**	44.2*	26.9	29.2
Systolic blood pressure (mm Hg)	127±24*	129±24†	132±21	128±22†
Diastolic blood pressure (mm Hg)	80±13**	82±14	86±12	85±12
Total cholesterol (mmol/l)	3.96±1.07**	3.88±1.10**	4.75±0.94	4.65±1.07
LDL cholesterol (mmol/l)	2.28±0.92**	2.13±0.87**	2.73±0.81	2.65±0.85
HDL cholesterol (mmol/l)	1.09±0.44**	1.06±0.33**	1.36±0.38	1.41±0.48

CLI, critical limb ischemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein. *p<0.05, **p<0.0005 vs controls; †p<0.05, ††p<0.0005 vs with. Probability values for comparison between cases and controls are derived from age and sex-adjusted data.

3.3.3 Age-related changes in arterial function

Across the early-to-middle-aged period of the adult lifespan, multivariate-adjusted aortic Zc, PWV, Pf and reciprocal of PP amplification (both peak aortic-to-brachial and early systolic aortic-to-brachial) (Figure 3.2), but not Pb, aortic PP or brachial PP (data not shown) were consistently increased in those with arterial events as compared to controls. Importantly, increases in Zc (Figure 3.2, panel B) were not accounted for by variations in aortic diameter (Figure 3.3, panel A). Moreover, increases in P_{QxZc} at peak aortic PP (Figure 3.2, panel D), but not Q or wave re-reflection (Figure 3.3, panels B and C respectively) accounted for the increases in Pf (Figure 3.2, panel C) noted in those with events.

3.3.4 Independent associations of arterial function with premature vascular events

Figure 3.4, and Tables 3.5 and 3.6 show multivariate-adjusted differences in aortic function between cases and controls in those experiencing premature events and in older age groups. Zc (Figure 3.4, panel B), PWV (Figure 3.4, panel A), Pf (Figure 3.4, panel C), the component pressure of Pf determined by the product of Q and Zc (P_{QxZc} at peak PP) (Figure 3.4, panel D) and reciprocal of PP amplification (both peak central arterial-to-brachial and early systolic central arterial-to-brachial) (Figure 3.4, panels E and F respectively), but not Pb or brachial PP (Table 3.5) were increased in those with premature arterial events. These increases in measures of arterial function in cases compared to controls, were similar irrespective of whether stroke or CLI were assessed (Table 3.6). Central arterial (aortic) PP was nevertheless only modestly increased in those with premature events (Table 3.5). At an older age however, Pf (Figure 3.4, panel C) and the component pressure of Pf determined by the product of Q and Zc (P_{QxZc} at peak PP) (Figure 3.4, panel D) as well as reciprocal of early systolic PP amplification (Figure 3.4, panel F), but neither PWV (Figure 3.4, panel A), Zc (Figure 3.4, panel B), Pb (Table 3.5), central arterial (aortic) PP (Table 3.5), brachial PP (Table 3.5), nor reciprocal of peak central arterial-to-brachial PP amplification (Figure 3.4, panel E) were increased in those with arterial events. Figure 3.5 shows the risk factor-independent relations (odds ratios) between aortic function and vascular events. Zc, PWV, Pf, the component pressure of Pf determined by the product of Q and Zc (P_{QxZc} at peak PP) and reciprocal of PP amplification (both peak central arterial-to-brachial and early systolic central arterial-to-brachial) (Figure 3.5, panels A and B), were independently associated with premature arterial events. Importantly, the relations between PWV, Zc or reciprocal of peak central arterial-to-brachial PP amplification and events were stronger for premature events as compared to for events in older age groups ($p<0.05$ to <0.005 for comparison of OR values) (Figure 3.5) and the relations between Pf, P_{QxZc} at peak P and early systolic central

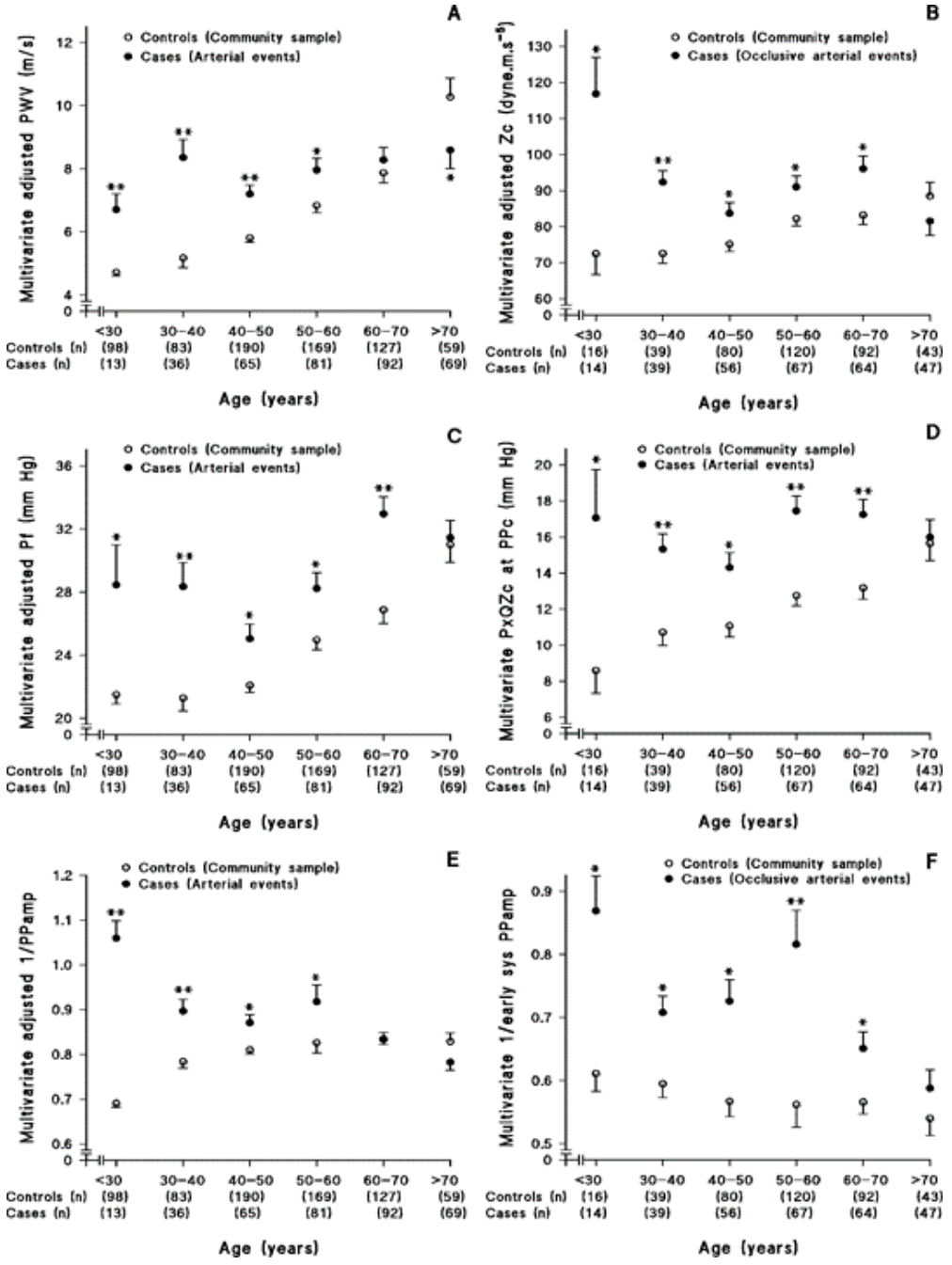

Figure 3.2. Multivariate-adjusted arteriosclerotic central arterial functional changes across the adult lifespan in cases with arterial events (stroke and critical limb ischemia) and ethnicity and age and sex-matched randomly selected community sample in Africa. Data shown are multivariate-adjusted means and SEM. Adjustments are for age in each age category, sex, hypertension, mean arterial pressure, diabetes mellitus, smoking and HDL cholesterol concentrations. PWV, carotid-femoral pulse wave velocity (panel A); Zc, proximal aortic characteristic impedance (panel B); Pf, forward wave pressures (panel C); PQxZc at PPc, contribution of pressures generated by the product of aortic flow (Q) and Zc at peak aortic PP (panel D); 1/PPamp, reciprocal of peak systolic central arterial to-brachial pulse pressure amplification (panel E); 1/early systolic PPamp, reciprocal of early systolic central arterial to-brachial pulse pressure amplification (panel F). *p<0.05, **p<0.0005 versus controls in each age category.

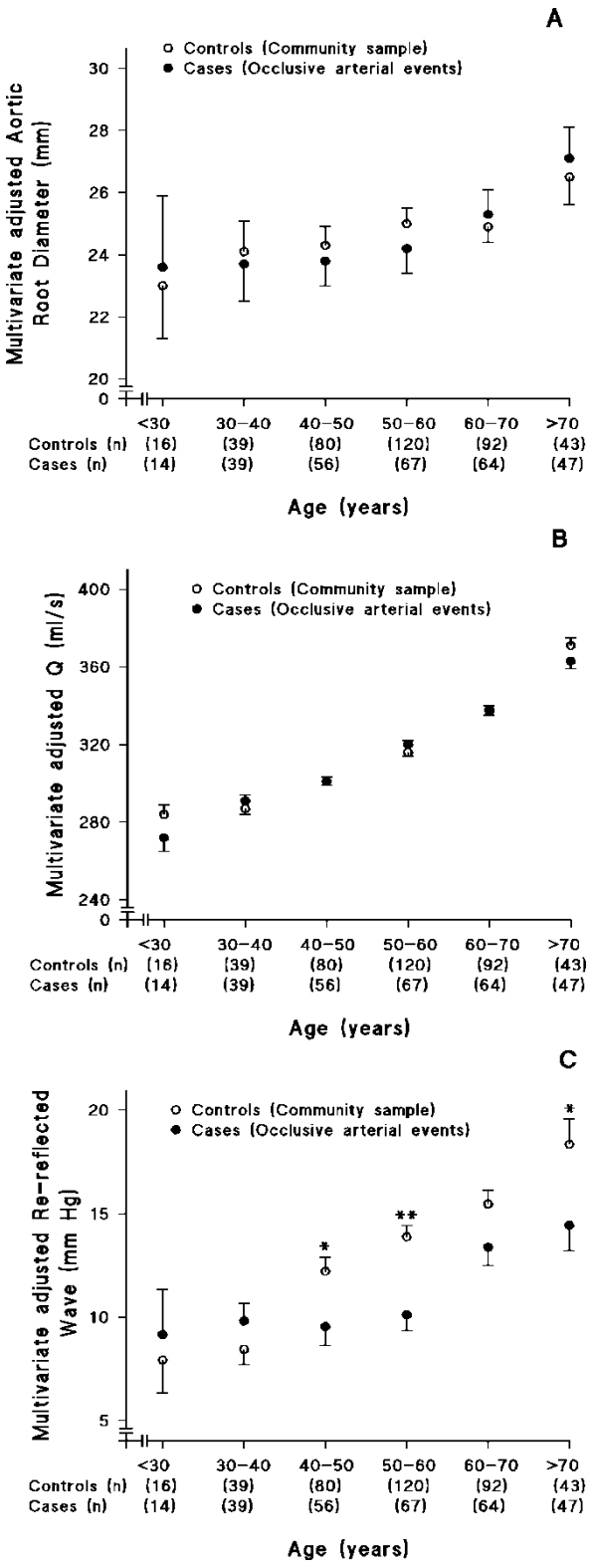

Figure 3.3. Multivariate-adjusted aortic root diameter (panel A), aortic flow (Q)(panel B) and the re-reflected pulsatile pressure component of aortic forward wave pressures (panel C) across the adult lifespan in cases with occlusive arterial events (stroke and critical limb ischemia) and ethnicity and sex-matched randomly selected community sample in Africa. Data shown are multivariate-adjusted means and standard error of the mean (SEM). Adjustments are for age in each age category, sex, hypertension, mean arterial pressure, diabetes mellitus, smoking and HDL cholesterol concentrations.*p<0.05, **p<0.0005 versus controls.

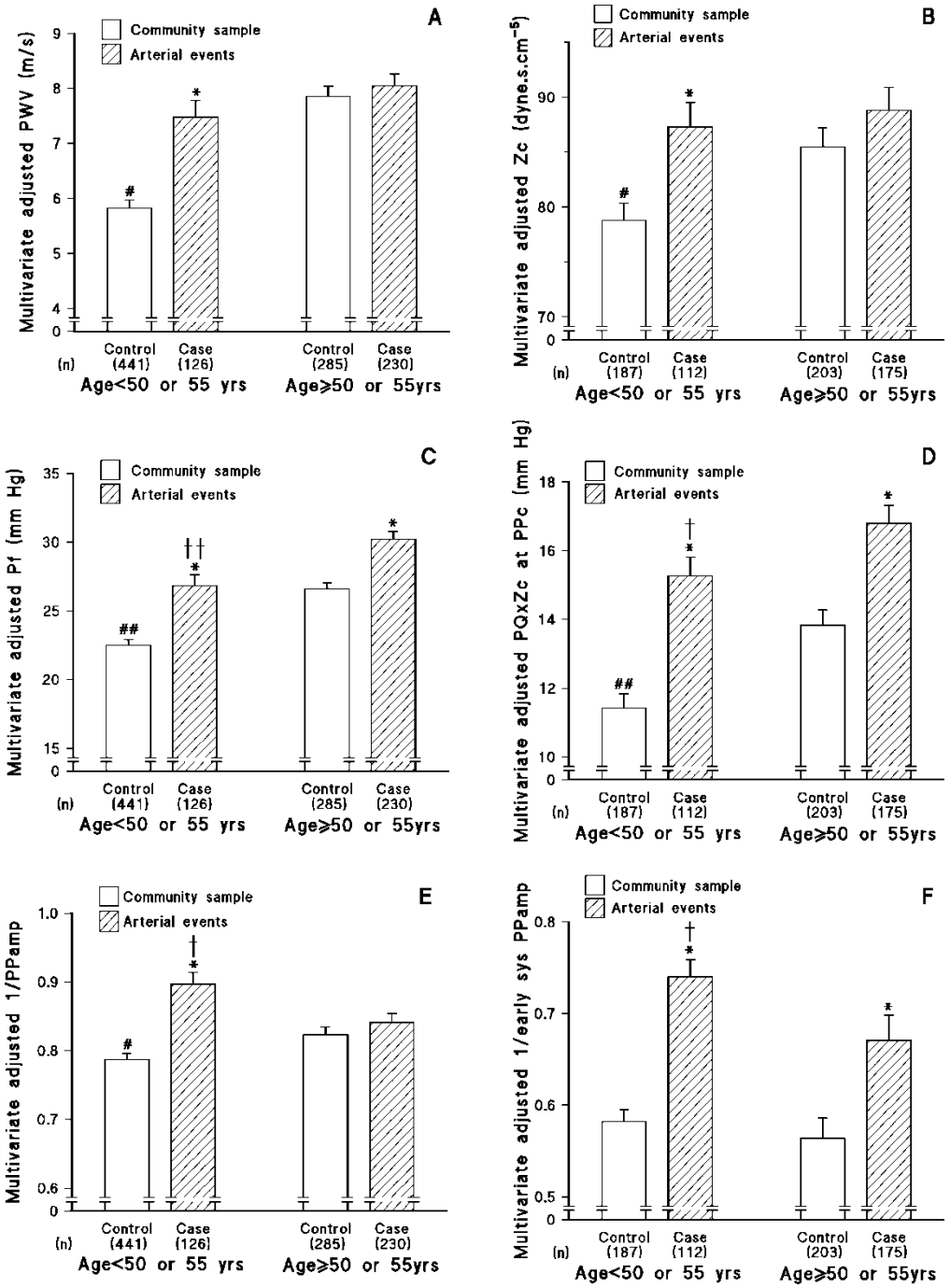

Figure 3.4. Multivariate-adjusted arteriosclerotic central arterial functional changes in those with premature arterial events (stroke and critical limb ischemia) (<50 and 55 years of age in men and women respectively), or arterial events at an older age as compared to age-, sex- and ethnicity-matched controls in Africa. Adjustments are for age in each age category, sex, hypertension, mean arterial pressure, diabetes mellitus, smoking and HDL cholesterol concentrations. PWV, carotid-femoral pulse wave velocity (panel A); Zc, proximal aortic characteristic impedance (panel B); Pf, forward wave pressures (panel C); P_{QxZc} at PPc, contribution of pressures generated by the product of aortic flow (Q) and Zc at peak aortic PP (panel D); 1/PPamp, reciprocal of peak systolic central arterial to-brachial pulse pressure amplification (panel E); 1/early systolic PPamp, reciprocal of early systolic central arterial to-brachial pulse pressure amplification (panel F).*p<0.0001 versus age and sex-matched controls, †p<0.005, ††p<0.0005 versus cases at an older age, #p<0.01, ##p<0.0001 versus healthy controls at an older age.

Table 3.5. Multivariate-adjusted pulse pressure and backward wave pressures in those with premature arterial events (stroke and critical limb ischemia) (<50 and 55 years of age in men and women respectively), or events at an older age as compared to age- and sex-matched controls in Africa.

	Age<50 or 55 years[a]		Age≥50 or 55 years[a]	
	Controls (n=441)	Cases (n=126)	Controls (n=285)	Cases (n=230)
Brachial pulse pressure (mm Hg)	42±1[#]	43±2[†]	50±1	53±1*
Central arterial pulse pressure (mm Hg)	33±1[#]	36±1*[†]	42±1	43±1
Aortic backward wave pressure (mm Hg)	16±1[#]	16±1[†]	21±1	20±1

[a]Refers to men < or ≥50 years of age or women < or ≥55 years of age. Adjustments are for age in each age category, sex, hypertension, mean arterial pressure, diabetes mellitus, smoking and HDL cholesterol concentrations. *p<0.05 versus respective age and sex-matched controls; [†]p<0.0001 versus cases at an older age, [#]p<0.0001 versus healthy controls at an older age.

Table 3.6. Multivariate-adjusted hemodynamic factors in those with premature (<50 and 55 years of age in men and women respectively) stroke or critical limb ischemia (CLI), or stroke or CLI at an older age as compared to age- and sex-matched controls in Africa.

	Stroke	Controls	CLI	Controls
		Age<50 or 55 years[a]		
n=	76	441	50	441
Aortic pulse wave velocity (m/sec)	7.49±0.24***	5.61±0.08	7.12±0.32***	5.64±0.09
Aortic forward wave pressures (mm Hg)	25±1**	22±1	27±1***	22±1
Aortic backward wave pressure (mm Hg)	15±1	15±1	16±1	16±1
n=	72	187	40	187
Aortic characteristic impedance (Zc)(dyne.s.cm^{-5})	87.2±2.8**	78.7±1.5	90.9±3.6**	78.3±1.4
Early systolic PP amplification	0.80±0.01***	0.58±0.01	0.72±0.02***	0.57±0.01
		Age≥50 or 55 years[a]		
n=	42	285	188 285	
Aortic pulse wave velocity (m/sec)	9.14±0.51	8.10±0.18	8.15±0.32	8.05±0.24
Aortic forward wave pressures (mm Hg)	30±1	28±1	32±1***	27±1
Aortic backward wave pressure (mm Hg)	19±1	22±1*	21±1	22±1
n=	37	203	138	203
Aortic characteristic impedance (Zc)(dyne.s.cm^{-5})	88.2±3.5	85.5±1.5	88.2±2.7	85.2±1.8
Early systolic PP amplification	0.74±0.02***	0.56±0.01	0,67±0.03*	0.56±0.02

[a]Refers to men < or ≥50 years of age or women < or ≥55 years of age. Adjustments are for age in each age category, sex, hypertension, mean arterial pressure, diabetes mellitus, smoking and HDL cholesterol concentrations. *p<0.05,**p<0.01,***p<0.0001 versus controls.

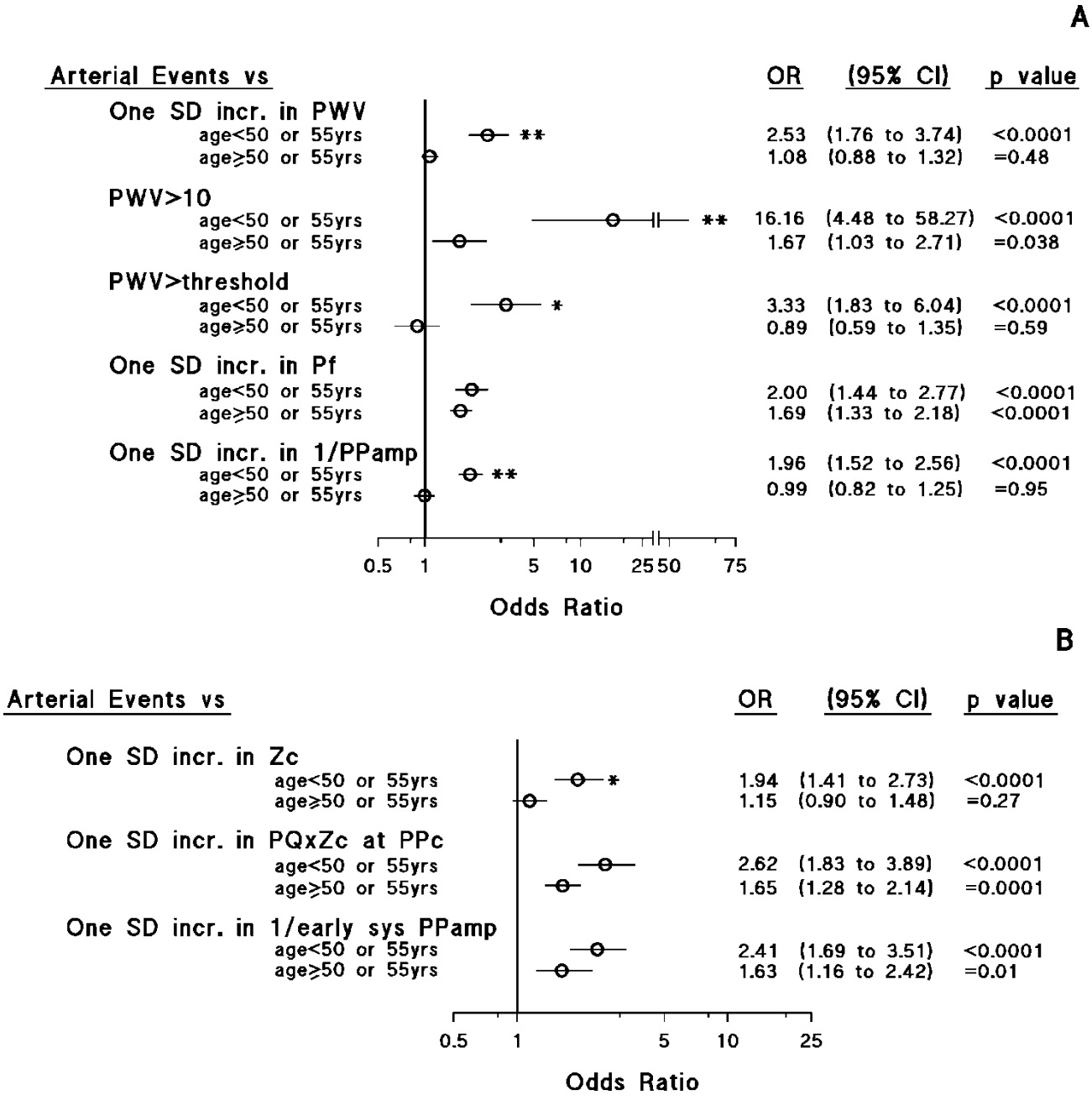

Figure 3.5. Multivariate-adjusted relations (odds ratios) between arteriosclerotic central arterial functional changes and either premature arterial events (stroke and critical limb ischemia) (<50 and 55 years of age in men and women respectively), or arterial events at an older age in Africa. Data are shown as one standard deviation (SD) effects or as PWV thresholds (>10 m/sec or age-specific). Adjustments are for age in each age category, sex, hypertension, mean arterial pressure, diabetes mellitus, smoking and HDL cholesterol concentrations. PWV, carotid-femoral pulse wave velocity (panel A); Pf, forward wave pressures (panel A); 1/PPamp, reciprocal of peak systolic central arterial to-brachial pulse pressure amplification (panel A); Zc, proximal aortic characteristic impedance (panel B); PQxZc at PPc, contribution of pressures generated by the product of aortic flow (Q) and Zc at peak aortic PP (panel B); 1/early systolic PPamp, reciprocal of early systolic central arterial to-brachial pulse pressure amplification (panel B). *p<0.05, **p<0.005 for comparison of odds ratio (OR) in those with premature events compared to those with events in older age groups.

arterial-to-brachial PP amplification and events were similar for premature events as for events in older age groups (Figure 3.5).

3.3.5 Relative contribution of aortic function versus conventional risk factors to associations with premature vascular events

In stepwise regression models, Zc, PWV, Pf or reciprocal of early systolic PP amplification independently contributed more than (comparison of standardized β-coefficients) diabetes mellitus or smoking, but less than hypertension to associations with premature vascular events (Table 3.7). Moreover, Zc, PWV, Pf or reciprocal of early systolic PP amplification independently contributed either more than (PWV, $p<0.0001$) or at least to a similar extent (Zc, Pf and reciprocal of early systolic PP amp) to premature events as to events at an older age (comparison of standardized β-coefficients) (Table 3.7).

3.3.6 Performance of aortic function for the detection of premature vascular events

Zc, PWV, Pf, the component pressure of Pf determined by the product of Q and Zc (P_{QxZc} at peak PP) and either reciprocal of peak central arterial-to-brachial or reciprocal of early systolic PP amplification showed a reasonable performance for the detection of premature vascular events (Figure 3.6). Importantly, PWV and reciprocal of peak central arterial-to-brachial PP amplification failed to show significant performance for event detection at an older age and the performance of PWV and reciprocal of peak central arterial-to-brachial PP amplification for event detection was markedly better for premature events than for events at an older age (Figure 3.6). Furthermore, Zc and reciprocal of early systolic PP amplification showed a better performance for event detection at a younger as compared to an older age (Figure 3.6).

3.4 Discussion

The main findings of the present study are as follows: First, 35.4% of the hospitalizations for the arterial events stroke and CLI, in those of African descent in a middle-income country were noted to be premature (<50 and 55 years of age for men and women respectively). Importantly, across the early-to-middle adult age range (including from 20-40 years of age), changes in multivariate-adjusted (including for steady-state pressures) PWV, Zc, Pf, the component pressure of Pf determined by the product of Q and Zc (P_{QxZc} at peak PP) and both reciprocal of peak central arterial-to-brachial and early systolic central arterial-to-brachial PP amplification (measures that index arteriosclerosis) were consistently noted in patients with arterial events.

Table 3.7. Relative contribution (standardized slopes [β-coefficients]) of arteriosclerotic central arterial functional changes versus conventional risk factors in associations with premature vascular events (<50 or 55 years for men and women respectively) or events occurring at an older age in Africa.

	Age<50 or 55 years[a]		Age≥50 or 55 years[a]	
Vascular event vs	β-coeff.±SEM	β-coeff.±SEM	β-coeff.±SEM	β-coeff.±SEM
Models with→	**PWV**	**Pf**	**PWV**	**Pf**
	cases=126, controls=441		cases=230, controls=285	
PWV	0.241±0.035**	-	0.033±0.043	-
Pf	-	0.152±0.035**	-	0.195±0.043**
Hypertension	0.413±0.037**	0.440±0.038**	0.106±0.043*	0.088±0.042*
Diabetes mellitus	0.059±0.033	0.077±0.034*	0.201±0.042**	0.177±0.041**
Smoking	0.112±0.032*	0.116±0.033*	0.263±0.041**	0.247±0.040**
HDL cholesterol	-0.188±0.033**	-0.196±0.034*	-0.193±0.041**	-0.193±0.040**
Models with→	**Zc**	**1/early systolic PP amp**	**Zc**	**1/early systolic PP amp**
	cases=112, controls=187		cases=175, controls=203	
Zc	0.160±0.007**	-	0.083±0.053	-
1/PP amplification	-	0.244±0.046***	-	0.146±0.048**
Hypertension	0.486±0.052***	0.441±0.051***	0.067±0.049	0.072±0.049
Diabetes mellitus	0.001±0.047	0.016±0.045	0.266±0.050***	0.258±0.050***
Smoking	0.123±0.046*	0.136±0.044**	0.270±0.049***	0.269±0.049***
HDL cholesterol	-0.242±0.048***	-0.238±0.045***	-0.170±0.050**	-0.178±0.050**

β-coeff., standardized β-coefficient. PWV, carotid-femoral pulse wave velocity; Zc, proximal aortic characteristic impedance; Pf, forward wave pressures; 1/early systolic PPamp, reciprocal of early systolic central arterial to-brachial pulse pressure amplification.[a]Refers to men < or ≥50 years of age or women < or ≥55 years of age. Also included in the regression models was age within each age category, sex and mean arterial pressure. *p<0.05, **p<0.005, ***p<0.0001 for relationship.

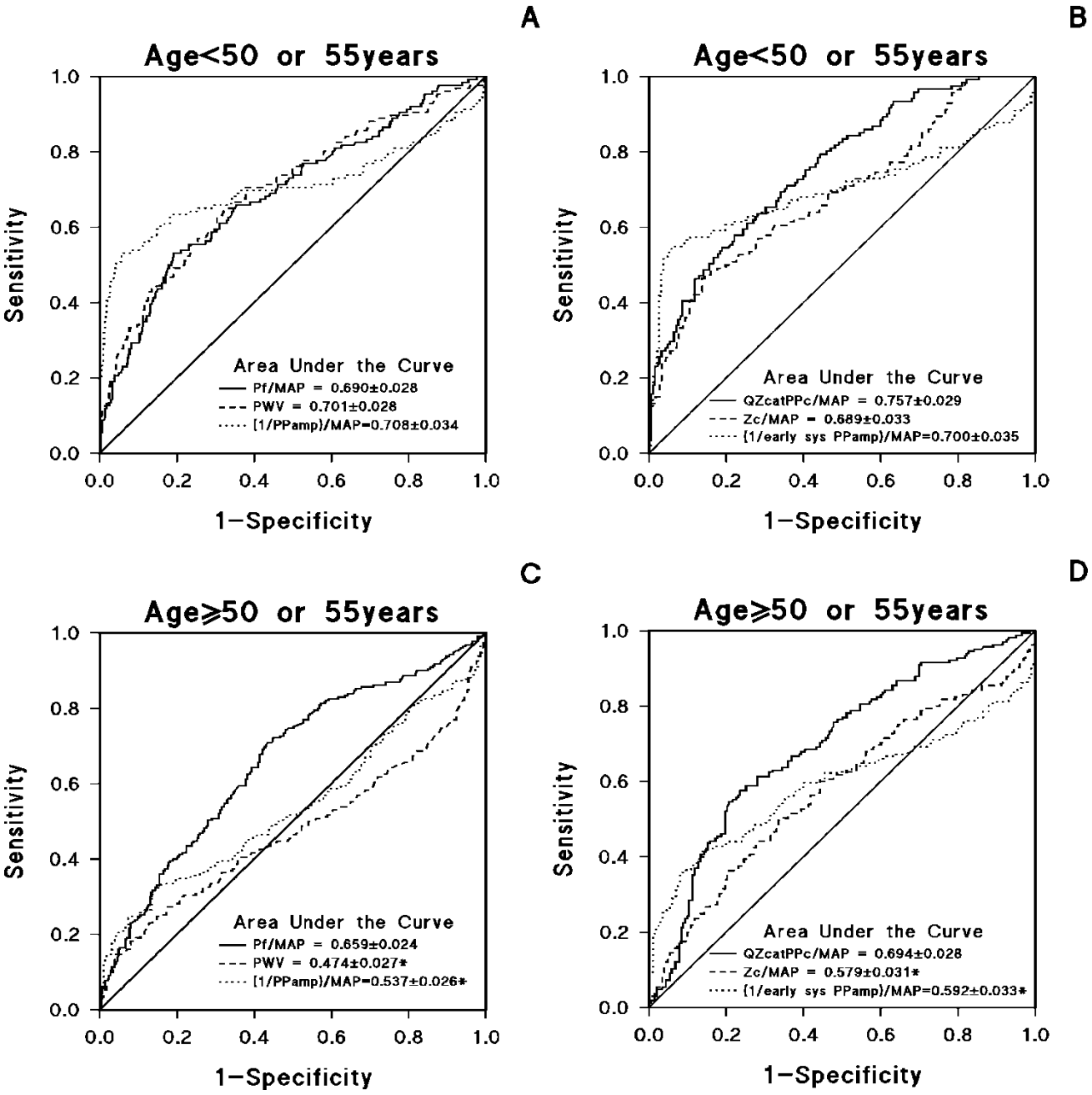

Figure 3.6. Receiver operator characteristic curves of arteriosclerotic central arterial functional changes for the detection of either premature occlusive arterial events (stroke and critical limb ischemia) (<50 and 55 years of age in men and women respectively), or arterial events at an older age in Africa. Several indexes strongly related to distending pressure effects are corrected for mean arterial pressure (MAP). PWV, carotid-femoral pulse wave velocity; Zc, proximal aortic characteristic impedance; Pf forward wave pressures; PQxZc at PPc, contribution of pressures generated by the product of aortic flow (Q) and Zc at peak aortic PP; 1/PPamp, reciprocal of peak systolic central arterial to-brachial pulse pressure amplification; 1/early systolic PPamp, reciprocal of early systolic central arterial to-brachial pulse pressure amplification. AUC values of all aortic function parameters in panels A and B and AUC for Pf/MAP and QZc at PPc/MAP in panels C and D respectively are significant at $p<0.0001$. (1/early sys PPamp)/MAP and Zc/MAP in panel D are significant at $p<0.005$ and $p<0.02$ respectively. *$p<0.05$ for comparison of AUC versus Pf/MAP in panel C and *$p<0.005$ for comparison of AUC versus QZc at PPc/MAP in panel D.

Second, in patients with precipitous events, PWV, Zc, Pf, P_{QxZc} at peak PP, and both reciprocal of peak and early systolic central arterial-to-brachial PP amplification, but neither Pb, brachial arterial PP nor central arterial PP were strongly and independently associated with vascular events. In multivariate models the relationships between PWV, Zc, Pf or reciprocal of early systolic PP amplification and premature events were at least as strong as the relationships between the presence of several conventional risk factors and premature events. Third, the relationship between PWV and premature events was stronger than that between PWV and events at an older age. Finally, PWV, Zc, Pf and reciprocal of early systolic or peak PP amplification showed reasonable performance (AUC of ROC) for premature event detection, whilst Zc, Pf and reciprocal of early systolic PP amplification, but not PWV or peak PP amplification, showed significant performance for event detection at an older age.

The present study is the first to show that the functional changes caused by arteriosclerosis that accompany arterial events in the elderly similarly occur and are as advanced in those with premature events at a very young adult age. Indeed, several central arterial functional measures well-recognized as being caused by arteriosclerosis were noted to show as striking changes in those experiencing premature events (at as low an age as 20-40 years), as those changes noted to occur in the elderly. As age is the dominant risk factor for arterial events, including stroke and CLI, these events usually occur over the age of 50 years for men and 55 years for women, at least in high-income countries. Therefore, little is understood as to what mediates premature arterial events, a frequent finding in low to middle income countries (Kolkenbeck-Ruh *et al.,* 2019b; Kolkenbeck-Ruh *et al.,* 2020). Although arterial events represent the end-stage of arterial vascular lesions, previous studies report on less extensive atherosclerotic vascular lesions and more events classified pathologically as thrombotic in nature in those with premature events (Kolkenbeck-Ruh *et al.,* 2020; Pineda *et al.,* 2008). In the present study we nevertheless show that despite the fact that at a population or community level, arteriosclerosis-induced arterial functional changes mainly occur after the age of 50 years (Namasivayam *et al.,* 2009; Mitchell *et al.,* 2010b; Namasivayam *et al.,* 2016; Hodson *et al.,* 2017; Hodson *et al.,* 2016), these aortic functional changes are strongly associated with premature events and are at least as advanced in younger patients as in patients with events at an older age.

Several longitudinal studies summarized in meta-analyses (Vlachopoulos *et al.,* 2010b; Ben-Shlomo *et al.,* 2014) have demonstrated that arteriosclerotic changes in central arteries as indexed by aortic PWV, predict events and that this occurs in the elderly. However, these meta-analyses also suggest that the ability of PWV to predict events may be more robust in younger rather than older age groups (Ben-Shlomo *et al.,* 2014). Indeed, in the present study little difference was noted in PWV between cases and controls at an older adult age. Importantly

however, consistent with the well-recognised effect of ageing on aortic stiffness (Mitchell *et al.,* 2010b; Hodson *et al.,* 2016), PWV in older persons without events was on average close to the accepted threshold (10 m/sec) indicative of a high-risk of events. Thus, a high proportion of elderly individuals in the control group were at a high-risk of an event. The limited relationship between PWV and events at an older age should therefore not be viewed as a finding to suggest that PWV does not predict events in the elderly, but rather that many elderly persons in the control group are at a high-risk of an event. Indeed, the same argument holds for the limited ability of hypertension to associate with events in the elderly. In this regard, the prevalence of hypertension in the elderly with events was striking, but a similarly high prevalence was noted in the control group at the same age. Thus, consistent with the findings of PWV, elderly persons in the control group should be considered as being at a high-risk of an event mediated by hypertension.

How may aortic stiffness and consequent increases in Pf contribute to stroke and CLI even at a young adult age? An increased aortic stiffness may enhance pulse pressure and hence pulsatile loads by increasing Zc and hence forward wave pressures. Although early systolic pulsatile load is increased centrally (through a reduced impedance mismatch between the aorta and peripheral vessels when aortic, but not peripheral arterial stiffness increases), PP amplification is reduced and the enhanced pulsatile load is not appropriately detected at the peripheral pulse. Although forward wave pressures generally do not increase to any marked extent below the age of 50 years (Namasivayam *et al.,* 2009; Mitchell *et al.,* 2010b; Namasivayam *et al.,* 2016; Hodson *et al.,* 2017; Hodson *et al.,* 2016), the present study suggests that in those with precipitous events, forward wave pressures may be sufficiently large that they associate with events before the age of 50 years. This is consistent with increases in forward wave pressures that accompany uncontrolled risk factors even in middle-aged community samples (Motau *et al.,* 2018). However, whether this is a cause and effect relationship is unclear as consistent with the Framingham Heart Study (Cooper *et al.,* 2015; Mitchell *et al.,* 2010b), and with a meta-analysis of several alternative studies (McEniery *et al.,* 2008), in the present study only weak independent relationships between central arterial PP and precipitous events were noted, whilst aortic PWV and forward wave pressures showed strong independent relationships with premature events. However, an increased heart rate reduces wave reflection and peak central arterial PP and in the present study participants with events had markedly higher heart rates than controls, a finding not unexpected in patients admitted with catastrophic events. Thus, peak central arterial PP in those with events may have been markedly higher prior to admission to hospital when heart rate values were more in keeping with resting, unstressed values. Alternatively, through an increased aortic impedance, a decrease in the impedance mismatch between the aorta and more distal vessels may occur, and this may cause vascular damage by

increasing the transmission of pulsatile energy into vascular beds beyond the aorta without necessarily modifying aortic PP (O'Rourke *et al.,* 2010; Hashimoto and Ito, 2011; Mitchell *et al.,* 2011; Webb *et al.,* 2012; Smulyan *et al.,* 2016). Irrespective of the mechanisms through which increases in aortic stiffness and Pf account for premature events, the results of the present study challenge the notion that mainly age-related arteriosclerotic arterial functional alterations are involved in mediating vascular events, and are in keeping with evidence that these arterial functional changes contribute to events even in younger age groups (Ben-Shlomo *et al.,* 2014).

The arteriosclerotic changes noted in the present study at a young adult age in participants with arterial events are consistent with the high prevalence of risk factors, particularly hypertension, diabetes mellitus and smoking in those with events. In this regard, these risk factors are well-recognized as causing arteriosclerotic changes in large arteries and the functional effects of arteriosclerosis cause progressive damage to large and small arteries leading to stroke and CLI (Nichols *et al.,* 2011). Although arteriosclerotic aortic functional changes in the present study showed a stronger relationship than diabetes mellitus or smoking with events, these functional changes were not more strongly related to events than hypertension *per se*. Thus, premature arteriosclerosis should not be seen as the primary cause of the event, but rather as a vascular change that in-part mediates the cardiovascular damage produced by risk factors such as hypertension, diabetes mellitus and smoking, and that this effect is in-part through enhanced central arterial pulsatile load. Hence, the present study highlights the possibility that the identification of premature arteriosclerotic changes, using measures such as PWV or Pf may enhance risk prediction in those with existing risk factors not only at an older age (Vlachopoulos *et al.,* 2010b; Ben-Shlomo *et al.,* 2014; Cooper *et al.,* 2015), but also at a younger adult age (Ben-Shlomo *et al.,* 2014).

The limitations of the present study include the study design (a case-control analysis) and hence the results may be subject to population stratification. In this regard, longitudinal studies are unlikely to capture a significant number of precipitous events across the full adult age range as they are infrequent in those between 20 and 40 years of age. However, data for a large control sample were obtained from age- sex- and ethnicity-matched controls derived from a randomly selected community sample and data from a relatively large case sample were obtained from sequentially admitted patients of the same ethnicity and with the same demographic features. Because lipid-lowering therapy was initiated on admission to hospital, we could not obtain fasting lipid profiles off therapy in these participants. Hence, we were unable to adjust for LDL cholesterol concentrations. However, dyslipidemia in groups of African ancestry in Africa is more frequently associated with reduced HDL cholesterol concentrations, which are unaffected by lipid-lowering therapy and hence could be included in adjustments. Moreover, no independent relations between LDL cholesterol concentrations and central arterial function were

noted in the community-based study. We assessed two major types of arterial events (stroke and CLI) and excluded MI, as at the time of initiating the present study few premature MI were noted to occur in individuals of African ancestry. Hence, further studies are required to evaluate relations between central arterial function and premature MI. Based on the TOAST classification, a significant number of patients (58.5%), had a stroke of either cardio-embolic (often not associated with ischemic heart disease) or undetermined origins. Under these circumstances, we may have underestimated the extent of the change in arterial function in those with an arterial cause of precipitous events. However, in sensitivity analysis similar changes in arteriosclerotic arterial function were noted in those with stroke as CLI, and CLI is recognised as largely being attributed to arterial disease. Lastly, in order to avoid a selection bias, we did not exclude cases or controls receiving either antihypertensive therapy or lipid-lowering therapy and hence differences in central arterial function may have been confounded by treatment differences.

In conclusion, in the present study we show that independent of conventional risk factors and steady-state pressures, arterial events over the young adult age range are strongly associated with early arteriosclerosis as indexed by increases in aortic stiffness (carotid-femoral PWV), proximal aortic characteristic impedance (Zc) (attributed to differences in proximal aortic stiffness rather than diameter), forward wave pressures (attributed to pressures generated by stiffness-associated increases in Zc and not flow or wave re-reflection), and decreases in central arterial-to-brachial PP amplification (explained by alterations in impedance mismatching rather than changes in central arterial reflected wave pressures). Importantly, the relations between arteriosclerotic central arterial functional changes and arterial events at a younger adult age were at least as strong as the relations in those with events at an older age. Thus, arteriosclerosis may contribute to events in Africa from as early as 20-to-40 years of age.

Chapter 4 Relations of Aortic Stiffness with Arterial Damage Beyond Brachial Pressure are both Dependent and Independent of Central Arterial Pulsatile Load

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4.0 Abstract

Aim: We aimed to determine whether the impact of aortic stiffness on atherosclerotic or small vessel end organ damage beyond brachial BP depends in-part on stiffness-induced increases in central arterial pressures produced by an enhanced resistance to flow (characteristic impedance, Zc).

Methods: We studied 1021 participants, 287 with stroke or critical limb ischaemia (CLI), and 734 from a community sample with atherosclerotic or small vessel end organ measures. Central arterial haemodynamics were determined from arterial pressure (SphygmoCor) and velocity and diameter assessments in the outflow tract (echocardiography).

Results: Although Zc and carotid-femoral PWV were correlated ($p<0.0001$), these relations were not independent of confounders ($p=0.90$). Both Zc and hence central arterial pressures generated by the product of Zc and aortic flow (Q) (P_{QxZc}), as well as PWV were independently associated with carotid intima-media thickness, estimated glomerular filtration rate (eGFR), endothelial activation markers (V-CAM-1) and events. With further adjustments for brachial pulse pressure (PP) or systolic BP (SBP), PWV and P_{QxZc} were both associated with eGFR and V-CAM-1. Relationships between PWV and eGFR or V-CAM-1 were independent of P_{QxZc} ($p<0.05$) and relationships between P_{QxZc} and eGFR and V-CAM-1 were independent of PWV ($p<0.005$). Similarly, with adjustments for confounders and brachial PP or SBP, across the full adult lifespan, both aortic PWV and P_{QxZc} were increased in those with arterial events ($p<0.0005$). Relationships between PWV and events were again independent of P_{QxZc} ($p<0.005$) and between P_{QxZc} and events were independent of PWV ($p<0.005$).

Conclusion: Beyond brachial BP, the impact of aortic stiffness on arterial damage involves effects that are both dependent (proximal aortic Zc and hence P_{QxZc}) and independent (full aortic length indexed by PWV) of central arterial pulsatile load. Hence, PWV and brachial PP may be insufficient to account for all of the damage mediated by increases in aortic stiffness.

4.1 Introduction

Aortic stiffness, as indexed by increases in carotid-femoral (aortic) pulse wave velocity (PWV), predicts arterial events beyond brachial blood pressure (BP) (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014). The measurement of both aortic PWV and brachial pulse pressure (PP) are therefore recommended by hypertension guidelines as measures of aortic end organ damage that may be employed to enhance risk prediction (Mancia *et al.*, 2013). Several mechanisms may explain the impact of aortic stiffness beyond brachial BP. In this regard, increases in aortic stiffness inthe proximal portion of the aorta may enhance aortic characteristic impedance (Zc, resistance to flow in a pulsatile system in the absence of wave reflection) and thus PP(Nichols *et al.*, 2011; Chirinos *et al.*, 2019). However, through an impedance mismatch between central and peripheral arteries, the central arterial effect of Zc on PP may not be adequately detected at the brachial pulse(Nichols *et al.*, 2011; Chirinos *et al.*, 2019). Nonetheless, an impact of aortic stiffness beyond central arterial PP may also occur. Indeed, by reducing the normal impedance mismatch that exists between several areas of the aorta and more distal vessels, increases in aortic stiffness may promote the transmission of pulsatile energy independent of central PP (O'Rourke *et al.*, 2010; Hashimoto and Ito, 2011; Mitchell *et al.*, 2011; Woodard *et al.*, 2015; Cooper *et al.*, 2016b; Webb *et al.*, 2012; Cooper *et al.*, 2016a; Smulyan *et al.*, 2016). Moreover, the interstitial changes responsible for an increased vascular stiffness (decreased elastin and increased collagen) or an increased vascular stiffness *per se* may decrease endothelial function through alterations that are beyond BP effects (Peng *et al.*, 2003; Orr *et al.*, 2009; González-Santiago *et al.*, 2002). Although at present the impact of aortic stiffness on arterial events beyond brachial BP is well-recognized, there is uncertainty as to the role beyond central arterial pulsatile load.

In the Framingham Heart Study, an increased aortic PWV, but not central arterial PP predicted events beyond brachial PP(Mitchell *et al.*, 2010b). Moreover, while meta-analyses of studies reporting on aortic PWV show a clear independent ability to predict arterial events beyond brachial BP (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014), a meta-analysis of studies on central arterial PP or SBP failed to show similar robust effects beyond brachial BP (Vlachopoulos *et al.*, 2010). Nevertheless, in the Framingham Heart Study, the forward travelling pressure wave, that component wave of the central arterial pulse that is strongly determined by proximal aortic stiffness and hence Zc, predicted events independent of brachial BP (Cooper *et al.*, 2015). Thus, whilst stiffness-induced increases in forward travelling pressure waves may predict risk for arterial events beyond brachial BP (Cooper *et al.*, 2015), peak central arterial PP may show a limited ability to do the same(Mitchell *et al.*, 2010b). The uncertainty as to the extent to which aortic stiffness predicts adverse effects through an impact dependent (through effects in the proximal aorta and hence Zc) or independent (through effects

in several areas of the aorta) of central arterial pulsatile load therefore prompted us to further explore these relationships with both small vessel (estimated glomerular filtration rate, eGFR) and atherosclerotic (carotid intima-media thickness and endothelial activation markers) end organ measures and events (stroke and critical limb ischaemia) in large community and hospital-based studies(Motau *et al.*, 2020). In this regard, we employed aortic PWV as an index of stiffness across the full length of the aorta. To evaluate the effects of proximal aortic stiffness on central arterial pressures we determined the impact of Zc beyond aortic root diameter and the effects of pulsatile pressure generated by the product of Zc and aortic flow (Q)the principle central arterial pressure effect of increases in Zc (Phan *et al.*, 2016).

4.2 Methods

4.2.1 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 protocols (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. The present study design has previously been described (Motau *et al.*, 2020; Woodiwiss *et al.*, 2009; Mmopi *et al.*, 2020; Bello *et al.*, 2020). In a community-based sample where in 734 participants from randomly recruited (population census figures of 2001) families of black African descent (Nguni and Sotho chiefdoms) from the South Western Townships (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years of age, with high-quality aortic velocity measurements in the outflow tract and pulse wave velocity, we assessed independent relations between aortic function and several arterial vascular end organ measures. In these participants, we assessed eGFR, an index of microvascular damage, in 709, circulating endothelial activation markers, an index of early atherogenesis, in 485, and carotid intima-media thickness (IMT) an index of structural changes in large vessels, in 543.The smaller sample sizes of those with IMT and endothelial activation marker measurements is attributed to the carotid ultrasound software only being available some years after initiating the study, and to the limited availability of serum samples in all participants. Thereafter, we compared aortic function in patients with arterial events to that obtained in age- and sex-matched controls from the community participants. In this regard, 287 consecutive black South Africans with stroke (n=109) who did not have atrial fibrillation at the time of assessment or critical limb ischaemia (CLI) (n=178) with high-quality aortic velocity assessments in the outflow tract were recruited from the Charlotte Maxeke Johannesburg Academic Hospital, South Africa. The presence of CLI or stroke was identified as described (Motau *et al.*, 2020). Data obtained in patients with arterial events were compared with data acquired over the same time-period in

390 age- and sex-matched randomly recruited participants from the community-based study with high-quality aortic velocity measurements in the outflow tract. 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.

4.2.2 Clinical and demographic information

A questionnaire was administered to obtain demographic and clinical data as described (Motau *et al.*, 2020; Woodiwiss *et al.*, 2009; Mmopi *et al.*, 2020; Bello *et al.*, 2020). Clinical information was also extracted from the hospital records and confirmed by the attending physician (Motau *et al.*, 2020). Clinical data included the presence of risk factors and the therapy thereof. Height and weight were measured using standard approaches and participants were considered to be obese if their body mass index (BMI) was ≥30 kg/m^2. Laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA1c) were performed. Diabetes mellitus (DM) was defined as the use of insulin or oral glucose-lowering agents, a fasting plasma glucose concentration ≥7mmol/l or an HbA1c value greater than 6.5%. High-quality office brachial blood pressure (BP) measurements were obtained in the seated position and after 5 minutes of rest, by a trained nurse-technician using a standard mercury sphygmomanometer as previously described (Woodiwiss *et al.*, 2009)and according to guidelines. 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 mm Hg systolic or ≥90 mm Hg diastolic BP or the use of antihypertensive medication.

4.2.3 Arterial haemodynamic assessments.

Arterial function was determined from pulse wave analysis and aortic velocity and diameter assessments obtained in the outflow tract (Motau *et al.*, 2020; Mmopi *et al.*, 2020; Bello *et al.*, 2020). 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 as recommended(O'Rourke, 2017) by manual measurement (auscultation) of brachial systolic and diastolic BP taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalised 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. Immediately after central arterial pressure waveforms were obtained, aortic velocity and diameter measurements (Figure 4.1) were acquired by an experienced observer (AJW) in participants in the left lateral decubitus position using an Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.). Velocity waveforms were obtained in the 5-chamber view. High-quality velocity assessments were identified as those with a smooth velocity waveform with a dense leading (outer) edge and a clear maximum velocity. Aortic diameter measurements were obtained just proximal to the aortic leaflets in the long axis parasternal view. The largest diameter recorded in early systole was used to construct the aortic flow waveform. As indicated in Figure 4.1, aortic flow waveforms were generated from aortic velocity and diameter measurements. Taking care to avoid any overshoot of the image, the leading (outer) edge or the most dense, or brightest, portion of the spectral image of the velocity waveform was outlined using graphics software and using aortic diameter measurements employed to construct a flow waveform (Figure 4.1). Characteristic impedance (Zc) was determined in the time domain using approaches previously described (Segers *et al.*, 2007; Mitchell *et al.*, 2010b) and validated against invasive pressure measurements (Kelly and Fitchett, 1992). In addition to generating a complete flow waveform (Figure 4.1), to determine Zc, serial pressures in the cardiac cycle up until the dicrotic notch were extracted from ShygmoCor data and a central arterial pressure waveform was also generated (Figure 4.1). The volume flow waveform was paired with central arterial pressure waveforms by aligning the foot (t_0) of the respective signal averaged waveforms (Figure 4.1). The point at which flow achieves 95% of its peak (t_{Q95}) was identified (Figure 4.1). The corresponding pressure change between t_0 and t_{Q95} was determined (Figure 4.1). Characteristic impedance was calculated as the ratio of change in pressure to change in flow in the window t_0 to t_{Q95} (Figure 4.1). As the accuracy of this approach is determined by the similarity of ejection durations in sequential pressure and then flow assessments, we first determined the differences in ejection duration observed during pressure (foot of the wave to the dicrotic notch) versus flow (duration of velocity) measurements. In this regard, Bland Altman analysis showed a 2.8 msec difference over an average 320.4 msec period, representing a 0.73% difference in ejection duration. The peak pressure generated by the product of flow (Q) and Zc (P_{QxZc}) was determined from the product of peak aortic Q and Zc. Systemic vascular resistance (SVR) was calculated from mean arterial pressure (MAP, average arterial pressure for the peripheral wave form using the formula $MP = \frac{\sum_{i=T_0}^{T_F} P_i}{n}$, where T_0=start of waveform; T_F=end of waveform; calculated from mean arterial pressure (MAP, average arterial pressure for the P_i=pressure points, n= number of pressure points) and cardiac output (SVR=[MAP-RAP]/CO), assuming right atrial pressure

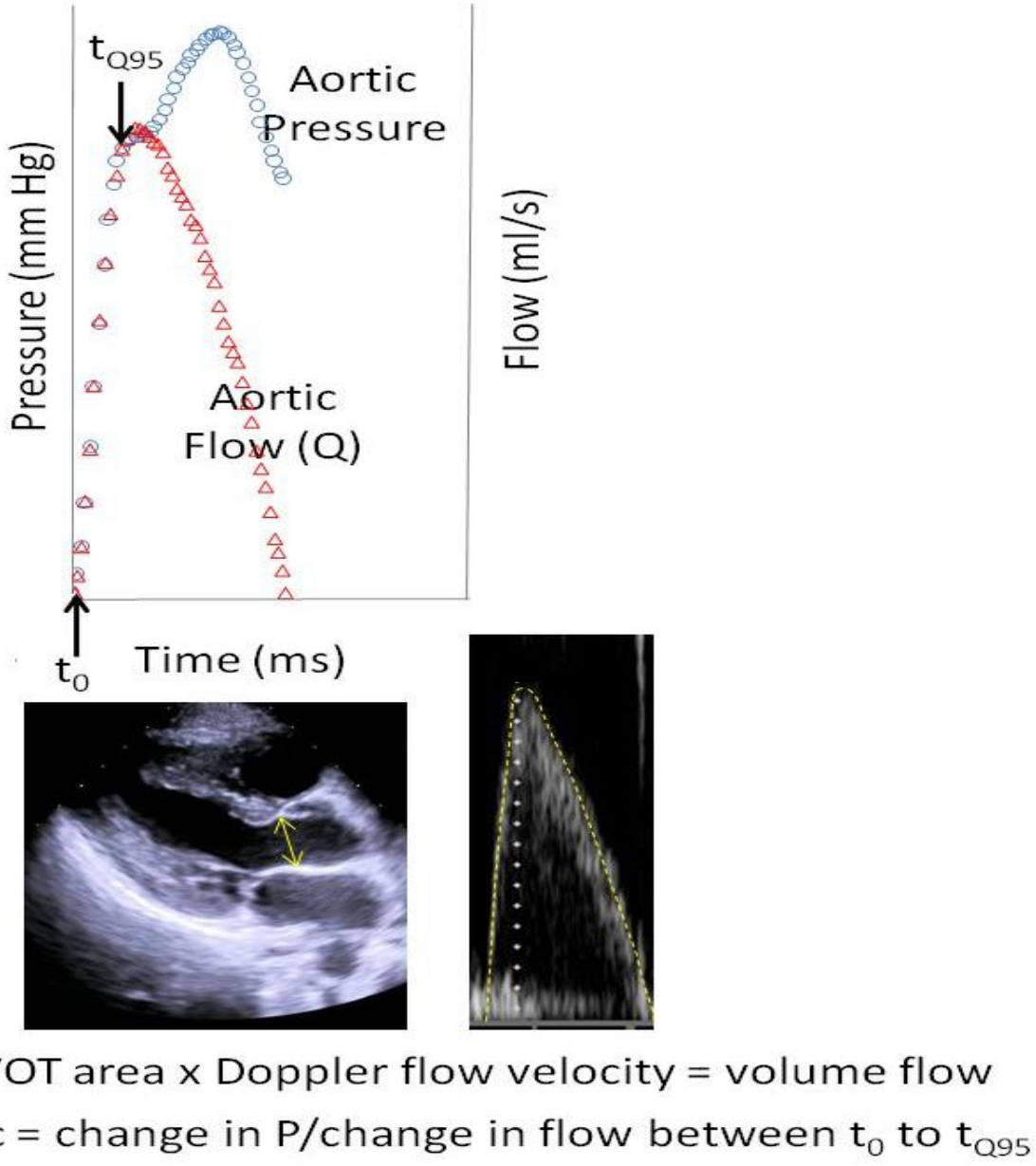


Figure 4.1. Approach to the assessment of aortic characteristic impedance (Zc). Aortic velocity and diameter measurements are obtained in the outflow tract (ultrasound images) and the velocity waveform is derived from image software (dashed line superimposed on the velocity image). An aortic flow waveform is generated from the product of aortic diameter and velocity. (individual points illustrated in the graph). A central pressure waveform is generated from pressures (P) determined from SphygmoCor software (individual points illustrated in the graph) The pressure and flow waveforms are paired as described in the methods and Zc is calculated as indicated from the change in P/change in flow up until 95% of peak flow. t0, the time at the foot of each of the signal averaged waveforms; t_{Q95}, the time at which flow is 95% of its peak; LVOT, left ventricular outflow tract.

(RAP) = 0 mm Hg. Cardiac output was determined from stroke volume (SV) x heart rate where SV was assessed from the product of aortic velocity-time integral and aortic root diameter. Heart rate (HR) was determined from the length (PD) of an averaged peripheral waveform captured over a 10 second period, using the formula: HR=1000/PDx60. Total arterial compliance (TAC) was calculated as SV/aortic pulse pressure. Carotid-femoral (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.

4.2.4 Atherosclerotic or small vessel end organ measures

Carotid IMT was determined using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington) as previously described (Kolkenbeck-Ruh *et al.*, 2019b) 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. Serum creatinine concentrations were measured using the Advia Chemistry systems (Siemens) with calibration traceable to isotope dilution mass spectrometry (IDMS) (Kolkenbeck-Ruh *et al.*, 2019b). The 4-variable CKD-EPI equation was employed to estimate eGFR. To determine concentrations of endothelial activation markers, blood samples were centrifuged and immediately stored at -80^{o}C. Plasma concentrations of vascular cell adhesion molecule-1 (V-CAM-1), intercellular adhesion molecule-1 (I-CAM-1), and E-selectin concentrations were measured using enzyme-linked immunosorbent assays (Quantikine, R&D Systems Inc, Minneapolis, MN, USA).

4.2.5 Data analysis

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 or SEM). Dichotomous variables are expressed as percentages. To improve on the distribution of data concentrations of endothelial activation markers were logarithmically (V-CAM-1) or square root (I-CAM-1, E-selectin) transformed, and transformed data was used in regression analyses. To determine relationships multivariate-adjusted linear regression analysis was performed. In regression analysis, adjustments were for mean arterial pressures (MAP), age, sex, regular alcohol intake, regular tobacco intake, BMI and diabetes mellitusm andheart rate. As stroke and

critical limb ischaemia are not necessarily caused by the same pathophysiological mechanisms, sensitivity analysis was conducted in stroke and critical limb ischaemia separately.

4.3 Results

4.3.1 Participant characteristics

The participant characteristics are given in Table 4.1. The characteristics of the participants from the community sample with and without carotid imaging or endothelial activation marker measurements are given in Tables 4.2 and 4.3. Modest differences in brachial BP and the proportion with diabetes mellitus were noted in those with as compared to without carotid imaging (Table 4.2). Modest differences in the proportion who regularly drank alcohol were noted in those with as compared to without endothelial activation marker measurements (Table 4.3). In the community sample a striking prevalence of participants had hypertension (Table 4.1). Nevertheless, as compared to age- and sex-matched participants (same ethnicity) from the community sample, patients with arterial events had a greater prevalence of risk factors (Table 4.1). Patients (with events) showed similar brachial BP values as community participants with lower brachial BP values compared to age- and sex-matched participants (Table 4.1).

4.3.2 Relations of proximal aortic Zc and carotid-femoral PWV

Although in the community sample independent of aortic root diameter, Zc was correlated with carotid-femoral PWV (partial $r=0.15$, $p<0.0001$) this relationship did not persist with adjustments for confounders including MAP, age, sex, regular alcohol intake, regular tobacco intake, BMI and diabetes mellitus and heart rate (partial $r=0.005$, $p=0.90$).

4.3.3 Relations of indices of aortic stiffness with small vessel or atherosclerotic end organ measures

Beyond mean arterial pressure (MAP), brachial PP showed only weak and inconsistent independent relations with eGFR and V-CAM-1 concentrations, but strong relations with carotid IMT (Figures 4.2 and 4.3). In contrast to brachial PP, aortic PWV and P_{QxZc} (Figures 4.2 and 4.3 and Table 4.4), showed strong independent relations with all small vessel or atherosclerotic end organ measures, while TAC failed to show independent relations with these same end organ measures (Table 4.4). Importantly, in the same regression models, Zc showed independent relations with all small vessel or atherosclerotic end organ measures, whereas Q showed relations with some measures (Table 4.4).

Table 4.1. Characteristics of study participants.

	Community sample	Age- and sex-Arterial events	Matched controls	p-value
Sample size (% women)	734 (68.0%)	287 (39.4)	390 (43.1)	=0.34
Events (% stroke/CLI)	-	38.0/62.0	-	
Age (years)	46.7±18.2	54.5±14.7	54.3±12.9	=0.85
Body mass index (kg/m^2)	29.4±7.5	27.9±7.3	28.4±6.3	=0.35
Hypertensive n, (%)	346 (47.1)	237 (82.5)	220 (56.4)	<0.0001
Diabetes mellitus n, (%)	95 (12.9)	92 (31.9)	48 (12.3)	<0.0001
Regular smoking n, (%)	118 (16.1)	115 (38.5)	88 (22.6)	<0.0001
Regular alcohol n, (%)	143 (19.5)	159 (55.3)	105 (26.9)	<0.0001
Brachial SBP (mm Hg)	129±22	127±24	132±21	<0.01
Brachial DBP (mm Hg)	83±12	80±13	86±12	<0.0001
MAP (mm Hg)	99±15	98±15	104±15	<0.0001
Brachial PP (mm Hg)	46±16	50±14	47±15	<0.01
Heart rate (beats/min)	67±12	84±17	65±11	<0.0001
Stroke volume (mls/beat)	81±25	87±39	86±40	=0.88
Cardiac output (l/min)	5.4±2.4	6.9±3.3	5.7±2.9	<0.0001
SVR (mm Hg/l/min)	20.4±8.9	17.9±11.7	21,7±10.1	<0.0005
Unadjusted central arterial haemodyamics				
Aortic SBP (mm Hg)	120±22	118±20	126±21	<0.0001
Aortic PP (mm Hg)	36±15	38±14	39±15	=0.57
Aortic root diameter (mm)	25.1±4.4	26.1±4.1	25.7±6.3	=0.35
Characteristic impedance (Zc)(dynes.s/cm^5)	85.9±43.5	87.0±24.6	83.1±22.7	=0.03
Peak aortic flow (Q) (ml/s)	350±170	397±167	377±175	=0.21
Max $P_{Qx\ Zc}$ (mm Hg)	25.6±8.3	27.9±8.4	26.5±8.0	=0.03
Aortic PWV (m/sec)	6.08±2.70	7.14±3.46	6.65±2.47	=0.04
TAC (ml/mm Hg)	2.36±0.99	2.58±1.69	2.42±1.38	=0.19
Arterial end organ measures				
Carotid IMT (mm) (n=543)	0.65±0.13	-	-	
eGFR (ml/min/1.73m^2)(n=709)	96.7±22.2	-	-	
V-CAM-1 (ng/ml)(n=485)	964±290	-	-	

Data are shown as mean±SD or percentages. p-values are for comparisons between high-risk patients and age- and sex-matched controls from the community sample. SBP, systolic blood pressure; DBP, diastolic BP; MAP, mean arterial pressure; SVR, systemic vascular resistance; Max $P_{Qx\ Zc}$, central arterial compression wave pressures; PWV, pulse wave velocity; TAC, total arterial compliance; IMT, intima-media thickness; eGFR, estimated glomerular filtration rate, V-CAM-1, vascular cell adhesion molecule-1.

Table 4.2. Characteristics of community study participants with and without IMT measurements.

	With	Without	p-value
Sample size (% women)	543 (68.5)	191 (66.5)	
Age (years)	46.9±17.9	46.3±19.1	
Body mass index (kg/m^2)	29.6±7.6	28.8±7.4	
Hypertensive n, (%)	246 (45.3)	100 (52.4)	=0.09
Diabetes mellitus n, (%)	62 (11.4)	33 (17.3)	<0.05
Regular smoking n, (%)	88 (16.2)	30 (15.7)	
Regular alcohol n, (%)	108 (19.9)	35 (18.3)	
Brachial SBP (mm Hg)	127±21	133±23	<0.005
Brachial DBP (mm Hg)	82±12	85±11	<0.005
MAP (mm Hg)	98±15	102±15	<0.005
Brachial PP (mm Hg)	45±16	48±17	<0.05

Data are shown as mean±SD or percentages. Probability values given are only those that are either significant or show trend effects. See table 4.1 for abbreviations.

Table 4.3. Characteristics of community study participants with and without endothelial activation marker measurements.

	With	Without	p-value
Sample size (% women)	485 (66.2)	249 (71.5)	
Age (years)	46.3±18.4	47.7±18.0	
Body mass index (kg/m^2)	29.3±7.5	29.7±7.6	
Hypertensive n, (%)	217 (44.7)	129 (51.8)	=0.07
Diabetes mellitus n, (%)	63 (13.0)	32 (12.9)	
Regular smoking n, (%)	83 (17.1)	35 (14.1)	
Regular alcohol n, (%)	107 (22.1)	36 (14.5)	<0.05
Brachial SBP (mm Hg)	128±23	130±22	
Brachial DBP (mm Hg)	82±12	83±13	
MAP (mm Hg)	99±15	100±16	
Brachial PP (mm Hg)	46±16	46±16	

Data are shown as mean±SD or percentages. See table 4.1 for abbreviations. Probability values given are only those that are either significant or show trend effects.

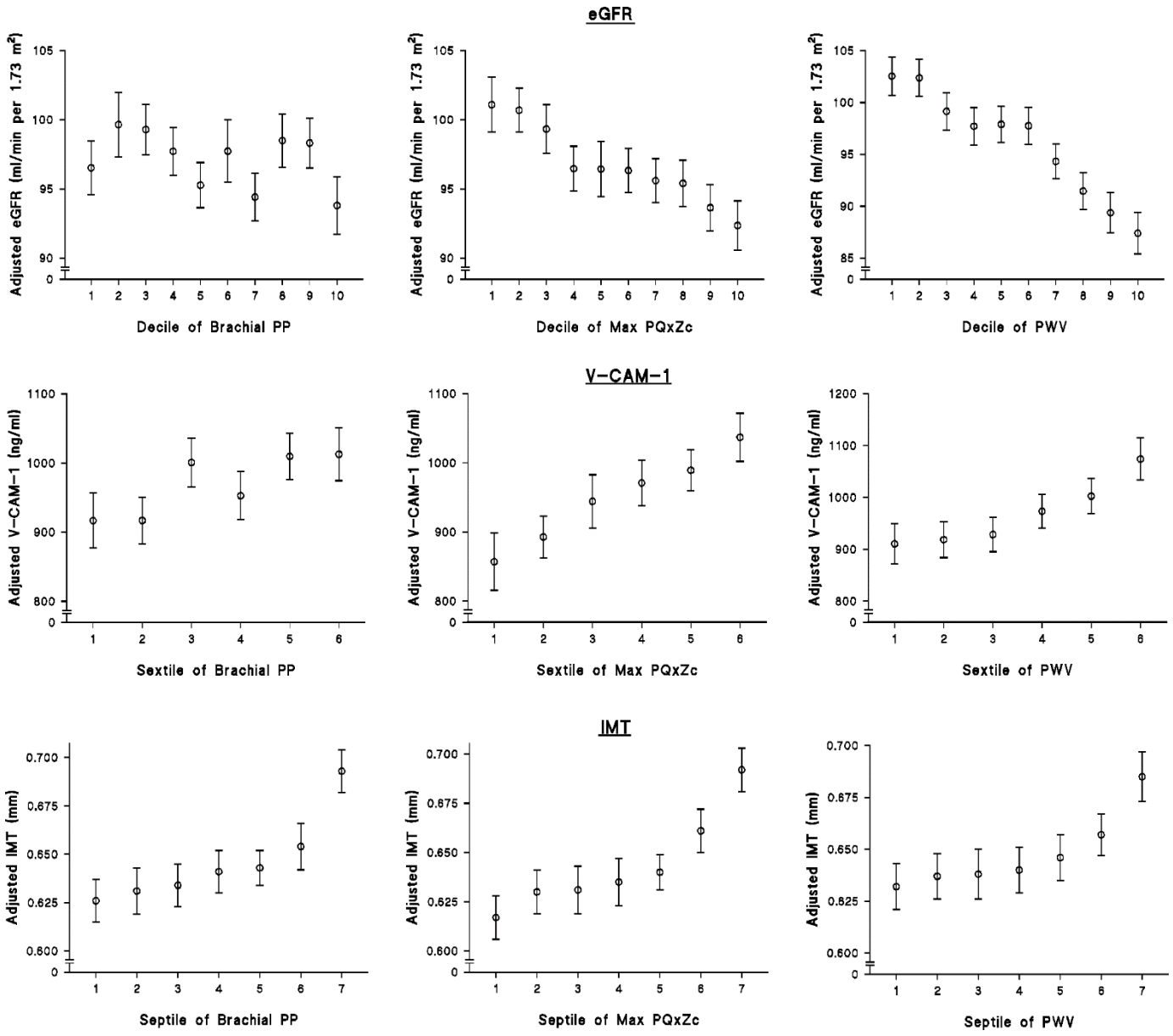
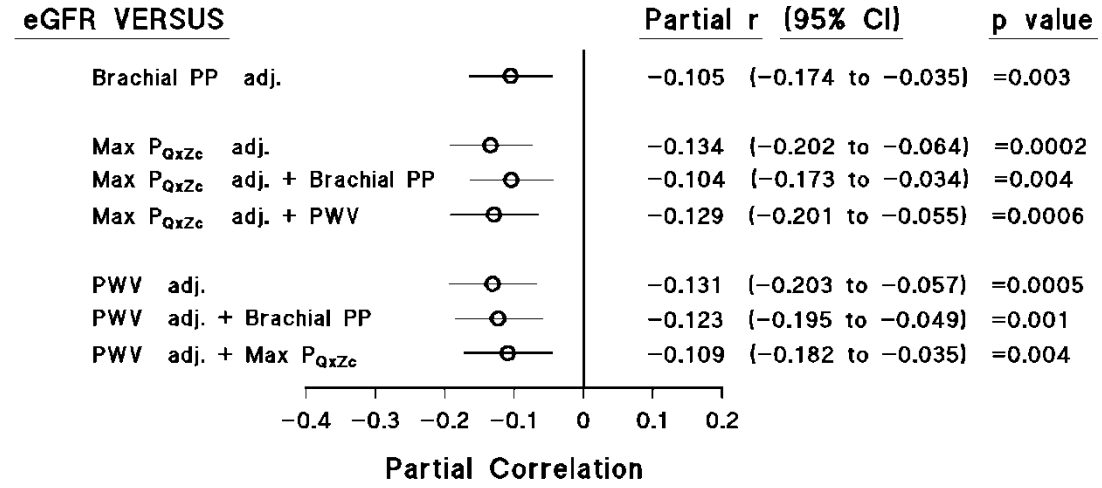
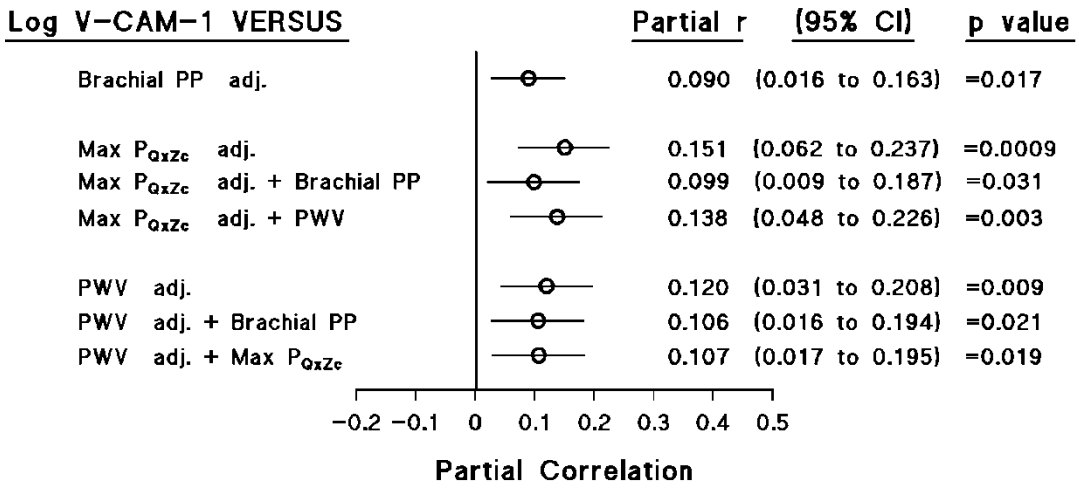


Figure 4.2. Multivariate-adjusted values of arterial end organ measures (eGFR, V-CAM-1 and IMT) across incremental values of brachial pulse pressure (PP) or indices of aortic stiffness (aortic PWV and PQxZc) in a community sample. Adjustments are for variations in age, sex, mean arterial pressure, body mass index, regular smoking, regular alcohol intake, diabetes mellitus and heart rate. See table 4.1 for abbreviations and figure 4.2 for relationships.





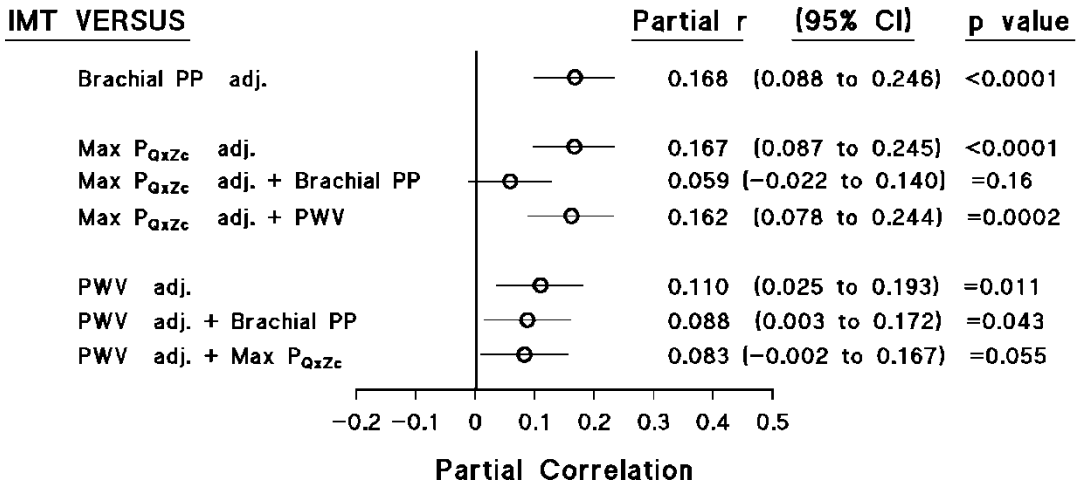


Figure 4.3. Impact of adjustments for brachial pulse pressure (PP) or alternative indices of aortic stiffness on multivariate-adjusted relations (partial r) between indices of aortic stiffness (aortic PWV and P_{QxZc}) and end organ measures (eGFR, V-CAM-1 and IMT) in a community sample. Adjustments are for variations in age, sex, mean arterial pressure, body mass index, regular smoking, regular alcohol intake, diabetes mellitus, heart rate and brachial PP as indicated. See table 4.1 for abbreviations.

Table 4.4. Multivariate-adjusted relations between blood pressures or indices of aortic stiffness and measures of arterial end organ changes in a community sample.

	Adjustments	partial r(95% CI)	p-value
	eGFR versus (n=709)		
Aortic PP	*	-0.091 (-0.161 to -0.021)	=0.01
Aortic PP	* + brachial PP	0.047 (-0.023 to 0.117)	=0.19
Peak aortic Q	* + Zc	-0.097 (-0.169 to -0.024)	=0.01
Aortic Zc	* + Q	-0.097 (-0.169 to -0.024)	=0.01
Aortic Zc	* + Aort diameter	-0.162 (-0.232 to -0.089)	<0.0001
Aortic Zc	* + Q + P_{QxZc}	0.016 (-0.058 to 0.089)	=0.68
P_{QxZc}	*	-0.134 (-0.202 to -0.064)	<0.0005
P_{QxZc}	* + brachial SBP	-0.109 (-0.176 to -0.037)	=0.003
Aortic PWV	*	-0.131 (-0.203 to -0.037)	=0.0005
Aortic PWV	* + brachial SBP	-0.123 (-0.196 to -0.049)	<0.002
TAC	*	-0.092 (-0.161 to -0.022)	<0.01
TAC	* + brachial SBP	-0.117 (-0.185 to -0.047)	<0.005
	V-CAM-1 versus (n=485)		
Aortic PP	*	0.117 (0.028 to 0.205)	=0.01
Aortic PP	* + brachial PP	0.019 (-0.071 to 0.109)	=0.67
Peak aortic Q	* + Zc	0.133 (0.040 to 0.224)	=0.005
Aortic Zc	* + Q	0.114 (0.021 to 0.205)	=0.02
Aortic Zc	* + Aort diameter	0.109 (0.015 to 0.200)	<0.05
Aortic Zc	* + Q + P_{QxZc}	0.014 (-0.079 to 0.108)	=0.77
P_{QxZc}	*	0.151 (0.062 to 0.237)	<0.001
P_{QxZc}	* + brachial SBP	0.091 (0.001 to 0.179)	<0.05
Aortic PWV	*	0.110 (0.025 to 0.193)	=0.01
Aortic PWV	* + brachial SBP	0.084 (-0.001 to 0.168)	<0.05
TAC	*	0.022 (-0.067 to 0.112)	=0.63
TAC	* + brachial SBP	0.051 (-0.039 to 0.140)	=0.27
	IMT versus (n=543)		
Aortic PP	*	0.184 (0.104 to 0.261)	<0.0001
Aortic PP	* + brachial PP	0.084 (0.003 to 0.165)	=0.043
Peak aortic Q	* + Zc	0.078 (-0.007 to 0.161)	=0.07

Aortic Zc	* + Q	0.129 (0.045 to 0.211)	<0.005
Aortic Zc	* + Aort diameter	0.102 (0.018 to 0.185)	<0.02
Aortic Zc	* + Q + P_{QxZc}	0.022 (-0.063 to 0.106)	=0.61
P_{QxZc}	*	0.167 (0.087 to 0.245)	<0.0001
P_{QxZc}	* + brachial SBP	0.096 (0.015 to 0.176)	<0.05
Aortic PWV	*	0.110 (0.025 to 0.163)	<0.05
Aortic PWV	* + brachial SBP	0.084 (-0.001 to 0.168)	=0.05
TAC	*	-0.043 (-0.123 to 0.039)	=0.31
TAC	* + brachial SBP	-0.017 (-0.098 to 0.064)	=0.68

See table 4.1 for abbreviations and Aort diameter, aortic root diameter. *Adjustments are for variations in age, sex, mean arterial pressure, body mass index, regular smoking, regular alcohol intake, diabetes mellitus and heart rate. Probability values are further adjusted for the non-independence of family members.

Thus, the impact of P_{QxZc} on small vessel or atherosclerotic end organ measures is attributed in-part to Zc effects. In addition, with adjustments for aortic root diameter, the relations between Zc and small vessel or atherosclerotic end organ measures persisted (Table 4.4). Thus, the relationships between Zc and small vessel or atherosclerotic end organ measures cannot be attributed to an impact of aortic diameter on Zc, and hence are likely to be through effects mediated by proximal aortic stiffness. Moreover, the relationships between Zc and small vessel or atherosclerotic end organ measures were entirely accounted for by an impact of Zc on P_{QxZc} as adjustments for P_{QxZc} abolished relationships between Zc and these end organ measures (Table 4.4) while adjustments for Zc failed to modify relationships between P_{QxZc} and these end organ measures (data not shown). Importantly, the PWV-eGFR or V-CAM-1 and the P_{QxZc}-eGFR, or V-CAM-1 relations remained after adjustments for brachial PP or SBP (Figure 4.3 and Table 4.4). Also, importantly, the relations between PWV and small vessel or atherosclerotic end organ measures remained after adjustments for P_{QxZc} and the relations between P_{QxZc} and these organ measures remained after adjustments for PWV (Figure 4.3). In contrast to P_{QxZc}, peak aortic PP was not independently related to small vessel or atherosclerotic end organ measures beyond brachial PP (Table 4.4). No independent relations between peripheral or central arterial pulsatile haemodynamics and I-CAM-1 and E-selectin concentrations were noted (data not shown).

4.3.4 Relations of indices of aortic stiffness with events

Consistent with in-hospital treatment for hypertension, brachial PP in those with arterial events was similar across the adult lifespan as compared to age- and sex-matched controls (Figure 4.4). However, both aortic PWV and P_{QxZc} showed marked increases in those with events across the adult lifespan (Figure 4.4) which remained after adjustments for brachial PP and SBP (Figure 4.5, Tables 4.6 and 4.7). In contrast, TAC failed to show independent relations with arterial events (Tables 4.6 and 4.7). Importantly, the increases in P_{QxZc} in those with arterial events are attributed entirely to increases in Zc, with no differences in peak aortic Q noted between groups (Tables 4.6 and 4.7). In addition, no differences in aortic root diameter were noted between those with and without arterial events (Tables 4.6 and 4.7). Thus, the relationships between Zc and arterial events cannot be attributed to an impact of aortic diameter (and hence area) on Zc, and hence is likely to be through effects mediated by proximal aortic stiffness. Moreover, the relationships between Zc and arterial events were entirely accounted for by an impact of Zc on P_{QxZc} as adjustments for P_{QxZc} abolished relationships between Zc and events (Tables 4.6 and 4.7) while adjustments for Zc failed to modify relationships between P_{QxZc} and arterial events (data not shown). Importantly, the relations between PWV and events were independent of P_{QxZc} and the relations between P_{QxZc} and events were independent of PWV (Figure 4.5). With (Tables 4.6 and 4.7), but not without

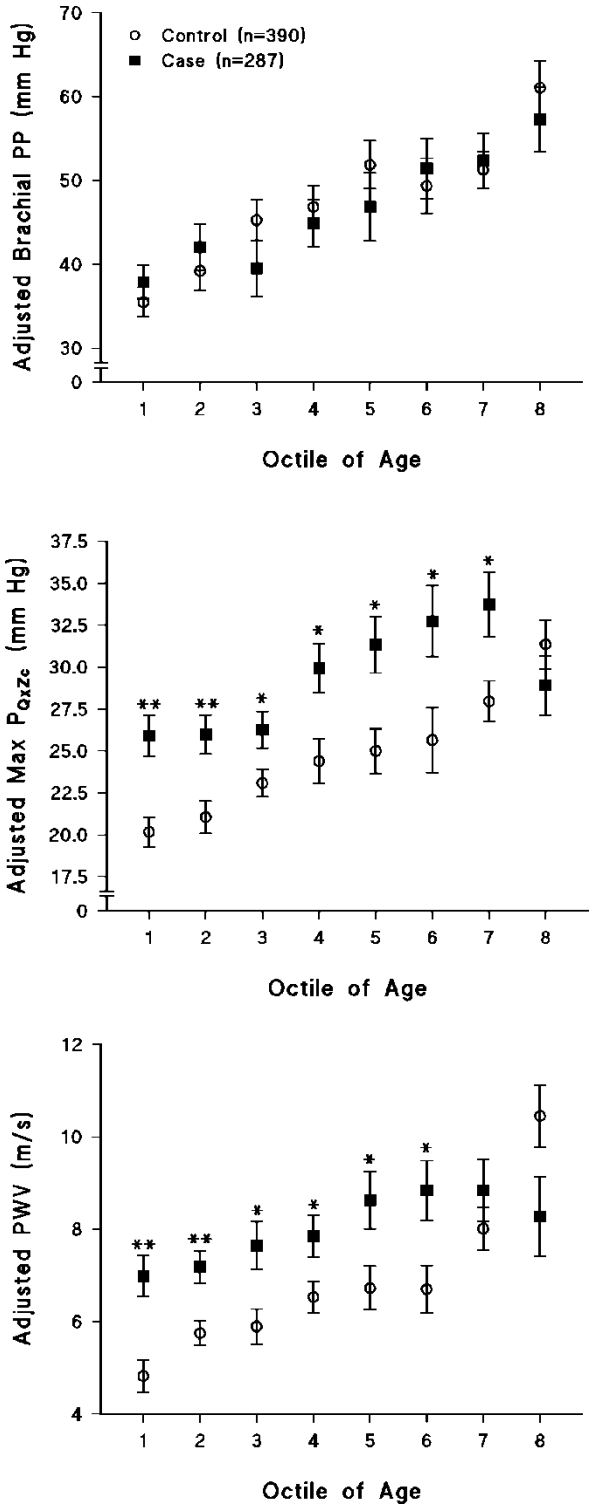


Figure 4.4. Multivariate-adjusted values of brachial pulse pressure (PP) and indices of aortic stiffness (aortic PWV and P_{QxZc}) across the adult lifespan in cases with arterial events as compared to age-and sex-matched controls from a community sample. Data shown are comparisons over the age range 20-80 years (see table 4.5). Adjustments are for variations in age, sex, mean arterial pressure, body mass index, regular smoking, regular alcohol intake, diabetes mellitus and heart rate. See table 4.1 for abbreviations. *p<0.02, **p<0.005 for cases versus controls.

Table 4.5. Octiles of age and sample sizes at reach age for comparisons of aortic function between those with arterial events (cases) and community participants (controls) shown in Figure 4.3.

	Stroke and CLI		Controls	
	Mean±SD age (years)	n=	Mean±SD age (years)	n=
Octiles of age				
1	31.5±4.9	42	31.4±2.4	47
2	41.8±2.2	38	43.5±3.2	51
3	48.1±1.4	36	49.3±0.9	57
4	53.3±1.8	39	52.2±0.9	46
5	59.1±1.3	32	56.4±1.4	48
6	63.4±1.1	38	61.4±1.4	43
7	69.0±2.1	25	66.7±1.6	49
8	78.4±4.7	37	74.8±5.4	49

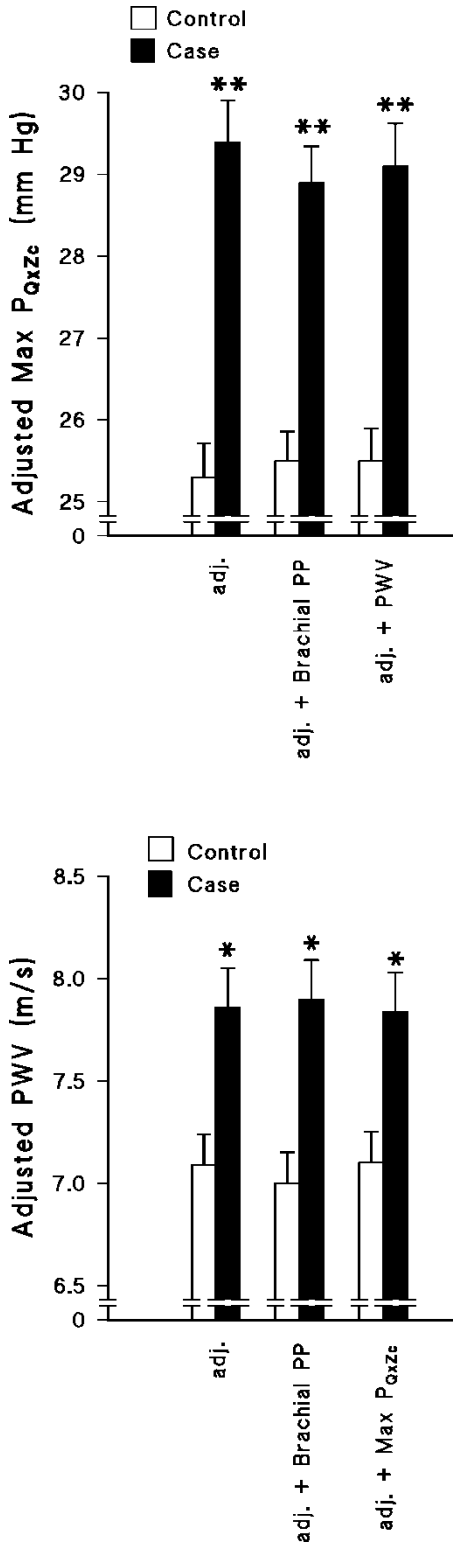


Figure 4.5. Impact of adjustments for brachial pulse pressure (PP) or alternative indices of aortic stiffness on indices of aortic stiffness (aortic PWV and P_{QxZc}) in cases with arterial events as compared to age-and sex-matched controls from a community sample. Adjustments are for variations in age, sex, mean arterial pressure, body mass index, regular smoking, regular alcohol intake and diabetes mellitus. See table 4.1 for abbreviations. *p<0.005, **p<0.0005 for cases versus controls.

Table 4.6. Multivariate-adjusted comparisons of blood pressures or indices of aortic stiffness in cases with arterial events versus age- and sex-matched controls.

	Adjustments	Stroke and CLI n=287	Controls n=390	p-value
Aortic PP (mm Hg)	*	41.6±12.0	37.5±11.4	<0.0001
Aortic PP (mm Hg)	* + brachial PP	40.7±5.9	38.1±5.5	<0.0001
Peak aortic Q (mls/sec)	*	374±163	355±160	=0.17
Aortic Z (dynes.s/cm^5)	*	91.9±24.4	79.7±23.5	<0.0001
Aortic Z (dynes.s/cm^5)	* + P_{QxZc}	84.7±13.2	84.9±12.6	=0.85
Aortic root diameter (mm)	*	26.7±6.3	25.7±5.1	=0.40
P_{QxZc} (mm Hg)	*	29.9±8.5	25.3±8.1	<0.0001
P_{QxZc} (mm Hg)	* + brachial SBP	27.5±4.7	26.1±4.5	<0.001
Aortic PWV (m/sec)	*	7.86±3.38	7.09±2.94	=0.005
Aortic PWV (m/sec)	* + brachial SBP	7.72±3.39	7.12±2.96	<0.05
TAC (ml/mm Hg)	*	2.55±1.86	2.44±1.58	=0.43

Data are shown as multivariate-adjusted mean±SD. See table 1 for abbreviations. *Adjustments are for variations in age, sex, mean arterial pressure, body mass index, regular smoking, regular alcohol intake, diabetes mellitus, and heart rate.

Table 4.7. Multivariate-adjusted comparisons of blood pressures or indices of aortic stiffness in cases with stroke or critical limb ischaemia (CLI) versus age- and sex-matched controls.

	Adjustments			p-value
		Stroke (n=109)	Controls (n=390)	
Aortic PP (mm Hg)	*	40.4±10.4	37.2±9.5	=0.007
Aortic PP (mm Hg)	* + brachial PP	42.8±4.4	36.5±3.9	<0.0001
Peak aortic Q (mls/sec)	*	381±146	355±160	=0.13
Aortic Zc (dynes.s/cm^5)	*	92.6±20.1	80.9±21.5	<0.0001
Aortic Zc (dynes.s/cm^5)	* + P_{QxZc}	83.9±9.0	84.1±9.5	=0.88
Aortic root diameter (mm)	*	25.4±4.9	25.7±4.9	=0.56
P_{QxZc} (mm Hg)	*	29.3±6.9	25.5±7.3	<0.0001
P_{QxZc} (mm Hg)	* + brachial SBP	28.8±5.4	25.7±5.9	<0.0001
Aortic PWV (m/sec)	*	8.62±2.40	6.96±1.97	<0.0001
Aortic PWV (m/sec)	* + brachial SBP	8.42±2.40	6.93±1.97	<0.0001
TAC (ml/mm Hg)	*	2.56±1.46	2.46±1.20	=0.57
		CLI (n=178)	Controls (n=390)	
Aortic PP (mm Hg)	*	43.7±11.2	38.4±11.8	<0.0001
Aortic PP (mm Hg)	* + brachial PP	40.5±5.7	39.0±5.3	=0.009
Peak aortic Q (mls/sec)	*	358±173	368±154	=0.59
Aortic Zc (dynes.s/cm^5)	*	93.2±27.3	80.6±23.9	<0.0001
Aortic Zc (dynes.s/cm^5)	* + P_{QxZc}	84.0±16.0	84.6±13.8	=0.70
Aortic root diameter (mm)	*	26.6±8.8	25.8±4.1	=0.27
P_{QxZc} (mm Hg)	*	30.1±9.5	25.9±8.3	<0.0001
P_{QxZc} (mm Hg)	* + brachial SBP	29.1±7.7	26.3±6.9	=0.0002
Aortic PWV (m/sec)	*	7.76±3.20	7.08±2.90	=0.037
Aortic PWV (m/sec)	* + brachial SBP	7.67±3.20	7.09±2.90	=0.047
TAC (ml/mm Hg)	*	2.29±1.60	2.45±1.38	=0.22

Data are shown as multivariate-adjusted mean±SD. See table 1 for abbreviations. *Adjustments are for variations in age, sex, mean arterial pressure, body mass index, regular smoking, regular alcohol intake, diabetes mellitus, and heart rate.

adjustments for heart rate (data not shown), peak aortic PP was also independently associated with arterial events.

4.4 Discussion

The main findings of the present study are as follows: In a large community-based sample, independent of brachial PP or SBP and confounders, striking relations between aortic stiffness across the full length of the aorta (PWV) or the central arterial pulsatile load determined from the product of Zc (which is determined in-part by increases in proximal aortic stiffness) and peak aortic Q (P_{QxZc}) and several small vessel or atherosclerotic end organ changes (eGFR and V-CAM-1 concentrations) were noted. The relationships between P_{QxZc} and small vessel or atherosclerotic end organ measures were accounted for in-part by Zc effects beyond aortic root diameter and thus are attributed to an increased stiffness in the proximal aorta. Importantly, relations between PWV and small vessel or atherosclerotic end organ measures were independent of P_{QxZc} and between P_{QxZc} and these same end organ measures were independent of PWV. Similarly, in comparison to age- and sex-matched controls from the community sample, participants with the arterial events, stroke and CLI showed striking increases in both PWV and P_{QxZc} well beyond brachial PP and SBP. The relationships between P_{QxZc} and arterial events were also accounted for entirely by Zc effects and not by differences in Q or in aortic root diameter and thus are attributed to an increased stiffness in the proximal aorta. Again, increases in PWV were independent of P_{QxZc} and increases in P_{QxZc} were independent of PWV.

Indices of aortic stiffness across the full length of the aorta (aortic PWV) are well-recognised as predicting arterial events beyond brachial BP (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014), and hence are included in risk prediction (Mancia *et al.*, 2013). However, the extent to which the impact of aortic stiffness anywhere in the aorta on small vessel or atherosclerotic end-organs is explained by increases in central arterial pressures beyond brachial BP, is unclear. Indeed, stiffness in the proximal aorta mediates adverse effects on pulsatile load in-part by increasing Zc and hence contributing to increases in forward wave pressures (Nichols *et al.*, 2011). In this regard, while peak central arterial PP may not predict events much beyond brachial BP (Mitchell *et al.*, 2010b; Vlachopoulos *et al.*, 2010), forward wave pressures, which are strongly determined by proximal aortic stiffness-induced increases in Zc, predict events beyond brachial BP (Cooper *et al.*, 2015). Thus, the possibility exists that some of the adverse effects of increases in aortic stiffness, at least in the proximal portion of the aorta, beyond brachial BP may be through central arterial pulsatile load. The present study nevertheless provides evidence to suggest that a considerable impact of aortic stiffness as indexed by carotid-femoral PWV on small vessel or atherosclerotic end-organs and events is indeed independent of increases in central arterial pulsatile load induced by increases in aortic stiffness and hence Zc in the proximal aorta (P_{QxZc}). Importantly however, the

present study also suggests that brachial BP-independent effects of increases in proximal aortic stiffness may additionally depend on an impact of increases in Zc beyond aortic root diameter and hence P_{QxZc} and that aortic PWV fails to appropriately index this effect. The present study therefore supports a role for central arterial pulsatile load-dependent (proximal aortic increases in Zc independent of aortic diameter and hence P_{QxZc}) and -independent (full length of the aorta as indexed by PWV) pathways of aortic stiffness that may mediate cardiovascular damage beyond brachial BP.

More recent studies have identified that much of the excess pressure generated by increases in forward wave pressures is through wave re-reflection(Phan *et al.*, 2016). Importantly, the extent of wave re-reflection depends on the amplitude of reflected (backward) pressure waves. Through Newton's Laws of Motion (inertial effects and for every action there is an equal and opposite reaction), increases in forward wave pressures enhance backward wave pressures. However, a significant portion of the variation of backward wave pressures is also determined by vascular changes that enhance wave reflection (Nichols *et al.*, 2011). These effects will therefore also influence wave re-reflection and the forward travelling pressure wave. Thus, forward wave pressures may be a limited surrogate of Zc and hence aortic stiffness-induced effects on central arterial pressure load beyond aortic root diameter, particularly when wave reflection is marked. To avoid the addition of the pressures generated by wave re-reflection to forward wave pressures (Phan *et al.*, 2016), in the present study we therefore evaluated arterial stiffness effects on central arterial pulsatile load from the pulsatile pressures generated by the product of peak aortic Q and Zc (P_{QxZc}), rather than forward wave pressures. Thus, a strength of the present study is the use of a central arterial pulsatile pressure measure that best indexes the impact of increases in proximal aortic stiffness.

Whether the brachial BP-independent relations between central arterial pressures generated by increases in proximal aortic stiffness and hence Zc beyond aortic root diameter (P_{QxZc}) and small vessel or atherosclerotic end organ measures and events noted in the present study are causal relationships, is uncertain. In this regard, a question that has remained unanswered is why forward wave pressures(Cooper *et al.*, 2015) (which are in-part determined by increases in proximal aortic stiffness and hence P_{QxZc}), but not peak aortic PP (Mitchell *et al.*, 2010b; Vlachopoulos *et al.*, 2010) predict events beyond brachial BP? Even in the present study the brachial BP-independent relationships between P_{QxZc} and either small vessel or atherosclerotic end organ measures or events were more robust than those between peak aortic PP and end organ measures and events. Indeed, alterations in central arterial pulsatile pressures generated by Zc (P_{QxZc} or forward wave pressures) may simply be an alternative measure that indexes the adverse effects of increases in aortic stiffness beyond central arterial pulsatile load. Nevertheless, in the present study the

relationships between Zc and small vessel or atherosclerotic end organ measures or events beyond aortic root diameter were fully accounted for by the impact of P_{QxZc}. This suggests that the impact of proximal aortic stiffness on arterial damage is a central arterial load effect and not simply another index of stiffness effects independent of central arterial load. Irrespective of the explanation for the relationship between P_{QxZc} and small vessel or atherosclerotic end organ measures or events, in the present study this effect was beyond carotid-femoral PWV. Thus, whether PWV effectively indexes all brachial BP-independent effects of increases in aortic stiffness is a question that requires further evaluation.

The possible mechanisms that may explain the ability of forward wave pressures (which are determined by increases in proximal aortic stiffness and Zc independent of aortic root diameter) to predict events beyond brachial BP (Cooper *et al.*, 2015), without translating into similar effects of peak aortic PP (Mitchell *et al.*, 2010b; Vlachopoulos *et al.*, 2010), requires consideration. In this regard, higher heart rates reduce reflected wave pressures and hence central arterial PP (Xiao *et al.*, 2018). Thus, at high heart rates Zc (proximal aortic stiffness independent of aortic root diameter)-induced increases in forward travelling pressure waves may not, through inertial effects, translate into similar increases in reflected wave pressures. The consequence may therefore be that peak aortic PP fails to increase in parallel with increments in aortic stiffness. It is nevertheless possible that increases in proximal aortic stiffness and hence P_{QxZc} translate into increases in peak aortic PP at alternative times of the 24-hour period, such as at night when heart rate and vascular tone decrease. Further studies are therefore required to evaluate the impact of proximal aortic stiffness-induced increases in P_{QxZc} on peak aortic PP over a 24-hour period. An alternative explanation for the limited ability of peak aortic PP as opposed to P_{QxZc} or forward wave pressures to predict events, is that the energy generated during ventricular ejection, which occurs largely in early systole, may play a dominant role in mediating arterial vascular damage. Hence peak aortic PP, which through summation of reflected and re-reflected waves with arterial stiffness-induced increases in P_{QxZc}, often occurs much later than peak P_{QxZc}, may be less important than P_{QxZc} in generating vascular damage.

In the present study although PWV and P_{QxZc} associated with endothelial activation (V-CAM-1 concentrations) independent of brachial BP, the association between PWV or P_{QxZc} and IMT, an index of atheroma formation, was not independent of brachial BP. This apparent inconsistency in relations between aortic stiffness indices and IMT may be explained by the fact that IMT may reflect in-part compensatory medial hypertrophy in response to a high wall stress rather than atherosclerotic changes in the intima (Wannarong *et al.*, 2013; Insull, 2009). In contrast, the extent of endothelial activation (V-CAM-1 concentrations) is likely to herald an impact on early atherogenesis, and hence effects that are specifically intimal in nature and independent of medial

hypertrophy. Nevertheless, IMT should not be seen as a less important index of arterial events as IMT complements atherosclerotic plaque in identifying those at risk in the present population (Kolkenbeck-Ruh *et al.*, 2020).

There are several limitations to the present study that warrant consideration. First, the present study was cross-sectional in design. Hence no conclusions regarding causality can be drawn and further longitudinal studies are required. However, the consistency in the findings across several small vessel or atherosclerotic end organ measures in a community sample and arterial events in case-control analysis suggests that residual confounding or population stratification are unlikely to explain these relations. Second, in order to avoid the impact of cardiac systolic dysfunction on peak aortic Q and hence P_{QxZc}, we did not assess relations between aortic function and myocardial infarction or heart failure of non-infectious or non-valvular origins. Thus, further studies are required to identify these relations. 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 and thus Zc may have been underestimated using the current approach. Last, the present study was conducted in one ethnic group. Our findings may therefore not be translatable to other ethnic groups.

In conclusion, in the present study we demonstrate that stiffness across the full length of the aorta, as indexed by aortic PWV, and the increased central arterial pulsatile pressure load produced by proximal aortic stiffness effects on characteristic impedance beyond aortic root diameter (P_{QxZc}), associate with several small vessel or atherosclerotic end organ measures and events beyond brachial BP. Importantly, the impact of aortic PWV was independent of proximal aortic stiffness-induced central arterial pulsatile load and the effect of proximal aortic stiffness-induced central arterial pulsatile load was independent of aortic PWV. Thus, independent of brachial BP (PP or SBP), the impact of aortic stiffness on arterial damage may involve effects that are both dependent (increased proximal aortic stiffness and hence Zc and P_{QxZc} independent of aortic root diameter) and independent (stiffness across the full length of the aorta as indexed by aortic PWV) of central arterial pulsatile load. Whether all of the damage mediated by increases in aortic stiffness is appropriately detected by PWV and brachial PP therefore requires re-evaluation. Moreover, whether targeting proximal aortic stiffness-induced central arterial pulsatile load has beneficial effects on risk reduction requires further study.

Chapter 5 Contextual Narrative and Conclusions

5.0 Introduction

In the present thesis I explored the possibility that aortic dysfunction mediated by arteriosclerotic changes in central arteries could contribute to premature arterial events (stroke and CLI) in South Africa. My interest in this topic was generated by several important issues described in the introductory chapter of the present thesis which are briefly summarised as follows. First, it is of concern that populations of low to middle-income countries such as many African countries, now experience the double burden of communicable and non-communicable disease and that of all non-communicable diseases, cardiovascular disease plays the dominant role. Second, it is of particular concern that in Africa, the arterial events stroke and CLI often occur over a younger adult age range, and in some instances in the 20-30 year age range. As highlighted in the introductory chapter, although it is well-recognised that when these events occur at a younger adult age, the prognosis is generally better, the impact on years spent disabled, and the associated effects thereof (unemployment, psychosocial problems, isolation, depression, a reduced ability to find significant others and marry, a limited ability to provide for and educate children, dependence on the state for survival, etc) when experiencing these events at a young adult age by far exceed that of the occurrence of a similar event at an older age. Third, when considering the overall risk of those experiencing premature events before the event had occurred, these patients do not have exceptional risk profiles (Kolkenbeck-Ruh *et al.*, 2019a; Kolkenbeck-Ruh *et al.*, 2020). That is, over a half of patients with arterial events (stroke or CLI) at a young adult age would have been considered as being at an unremarkable risk prior to the event (none or one risk factor only, no severe or refractory hypertension, no diabetes mellitus, and no prior event). Thus, the ability to identify those at risk of a premature arterial event using conventional risk factor analysis is limited and how to manage the risk at a young age is unclear. Thus, further work is required to identify the pathogenesis of premature events in Africa.

Importantly, previous studies in those with premature arterial events had provided unequivocal evidence for a striking impact of atherosclerotic changes as possible determinants of premature events (Kolkenbeck-Ruh *et al.*, 2019a; Kolkenbeck-Ruh *et al.*, 2020). These changes were identified using both indirect (ultrasound of the carotid arterial bed) and direct (histology of amputated limbs) approaches (Kolkenbeck-Ruh *et al.*, 2019a; Kolkenbeck-Ruh *et al.*, 2020). However, no particular conventional risk factor emerged in these studies as contributing more to premature events (Kolkenbeck-Ruh *et al.*, 2019a; Kolkenbeck-Ruh *et al.*, 2020). Thus, where the focus for intervention (detection of hypertension, diabetes mellitus, increased LDL cholesterol concentrations, etc that were previously undetected or where poor control was particularly obvious) is required, was unclear. Thus, alternative factors required identification. In this regard, as highlighted in the introduction, one pathophysiological alteration previously overlooked is the

possibility that arteriosclerosis could account for at least some of these effects. As also indicated in the introductory chapter this was almost counterintuitive to current views and certainly represents a view against almost all existing dogma. In the present thesis I nevertheless provide the evidence to suggest that central arterial functional changes produced by arteriosclerosis are indeed associated with premature arterial events and hence that a paradigm shift in how we view arteriosclerosis may therefore be required. Importantly, the main finding in this regard has been published in the high impact *American Heart Association* journal *Arteriosclerosis Thrombosis and Vascular Biology* (*ATVB*) (Motau *et al.*, 2020) and several additional manuscripts either preceding this work or following on from this work have either been published or are in-press (Motau *et al.,*2020, *J Hypertens*). In the present chapter I will first underscore the main finding of the present thesis and place it in the context of the limited evidence prior to these studies that suggested that arteriosclerotic aortic dysfunction could perhaps contribute to arterial events at a young adult age. I will then remind the reader why I developed this hypothesis despite existing dogma being so strongly against the notion. In this process I will place the evidence provided in the present thesis in the context of contemporary views of the impact of arteriosclerotic aortic functional changes as causes of arterial events. I will then suggest several possible implications for the findings in the present and alternative studies from both a clinical perspective and a perspective of possible further research required.

5.1 A paradigm shift in the role of arteriosclerotic arterial dysfunction at a young adult age

As reviewed in the introductory chapter of the present thesis the evidence prior to the work contained herein to support a role for arteriosclerotic changes as a possible determinant of premature arterial events was limited. In this regard, in a meta-analyses of several major studies, demonstrating an independent ability of aortic PWV to predict events, the effect of PWV was noted not only in the elderly, but also over a younger adult age range (below 61 years of age) (Ben-Shlomo *et al.*, 2014). Nevertheless, the number of events in the younger age group in the meta-analysis was too small to draw meaningful conclusions as the original studies were performed in developed countries where arterial events seldom occur in the young. Importantly, the age threshold employed (61 years) incorporated participants in their middle age (Ben-Shlomo *et al.*, 2014) and thus most of the events in this age range may have occurred at an age where clinically significant arteriosclerotic changes are not necessarily unexpected (middle age). Nevertheless, significant evidence for an association between risk factors and arteriosclerotic aortic functional changes (PWV) in younger individuals had been reported (Townsend *et al.*, 2015), and thus the possibility that arteriosclerosis may contribute to events over a young adult age range required further consideration. Indeed, at this time one study had reported on a high prevalence of

arteriosclerotic vascular abnormalities (PWV) in stroke in the young (Saeed *et al.*, 2014), but failed to compare aortic functional changes with age-matched controls, this preventing the identification of those in whom PWV changes were most marked, and did not report on all central arterial functional changes that characterise arteriosclerotic effects. In this regard, as reported on in the present thesis (Motau *et al.,*2020, chapter 3), I provide clear evidence for a robust association across most of the early adult lifespan between the arterial events stroke and CLI and several arteriosclerotic aortic functional changes including PWV, Zc, forward wave pressures and PP amplification. Strikingly these relations were most marked at a lower adult age as compared to randomly selected age, sex and ethnicity-matched controls, often being most robust in the 20-40 year age range. Additionally, I show that changes in Zc, forward wave pressures and PP amplification noted in those with events were attributed to aortic stiffness effects, rather than to an impact of aortic root diameter, wave reflection or wave re-reflection. In the absence of longitudinal follow-up studies which are unlikely to be easily performed in the near future in young adults, these data therefore provide compelling evidence for consideration of a possible role of arteriosclerotic aortic dysfunction in the pathogenesis of premature arterial events. These data support contemporary views on arteriosclerotic effects and highlight the major limitations with current dogma that arteriosclerotic changes translate into events only in geriatric populations with some effect noted in middle age. Importantly, how do contemporary views challenge conventional thought and how does the current data advance these contemporary views?

5.2 Challenging the dogma against a role for arteriosclerosis in the young

Prior to the past two decades, although several studies had provided evidence that arteriosclerosis may contribute to producing adverse vascular effects beyond pulsatile load, the exact effect and how these changes contribute to events was entirely unclear. Thus, at this time and even in current times, basic texts only referred to the adverse effects of arteriosclerosis as that produced by an impact of BP. As striking increases in PP and hence SBP are seldom seen in patients under 50 years of age, the notion that arteriosclerosis could contribute to cardiovascular events in the young has largely been viewed as indefensible. In this regard, the effects of arteriosclerosis on central arterial stiffness and hence PP and SBP were well described. The favoured approach to explaining this effect was analogous to that often employed to describe the impact of the Windkessel in devices designed to fight fires centuries ago and this approach was popularised by Otto Frank in the first half of the previous century. Thus, most texts described an impact of arteriosclerosis as that produced by a reduced ability of the proximal aorta to cushion blood ejected into the aorta which then increased PP and hence SBP. However, as discussed in the introductory chapter, important concepts around the Windkessel model considerably expanded in the latter half of the previous century, to include effects of a reduced aortic stiffness on Zc and TAC. These concepts

although appearing in several major scientific papers and texts(Nichols *et al.*, 2011; Chirinos *et al.*, 2019), unfortunately never caught on in basic undergraduate or postgraduate medical curricula and were largely misinterpreted when they appeared. As a consequence, although the basic molecular mechanisms of arteriosclerosis have been well studied in the recent past, much of the fundamental information surrounding the impact of arteriosclerosis has only more recently emerged. As basic texts generally only refer to the adverse effects of arteriosclerosis as that produced by an impact of BP and striking increases in PP and hence SBP are seldom seen in patients under 50 years of age, the notion that arteriosclerosis is a cause of cardiovascular disease in the young has largely been seen as untenable. What is the argument that PP and hence SBP is not an appropriate index of arteriosclerotic effects and how has the present thesis contributed to this understanding?

5.3 Is brachial pulse pressure an appropriate index of arteriosclerotic effects in the young?

Over the past two decades the limited ability of brachial PP as an appropriate index of increases in central arterial stiffness has been highlighted. In this regard, as discussed in the introductory chapter, through an impedance mismatch that exists between the aorta and more distal vessels, PP is amplified from central to peripheral arteries (Nichols *et al.*, 2011; McEniery *et al.*, 2008). This effect is greatest at a younger age when the aorta has the highest degree of elasticity and decreases at older ages when the aorta loses its elasticity (Nichols *et al.*, 2011; McEniery *et al.*, 2008). When alterations in the central arterial pulse occur as consequence of an increased aortic stiffness, the same effects do not occur in the peripheral pulse and hence the peripheral pulse has a limited ability to detect these changes. Thus, a younger age is most likely to render brachial PP as an inadequate index of arteriosclerotic effects. However, before the findings of the present thesis it was uncertain to what extent arteriosclerotic aortic dysfunction including central arterial pulsatile load could occur in association with the common risk factors for this arterial change without producing brachial PP changes. Furthermore, before the findings of the present thesis it was uncertain to what extent arteriosclerotic aortic dysfunction could occur including central arterial pulsatile load in association with arterial events without producing brachial PP changes. What has the present thesis contributed to our understanding of these questions?

In the present thesis I show that whilst at a community level forward wave pressures are indeed associated with common risk factors in a group of African ancestry living in South Africa, that similar relations with brachial PP did not occur (Motau *et al.,*2018; chapter 2). Moreover, I show that as compared to randomly selected ethnicity and age- and sex-matched controls from a community sample, patients admitted to a referral hospital with stroke or CLI across most of the adult age range have a marked increase in forward wave pressures but not brachial PP (Motau *et al.,* 2020; chapter 3). Importantly, the increase in forward wave pressures in those with arterial

events were attributed to increases in the pressures generated by the product of Zc and Q and not to the contribution of pressure re-reflection (Motau *et al.,*2020; chapter 3). Of critical importance is the finding that all of these changes resulted in differences as compared to age-matched controls which were most marked over a younger as compared to an older adult age. Although not reported on as data were not available at the time, the associations between forward wave pressures and uncontrolled hypertension and diabetes mellitus published in 2018 (Motau *et al.*, 2018), were indeed also accounted for by an enhanced pressure generated by the product of Zc and Q (uncontrolled hypertension, max P_{QxZc}, 27.4±0.64 versus normotensives of 24.7±0.40 mm Hg, $p<0.005$. and diabetes mellitus, max P_{QxZc}, 27.3±0.79 versus non-diabetics of 25.3±0.29 mm Hg, $p<0.02$) and not to the contribution of pressure re-reflection. In this regard, previous studies have highlighted the possibility that any excess in the forward wave pressure may be caused by wave re-reflection (Phan *et al.,*2016) and thus not necessarily to increases in aortic stiffness and hence Zc. This was clearly not the case in the present studies. Also importantly, the increase in the pressure generated by the product of Q and Zc in those with arterial events was associated with an increase in Zc and not in Q (Motau *et al.,* 2020, chapter 3; Motau *et al*., 2020 in-press, chapter 4). Thus, arterial and not flow-dependent mechanisms accounted for the increase in forward wave pressures. Moreover, the increase in Zc noted in those with arterial events could not be accounted for by differences in aortic root diameter (Motau *et al.,* 2020, chapter 3; Motau *et al*., 2020 in-press, chapter 4) and hence can only be attributed to increases in proximal aortic stiffness. Importantly, in both studies I show that aortic stiffness along the full length of the aorta (PWV) was associated with risk factors (diabetes mellitus) (Motau *et al.,* 2018, chapter 2) and arterial events (Motau *et al.,* 2020, chapter 3; Motau *et al*., 2020 in-press, chapter 4) and the association with arterial events was again most marked over a younger adult age. Thus, the association between risk factors or events and forward wave pressures is most likely attributed to arteriosclerotic effects on aortic stiffness and hence Zc. Nevertheless, an important question that arises is whether the inability of brachial PP to index arteriosclerotic effects in the young is because of a decrease in the impedance mismatch between the aorta and more distal vessels or because of increases in backward wave pressures often noted to increase dramatically over a younger adult age range? In this regard, as discussed in chapter 1, a decline in PP amplification may occur because of a decrease in impedance mismatches between central and peripheral arteries caused by arteriosclerotic changes or because of enhanced backward wave pressures. The question is therefore which one of these mechanisms explained differential relations between risk factors or events and central arterial versus brachial PP?

5.4 Explanation for an attenuated ability of brachial pulse pressure to index arteriosclerosis

Although in the present thesis insufficient data were available to provide an explanation for the relationships between risk factors and forward wave pressure without parallel relations also noted between risk factors and brachial PP (Motau *et al.*, 2018), I did have sufficient data to explore this issue for relations with arterial events (Motau *et al.*, 2020). In this regard, as discussed in chapter 3 (Motau *et al.*, 2020) marked decreases in central arterial-to-brachial PP amplification (increases in the reciprocal of PP amp, i.e., 1/PP amp) were noted and consistent with the normally high PP amplification that occurs at a younger adult age, the differences in PP amplification between those with events and age-matched controls was most marked over a younger adult age. Importantly, as discussed in chapter 1, peak brachial PP is largely determined by forward travelling pressure waves (excluding re-reflected waves) and hence the pressure generated by the product of Zc and Q, the peak of which occurs in early systole. Whilst backward waves generate the second systolic shoulder of the brachial pulse, the only impact of backward waves on peak brachial PP is from local vascular beds in the forearm. Therefore, the ratio of peak brachial PP to early systolic central arterial PP (peak pressure generated by the product of Zc and Q) (early systolic PP amplification) is likely to largely index the impact of the impedance mismatch on PP amplification. In this regard, in the present thesis I demonstrated that decreases in PP amplification in those with arterial events over a young adult age were largely determined by decreases in early systolic PP amplification. Therefore, the inability of brachial PP to appropriately index the impact of arteriosclerotic changes on central arterial pulsatile load is likely to be because of a reduced impedance mismatch between central and peripheral arteries. This finding has implications for a possible mechanism that may explain the adverse effects of arteriosclerosis at a young adult age on arterial events, an issue that will be discussed in subsequent sections. Although the issue of whether brachial PP is an adequate index of arteriosclerotic effects on central arterial pulsatile load has been a high priority for several decades, what has received considerably less attention is whether peak aortic PP is an adequate index of arteriosclerotic effects on central arterial pulsatile load?

5.5 Does peak central arterial pulse pressure index of the adverse effects of arteriosclerosis?

An important observation, one which was largely perceived as the novelty in the work described in the present thesis demonstrating a relationship between risk factors and central aortic dysfunction (Motau *et al.,* 2018, chapter 2) was that despite consistent relations between risk factors and forward wave pressures, similar relations between risk factors and peak central arterial PP (PPc) were not observed. Although not highlighted in the work demonstrating relations between arterial events and aortic dysfunction over a younger adult age, again despite consistent relations between

PWV, Zc and forward wave pressures and events being observed, similar relations between peak central arterial PP (PPc) and events were not observed (Motau *et al.,* 2020, chapter 3). These data are consistent with several published observations and these have largely been reviewed in the introduction to the present thesis (Chapter 1) but are worth summarising at this point.

Whilst, as discussed in above sections, in meta-analyses of several major studies, aortic PWV has been shown to be an independent predictor (beyond brachial BP) of cardiovascular events (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014), in a meta-analysis, the ability of aortic PP or SBP to predict risk beyond peripheral PP or SBP failed to achieve significance (Vlachopoulos *et al.,* 2010). Moreover, the Framingham Heart Study failed to show an ability of aortic BP to predict events beyond brachial BP despite an ability of PWV to predict events independent of brachial BP (Mitchell *et al.,* 2010b). Furthermore, in several other studies, whilst PP amplification better predicted cardiovascular mortality or events (Benetos *et al.,* 2010; Bursztyn *et al.,* 2016; Regnault *et al.,* 2012; Safar *et al.,* 2002), and heart disease (Salvi *et al.,* 2010) than brachial BP and added to brachial PP in risk prediction (Bursztyn *et al.,*2016), these studies failed to show a better effect of central PP or SBP in risk predicting than brachial PP or SBP. The results of many of these studies have been interpreted to indicate that the brachial BP-independent effects of increases in aortic stiffness are independent of even central arterial pulsatile load. However, as also indicated in chapter 1, whilst the authors of the Framingham Heart Study demonstrated an inability of PPc to predict events beyond brachial BP (Mitchell *et al.,* 2010b), they were able to show an ability of both PWV (Mitchell *et al.,* 2010b) and forward wave pressures(Cooper *et al.*, 2015)to predict vents beyond brachial BP. Thus, several lines of evidence show relations between the central arterial pulsatile wave driven by increases in aortic stiffness (forward wave pressures) and either risk factors or events beyond brachial BP despite a lack of relationship between these same risk factors and events and PPc. In this regard, as shall be discussed these findings have a number of implications. However, as increases in forward wave pressures are, through Newton's Laws of Motion, strong determinants of backward wave pressures, and the summation of forward and backward wave pressures determine peak PPc, an important question is why does aortic stiffness not increase PPc when forward wave pressures are enhanced?

There are several explanations for the findings that aortic stiffness causes an increased forward wave pressure without altering PPc. In this regard, increases in central arterial stiffness decrease baroreceptor function (Xiao *et al.*, 2018; Wilkinson *et al.*, 2000). By decreasing the sensitivity of baroreceptors, an increase in heart rate will occur. This will decrease the amplitude of backward travelling pressure waves, an effect mediated by high frequency oscillations (harmonic effects) and to some degree changes in the viscoelastic properties of arteries (Xiao *et al.*, 2018). As a consequence of a decrease in backward wave pressures a reduced augmentation of central

arterial pressures will occur. Hence, even though the forward travelling pressure wave is increased, peak central arterial pressure may fail to increase in parallel. In addition, it is possible that through a reduced impedance mismatch between central and more peripheral arteries when central arterial stiffness increases, wave reflection is reduced. Once again, a reduced augmentation of the central arterial pulse will occur and a limited correspondence between increases in forward wave pressures and peak central arterial PP will be noted. Although this hypothesis is tenable as wave reflection is greatest at points of impedance mismatch, at present there is little direct evidence to support the contention that arterial stiffness-induced decreases in the impedance mismatch cause a reduction in wave reflection. Irrespective of the mechanism that explains the effect, the observations that despite increases in forward wave pressures generated by risk factor-related arteriosclerotic changes in aortic stiffness, PPc shows no similar changes, raises an essential question. If the adverse effects are not through an impact on peak aortic PP, what is the relevance of arteriosclerotic changes, and in particular, what is the role of an enhanced forward wave pressure without increases in PPc?

5.6 Arteriosclerotic effects beyond peak aortic pulse pressure? Management and therapeutic implications

This question has stimulated several approaches to identifying possible mechanisms responsible for the impact of arteriosclerosis on events beyond that produced by pulsatile load, research which has spanned a 30-year period. As indicated in chapter 1, earlier studies were focussed on attempting to identify direct effects of arteriosclerosis or vascular stiffness on endothelial function beyond that produced by pulsatile load, studies often demonstrating *ex vivo* effects on endothelial function either in the absence of pulsatile loads or at controlled pulsatile loads (Peng *et al.*, 2003; Orr *et al.*, 2009; González-Santiago *et al.*, 2002). Although these studies provide one explanation as to why arterial stiffness in a particular vascular bed corresponds with atherosclerosis in that same vascular bed, arteriosclerotic large artery dysfunction occurs mainly in the aorta and the atherosclerotic effects are noted in the distal arterial sites (coronary, cerebral, femoral, popliteal, etc). Hence, alternative explanations are required. Later work, also largely discussed in chapter 1, supported mechanical effects that may occur beyond either brachial or central arterial PP. Of all of the potential mechanisms that have been proposed, the mechanism that has received possibly the most attention is that of a possible increase in the transmission of pulsatile energy into distal arterial beds when the impedance mismatch between central and peripheral arteries is decreased by an increased central arterial stiffness (O'Rourke *et al.*, 2010; Hashimoto and Ito, 2011; Mitchell *et al.*, 2011; Woodard *et al.*, 2015; Webb *et al.*, 2012; Cooper *et al.*, 2016b; Cooper and Mitchell, 2016; Smulyan *et al.*, 2016). Although this mechanism has been employed to explain largely arterial damage in vascular beds that receive pulsatile flow (brain and kidney), the ratio of arterial

impedance between the carotid and the aorta is strongly and inversely associated with the carotid pulsatility index (flow in the carotid versus flow in the aorta) (Mitchell *et al.*, 2011). This suggests that any arterial bed distal to the aorta may be susceptible to pulsatile damage when aortic stiffness increases. Importantly, this hypothesis is one of the many which has been employed to explain the marked ability of aortic PWV to predict events beyond brachial BP, even when peak central arterial PP fails to do so (see aforementioned sections). However, what has not received due consideration is the role of increases in forward wave pressures associated with arteriosclerotic changes even when peak aortic PP is not elevated.

The proposal is that distal arterial damage does not require increases in peak aortic PP as at these distal sites, backward wave effects are largely determined by wave reflection off local vascular beds, whereas in central arteries backward wave pressures are a sum of numerous backward waves derived from wave reflection off all distal arterial sites, but mainly those from the lower part of the body. In short, this hypothesis proposes that energy from forward wave pressures is transmitted to distal arterial sites, whereas the backward wave pressures noted centrally are largely derived from wave reflection that occurs proximal to these sites and hence is not involved in mediating damage at these sites. If the latter hypothesis is correct, then targeting central PP is of little relevance to arterial damage but targeting forward wave pressures with antihypertensive therapy may reduce the adverse effects of aortic stiffness on arterial damage. Do the data in the present thesis support a role for an impedance mismatch and do the data provide significant evidence to support a role for forward wave pressures, but not peak aortic PP in mediating vascular damage or arterial events?

As discussed in aforementioned sections, in chapter 3 (Motau *et al.,* 2020) I describe the mechanisms that explain the relationship between arterial events and the reciprocal of PP amplification. As discussed, a decreased PP amplification in those with arterial events is attributed to increases in central arterial stiffness and hence a reduced impedance mismatch between central arterial and peripheral vessels. Although the peripheral vessel that this effect was demonstrated in is the brachial artery there is no reason to believe that similar changes in impedance mismatches would not occur between central arteries and extracranial or intracranial vessels or vessels supplying the lower limbs. As such, it is indeed possible that arteriosclerotic changes in central arteries has caused an increased transmission of pulsatile energy into these more distal vascular beds, the consequence being an accentuated atheroma formation, with stroke and CLI following. What of the role of arteriosclerotic-induced changes in forward wave pressures?

In chapters 2 and 3 (Motau *et al.,* 2018; 2020) I show consistent relations between risk factors or arterial events and increases in forward wave pressures. Moreover, I show that these forward wave pressure effects are likely to be attributed largely to increases in aortic stiffness. In chapter 4

(Motau *et al*., 2020 in-press) I show clear separate and independent relations between PWV or that component of the pressure generated by increases in aortic stiffness-induced increases in Zc (P_{QxZc}) and several vascular end organ changes including that of endothelial activation, the precursor of atherosclerosis. These relationships with several end organ change (eGFR and V-CAM concentrations), were independent of brachial BP and similar relations with peak central arterial PP were not observed. In chapter 4 (Motau *et al*., 2020 in-press) I also show clear and striking separate and independent relations between PWV or P_{QxZc} and the presence of the arterial events, stroke and CLI. The consistency of the differences between PWV or P_{QxZc} in those with as compared to without events was quite remarkable. As with relations with end organ measures these relationships with arterial events were independent of brachial BP. These data therefore provide strong evidence that the adverse effects of increases in aortic stiffness are mediated by a pressure dependent (P_{QxZc} and hence forward wave pressures) and independent (PWV) pathway. These separate effects may suggest that whilst PWV indexes the impact of the impedance mismatch, that the P_{QxZc} effect indexes the pressure effect that amplifies the transmission of pulsatile energy into more distal arteries generated by the impedance mismatch, a pressure effect not adequately detected at the brachial pulse. These findings suggest that both measurements are required to adequately index the adverse effects of increases in arterial stiffness beyond brachial BP, even though peak aortic PP adds only inconsistent information. In this regard, pressure up until the inflection point (also called incident wave pressure or Pi) of the central arterial pulse is a close surrogate of P_{QxZc} and is readily available in most non-invasive devices designed to determine central arterial pressure. Thus, increases in either PWV or Pi may better predict events caused by arteriosclerosis. If P_{QxZc} is indeed causally related to events, unlike increases in aortic stiffness, as indexed by PWV, for which there is no therapeutic approach that will diminish the effect, P_{QxZc} or Pi is well-recognised as being readily targeted by conventional antihypertensive therapy. This therapy should be titrated against Pi, rather than PPc or brachial PP.

5.7 Limitations, further implications, and future studies

There are several limitations of the studies reported on in the present thesis (described in detail in the discussion section of each chapter), the most important being the cross-sectional nature of the study designs and the lack of longitudinal follow-up with outcomes data. This would suggest that the present findings fail to provide sufficiently high enough evidence to support the notion that arteriosclerosis contributes to arterial events at a young adult age. Nevertheless, as previously highlighted, because premature events are uncommon in countries where adequate follow-up can be performed and causes of death or hospitalisations are well documented (in Africa post-mortems are not required in cases of uncertain causes and often imaging is unavailable to confirm the cause of an event), the chances of better data emerging in the near future are unlikely. As the present

study was carefully controlled for (large randomly selected control sample across the adult age range in the same ethnic group thus reducing the chances of population stratification) and showed robust and consistent effects over a variety of ages, and all events were documented by specialist neurologists or vascular surgeons and conformed with imaging data, the present data represents the highest level of evidence possible to support a need to measure PWV to add to risk prediction at a young adult age. This is not counter to current guidelines which do not specify the age at which this should occur, but rather the present data emphasise the need to perform these measurements across the full adult age range in any individual with risk factors. In this regard, until the dogma that arteriosclerosis is only of concern in the very elderly or in those with an increased brachial PP, guidelines must emphasise the need to perform these measurements in any young adult with risk factors. Nevertheless, to show causality, at least for the forward wave pressure effects, randomised studies with outcomes are required where antihypertensive therapy targets increases in P_{QxZc} or Pi versus just brachial BP. In the meanwhile, the current results support the view that young adults with risk factors should have BP controlled to lower BP targets than 140/90 mm Hg. Indeed, it is only at these lower BP values that clinicians can ensure that forward wave pressures (P_{QxZc} component) are sufficiently low that transmission of pulsatile energy into distal vascular beds is limited. This is the only approach at present that will guarantee that premature events do not occur in response to the striking arteriosclerotic aortic functional changes noted often in the very young with events. However, further studies should identify the exact brachial BP threshold that should be achieved where forward wave pressure effects are no longer considered to cause arterial damage.

5.8 Conclusions

In conclusion, I provide evidence published in the high impact journals *Art Thromb Vasc Biol (ATVB)*, *J Hypertension* and *Am J Hypertens* to advance our understanding of arteriosclerosis as a possible cause of arterial disease. In this regard, I show that in contrast to what has previously been thought, that arteriosclerotic aortic dysfunction is as strongly associated with arterial events over a younger as over an older adult age. Moreover, I demonstrate that these effects go undetected because the impact on arterial function produced by risk factors is largely on central arterial forward wave pressures with little impact on brachial or peak central arterial pressures. Further, I show that the impact of arteriosclerosis on arterial damage may occur through both forward wave pressure (P_{QxZc}) effects and pressure-independent effects (as indexed by aortic PWV).

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Appendices

Appendix A Human Research Ethics Clearance Certificate (M170401)

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

Appendix B Human Research Ethics Clearance Certificate(M140429)

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 C Human Research Ethics Clearance Certificate (M160411)

R14/49 Mr Tshegofatso Motau et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160411

NAME: (Principal Investigator)	Mr Tshegofatso Motau 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

Appendix D TurnItIn Report

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ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

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4	Tshegofatso H Motau, Gavin R Norton, Pinhas Sareli, Angela J Woodiwiss. "Aortic Pulse Pressure Does Not Adequately Index Cardiovascular Risk Factor-Related Changes in Aortic Stiffness and Forward Wave Pressure", American Journal of Hypertension, 2018 Publication	<1%
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