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Central Aortic Pressure Waveforms in Hypertension

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, for the degree of Doctor of Philosophy

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Abstract

The impact of hypertension on cardiovascular end organs is mediated by increases in steady-state and pulsatile components of blood pressure (BP). The pulsatile component of BP is determined by both a forward travelling pressure wave produced by ventricular ejection and a backward pressure wave generated by wave reflection off points of vascular tapering. Although the backward pressure wave contributes to events, the factors that influence the impact and the most appropriate method of measuring the adverse effects thereof are unclear. In the present thesis I explored several aspects of the determinants, impact and measurement of backward travelling pressure waves.

An extended time of the forward pressure wave and increased speed of wave travel with an earlier return of the backward wave enhance overlap of these waves and hence augment pulse pressure (PP). The extent to which these factors influence the impact of backward wave pressures on end organ is nevertheless unknown. In 701 participants randomly selected from a community sample of African descent, I demonstrated that neither the time to the peak of the forward wave nor the speed of wave reflection were independently associated with echocardiographic left ventricular mass (LVM). However, there was a strong interaction between the time to the peak of the forward wave and central aortic PP (PPc) or peak backward wave pressure (Pb) to determine LVM and LV hypertrophy. Thus, the time to the peak of the forward pressure wave is a major determinant of the impact of backward wave pressures and hence PPc on LVMI.

Augmentation of PPc (augmentation index [AIx]) is increased in women as compared to men. However, the contribution of the peak of the forward wave pressure (Pf) and Pb to the increases in AIx in women is uncertain. In some populations, Pf contributes more than Pb to the increases in AIx in women, but the role of Pf or Pb in determining sex differences in AIx in populations where Pb plays a major role in explaining variations in PPc, is unknown. In 1097

participants randomly selected from a community sample of African descent, I showed that as compared to men, women have an increased AIx. The most important change associated with sex differences in AIx was an increased reflected wave magnitude (RM=PPb/Pf x 100). Thus, in groups of African ancestry an increased wave reflection rather than forward wave pressures largely accounts for sex differences in AIx.

The β-adrenergic receptor blocker, atenolol, is not as effective as alternative antihypertensive agents at preventing cardiovascular events, an action attributed to increases in AIx. However, whether this is a class effect of all β-adrenergic receptor blockers or an effect of atenolol on wave reflection is uncertain. In the present thesis, using simultaneous carotid pressure and aortic outlet tract velocity and diameter assessments and subsequently performing wave separation analysis, I showed that across a range of steady-state pressures (induced by phenylephrine administration), atenolol given to spontaneously hypertensive rats for 3 months resulted in increases in AIx associated with an increased time to peak Pf and an enhanced RM. Thus the deleterious effect of atenolol on AIx is attributed to both a class effect (heart rate-dependent increases in the time to peak Pf) as well as an effect specific to the vascular effects of atenolol on wave reflection.

Although aortic backward wave function (RM and Pb) predicts events, the assessment requires wave separation analysis. Whilst AIx has been employed as an easy and reproducible approach to reflected wave function, it underestimates backward wave effects. In this present thesis, in 1367 participants from a community sample I assessed whether the second systolic shoulder of the peripheral pulse waveform [pP_2]) more accurately indexes Pb effects than aortic pressure augmentation (Pa). Importantly, pP_2 was more closely correlated with Pb than Pa. The largest difference was at the highest tertile of the reflected wave foot time, where the speed of wave reflection was lowest. Consequently, independent of brachial BP and additional confounders, Pb or pP_2 but not Pa were independently associated with LVM. Thus, pP_2 which is readily identified on a peripheral pulse wave, may be a useful surrogate of Pb effects.

In conclusion, in the present thesis I show that an extended time to the peak of the forward wave pressure determines the impact of backward wave pressures on LVM; that atenolol-based antihypertensive therapy not only produced adverse effects on aortic function by extending the time to the peak of the forward wave through heart rate-related effects (class effect), but also increases wave reflection (specific to non-vasodilator β-adrenergic receptor blockers); that the adverse effects of female gender on aortic function in groups of African ancestry are through enhanced backward pressure waves and that backward wave pressure effects, although inadequately indexed by aortic augmented pressure, can be appropriately indexed by the second systolic shoulder of the peripheral pulse. These data provide further insights into the adverse effects and appropriate measures of central arterial wave reflection.

Declaration

I declare that this 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.

……

Oluwatosin Grace Tade …… day of ……., 2019

I certify that all human and animal studies contained in this thesis have the approval of the Human Research Ethics Committee (Medical) and Animal Ethics Screening Committee of the University of the Witwatersrand respectively.

……

Oluwatosin Grace Tade …….. day of ……., 2019

Dedication:

To my sons, Gbeminiyi and Temitope, who gave me company, courage and for whose sake, I chose to finish strong.

Publications and Presentations arising from this Thesis

Publications

1) **Tade, G**., Norton, G.R., Booysen, H.L., Sibiya, M.J., Ballim, I., Sareli, P. and Woodiwiss, A.J. (2017). Enhanced aortic reflected wave magnitude accounts for the impact of female gender on aortic pressure augmentation in a Group of African Ancestry. *American Journal of Hypertension*, 30(8), pp.781–790.
2) **Tade, G**., Norton, G.R., Booysen, H.L., Sibiya, M.J., Ballim, I., Sareli, P., Libhaber, E., et al. (2017). Time to the peak of the aortic forward wave determines the impact of aortic backward wave and pulse pressure on left ventricular mass. *Journal of Hypertension*, 35(2), pp.300–309.

Oral presentations

1) **Tade, G**., Norton, G.R., Libhaber C.D, Sareli, P. and Woodiwiss, A.J.The second systolic peak of the peripheral pulse more closely indexes the adverse effects of reflected waves than central aortic augmentation pressures." Stroke and Hypertension Congress 2018 (Stellenbosch, South Africa 3rd – 5th August 2018).
2) **Tade, G**., Norton, G.R., Booysen, H.L., Sibiya, M.J., Ballim, I., Sareli, P. and Woodiwiss, A.J. (2017). Time to the peak of the aortic forward wave determines the impact of backward wave and pulse pressure on left ventricular Mass. 44th Conference of the Physiology Society of Southern Africa (PSSA), **(**Cape Town, South Africa, 28-31 August 2016).
3) **Tade, G**., Norton, G.R., Booysen, H.L., Sibiya, M.J., Ballim, I., Sareli, P. and Woodiwiss, A.J. (2017). Time to the peak of the aortic forward wave determines the impact of backward wave and pulse pressure on left ventricular Mass. Stroke and

Hypertension congress 2016 (Misty Hills Hotel, Cradle of Humankind, South Africa 19-21 August 2016).

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List of Abbreviations

AIx	augmentation index
AP	augmentation pressure
BMI	body mass index
BP	blood pressure
CAFE	Conduit Artery Function Evaluation
CHD	coronary heart disease
CI	confidence interval
Cm	centimetre
DBP	diastolic blood pressure
DM	diabetes mellitus
EF	ejection fraction
F(t)	flow wave
GBD	global burden of disease
GTF	generalized transfer function
HbA1C	glycated haemoglobin
HDL	high density lipoprotein
IMT	carotid intima-media thickness
ISH	isolated systolic hypertension
kg	kilogram
kg/m^2	kg per $meter^2$
LV	left ventricular
LVED	LV end diastolic
LVEDD	LV end diastolic diameter
LVEDV	LV end diastolic volume

LVESD	LV end systolic diameter
LVESV	LV end systolic volume
LVH	LV hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass index
m/sec	meter per second
MAP	mean arterial pressure
ml/min	milliliter per minute
ms	milliseconds
mm	millimeter
mm Hg	millimetres of mercury
mmol/l	millimole per litre
mmol/day	millimoles per day
n	number (sample size)
P1	first systolic peak or inflection point
P2	second systolic peak
Pa	augmentation pressure
Pb	backward wave pressure
Pf	forward wave pressure
Pi	incident wave pressure
PPb	brachial pulse pressure
PPc	central aortic pulse pressure
PP	pulse pressure
p value	probability value
PWV	pulse wave velocity
r partial	correlation coefficients

RI	reflected wave index
RM	reflected wave magnitude
SAS	statistical analyses software
SBP	systolic blood pressure
SBPc	central aortic systolic BP
SD	standard deviation
SEM	standard error of the mean
Statistics SA	statistics South Africa
WHO	World Health Organization
Zc	characteristic impedance

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Preface

The global burden of cardiovascular disease is predicted to increase over the next 10-15 years until over 23 million people will die per year by the year 2030. Importantly, most cardiovascular-related deaths occur in low- and middle-income countries such as those in sub-Saharan Africa. That risk factor that accounts for most population attributable risk for cardiovascular events in all populations, including South Africa, is hypertension. Traditionally the adverse effects of hypertension have always been evaluated by the assessment of blood pressure at the brachial artery. However, in many studies the pulsatile component of central arterial pressures has been shown to be more closely associated with cardiovascular events and end organ damage than pressure at the brachial artery. In this regard, the pulsatile component of central arterial pressure is produced by both forward and backward travelling pressure waves. Indeed, whilst the forward travelling pressure wave is generated by ventricular ejection, the backward pressure wave is generated by reflection of forward travelling pressure waves off sites of vascular tapering. Although through Newton's Laws of Motion variations in backward wave pressures are dependent on the magnitude of forward travelling waves, there is large component of backward wave pressures that is determined by vascular tone and not always by arteriolar tone. There is however an ongoing debate as to the role of wave reflection as a determinant of the adverse effects of increases in pulsatile pressures. As wave reflection has, compared to several populations around the world, been demonstrated to be the highest in urban communities in Africa, in the present thesis I explored several aspects of the determinants and impact of wave reflection on end organ measures in an African population. To complement these studies, I also, in the present thesis, assessed reflected wave function in animal models of hypertension where similar studies in humans were not feasible.

The present thesis consists of a series of semi-independent chapters, each with its own introduction, methods, results and discussion section. The thesis begins with a chapter where I

critically review the available evidence in the field and lead the reader through a series of arguments in support of conducting the studies described in the present thesis. Furthermore, the present thesis concludes with a summary chapter which consolidates the findings of each chapter and underscores the novelty of the findings by placing the studies in the context of our present understanding of the field. In support of the present thesis, the data presented in chapters 2 and 3 have been published in the *Journal of Hypertension* (Tade et al 2017a), and *American Journal of Hypertension* (Tade et al 2017b) respectively. The data in the other chapters (chapters 4 and 5) are currently in preparation for submission to international journals for review.

Chapter 1

Introduction and Critical Review

1.0 Introduction

Cardiovascular disease (CVD) incorporates a wide variety of clinical conditions of the heart or vessels. More commonly occurring conditions include coronary heart disease (CHD) (otherwise referred to as coronary artery [CAD], or ischaemic heart [IHD] disease) often presenting as myocardial infarction [MI]), cerebrovascular disease (stroke), peripheral arterial disease, rheumatic heart disease with valvular disorders, congenital heart disease, heart failure (HF) of any cause, and renal insufficiency. On average as at 2015, 17.7 million people die from CVD annually (Narayan et al., 2010). Deaths caused by CVD represent 31% of all global deaths (Murray et al., 1996; Laslett et al., 2012; Laslett et al., 2012; Murray and Lopez, 1996) of which 12.9 million of these deaths are due to MI and stroke alone (Mensah et al., 2015).

Globally, each year, one or more of these conditions is therefore the principal cause of death and a leading cause of disability-adjusted life years (Reis et al., 2016). Of the 17.7 million annual deaths around the world due to CVD, 82 % are in low- and middle-income countries. Should this trend continue, it is predicted that the burden of CVD will increase significantly until over 23.6 million people die from CVD per year by the year 2030 with most dying from CVD in low- and middle-income countries (Heidenreich et al., 2011; Mosca et al., 2011, 1997; Vaitkevicius et al., 1993).

1.1. Cardiovascular disease in Africa

Historically, nutritional and infectious diseases have been the main causes of death in Africa. The African continent is however, presently also burdened with deaths from CVD with mortality rates that are higher than developed countries. Indeed, for example, age adjusted stroke mortality for individuals over the age of 15 years in Tanzania is markedly higher than that in the

United Kingdom (Walker et al., 2000). Projections from the Global Burden of Disease project suggest that the burden of CVD faced by African countries would be doubled between 1900 and 2022, with a large proportion of the affected being middle aged (Mbewu and Mbanya, 2006).

In South Africa, CVD is a leading cause of death after infectious diseases and it is responsible for 17.8% of deaths in South Africa (GBD 2015 HIV Collaborators, 2016). Despite the sustained epidemic of infectious diseases causing 19.5% of deaths in South Africa, stroke, hypertensive heart disease and CHD account for more than one-third of deaths in people older than 65 years of age (Sliwa et al., 2008). Within the age group 55-74 years, stroke is the leading cause of death and ranks second in the age group 34-54 years (Connor et al., 2007) with a 10% greater prevalence among women compared to men (Mensah et al., 2015).

1.1.2. Cardiovascular disease in women

Previously, CVD was perceived as a disease predominantly affecting males. This is in part because men generally develop CVD at a younger age than women, and thus women were thought to have a lower risk of CVD than men (Mosca et al., 2011). However, women, at least when reaching menopause, may be at a higher risk of CHD and stroke or the deaths caused by these events than men, both in developed and developing countries (Garcia et al., 2016; Mosca et al., 2011; Sliwa, 2012). In this regard, CVD mortality in women was earlier reported to be responsible for 8.5 million deaths annually (Mozaffarian et al., 2015) and in 2013, data from the Global Burden of Disease study showed that IHD and stroke were the leading causes of death and disability adjusted life years in women worldwide. More women die from CAD (Roger et al., 2012) and MI (Bairey Merz et al., 2006; Moriel et al., 1995; Shaw et al., 2008, 2006; Smilowitz et al., 2011) compared to age-matched men. Women under the age of 50 years experience a 2-fold increase in mortality after acute MI (Vaccarino et al., 1999). Between the age range of 45-64 years, more women develop HF within 5 years of MI (Roger et al., 2012) and

among those who are 65 years and over, more women die within the first year of developing MI (Roger et al., 2012). Overall, women are twice more likely than men to develop HF with a preserved ejection fraction (Garcia et al., 2016). In contrast to men having a linear increase in the incidence of CAD with age, there is a more exponential increase of CAD incidence in women with a marked increase after the age of 60 years (Wenger et al., 1993). Importantly, there is an increased risk of CVD associated with hypertension and other risk factors in women. In this regard, for every 10 mm Hg increase in systolic blood pressure (SBP), the risk of CVD is higher in women after adjusting for age and baseline SBP compared to age-matched men (Wei et al., 2017). Of note, the death rate from cardiovascular events is on the rise in women (Mosca et al., 1997; Mozaffarian et al., 2015). Of concern is that two-thirds of sudden deaths due to CAD in women in the Framingham Heart Study occurred in those with no previous symptoms of disease compared with half the sudden deaths in men (Mosca et al., 2010). It is well accepted that efforts to prevent major cardiovascular events should be directed at managing the well accepted risk factors for CVD including abnormal blood lipid profiles, smoking, hypertension, diabetes mellitus, abdominal obesity, psychosocial factors and poor dietary habits. In this regard, these risk factors are present in both men and women across all age and ethnic groups (Keates et al., 2017). Importantly however, as will be discussed below, hypertension stands out as a major modifiable risk factor for CVD accounting for more population attributable risk of events than alternative risk factors (Turnbull and Blood Pressure Lowering Treatment Trialists' Collaboration, 2003) and in women, this occurs alongside diabetes mellitus (Cheng et al., 2014).

1.2. Contributions of hypertension to cardiovascular disease.

Hypertension affects approximately 30% of the world's population and has become a major public-health challenge (Kearney et al., 2005) and a leading risk factor for CVD worldwide (Chobanian et al., 2003; Heidenreich et al., 2011). Hypertension is a major risk factor

for end-stage renal disease in developed countries (Chobanian et al., 2003) and most likely also in Africa. One in every five cardiovascular deaths is attributed to the presence of hypertension (Chobanian et al., 2003). In Africa, the incidence of HF (Stewart et al., 2008), CAD (Steyn et al., 2005) and stroke (Connor et al., 2009; Ezzati et al., 2006; Lewington et al., 2002) is often attributed to the presence of hypertension. Hypertension is therefore globally, a major driver of CVD (Narayan et al., 2010; Schmidlin et al., 2007). In urban, developing communities of African ancestry, 26% of the population are hypertensive (Guwatudde et al., 2015). Unfortunately, 50% of these cases are undiagnosed and therefore untreated (Ataklte et al., 2015; Cappuccio Francesco P. et al., 2004; Guwatudde et al., 2015; Hendriks et al., 2012; Musinguzi et al., 2015; Musinguzi and Nuwaha, 2013). Although for practical reasons, the diagnosis of hypertension and antihypertensive therapy use is based on thresholds (cut-points) for BP, observational studies demonstrate a continuous relationship between BP and cardiovascular morbidity and mortality (Lewington et al., 2002; Stewart et al., 2008) both in the elderly and the young (Franklin and Wong, 2013; Miura et al., 2001), Consequently, a better understanding of the relationship between BP and the risk of CVD or CVD-related mortality is critical to directing efforts to improve global public health.

1.2.1 Systolic or diastolic blood pressure. Which is more important?

Although as a consequence of contemporary clinical trials some countries have recently adopted much lower threshold values for the diagnosis of hypertension, hypertension is generally defined as the consistent increase of BP above 140 mm Hg SBP and 90 mm Hg diastolic BP (DBP). Normal or optimal BP is classified as being a SBP less than 120 mm Hg and a DBP less than 80 mm Hg. Over the years, there have been several debates as to whether SBP or DBP best predicts CVD. In this regard, DBP was previously thought to have the better prognostic value (Pickering, 1955). Clinical practice and trials placed a greater importance on DBP largely

because it was assumed that increased SBP was mainly due to a loss of elasticity of the major arteries, a change that is largely age-dependent and therefore unavoidable. On the other hand, DBP is a function of peripheral vascular resistance and was therefore perceived as easily modified by antihypertensive agents that target arteriolar tone. However, age is the major risk factor for hypertension, and with advancing age, increases in SBP occur across the full adult lifespan, whilst DBP increases from youth to middle age and then declines thereafter (Kannel, 2000). Indeed, isolated systolic hypertension (ISH) occurs in 0.8 % of 50 year olds, 5% of 60 year olds, 12.6% of 70 year olds and up to 23.6% of 80 year olds (Silagy and McNeil, 1992), whilst the prevalence of hypertension defined according to an increased DBP increases until 50-60 years of age and remains constant thereafter. Better insights into the relative importance of DBP versus SBP was obtained from early follow-up data of participants in the Framingham Heart Study which showed "a trend of declining relative importance of DBP and a corresponding increase in the importance of SBP with advancing age" (Kannel et al., 1971). More clinical and observational studies in the elderly have subsequently revealed that an elevated SBP predicts adverse cardiovascular outcomes better than DBP; that SBP predicts events independent of DBP; and that there is a reduction in cardiovascular events in the elderly with treatment of isolated systolic hypertension (ISH). Some of the studies that show SBP to predict cardiovascular events independent of DBP and in a continuous and graded manner are summarized in Table 1.1. Importantly, SBP is now accepted as being a stronger predictor of events than DBP after the age of 50 years (Franklin et al., 1997; Vishram et al., 2012).

1.2. Pulse pressure in risk prediction

The adverse effects of BP are now recognised as being caused by both steady-state pressure effects, best indexed by mean arterial pressure (MAP) and pulsatile pressure effects, as indexed by pulse pressure (PP) measured at the brachial artery (the difference between brachial

Table 1.1. SBP rather than DBP predicts risk

References	Conclusion	Sample size	Number of events	Outcomes	Duration of study
(Liu et al., 1999)	Reduced SBP improves cardiovascular health	2394		Stroke, all-cause mortality, cardiovascular mortality,	2 years.
(He and Whelton, 1999)	SBP ≥ DBP			Coronary heart disease, stroke, total cardiovascular mortality, all-cause mortality	4 years
(SHEP study, 1991)	Reduced SBP reduces cardiovascular risk	4736		Stroke, cardiovascular complications	4.5 years
(Nielsen et al., 1997)	SBP ≥ DBP	6545			12 years
(Franklin et al., 2001)	SBP and PP ≥DBP in older and elderly	6539	807	Incident fatal and non-fatal coronary heart diseases	17 years
(Wang et al., 2000)	Reduced SBP = reduced cardiovascular events	2394	547	Total or cardiovascular mortality, all fatal and non-fatal endpoints (stroke, cardiac end points)	3 years
(Forette et al., 2002)	Reduced SBP = reduced cardiovascular events	2902	64	Dementia	3 – 4 years

(Staessen et al., 2004)	Reduced SBP = reduced cardiovascular events	4695	286	Stroke and cardiovascular events	6 years
(Hulley et al., 1985)	Increased SBP is associated with cardiovascular risk	551	540	To reduce SBP	1 year
(Wang et al., 2010)	SBP effects are independent of DBP	1272	222	All-cause mortality	15 years
(Kostis et al., 1997)	Reduced SBP prevents the incidence of heart fail	4736	160	Fatal and non-fatal heart fail	4.5 years
(Staessen et al., 1997)	SBP reduction reduced incidence of CV events	4695		Fatal and non-fatal cardiac endpoints, sudden death and cardiovascular mortality	2 years
(Staessen et al., 1999)	SBP predicts cardiovascular events	808		Total and cardiovascular mortality, all cardiovascular end points, fatal and non-fatal stroke, fatal and non-fatal end points.	4.4 years

SBP and DBP). Hypertension, defined according to any threshold is characterized by increases in both the steady-state and pulsatile components of BP (Cheng Susan et al., 2012). Importantly; SBP reflects a composite of steady-state pressure and pulsatile pressure effects. However, the ability of SBP to predict events beyond DBP is thought to largely reflect PP effects. As SBP predicts events beyond DBP (see above discussion) and hence MAP (both DBP and MAP are largely driven by vascular resistance), so too does an increased or widened PP as estimated from usual brachial BP measurements, predict risk and all-cause mortality beyond MAP (Avolio et al., 2009; Laurent et al., 2006). Indeed, an increased brachial PP is associated with cardiovascular risk in different prediction models (Safar, 2008). Increases in PP is related to the risk of cardiovascular events in the middle aged and elderly (Assmann et al., 2005; Franklin et al., 2001), in patients with CAD (Fontes et al., 2008) and mortality in middle-aged men with a low cardiovascular risk (Benetos et al., 1997). As changes in SBP with age are attributed mainly to increases in PP rather than MAP with advancing age, there has therefore been a gradual shift in the importance in risk prediction from DBP to SBP and then to PP (Meaume et al., 2001).

1.3.1. Brachial versus central aortic PP: Differences and ability to predict risk

In contrast to MAP, which remains nearly constant along the arterial tree, PP increases significantly from the central aorta to peripheral arteries, such as the brachial or femoral arteries (O'Rourke and Nichols, 2005). This difference between central aortic and peripheral PP, a phenomenon known as PP amplification, is thought to be driven to some extent by a gradient in vascular stiffness and diameter and hence an increased impedance (resistance in a pulsatile system) from the aorta to more peripheral arteries (Hashimoto and Ito, 2010; Pierce et al., 2013; Sweitzer et al., 2007). In this regard, moving away from the heart, there is a gradual decrease in arterial diameter (generating points of impedance mismatch) and a gradual increase in arterial stiffness (due to differences in elastin to collagen ratio) causing amplification of PP as the wave

travels outward (McEniery et al., 2014; Payne et al., 2010). Aging and the presence of several major risk factors including uncontrolled hypertension, diabetes mellitus and smoking are associated with a loss of arterial elasticity which occurs more rapidly in the central aorta than the peripheral arteries (Vlachopoulos et al., 2010). Indeed, the muscular peripheral arteries such as the brachial artery are less influenced by these aging changes (Agabiti-Rosei et al., 2007; Laurent et al., 2006; O'Rourke and Nichols, 2005). Thus, in time (aging and risk factors), although aortic PP may be increased, the peripheral pulse wave is not amplified as much as in youth, and peripheral PP tends to be a better reflection of central aortic PP (McEniery et al., 2008; Nichols et al., 2011). In other words, through increases in aortic stiffness, aortic PP increases, but because of a reduction in the stiffness gradient and impedance mismatch between peripheral and central arteries, PP amplification is markedly reduced and peripheral PP begins to reflect aortic PP (Nichols et al., 2008; Nijdam et al., 2008). The much higher PP amplification in healthy young individuals, at a given MAP (Agabiti-Rosei et al., 2007; O'Rourke, 2002; Pauca et al., 1992; Roman et al., 2007; Safar et al., 2002) thus diminishes with age and risk factors (Wilkinson et al., 2001). Low PP amplification and a disproportional increase of central over peripheral PP (Nichols et al., 2011) is therefore a risk predictor, particularly in the elderly and in patients with renal failure and hypertension (Agabiti-Rosei et al., 2007; Roman et al., 2007; Safar et al., 2002).

Based on the fact that the aorta is closer in proximity to cardiovascular end organs such as the heart, brain and kidney, than the brachial artery, brachial PP may not always be an appropriate measure of aortic PP. Indeed, it is the aortic SBP that the left ventricle encounters during systole (afterload) and the aortic pressure during diastole that determines coronary perfusion (Vlachopoulos et al., 2010). Large artery stiffening involved in the pathogenesis of hypertension (Laurent et al., 2006; Nichols et al., 2011) may thus mediate adverse cardiovascular events in-part by increasing left ventricular afterload (Laurent et al., 2006; Nichols et al., 2011) and promoting hypertrophy and damage to the myocardium through increases in central aortic

PP (Mitchell et al., 2010a), an effect not always well indexed by peripheral PP. Central aortic SBP or PP rather than brachial SBP or PP may therefore be required to adequately predict risk or at least add to the ability of brachial BP to predict cardiovascular events. Nevertheless, although several studies have demonstrated independent relations between aortic SBP or PP and cardiovascular end organ measures, outcome-based studies have shown inconsistent evidence for a role of central aortic SBP or PP in risk predicting. What is this evidence?

With respect to independent associations between aortic SBP or PP and end organ measures, central SBP or PP independently determines carotid intima media thickness (Boutouyrie et al., 1999; Neisius et al., 2012), left ventricular mass and hypertrophy (Covic et al., 2000; Neisius et al., 2012; Roman et al., 2010; Wohlfahrt et al., 2012) and pulse wave velocity (Neisius et al., 2012). Reviews and meta-analysis show that central SBP or PP have better relations with cardiovascular target organ damage over brachial pressures (Kollias et al., 2016; Roman and Devereux, 2014) and hence may be a potential mechanism to explain clinical outcomes (Williams et al., 2006). Do these better relationships between central arterial as compared to peripheral SBP or PP and end organs translate into superiority of central as compared to peripheral arterial BP with cardiovascular events?

With regards to the ability of aortic SBP or PP to predict outcomes, 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 PP to predict events beyond brachial SBP (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 central PP 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. Other studies have however demonstrated that aortic PP, but not PP amplification independently predict events (Temmar et al., 2010) and the Framingham Heart Study also failed to show an ability of PP amplification to independently predict events (Mitchell et al., 2010b). Importantly, few studies have assessed whether targeting central aortic as compared to brachial BP better predicts outcomes. In this regard, however, one study suggested that treatment-induced reductions in central aortic SBP may better reduce the risk of cardiovascular events (Williams et al., 2006).

In summary, the evidence to show an ability of aortic SBP or PP to predict risk beyond brachial BP is inconsistent. The question however arises as to whether more specific determinants of aortic, as opposed to brachial, PP should be evaluated when adding to risk prediction using central aortic haemodynamic assessments? Because aortic PP or SBP may not add to risk prediction beyond brachial BP, several groups including our own have therefore turned our attention to the individual determinants of central aortic SBP or PP to attempt to identify whether these individual determinants rather than the composite effects (increases in aortic PP) improve on risk prediction. What are the determinants of aortic PP?

1.3.2. Aortic forward wave pressures and pulse pressure

Pulse pressure waveforms in many arterial beds, including that of the aorta are generally composed of two waves; the incident or forward (Pf) and the reflected or backward (Pb) pressure waves (Murgo et al., 1981; Nichols et al., 2011) (Figure 1.1). The incident pressure wave is generated by left ventricular ejection into the aorta. Factors that determine aortic flow (stroke volume), such as cardiac contractility, afterload to the left ventricle and ventricular filling (Frank-Starling effect) therefore make a major contribution to the magnitude of the forward pressure wave (Nichols et al., 2011). As the aorta has a large diameter and at a young age is

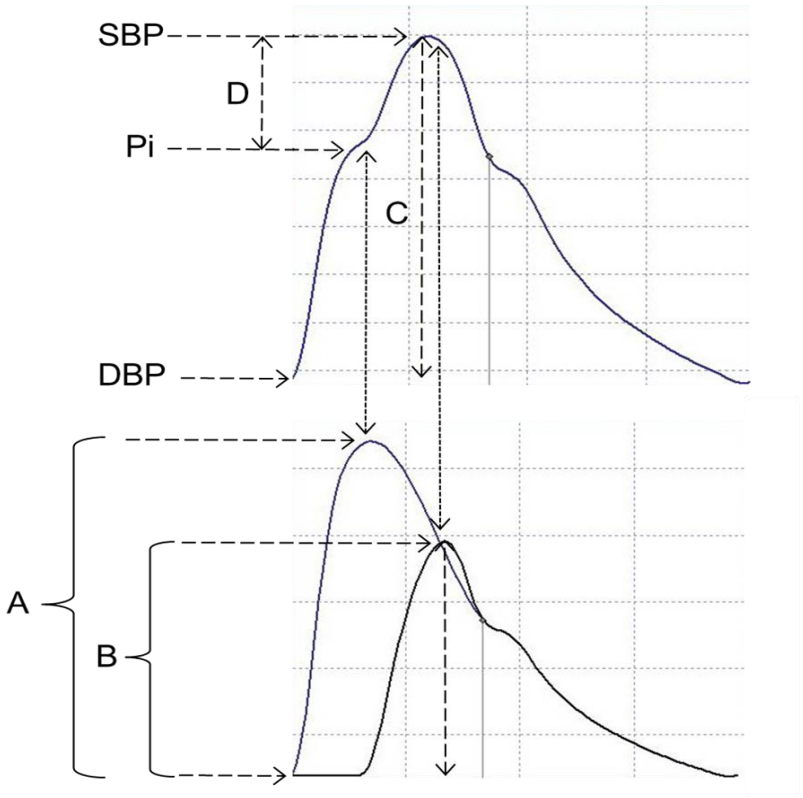

Figure 1.1. Central aortic pressure waveform (upper panel) and its component waves. The upper panel shows the summation of the forward and backward waves to produce one pressure wave. The lower panel shows the component forward and backward pressure waves derived from wave separation analysis. A: Forward wave pressure (Pf); B: Reflected wave pressure (Pb); C: Pulse pressure (PP); D: Augmented pressure (Pa); Augmentation index (AIx) = Pa/PP

elastic and hence compliant, it creates a cushioning effect during ventricular ejection and hence generates little impedance to flow (Mitchell et al., 2010b; Nichols et al., 2011). The high compliance of the aorta in a young, healthy individual allows for expansion during systole to such an extent that storage of 60 to 70% of the ejected stroke volume occurs. The subsequent elastic recoil during diastole propels blood into distal vessels. However, with aging and the presence of several risk factors including hypertension and diabetes mellitus, elastic fibres thin, fray, fracture and fragment, collagen content increases in quantity and becomes more cross-linked, and calcification of the intima occurs (Nichols et al., 2011). These structural alterations in the aorta, which may progress to advanced changes including cystic medial necrosis, are generally considered to reflect arteriosclerotic changes, and increase the stiffness of the aorta (decrease the compliance) and alter aortic dimeter. Changes in aortic stiffness and diameter generate a greater impedance (referred to as characteristic impedance as it specifically denotes impedance generated in the absence of reflected waves [which is input impedance]) to flow (Nichols et al., 2011). Under these circumstances, the magnitude of the incident pressure wave depends less on flow itself, and more on the aortic characteristic impedance to flow. With increases in aortic characteristic impedance produced by changes in stiffness and diameter of the aorta, the aorta is unable to expand as much as normal during systole and this reduces the extent of the elastic recoil, with a consequent drop in flow during diastole, a change that may explain reductions in DBP at an older age.

Whilst aortic stiffness effects on aortic impedance are easily grasped, the impact of aortic diameter on aortic impedance is complex and less easy to comprehend. It is acknowledged that the aortic root increases in diameter with age (Mao et al., 2008; Lam et al., 2010; Devereux et al., 2012) an effect that should theoretically reduce resistance and hence impedance to flow. However, the large diameter of the aorta may contribute little to resistance to flow at any age (it is not a resistance vessel). Rather, dilatation of the proximal aorta is thought to transfer the load from less stiff elements such as elastin to stiffer elements such as collagen. Thus, diameter

changes with aging may increase forward wave pressures by enhancing stiffness and thus impedance to flow. Nevertheless, the debate as to whether aortic diameter increases or decreases aortic PP is presently unsettled and several studies suggest that a relative reduction in aortic diameter (less of an increase with aging) is associated with increases in aortic PP (Farasat et al., 2008; Vasan et al., 1995). Although aortic forward wave pressures have been viewed as determined largely by factors that are not modified to any marked degree by antihypertensive therapy, more recent evidence now also points to a contribution of re-reflection of backward waves (see below) to aortic forward waves and hence aortic PP (Berger et al., 1993; Phan et al., 2016) and backward wave pressures are modified by antihypertensive therapy. Further, what is frequently ignored by authors in published work is the fact that aortic stiffness is determined not only by structural changes in the aorta, but by distending pressure effects (passive effects) as well. Hence, for a higher degree of aortic distension, stiffness of the aortic wall is greater than at lower levels of distension (stiffness relations are exponential and hence at low diameters, stiffness is low and at high diameters stiffness is high). These effects are in-turn driven by MAP or steady-state pressures which determine the degree of distension of the aorta and are in-turn readily influenced by acute changes in BP produced by conventional antihypertensive therapy. Thus, forward wave pressures, through changes in aortic stiffness, are also strongly influenced by acute changes in steady-state pressures and may be modified by therapy.

1.3.3. Aortic reflected waves and pulse pressure.

The aortic forward or incident pressure wave travels along the arterial wall from the ascending aorta to the peripheral arteries at a speed that is determined by the stiffness of the aorta. In this regard, it is important to note that whilst forward flow waves may take several cardiac cycles to reach peripheral vessels, forward pressure waves are transmitted to these arteries almost immediately (Nichols et al., 2011). As the incident pressure waves encounter sites

of high impedance such as artery-arteriolar junctions and arterial branch points, including the interface between muscular arteries and arterioles, they are reflected and travel back to the ascending aorta. These reflected pressure waves therefore generate a backward pressure wave (the peak of which is Pb in Figure 1.1). It is likely that innumerable small reflections from these various reflecting sites summate to form a relatively discrete composite reflected wave. As long as backward wave pressures are not out of phase (at pressure nodes rather than antinodes) with forward wave pressures (which will subtract from the forward wave), because the speed of wave travel along the aorta is normally very fast, the reflected wave may arrive at the ascending aorta sufficiently early that the arrival coincides with the late systolic period of the same cardiac cycle and overlaps with the incident wave to boost aortic PP (Murgo et al., 1980) (Figure 1.2). That is, with an earlier time of reflected wave return, the reflected wave will arrive closer to the forward pressure wave antinode than node and the extent of summation of the forward and backward pressure waves will increase, This increase in aortic PP generated by aortic reflected waves returning early in the cardiac cycle is called aortic augmentation pressure (AP or Pa) and is often expressed as augmentation index (AIx=AP/aortic PP or Pa/aortic PP) in order to separate the impact of forward on backward travelling pressure waves (see below). This is illustrated in Figure 1.1.

Through inertial effects and Newton's 3rd Law of Motion (for every action there is an equal and opposite reaction), the magnitude of the forward wave pressure has a major effect on the amplitude of the backward or reflected wave pressure. Hence, in-part to identify the extent of the increase in AP (Pa) produced by Pb independent of forward wave pressures, AP (Pa) is divided by aortic PP (AP or Pa/Aortic PP=AIx). It is important to note therefore, that not only are forward wave pressures in-part determined by backward wave pressures through re-reflection of backward waves, but backward wave pressures are driven by forward wave pressure through the impact of Newton's Laws of Motion. Nevertheless, independent of forward wave pressures,

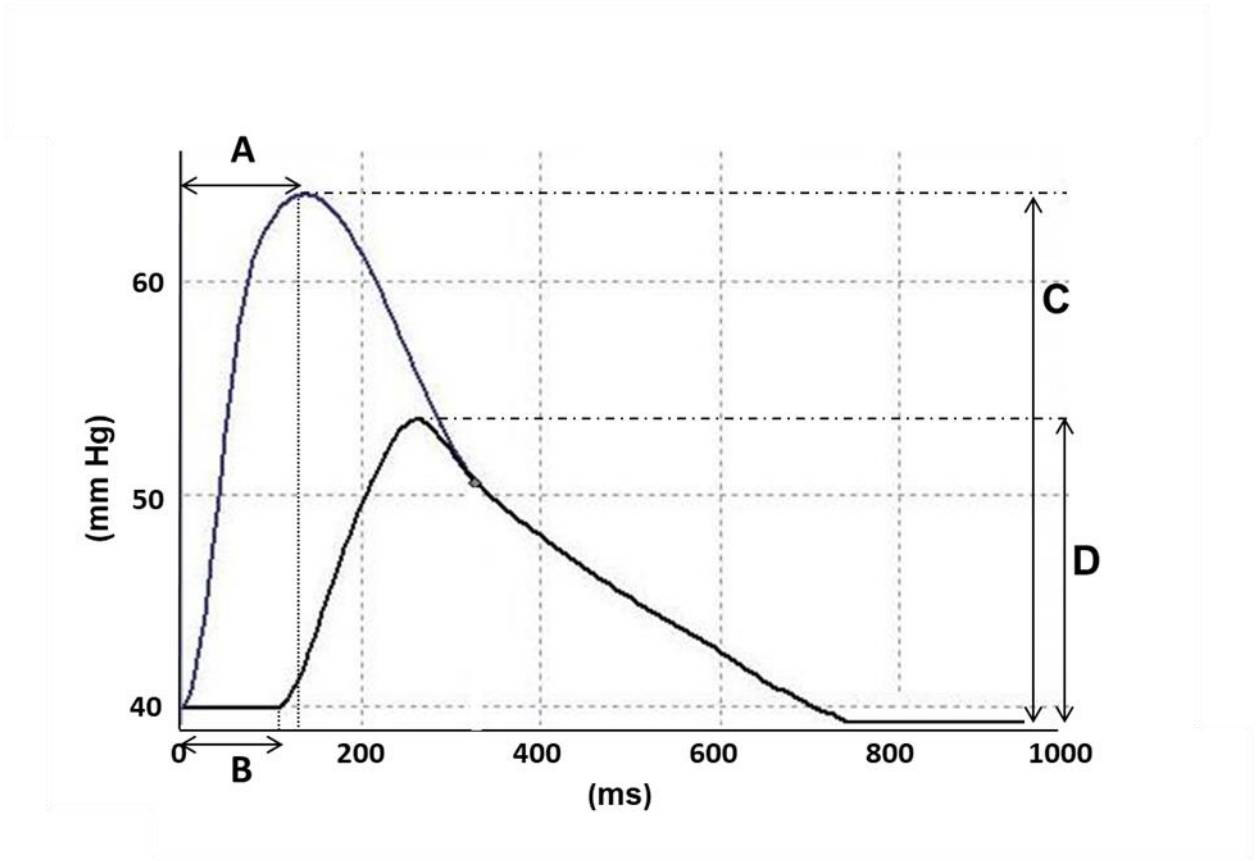

Figure 1.2. Central aortic pressure waveforms showing the time to the peak of the forward wave and the reflected wave foot time. A: Time to the peak of the aortic forward wave (Ft); B: Reflected wave foot time (Rt); C: forward wave pressure; D: Reflected wave pressure.

increases in arteriolar resistance (which determines total peripheral resistance and hence MAP) may enhance wave reflection thus increasing backward wave pressures (Nichols, 2005) Moreover, changes in larger muscular artery tone is also now thought to increase wave reflection and hence modify the extent of backward wave pressures beyond arteriolar resistance or MAP (Cecelja et al., 2012). Indeed, backward wave pressures increase dramatically from 20 years of age, and this effect is independent of arteriolar function (as indexed by MAP) or forward wave pressures (Hodson et al., 2016). Moreover, changes in aortic AP (Pa) and hence PP may occur with the use of nitroglycerine at doses that do not change total peripheral resistance and MAP, an effect that may in-part be explained by decreases in reflected waves independent of arteriolar function or Pf (Fok et al., 2014a; Smulyan, 2007). Although the factors that determine muscular artery tone and hence backward wave pressures, beyond arteriolar tone, are largely unknown, antihypertensive therapy, either through an impact on arteriolar function or by decreasing forward wave pressure (through distending pressure effects), may produce dramatic decreases in the magnitude of backward wave pressures.

1.3.4 Relative contribution of forward and backward wave pressures to aortic pulse pressure.

Pulse pressure increases with age from early adulthood (McEniery et al., 2008; Mitchell, Wang, et al., 2010; Hodson et al., 2016). The contributions of the forward or backward waves to variations in age-related increases in PP are dependent on age itself and perhaps circumstances. In many studies, age-related increases in PP are closely matched with increases in aortic backward wave pressures across the adult age spectrum (Hodson et al., 2016; Namasivayam et al., 2009). Reflected waves assessed from either indices of pressure augmentation or from wave separation analysis, have on several occasions been demonstrated to contribute more to age-related increases in aortic pulse PP in the general community (Booysen et al., 2015; Cecelja et

al., 2009; McEniery et al., 2008; Namasivayam et al., 2009) in women (Cecelja et al., 2009) and in hypertensives (Sibiya et al., 2015). In this regard, while backward wave pressure contributes to age-related increases in PP across the adult life span, forward wave pressure together with backward wave pressure contributes to increases in aortic PP in the elderly (Hodson et al., 2017, 2016; Namasivayam et al., 2016, 2009). In contrast, the Framingham heart study reported that the forward wave contributes more to age-related increase in PP (Mitchell et al., 2010b). Possible reasons for variations in findings between studies are discussed below in section 1.4.1 but in short may be attributed to the high proportion of Framingham Heart Study participants with well-controlled BP values, whilst alternative samples have a high prevalence of those with an uncontrolled BP. Indeed, in a community based study comprised of individuals not receiving antihypertensive therapy, independent of mean arterial pressure, backward wave pressures contributes more than the forward wave pressures to age-related increases in pulse PP (Booysen et al., 2015; Hodson et al., 2017). Importantly, across the adult lifespan when assessing the relative contribution of forward and backward wave pressures to variations in aortic PP with both forward and backward wave pressures included in the same regression models, backward wave pressures account for a far greater effect (Booysen et al 2015). This effect is noted even over an older age range where forward wave pressures increase, but backward wave pressures although increasing, do not increase more than forward wave pressures (Booysen et al, 2015). Thus, even if forward wave pressures are driving backward wave pressures, the main determinant of aortic PP is still the backward wave, an effect that may be explained by the impact of wave re-reflection (see below).

Although, several studies support an important contribution of backward waves to increases in aortic PP, it is important to note that this depends on circumstance. Indeed, in exercise, increases in aortic PP are driven by an enhanced forward wave pressure, whilst the reflected wave does not increase in magnitude (Schultz et al., 2013). Whether this means that with exercise reflected wave magnitude (backward wave pressures beyond the impact of forward

wave effects [inertial forces and Newton's 3rd Law]) decreases is unknown. The increase in aortic PP during exercise is in response to an increased left ventricular contraction and ejection and hence aortic flow, factors that are well recognised as influencing forward wave pressure (Nichols et al., 2011). Moreover, by comparing the relative impact of dobutamine and norepinephrine on aortic pressure waves, forward wave pressures were noted to contribute more than backward wave pressures to increases in aortic PP (Fok et al., 2014). However, that study only assessed effects of pharmacological agents over a low BP range (Fok et al., 2014) and hence what occurs at higher pressures is unknown.

1.3.5. Impact of timing and duration of pressure waves on pulse pressure

As implied in the aforementioned discussion, the effect of the backward wave on aortic PP is not only determined by the magnitude of Pb, but also the ability of the reflected wave to return sufficiently early to augment pressures generated by the forward pressure wave. In young individuals, the speed of wave return may not be sufficiently fast to result in the backward pressure wave returning during systole to enhance forward wave pressures. Rather, the reflected wave may only return during diastole, thus increasing DBP (London and Pannier, 2010). This may be beneficial as it will enhance coronary blood flow, which largely occurs in diastole and is strongly influenced by pressure gradients across the coronary artery (London and Pannier, 2010). However, by early adulthood, the speed of wave reflection is sufficiently fast that the backward pressure wave returns in systole and augments systolic rather than diastolic BP (Figure 1.1), the consequence being an increased aortic PP (Nichols et al., 2011). Thus, it is not only the magnitude of pressure generated by wave reflection (Pb) that enhances aortic PP, but also the speed of wave reflection (Rt in Figure 1.2) that enhances aortic PP (independent of the magnitude). An increased speed of wave reflection with age is thought to be determined mainly by increases in aortic stiffness and hence age-related changes in aortic stiffness increase aortic

PP, not only through an impact on forward wave pressures (by changing characteristic impedance), but also by an impact on the speed of wave reflection increasing aortic pressure augmentation (Figure 1.1). Importantly however, an early return of reflected wave may also occur independent of the speed of wave reflection if reflection sites are closer to the proximal aorta. This may theoretically occur through vasoconstriction or in those with a shorter stature.

Several factors including the time to the peak of the forward pressure wave (Ft in Figure 1.2), the slope of the backward pressure wave and the pressure x time area integral of the forward or backward pressure waves have now been identified as contributing to several aspects of aortic PP (Torjesen et al., 2014). Most of these factors have not as yet been identified as being of clinical significance and hence will not be elaborated on in the present thesis. However, the time to the peak of the forward wave pressure (Ft in Figure 1.2) is of major clinical importance and further evaluated as part of the present thesis. A prolonged ejection duration caused by a multitude of factors results in a delay in the systolic peak of the forward wave pressure. That is, a prolonged ejection duration causes an extended time to the peak of flow and hence the forward wave pressure. The consequence of a prolonged ejection duration is that a greater overlap of forward with reflected waves returning early in systole occurs and this augments aortic PP to a greater extent. An additional workload (the augmented systolic pressure [AP or Pa]) is therefore generated for the left ventricle to overcome during ejection (Takahashi et al., 2003; Nichols and O'Rourke, 2005). The extended time to the peak of the forward wave may explain how the use of negative chronotropes, such as β-adrenergic receptor blockers may not decrease aortic augmented pressures and hence PP as well as alternative antihypertensive agents (Lindholm et al., 2005; Reboussin et al., 2018; Williams et al., 2006; Wiysonge and Opie, 2013). In this regard, for a given decrease in brachial BP, atenolol-based therapy is unable to reduce aortic PP and SBP as effectively as alternative agents (Boutouyrie et al., 2010; Sluyter et al., 2016; Williams et al., 2006). The inability of β-adrenergic receptor blockers to decrease central aortic PP as well as alternative antihypertensive agents is thought to in-part explain the inability of

these agents to prevent events as well as alternative antihypertensive agents (Williams et al., 2006). There are nevertheless several alternative factors other than an extended time to the peak of the forward pressure wave, that may nevertheless explain the inability of atenolol-based therapy to reduce aortic PP as well as other antihypertensive agents. All of the possible mechanisms responsible for the adverse effects of atenolol-based therapy on central arterial pressures will be discussed in a subsequent section below.

1.3.6 Summary of the determinants of aortic pulse pressure

In summary, pulsatile pressure waves in the proximal aorta are generated by the summation of forward (the peak of which is Pf) and backward (the peak of which is Pb) pressure waves (Hashimoto and Ito, 2009). Whilst Pf is determined by ventricular ejection, aortic stiffness and aortic diameter (stiffness and diameter changes determine impedance), Pb is determined by Pf (Newton's Laws of Motion), arteriolar and possibly larger muscular vessel (arterial) tone. Both Pf and Pb are influenced by distending pressures (MAP) (which increases aortic stiffness through passive processes) and aortic PP is determined not only by Pb (the magnitude of the reflected wave pressure), but also by the speed of wave reflection, and the time to peak ejection, both of which determine AP (Pa) without influencing the magnitude of the reflected wave.

1.4. Impact of the determinants of aortic pulse pressure on cardiovascular damage.

If aortic PP is unable to consistently enhance risk prediction beyond brachial BP, the question arises as whether some of the individual determinants of aortic PP enhance risk prediction beyond brachial BP. Previously, forward and backward wave pressures have been estimated using incident (Pi) and augmented (Pa) pressures identified as indicated in Figure 1.1

from the inflection point of the first systolic shoulder of the aortic pressure wave (Kelly et al., 1989). However, more recently studies assessing the role of forward and backward wave pressures derived from formal wave separation analysis have begun to emerge. In this regard, wave separation analysis has been performed using an actual aortic flow wave determined from velocity and diameter measurements in the left ventricular outflow tract (Figure 1.3) or assuming that the aortic flow wave either approximates a triangular shape (Figure 1.3) or has a "physiological" shape (Kips et al., 2009; Westerhof et al., 2006). Using these various approaches what is the evidence for a role of forward and backward wave pressures in mediating cardiovascular damage?

Using the contemporary method of assessing aortic backward wave function at the time, several studies showed a relatively consistent independent association of Pa and AIx with end organ changes such as increases in left ventricular mass index (LVMI) (Hashimoto et al., 2007, 2006; Sibiya et al., 2014), the presence of left ventricular diastolic dysfunction (Weber et al., 2006), or vascular pathology such as increases in carotid intima media thickness (Sibiya et al., 2014; Westerbacka et al., 2005). In addition, Pa or AIx showed an ability to predict all cause and cardiovascular mortality beyond brachial BP (Vlachopoulos et al., 2010). However, more recent studies including the Framingham Heart Study showed no independent associations of Pa and AIx with end organ changes (Kaess et al., 2016.) or the ability to predict cardiovascular events (Mitchell et al., 2010a). However, the use of Pa and AIx as indexes of wave reflection has being challenged (Davies et al., 2010). In this regard, Pa or AIx may better reflect changes in left ventricular function (Cheng et al., 2012) and through these effects index alterations in the effects of forward wave pressures rather than wave reflection. Indeed, because Pa is strongly driven by left ventricular systolic function (Cheng et al, 2012b) it could confound the ability to identify reflected wave effects (Fok et al., 2014b; Karamanoglu and Feneley, 1999). Moreover, Pa does

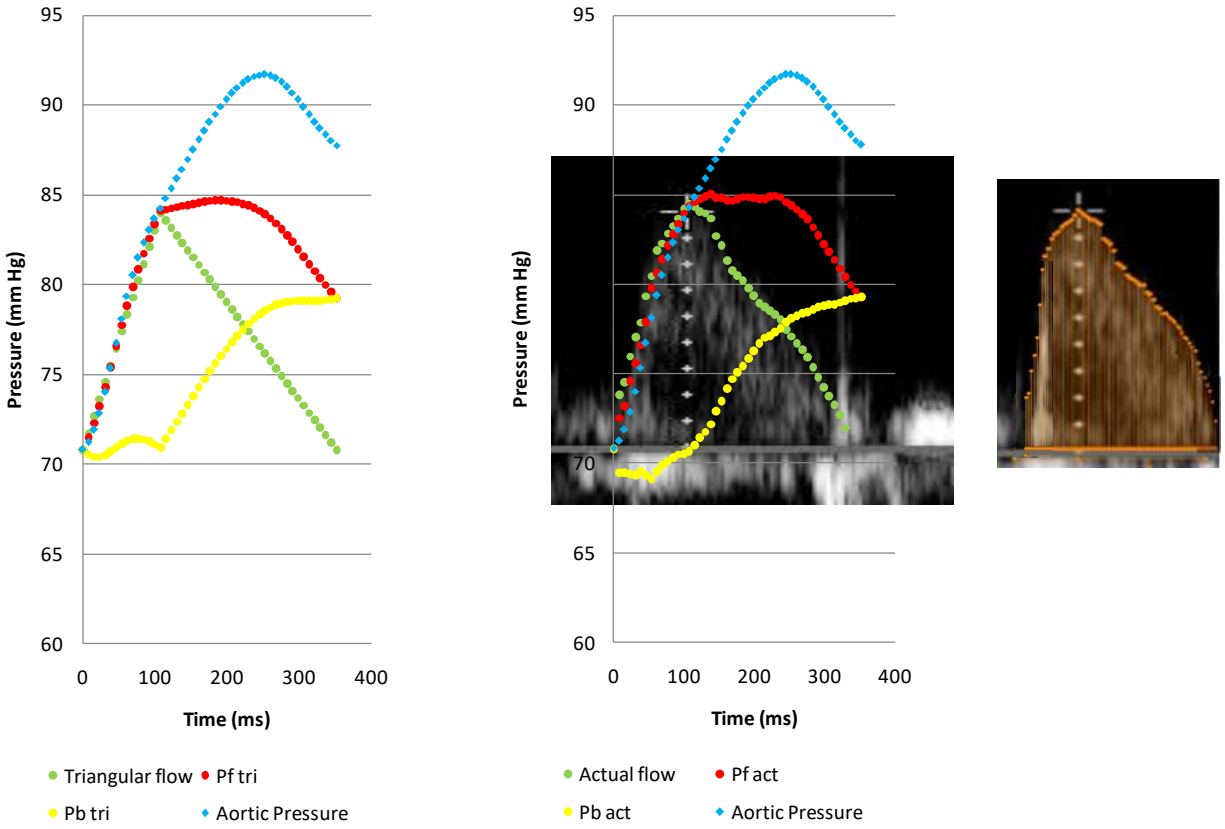

Figure 1.3. Wave separation analysis performed using an assumed triangular aortic flow wave (left panel) and using an actual aortic flow wave (right panel). The actual flow wave is derived from aortic velocity (shown in figure) and diameter measurements. The forward and backward pressure waves are superimposed on the aortic pressure wave. Pf tri or Pb tri, forward or backward pressure waves derived from wave separation using a triangular flow wave; Pf act or Pb act, forward or backward pressure waves derived from wave separation using an actual flow wave.

not account for the additional impact of wave re-reflection, driven by backward wave effects, on PP (Phan et al., 2016) and will therefore underestimate backward wave pressure effects. Indeed, it is now recognised that a significant portion of the forward pressure wave at the point where peak aortic PP occurs, is largely driven by wave re-reflection rather than the pressure generated by the product of flow and characteristic impedance (Zc)(Phan et al., 2016). Furthermore, the speed of wave reflection influences the timing of the backward wave and thus determines the overlap between forward and backward waves and thus the extent of summation of the backward with the forward travelling wave. Thus, the speed of wave reflection confounds the extent to which the forward wave is augmented by the backward wave. Indeed, the speed of wave reflection limits the ability of Pa to detect the absolute Pb required to produce a given increase in aortic PP. Thus Pb may not contribute as much as previously thought to Pa and AIx, with alternative factors playing a major role (Davies et al., 2010). Indeed, the use of Pa and AIx has recently been demonstrated by our group to markedly underestimate the impact of Pb on aortic PP and the end organ measure, LVMI (Booysen et al., 2015). Moreover, the ability of Pb or the reflected wave magnitude (RM=Pb/Pf, the forward wave pressure-independent index of backward wave pressures derived from wave separation analysis) to predict outcomes is often not as closely reproduced by the use of Pa or AIx (Chirinos et al., 2012; Wang et al., 2010). However, it may also be argued that the lack of ability of Pa and AIx to predict cardiovascular events in the Framingham Heart Study (Mitchell et al., 2010b) may be because a high proportion of participants had well managed hypertension, and through arteriolar or larger vessel dilation (Agabiti-Rosei et al., 2007), treatment may have abolished the impact of backward wave pressures.

As a consequence of the controversial use of Pa or AIx as an adequate index of aortic backward wave pressure effects, more recent studies have focused on measuring the magnitude of Pb using wave separation analysis described in Figure 1.3 or using a “physiological” rather than actual or “triangular” flow wave and assessed its associations with end organ changes and

the ability to predict events. In this regard, the authors of the Framingham heart study, using wave separation analysis from an actual flow wave, showed that Pf, but not Pb predicted cardiovascular events (Cooper et al., 2015). On the other hand however, the Multi-Ethnic Study in Atherosclerosis where wave separation analysis was conducted using a "physiological" flow wave showed that Pb and RM, but not Pf, independently predict cardiovascular events, with a major effect on incident heart fail (Chirinos et al., 2012; Zamani et al., 2014). Furthermore, using a "triangular" flow waveform to separate pressure waves, Wang et al. (2010) demonstrated that backward, but not forward wave effects predict outcomes independent of brachial BP. In addition, our group, using a "triangular" flow waveform to separate pressure waves have demonstrated that Pb rather than Pf is associated with several end organ changes independent of brachial pressure (Booysen et al., 2015; Sibiya et al., 2015).

Once again, it may be argued that the lack of ability of Pb to predict cardiovascular events in the Framingham Heart Study (Cooper et al., 2015) may be because a high proportion of participants had well managed hypertension and through arteriolar or larger vessel dilation (Agabiti-Rosei et al., 2007), treatment may have abolished the impact of backward wave pressures. Nevertheless, the fact that the role of forward and backward wave pressures are inconsistently associated with end organ changes or predict events beyond brachial BP raises the question of why these inconsistencies exist? In this regard, as indicated in the aforementioned discussion, the time to the peak of the forward pressure wave and the speed of wave reflection may influence the impact of Pb on aortic PP and hence on the extent of cardiovascular damage. In this regard however, there is a substantial lack of evidence to support an important role of these two factors in mediating cardiovascular damage. Therefore, as part of the present thesis, I hypothesized that either reflection time or time to the peak of the aortic forward wave impact on the contribution of aortic backward wave pressures and hence aortic PP to variations in LVM and LV hypertrophy (LVH). The results of these studies have been published in the *Journal of Hypertension* (Tade et al., 2016) and are described in chapter 2 of the present thesis.

1.5. Demographic and clinical determinants of aortic pulse pressure.

Multiple demographic and clinical factors such as age (Dart et al., 2008), gender (Gatzka et al., 2001), height (Smulyan et al., 1998), heart rate (Yasmin and Brown, 1999), ethnicity (Heffernan et al., 2008), smoking (Mahmud and Feely, 2003), exercise (Edwards and Lang, 2005; Tanaka et al., 2000) and several cardiovascular risk factors including hypertension and diabetes mellitus (McEniery et al., 2008) influence central aortic PP. The haemodynamic basis of the impact of these factors on aortic PP has been extensively investigated, with ongoing debate on several aspects. For example, although it is well known that age is an important determinant of aortic PP, the factors that determine age-related increase in aortic PP remain controversial (Cecelja et al., 2009; Hodson et al., 2017; Mitchell et al., 2010b; Namasivayam et al., 2016, 2009), but this topic goes beyond the scope of the present thesis. However, as indicated in the aforementioned sections of this chapter, women are at a high risk of CVD. In this regard, an important unresolved consideration is the mechanism responsible for the impact of female gender on aortic PP. In the following section, I will review this evidence highlighting the unresolved issue that led me to further pursue this question in the present thesis.

1.5.1 Haemodynamic determinants of sex differences in aortic pulse pressure.

Women have an increase PP as compared to men (Franklin et al., 1997; Segers et al., 2007). Importantly however, higher age-related increase in PP in women as compared to men are independent of menopause and its associated hormonal changes (Casiglia et al., 1996; O'Neill et al., 2013; Smulyan et al., 2001). Thus, gender differences in PP are not attributed to hormonal effects. In women, increases in PP have been attributed to several factors. These include an accelerated aortic wall stiffening (Berry et al., 2004; Gatzka et al., 2001; Goto et al., 2013; Mitchell et al., 2008, 2004; Noon et al., 2008; Shim et al., 2011), and reduced aortic diameter

(Mitchell et al., 2008, 2003), both of which will increase characteristic impedance (Zc) (Mitchell et al., 2004, 2003). A reduced aortic diameter, increased aortic stiffness and thus enhanced characteristic impedance (Zc) may account for an elevated forward pressure wave amplitude in women (Mitchell et al., 2008, 2004). In addition women may have an increased PP because of premature wave reflection resulting from a shorter stature (Gatzka et al., 2001). Importantly, height (Gatzka et al., 2001), aortic dimensions and stiffness (Dart et al., 2008) do not explicitly account for the sex differences in PP and hence alternative factors must play a role. Importantly, women have an increased augmented pressure and augmentation index as suggested by the Framingham Heart Study (Gatzka et al., 2001; Torjesen et al., 2014). The increased pressure augmentation although suggestive of enhanced reflected wave effects, could nevertheless be attributed to several factors. More recently an increased forward wave peak width, slope of the backward pressure wave, and forward wave amplitude, but not backward wave amplitude have been demonstrated to be the main determinants of an increased AIx in women of the Framingham Heart study (Torjesen et al., 2014). However, whether the same factors explain sex differences in AIx or Pa in alternative population groups is nevertheless uncertain.

Whilst increases in the magnitude of the forward pressure wave exceed that of the backward wave in explaining age-related increases in aortic PP in the Framingham Heart Study (Mitchell et al., 2010b), the magnitude of backward waves exceeds that of the forward wave in contributing to age-related variations in aortic PP in a community of African ancestry (Booysen et al., 2015). Furthermore, in groups of African ancestry backward wave pressures contribute more to variations in measures of cardiovascular end-organ damage than forward wave pressures (Booysen et al., 2015; Sibiya et al., 2015). However, whether increases in backward wave magnitude account for sex differences in AIx or Pa in groups of African descent has not been determined. As the factors that determine sex-related differences in AIx may assist in identifying targets for therapeutic interventions, it is important to assess whether backward wave magnitude explains sex differences in AIx in groups of African descent. Hence, in the present thesis I also

assessed whether increases in forward or backward wave pressures or differences in the time to the peak of the forward wave produced by a prolonged ejection duration or an earlier return of reflected waves account for sex differences in AIx or Pa in a relatively large community-based sample of African ancestry. These data have been published in the *American Journal of Hypertension* (Tade et al., 2017) and are described in chapter 3 of the present thesis.

1.6 Are there more readily available approaches to assessing the determinants of aortic pulse pressure?

Assuming that specific determinants of aortic PP, rather than aortic PP *per se* independently associate with cardiovascular damage, it is also important to identify the most appropriate (cost-effective) method of assessing these effects. Importantly the amplitude of forward and backward pressures are most accurately determined from wave separation analysis conducted with the use of aortic flow and pressure waveforms (Murgo et al., 1981; Nichols, 2005; Westerhof et al., 1972). However, this approach requires expertise and the availability of devices to accurately identify both pressure and flow waveforms. As indicated above, wave separation analysis may nevertheless also be conducted using an assumed triangular or "physiological" flow waveform. Although the approach that employs the triangular waveform can be embedded in software that allows for automated assessments, the approach that employs the "physiological" waveform is not readily incorporated in software that allows for automated assessments. In this regard, whilst the approach that employs the "physiological" waveform is accurate, there is some question as to the accuracy of the approach that employs the triangular waveform (Kips et al., 2009). Nevertheless, the height of the second systolic shoulder of the peripheral pulse (pP_2) (minus diastolic blood pressure) (Figure 1.4), which represents that component of the peripheral waveform generated by the backward travelling pressure wave, is

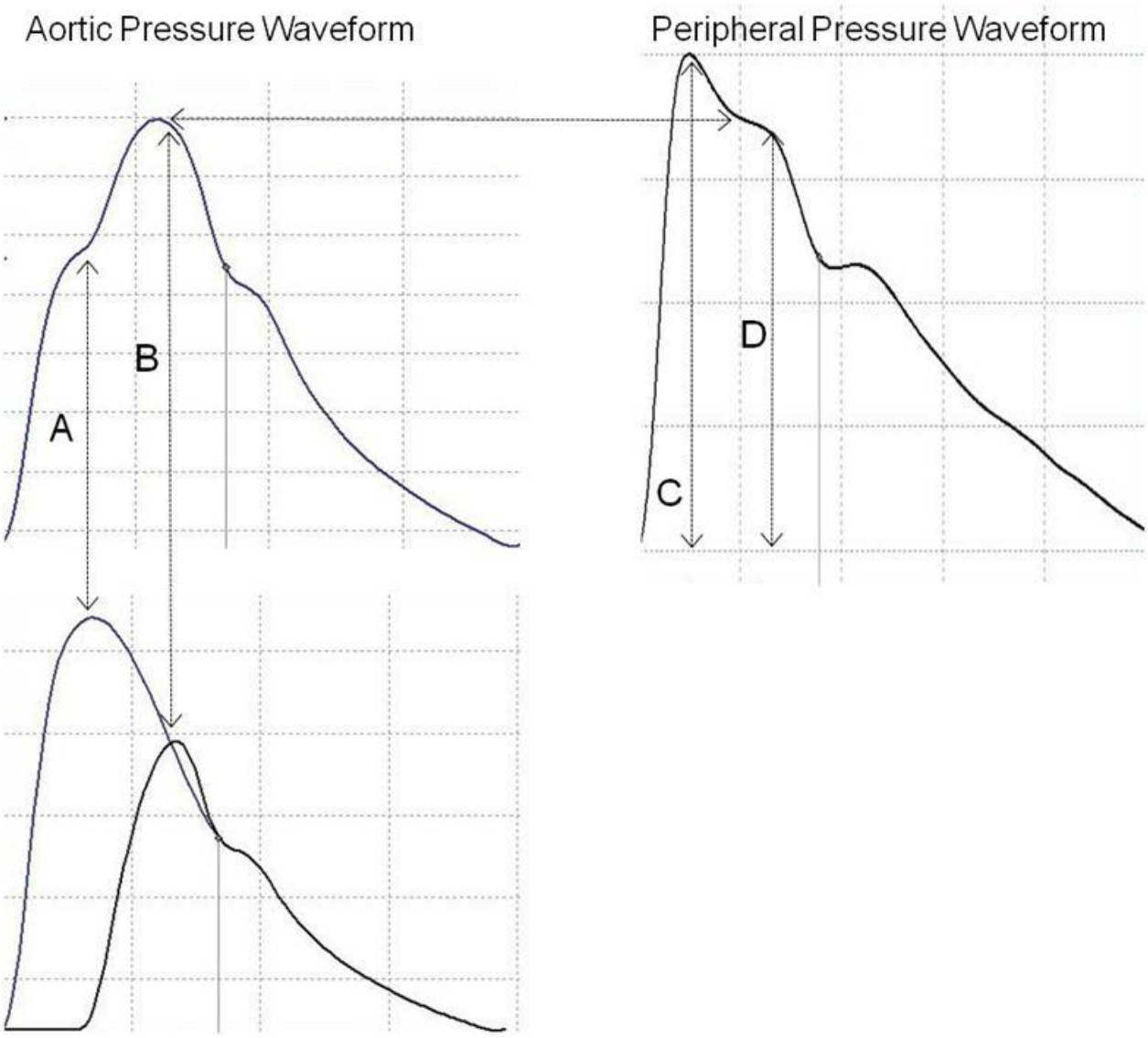

Figure 1.4. The second systolic shoulder on the peripheral pulse corresponds with the aortic reflected wave. A: Forward pressure wave of the aortic wave form; B: reflected pressure wave of the aortic wave form; C: Peripheral pulse pressure; D: Peripheral P_2 (pP_2) [pP_2– DBP]

an attractive alternative to the use of Pa. This second systolic shoulder of the peripheral pulse in the arm is thought to be derived from reflected waves transmitted from the lower part of the body, travelling up into the upper limb and following closely after the forward travelling pressure wave as it is transmitted into the arm (Nichols et al 2011). As Pb in the aorta is also thought to be derived largely from reflected waves returning from the lower part of the body, pP_2 indexes Pb. Importantly, pP_2 being derived from a peripheral pulse waveform alone, does not require the generation of an aortic pulse waveform and may not be subject to the same confounding effects as Pa. In this regard, there is some evidence that pP_2 may offer a more reliable index of the adverse effects of reflected waves than Pa. Indeed, whilst Pa may not predict on-treatment changes in left ventricular mass index (LVMI) beyond brachial BP, pP_2 may (Hashimoto et al., 2006). However, the extent to which pP_2 reflects the adverse effects of Pb and whether pP_2 is a better index of the adverse effects of Pb than Pa, is unknown. In the present thesis I therefore assessed the extent to which pP_2 reflects the adverse effects of Pb on PPc and LVMI and whether pP_2 is a better index of the adverse effects of Pb on PPc and LVMI than Pa. The results of this study are described in chapter 4 of the present thesis.

1.7 Mechanisms of the adverse effects of atenolol on aortic pressure.

As discussed in previous section, several large studies comparing the effects of alternative antihypertensive agents with that of therapy that includes the β-adrenoreceptor blocker atenolol, have established superiority of alternative agents over atenolol-based therapy (Lindholm et al., 2005; Reboussin et al., 2018; Wiysonge and Opie, 2013). Consequently, although atenolol is an effective antihypertensive agent, contemporary guidelines do not recommend atenolol as first-line therapy. As also indicated in the aforementioned section, one explanation for the attenuated efficacy of atenolol at reducing mortality is that for a given decrease in brachial BP, atenolol-based therapy is unable to reduce central arterial PP as

effectively as alternative agents (Boutouyrie et al., 2010; Sluyter et al., 2016; Williams et al., 2006). However, the mechanisms that explain the impact of atenolol on central arterial pressure are poorly understood. Whether this is a class effect of all β-adrenoreceptor blockers or an impact specific to atenolol or similar agents with limited direct vasodilator properties, is unknown. As there are compelling arguments for the use of this class of agents for the treatment of hypertension in several cardiovascular conditions including heart fail, coronary artery disease and arrhythmias, a better understanding of the impact of atenolol in hypertension is required.

As discussed above, the limited impact of atenolol on aortic PP is associated with an inability to reduce aortic augmentation index (AIx) as effectively as alternative agents (Chen et al., 1995; Manisty et al.,2009; Manisty and Hughes, 2013; Sluyter et al., 2016; Williams et al., 2006). However, as also indicated above, AIx is determined by several factors (Nichols et al., 2011) including backward and forward (determined by ventricular ejection and aortic impedance) wave pressures; or time to the peak of the forward wave or speed of wave reflection, either of which influence the chances of backward wave pressures augmenting systolic pressure. The impact of atenolol on AIx may be explained by a decreased heart rate, and hence an increased stroke volume (force-frequency relationship) and Pf. In this regard, in the face of a reduced heart rate, cardiac output is maintained by increasing stroke volume (Nichols and Edwards, 2001). The impact of atenolol on AIx may also be explained by a decreased heart rate, and hence an increased ejection duration and prolonged time to the peak of Pf (Williams and Lacy, 2009). Heart rate-related changes in central arterial function are effects that are shared by all β-adrenoreceptor blockers (class effects). If heart rate-related effects of atenolol explain the adverse effects on central arterial function, then all β-adrenoreceptor blockers are likely to produce the same adverse effects. Alternatively, as some β-adrenergic receptor blockers (e.g. atenolol) have no direct vasodilator properties they may not reduce wave reflection as much as other antihypertensive agents, the effect being that the reflected wave magnitude increases. The lack of vasodilator effects of atenolol may influence the magnitude of Pb through effects that are

independent of Pf and arteriolar function (i.e. medium-sized or small arteries). An impact of atenolol on wave reflection would be specific to some but not other β-adrenoreceptor blockers. This would therefore suggest that the adverse actions of atenolol on central arterial function would not be class-specific. However, whilst a number of studies have assessed the impact of atenolol monotherapy on AIx (Murgo et al., 1981; Nichols et al., 2011; Westerhof et al., 1972), the impact of atenolol on reflected wave function, independent of alternative agents and at comparable steady-state pressures (mean arterial pressure-MAP) using wave separation analysis has not been determined. In this regard, studies on the impact of atenolol monotherapy in humans are no longer ethically feasible (it is not recommended as first-line therapy, except in special circumstances). Consequently, using simultaneous carotid pressure and aortic velocity and diameter measurements, we developed an approach to assessing aortic function in rats over a range of steady-state pressures. Therefore, as part of the present thesis, I evaluated the impact of the β-adrenergic receptor blocker atenolol on aortic function in an animal model of hypertension. In that study, I assessed aortic function from a combination of central arterial pressure measurements and aortic velocity determined in the aortic outflow tract and aortic diameter measurements. Using wave separation analysis (see Figure 1.3), I subsequently determined the impact of atenolol therapy on aortic characteristic impedance and forward and backward waves and their timing (speed of wave reflection and time to the peak of the forward wave). The results of this study are described and the implications thereof discussed in chapter 5 of the present thesis.

1.8 Problem statement

Although indices of aortic pressure augmentation are independently associated with cardiovascular events, these indices reflect a composite of backward pressure waves and the timing of the aortic pressure waves. The exact role of the time to the peak of the aortic forward

wave or reflection time in determining backward wave pressure effects on cardiovascular outcomes or end organ changes is, however, unknown and requires clarification. In women, AIx is increased, but whether the forward or backward wave contributes to this increase is uncertain. Although the Framingham Heart Study report on a greater contribution of the forward wave to an increased AIx in women, in contrast to the Framingham Study, in groups of African ancestry a greater contribution of the backward wave to variations in PP occurs across the adult age range. Thus, the role of aortic wave reflection in sex-differences in PP in groups of African ancestry requires further study. As reflected wave function may be an important index to predict events and current indices such as aortic AIx may be limited, simpler approaches to assessing reflected wave function are required. Whether the second systolic shoulder of the peripheral pressure waveform (pP2), although amplified as it is transmitted peripherally, closely correlates with aortic backward wave pressures and hence that pP_2 may improve on the ability to detect sub-clinical cardiovascular disease as well as backward wave pressures is unknown. Although an increased AIx may in-part explain the inferiority of atenolol-based antihypertensive therapy in reducing events as compared to alternative agents, the exact mechanism explaining the impact of atenolol on AIx is uncertain. Whether this is a class-specific effect of all β blockers associated with heart rate-related increases in stroke volume or ejection duration or an impact at a vascular level on wave reflection, requires further study.

1.8 Aims

Hence as part of the present thesis I aimed:

1) To determine whether either reflection time or time to the peak of the aortic forward wave determine the contribution of aortic backward wave pressures to variations in left ventricular mass (LVM) and LV hypertrophy (LVH) in a large, community-based sample. These data

are described in chapter 2 and have been published in the *Journal of Hypertension* (Tade at al., 2017).

2) To determine the relative contribution of aortic forward and backward pressure waves and the timing thereof to the relationship between sex and augmentation index or augmented pressure in a relatively large community-based sample of African ancestry. These data are described in chapter 3 and have been published in the *American Journal of Hypertension* (Tade at al., 2017).
3) To determine the extent to which the second systolic shoulder of the peripheral pulse (pP_2) reflects the adverse effects of aortic backward waves on PP and LVMI and whether pP_2 is a better index of the adverse effects of aortic backward waves on aortic PP and LVMI than augmented pressures (Pa) in a large community-based sample. These data are described in chapter 4.
4) To determine the mechanisms responsible for the impact of atenolol-based monotherapy on AIx at controlled steady-state pressures in spontaneously hypertensive rats (SHR). These data are described in chapter 5.

Chapter 2

Time to the Peak of the Aortic Forward Wave Determines the Impact of Aortic Backward Wave and Pulse Pressure on Left Ventricular Mass

This chapter has been published in the *Journal of Hypertension* as follows;

Grace Tade, Gavin R. Norton, Hendrik L. Booysen, Moekanyi J. Sibiya, Imraan Ballim, Pinhas Sareli, Elena Libhaber, Olebogeng H.I. Majane, and Angela J. Woodiwiss. Time to peak of the aortic forward wave determines the impact of aortic backward wave and pulse pressure on left ventricular mass. *Journal of Hypertension* 2017; 22(6): 410-415.

2.0 Abstract

The extent to which the timing of component forward and backward wave pressures of aortic pulse pressure (PP) contribute to cardiovascular effects is uncertain. I therefore aimed to determine the degree to which an extended time to the peak of the aortic forward wave or early wave reflection time enhance associations between aortic backward wave pressure and hence central aortic PP (PPc) and left ventricular mass index (LVMI). In 701 adult participants from a community sample either receiving no antihypertensive therapy or receiving low-dose thiazide diuretic monotherapy for at least a year (the major therapy employed), we assessed aortic haemodynamics (SphygmoCor software and wave separation analysis; AtCor Medical, West Ryder, New South Wales, Australia) and LVMI (echocardiography). An interaction between time to the peak of the aortic forward wave and aortic backward wave pressure was independently associated with aortic augmented pressure ($P < 0.01$), PPc ($P < 0.005$), LVMI ($P < 0.01$), and LV hypertrophy (LVH; $P = 0.01$). The time to the peak of the aortic forward wave–aortic backward wave pressure interaction translated into a stepwise increase in the independent association between aortic backward wave pressure and aortic augmented pressure or PPc across quartiles of time to the peak of the aortic forward wave ($P < 0.05$ to < 0.0001 for comparison of slopes of relations). Furthermore, the time to the peak of the aortic forward wave aortic backward wave pressure interaction translated into an increase in the independent association between PPc or aortic backward wave pressure and LVMI ($P < 0.05$ to < 0.001 for comparison of slopes and strength of relations) or LVH ($P < 0.05$ for comparisons of odds ratios), but not between forward wave pressures and LVMI or LVH across quartiles of time to the peak of the aortic forward wave. A markedly better ability of aortic backward wave pressure and PPc, but not forward wave pressures to detect LVH was noted in the highest as compared with the first three quartiles of time to the peak of the aortic forward wave ($P < 0.05$). In contrast, reflection time failed to

influence the impact of aortic backward wave pressure or PPc on LVMI. In conclusion, the time to the peak of the aortic forward wave, but not early wave reflection markedly influences the impact of aortic backward wave pressure and hence aortic pulse pressure on LVMI and LVH in adults.

Keywords: Aortic pulse pressure, augmentation index, central blood pressure, left ventricular mass index, reflected waves.

2.1 Introduction

Pulse pressure is generally accepted as being a strong determinant of cardiovascular damage beyond steady-state pressures. Central aortic pulse pressure (PPc) may be considerably lower than brachial pulse pressure; controlled by different mechanisms to brachial pulse pressure; and possibly more closely associated with end-organ changes than brachial pulse pressure (Agabiti-Rosei et al., 2007; Avolio et al., 2009; Nichols et al., 2011). Although the fundamental determinants of PPc are well described (Agabiti-Rosei et al., 2007; Avolio et al., 2009), the contribution of each of these elements to cardiovascular damage is a topic of continuous debate. Despite several studies addressing the relative contribution of the component aortic forward and reflected (backward) waves of PPc to variations in end-organ changes (Booysen et al., 2015; Chirinos et al., 2012; Sibiya et al., 2015; Vlachopoulos et al., 2010; Wang et al., 2010; Weber et al., 2012), the contribution of the timing of these waveforms has received less attention.

Although reflected waves enhance coronary flow when returning in diastole, in adulthood early reflected waves arrive in systole and augment PPc (Nichols et al., 2011). Even in adulthood advancing age is associated with a gradual increase in the speed of wave reflection, a change which contributes to age-related increases in augmented pressures. Variations in the time of wave reflection may therefore contribute to cardio-vascular damage produced by backward waves in adulthood (Ueda et al., 2004). β-adrenergic receptor blockers, that are not as effective at reducing cardiovascular events as other antihypertensive agents, extend the time to the peak of the forward wave (Williams and Lacy, 2009) by slowing ventricular ejection. Assuming that reflection time remains unchanged, an extended time to the peak of the aortic forward wave may therefore increase the chances of the backward wave overlapping with the forward wave, thus enhancing the possibility of early backward waves augmenting PPc. Hence, an increased time to the peak of the aortic forward wave may also determine the adverse effects of backward wave

pressures on cardiovascular end organs. Indeed, heart fail with a preserved ejection fraction, a form of heart fail that may be determined in part by increases in aortic backward wave pressures, is associated with prolonged mechanical systole (Weber et al., 2006). Although indices of aortic pressure augmentation are independently associated with cardiovascular outcomes (Vlachopoulos et al., 2010), these indices reflect a composite of backward wave pressures and the timing of aortic pressure waves. The exact role of time to the peak of the aortic forward wave or reflection time in determining backward wave pressure effects on cardiovascular outcomes or cardiovascular end organs is unknown. In the present study, we therefore aimed to test the hypothesis that either reflection time or time to the peak of the aortic forward wave significantly determine the contribution of aortic backward wave pressures and hence PPc to variations in left ventricular mass (LVM) and LV hypertrophy (LVH) in a large, community-based sample.

2.3 Methods

2.3.1 Study group

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 and M12–04–108). Participants gave informed, written consent. The present study design has previously been described (Norton et al., 2012, 2008; Redelinghuys et al., 2010; Woodiwiss et al., 2009). Briefly, 1192 participants from families of black African descent (Nguni and Sotho chiefdoms) with siblings older than 16 years with central haemodynamic measurements were randomly recruited (based on the census Figures of 2001) from the South West Township (SOWETO) of Johannesburg, South Africa. In a sub-study, 812 participants had LVMI determined using echocardiography. Of these participants, 701 were either not receiving

antihypertensive therapy (n = 597) or were receiving low-dose thiazide diuretic monotherapy for at least a year (the recommended first line agent in South Africa).

2.3.2 Clinical, demographic, and anthropometric measurements

A standardized questionnaire was administered to obtain demographic and clinical data (Norton et al., 2012, 2008; Redelinghuys et al., 2010; Woodiwiss et al., 2009). Height and weight were measured using standard approaches and participants were identified as being overweight if their BMI was at least 25 kg/m^2 and obese if their BMI was at least 30 kg/m^2. High- quality blood pressure (BP) measurements were obtained by a trained nurse technician using a standard mercury sphygmomanometer according to guidelines and as previously described (Woodiwiss et al., 2009). Adult (24–32 cm), large adult (32– 42 cm), and extra-large adult (38–50 cm) cuffs were available for use and the cuff size employed was determined by arm circumference. Korotkov phases I and V were employed to identify SBP and DBP, respectively and care was taken to avoid auscultatory gaps. BP was taken as the mean of five measurements obtained at least 30 s apart in the seated position after 10 min of rest. Hypertension was defined as a mean BP at least140 and/or 90 mmHg or the use of antihypertensive medication. Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin were performed. Diabetes mellitus (DM) or an abnormal blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or a percentage glycated haemoglobin value greater than 6.1%.

2.3.3 Pulse wave analysis

Central aortic haemodynamics were estimated using radial applanation tonometry and SphygmoCor software as previously described (Booysen et al., 2015; Norton et al., 2012;

Redelinghuys et al., 2010; Sibiya et al., 2015). Central aortic pulse pressure (PPc) was determined as the difference between central aortic systolic BP and DBP and pulse pressure amplification was determined as brachial pulse pressure /PPc. Aortic backward and forward wave pressures, and time to the peak of the aortic forward wave and reflection time were determined using SphygmoCor software which separates the aortic waveform using a triangular flow wave (Westerhof et al., 2006)(Figure 2.1). Importantly, the use of a 'triangular flow waveform' to perform wave separation analysis assumes that the peak of the flow wave occurs at the same time as the point of inflection on the upstroke of the aortic pressure wave (P_1 time in the upper panel of Figure 2.1) (or at one-third ejection duration). However, this assumption is not always true as the peak of the flow wave may precede the pressure wave and hence, may occur earlier. We therefore assessed the extent to which a hypothetical earlier peak of the aortic flow wave would alter the ability to accurately evaluate the time to the peak of the forward pressure wave. In this regard, a hypothetical assumption that the peak of the aortic flow wave occurs at a point half the time to the inflection point produced little effect on the ability to identify the time to the peak of the aortic forward wave (Figures 2.2 and 2.3).

Because the ''triangular flow waveform'' approach generates a hypothetical waveform, in 392 participants we also separated the forward and backward waves using aortic flow waveforms derived from aortic velocity and diameter measurements obtained using echocardiography (five-chamber view). In this cohort, we validated the independent and interactive relations between aortic haemodynamic parameters generated using the 'triangular' flow waveform and LVMI. To perform wave separation analysis using an aortic flow waveform, 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 1.3). The foot of the flow waveform was aligned with the foot of the pressure waveform.

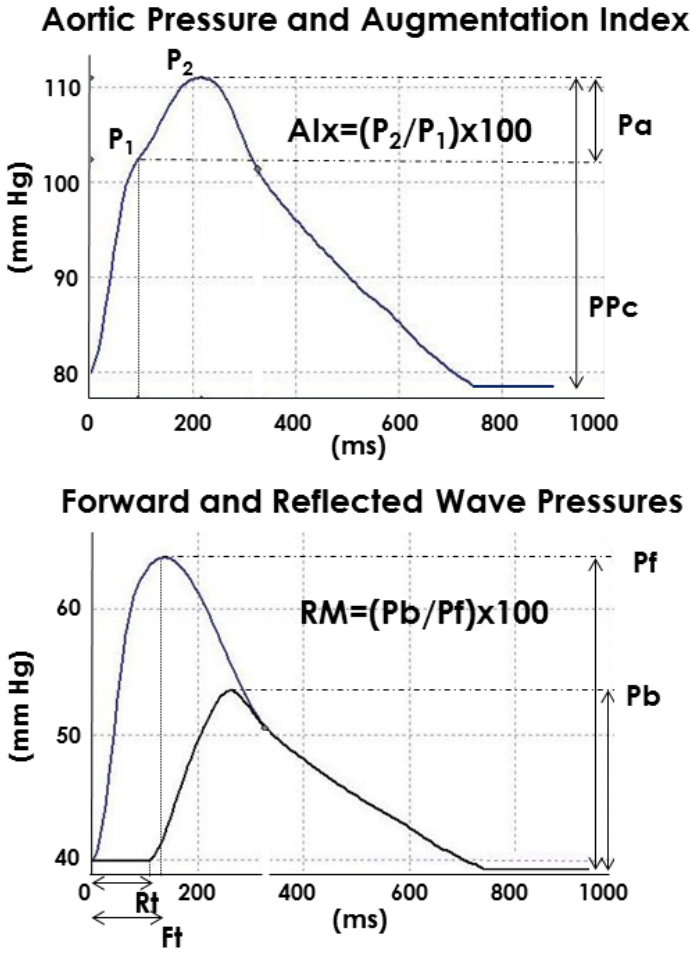

Figure 2.1. Derivation of aortic functional parameters from the aortic pressure waveform using SphygmoCor software. The aortic pressure wave was separated into the forward and backward waves assuming the aortic flow wave to have a triangular shape. Pa, augmented pressure; PPc, central aortic pulse pressure; AIx, augmentation index; Pf, forward wave pressure; Pb, backward (reflected) wave pressure; RM, reflection magnitude; Ft, time to peak forward wave; Rt, reflected wave time; P_1, first systolic peak; P_2, second systolic peak.

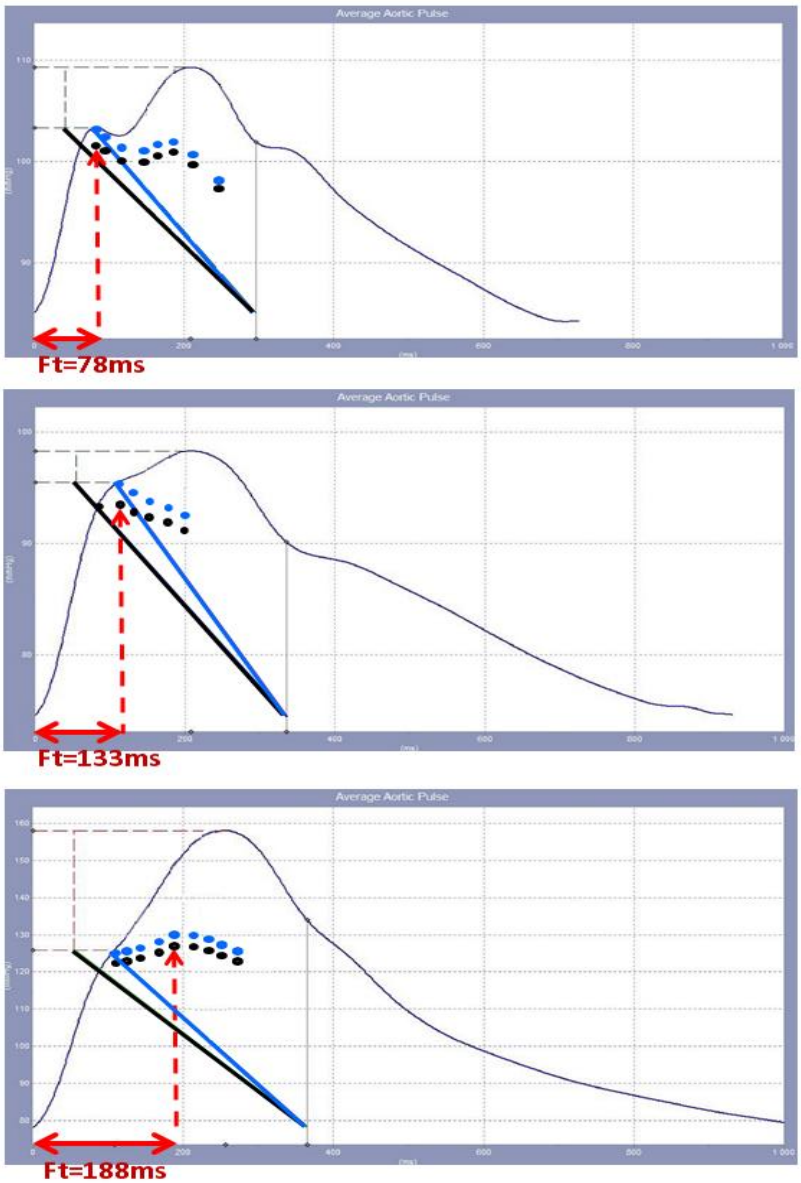

Figure 2.2. Approach to comparing the time to the peak of the aortic pressure wave (Ft) assuming the peak of the aortic flow wave occurs at either the inflection point (P_1 time) or at a point half of this time (half P_1 time). Three pressure waves with varying Ft values are shown for comparison. The dots represent the points used to construct the forward wave when wave separating using a triangular waveform with the peak at P_1 time (upper dots) or at half P_1 time (lower dots). Note that Ft is largely determined by the shape, duration and magnitude of the aortic pressure wave and not by differences in the pressure at the leading edge of the triangular flow wave.

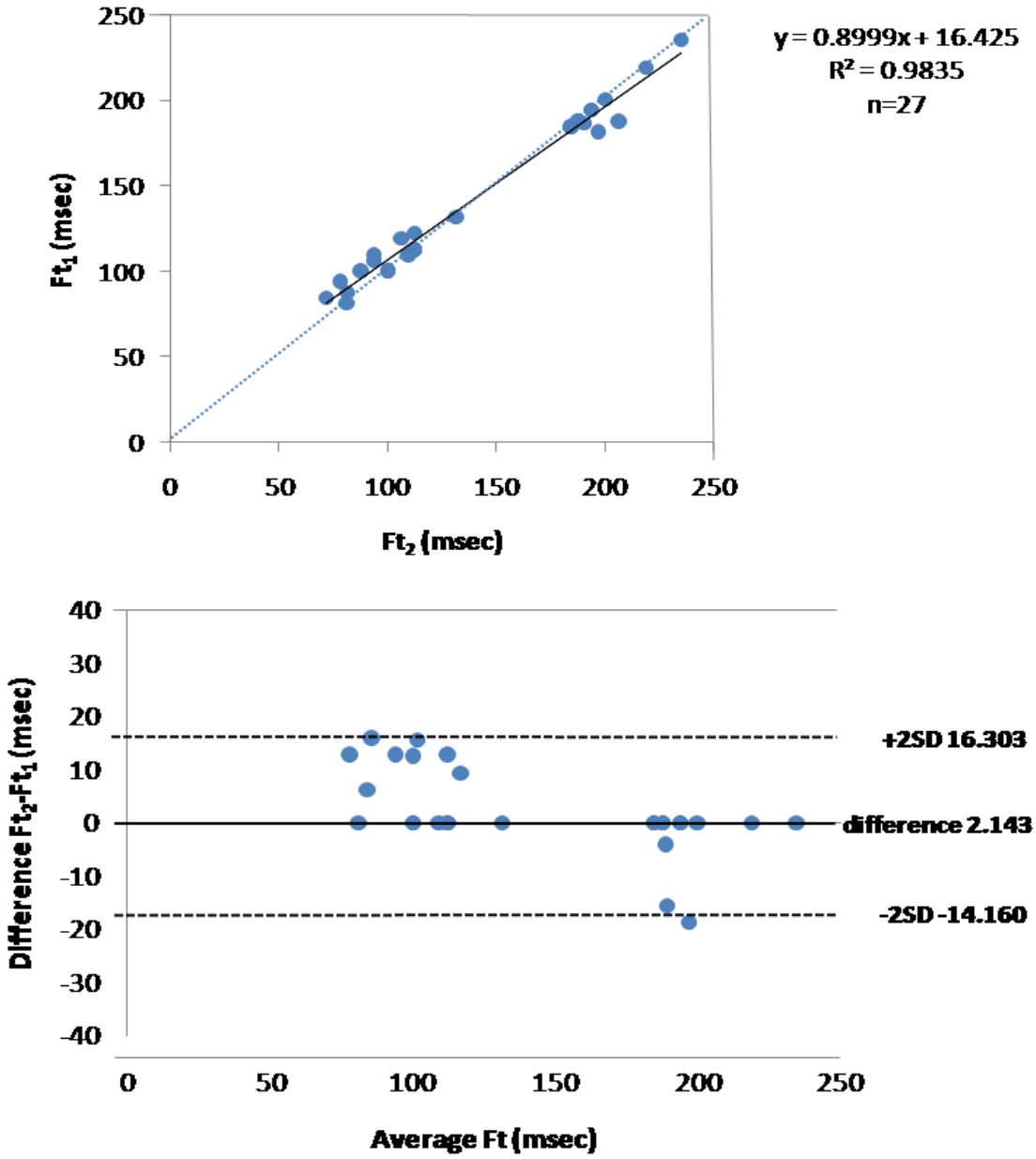

Figure 2.3. Correlation and difference (Bland-Altman plots) between the time to the peak of the aortic pressure wave (Ft) obtained assuming the peak of the aortic flow wave occurs at the inflection point (P_1 time) versus at a point half of this time (half P_1 time). Ft_1 is Ft obtained with the peak of the triangular flow wave placed at P_1 time and Ft_2 is Ft obtained with the peak of the triangular flow wave placed at a point approximately half P_1 time.

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). Using Zc values and the flow and pressure waveforms, wave separation analysis was performed as illustrated in Figure 1.3 and based on the following formulae: Forward wave pressure=(PPc + QxZc)/2 and Backward wave pressure=(PPc – QxZc)/2 (Murgo et al., 1981; Westerhof et al., 2006), where Q is flow. Wave separation analysis was performed in the time domain based upon the consensus that wave separation analysis can be done in either the time or the frequency domain (Segers et al., 2017)

Aortic augmented pressure was determined using SphygmoCor software and identified as the difference between central aortic systolic BP and pressure at the first systolic peak of the aortic pulse wave (Figure 2.1). To avoid obtaining negative aortic augmentation indexes (AIx) values in young participants, AIx was determined as the pressure at the second systolic peak (P_2) of the aortic pulse wave/the pressure at the first systolic peak (P_1) of the aortic pulse wave expressed as a percentage (Figure 2.1). LV ejection duration was also determined from SphygmoCor software (time from the beginning of the forward wave to end systole identified from the dicrotic notch).

2.3.4 Echocardiography

Left ventricular mass was determined from transthoracic two-dimensional targeted M-mode echocardiographic images obtained in the parasternal long-axis view as previously described (Booysen et al., 2015; Norton et al., 2012; Sibiya et al., 2015; Woodiwiss et al., 2009). Variables were analysed according to the American Society of Echocardiography convention (Sahn et al., 1978). LVM was determined using a standard formula (Devereux et al., 1986) and indexed (LVMI) to body surface area. LV hypertrophy (LVH) was identified as an LVMI more than 95 g/m^2 for women and more than 115 g/m^2 for men. LV ejection fraction was calculated

from [(end diastolic end systolic volume)/end diastolic volume] 100, where LV volumes were determined from the biplane Simpson rule as applied to LV internal dimensions assessed from a four-chamber view.

2.3.5 Statistical analysis

For database management and statistical analysis, SAS soft-ware, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) was employed. To in part control for variations in the type and duration of antihypertensive therapy in the primary analysis we included untreated participants together with participants receiving low-dose thiazide diuretic monotherapy for at least a year (the recommended first line agent in South Africa), which of those being treated, 48.4% were receiving. To ensure that relationships occurred independent of the use of antihypertensive therapy, sensitivity analysis was conducted in participants not receiving antihypertensive therapy. To determine relationships, multivariate linear (continuous analysis LVMI) or logistic (dichotomous LVH) regression analysis was performed with appropriate adjustments. Adjustments included in multivariate models were those correlated with central haemodynamic variables or LVMI in bivariate analysis. To determine probability values, further adjustments for non-independence of family members was performed using nonlinear regression analysis (mixed procedure as defined in the SAS package). Regression coefficients were compared with Z statistics. To compare the performance of aortic haemodynamic variables for LVH detection, the area under the receiver operating curve (AUC) was evaluated.

2.4 Results

2.4.1 Characteristics of participants

The clinical and demographic characteristics of the participants are shown in Table 2.1. A total of 1.9% of participants had a history of cardiovascular disease. Importantly, a high proportion (38.7%) of participants had hypertension, and 61.6% of hypertensive participants were not receiving therapy. Moreover, 32.5% of all participants and 58.7% of participants receiving antihypertensive therapy had uncontrolled hypertension. Of the participants with echocardiography 15.4% had LVH. Other than moderately greater proportions of participants treated for hypertension and hypertensive participants controlled to target BP, no differences in the clinical and demographic characteristics of the participants included in the echocardiographic sub-study were noted as compared with those not included in the sub-study (Table 2.2).

2.4.2 Factors associated with time to the peak of the aortic forward wave and reflection time

In stepwise regression models, the independent determinants of time to the peak of the aortic forward wave were age (partial $r = 0.31$, $P < 0.0001$), heart rate (HR) (partial $r = 0.20$, $P < 0.0001$) and female sex (partial $r = 0.13$, $P < 0.0001$). Moreover, the independent determinants of reflection time were age (partial $r = 0.50$, $P < 0.0001$), male sex (partial $r = 0.16$, $P < 0.0001$), HR (partial $r = 0.18$, $P < 0.0001$), and mean arterial pressure (partial $r = 0.14$, $P < 0.0001$).

Table 2.1. Characteristics of the study sample.

Characteristics	
Sample size (% Female)	701 (63.2)
Age (years)	42.5±17.6
Body mass index (kg/m^2)	28.9±7.5
% Obese	41.4
Regular tobacco (% subjects)	16.0
Regular alcohol (% subjects)	20.7
% with DM or HbA_{1c}>6.1%	22.2
% Hypertensive	38.7
% Treated for hypertension	14.8
% Hypertensives controlled to target BP	15.9
% of all with uncontrolled BP	32.5
Pulse rate (beats/min)	65.7±11.3
Brachial SBP/DBP (mm Hg)	128±22/83±12
Brachial pulse pressure (mm Hg)	43±15
Brachial mean arterial pressure (mm Hg)	99±15
Central aortic pulse pressure (PPc) (mm Hg)	34±14
Aortic forward wave pressure (Pf) (mm Hg)	23.5±8.4
Aortic reflected wave pressure (Pb)(mm Hg)	16.6±7.6
Aortic augmented pressure (Pa)	9.9±7.3
Aortic augmentation index (AIx) (%)	140.6±25.0
Forward peak pressure time (Ft) (ms)	125.6±36.9
Aortic pulse reflection time (Rt) (ms)	118.4±14.1
Pulse pressure amplification	1.31±0.18
Left ventricular mass index (g/m^2)	75.0±29.0

Data expressed as mean ± SD or proportions. DM, diabetes mellitus; HbA_{1C}, glycosylated hemoglobin; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

Table 2.2. Characteristics of the sample with and without echocardiography.

Characteristics	p-values vs Table 1		
Echocardiography→	Yes	No	
Sample size (% Female)	812 (65.3)	380 (65.0)	=0.93
Age (years)	45.0±18.1	42.9±18.8	=0.08
Body mass index (kg/m^2)	29.7±7.8	29.3±8.5	=0.45
% Obese	44.9	40.5	=0.15
Regular tobacco (% subjects)	14.8	16.6	=0.42
Regular alcohol (% subjects)	19.3	23.2	=0.13
% with DM or HbA_{1c}>6.1%	26.1	24.7	=0.61
% Hypertensive	47.0	42.9	=0.18
% Treated for hypertension	26.5	19.7	=0.01
% Hypertensives controlled to target BP	23.8	13.5	=0.01
% of all with uncontrolled BP	35.8	37.1	=0.67
Pulse rate (beats/min)	65.9±11.4	65.8±11.7	=0.83
Brachial SBP/DBP (mm Hg)	129±23/84±13	129±23/84±13	=0.70/=0.96
Brachial pulse pressure (mm Hg)	44.9±16.3	44.5±16.8	=0.67
Brachial mean arterial pressure (mm Hg)	100±16	100±16	=0.96
Central aortic pulse pressure (PPc) (mm Hg)	35.4±15.0	35.1±15.5	=0.74
Aortic forward wave pressure (Pf) (mm Hg)	24.3±9.3	23.9±8.4	=0.48
Aortic reflected wave pressure (Pb)(mm Hg)	17.3±8.0	17.3±8.5	=0.98
Aortic augmented pressure (Pa)	10.6±7.6	10.6±8.4	=0.97
Aortic augmentation index (AIx) (%)	141.7±24.8	141.4±25.4	=0.82
Forward peak pressure time (Ft) (ms)	127.6±37.2	128.5±37.8	=0.68
Aortic pulse reflection time (ms)	117.3±14.5	117.7±14.0	=0.68

Data expressed as mean ± SD or proportions. DM, diabetes mellitus; HbA_{1c}, glycosylated hemoglobin; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

2.4.3 Associations of time to the peak of the aortic forward wave or reflection time with aortic haemodynamics

In descending order of importance (comparison of partial r values using Z statistics), in a multivariate model, aortic backward wave pressure (partial $r^2 = 0.85$, $P < 0.0001$), forward wave pressure (partial $r = 0.02$, $P < 0.0001$), time to the peak of the aortic forward wave (partial $r^2 = 0.003$, $P < 0.0005$) and reflection time (partial $r^2 = 0.0008$, $P < 0.05$) were independently associated with PPc. With [or without (data not shown)] adjustments for age, aortic augmented pressure was associated with time to the peak of the aortic forward wave (partial $r = 0.25$ $P < 0.0001$), reflection time (partial $r = 0.43$, $P < 0.0001$), and aortic back-ward wave pressure (partial $r = 0.85$, $P < 0.0001$). Moreover, with [or without (data not shown)] adjustments for age, AIx was associated with time to the peak of the aortic forward wave (partial $r = 0.41$, $P < 0.0001$), reflection time (partial $r = 0.63$, $P < 0.0001$), and aortic backward wave pressure (partial $r = 0.41$, $P < 0.0001$). With adjustments for reflection time and the individual terms, an interaction between time to the peak of the aortic forward wave and aortic backward wave pressure was independently associated with aortic augmented pressure ($P < 0.01$). However, no interaction between reflection time and aortic backward wave pressure was independently associated with aortic augmented pressure ($P = 0.42$). Moreover, with adjustments for reflection time and aortic forward wave pressure and the individual terms, an inter-action between time to the peak of the aortic forward wave and aortic backward wave pressure was independently associated with PPc ($P < 0.005$). In analysis conducted in 392 participants, where aortic backward wave pressure was calculated, with adjustments for the individual terms and aortic forward wave pressure, no interactions between reflection time and aortic backward wave pressure were independently associated with aortic augmented pressure or PPc.

2.4.4 Time to the peak of the aortic forward wave determines relations between backward waves and aortic pressures

Although time to the peak of the aortic forward wave contributed to only a small proportion of the variation in aortic augmented pressure or PPc, with adjustments for reflection time (for aortic augmented pressure) or reflection time and aortic forward wave pressure (for PPc), the independent association of the time to the peak of the aortic forward wave–aortic backward wave pressure inter-action and aortic augmented pressure or PPc translated into incremental increases in the slope of the relationship between aortic backward wave pressure and aortic augmented pressure or between aortic backward wave pressure and PPc across quartiles of time to the peak of the aortic forward wave (Figure 2.4). In this regard, the highest quartile of time to the peak of the aortic forward wave showed a greater slope of the aortic backward wave pressure-aortic augmented pressure or aortic backward wave pressure –PPc relations than the lowest two quartiles of time to the peak of the aortic forward wave (Figure 2.4).

2.4.5 Associations of time to the peak of the aortic forward wave or reflection time with left ventricular mass and hypertrophy

In multivariate models, PPc, aortic backward wave pressure, aortic forward wave pressure, but not reflection time or time to the peak of the aortic forward wave were independently associated with LVMI (Tables 2.3, 2.4 and 2.5), with aortic backward wave pressure demonstrating stronger relations with LVMI than aortic forward wave pressure (Table 2.3 and 2.5). Importantly, similar relations were noted between aortic forward wave pressure or aortic backward wave pressure and LVMI when aortic backward wave pressure or aortic forward wave pressure were derived from wave separation analysis employing actual flow measurements versus aortic backward wave pressure or aortic forward wave pressure derived from the

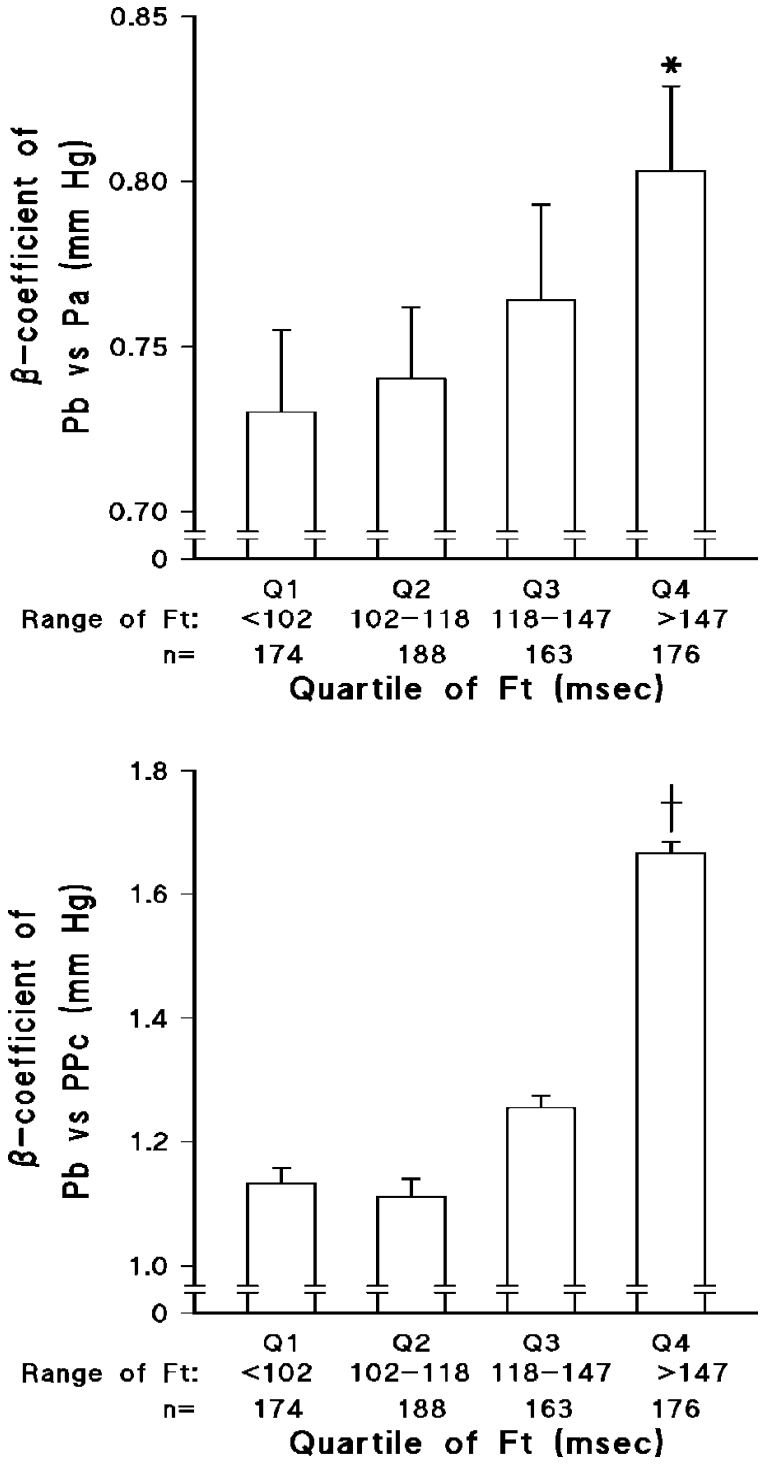

Figure 2.4. Slopes (β-coefficients) of the multivariate adjusted relations between Pb and Pa or central aortic pulse pressures (PPc) across quartiles of the time to the peak of the forward wave (n = 701). Adjustments are for the time of wave reflection in relations with Pa and both the time of wave reflection and aortic forward wave pressures in relations with PPc. All relations are significant at $P < 0.0001$. For comparison of slope: *$p < 0.05$ versus quartiles 1 and 2, $^{\dagger}p < 0.0001$ versus quartiles 1, 2 and 3. Ft, time to the peak of the aortic forward wave; Pa, aortic augmented pressures; Pb, aortic backward wave pressure.

Table 2.3. Multivariate adjusted relations between aortic haemodynamics and left ventricular mass index (LVMI).

		All participants (n=701)		Untreated participants (n=597)	
LVMI versus	Adjustors	Partial r† (95%CI)	p-value	Partial r† (95%CI)	p-value
		Individual terms			
PPc	†	0.20 (0.13 to 0.27)	<0.0001	0.25 (0.17 to 0.32)	<0.0001
Pb	†	0.20 (0.12 to 0.27)‡	<0.0001	0.26 (0.18 to 0.33)‡	<0.0001
Pf	†	0.11 (0.03 to 0.18)	<0.005	0.16 (0.08 to 0.24)	<0.0005
Ft	†	-0.01 (-0.08 to 0.06)	=0.79	-0.01 (-0.08 to 0.08)	=0.93
Rt	†	-0.01 (-0.08 to 0.06)	=0.78	-0.01 (-0.09 to 0.07)	=0.82
		Interactive terms			
PPc*Ft	PPc,Ft, †	0.09 (0.02 to 0.16)	=0.01	0.10 (0.01 to 0.18)	=0.02
Pb*Ft	Pb, Ft, †	0.11 (0.03 to 0.18)	<0.01	0.09 (0.01 to 0.17)	=0.02
Pb*Rt	Pb, Rt, †	-0.03 (-0.11 to 0.04)	=0.41	-0.02 (-0.10 to 0.07)	=0.70

PPc, central aortic pulse pressure; Pb, backward wave pressure; Pf, forward wave pressure; Ft, forward peak pressure time; Rt, aortic pulse reflection time; PPc*Ft, Pb*Ft, Pb*Rt indicate interactions between these terms. †Adjustments are for age, sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and the individual terms in interactive analysis (as indicated). ‡p<0.05 versus Pf.

Table 2.4. Stepwise regression analysis showing the contribution of aortic haemodynamic factors as compared to other factors to variations in left ventricular mass indexed to body surface area (LVMI).

LVMI versus	partial r^2	p value	partial r^2	p value	partial r^2	p value
	Model with PPc		Model with Pb		Model with Pf	
PPc	0.107	<0.0001	-	-	-	-
Pb	-	-	0.111	<0.0001	-	-
Pf	-	-	-	-	0.010	<0.005
Mean arterial pressure	-	-	-	-	0.064	<0.0001
Age	0.004	=0.07	0.004	=0.09	0.021	<0.0001
Male gender	0.030	<0.0001	0.031	<0.0001	0.023	<0.0001
Regular tobacco	-	-	0.005	=0.05	-	-
DM or HbA1c>6.1%	-	-	-	-	0.005	=0.06

Also included in regression models that failed to independently associate with LVMI were body mass index and regular alcohol intake.

Table 2.5. Multivariate adjusted relations between aortic haemodynamic parameters derived from wave separation analysis using actual aortic flow waveforms and left ventricular mass index (LVMI).

LVMI versus	Adjustors	Partial r† (95%CI)	p-value
		Individual terms	
Pb	†	0.24 (0.14 to 0.34)‡	<0.0001
Pf	†	0.13 (0.02 to 0.22)	<0.02
		Interactive terms	
Pb*Ft	Pb, Ft, †	0.10 (0.00 to 0.20)	=0.05
Pb*Rt	Pb, Rt, †	-0.07 (-0.17 to 0.03)	=0.18

Pb, backward wave pressure; Pf, forward wave pressure; Ft, forward peak pressure time; Rt, aortic pulse reflection time; Pb*Ft, Pb*Rt indicate interactions between these terms. †Adjustments are for age, sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and the individual terms in interactive analysis (as indicated). ‡$p<0.05$ versus Pf.

triangular waveform approach to wave separation analysis (Table 2.5 versus Table 2.3). Although aortic backward wave pressure was independently associated with LVH, neither PPc, aortic forward wave pressure, reflection time nor time to the peak of the aortic forward wave were independently associated with LVH (Table 2.6). Although neither reflection time nor time to the peak of the aortic forward wave were independently associated with LVMI or LVH, independent of the individual terms and confounders, an interaction between time to the peak of the aortic forward wave and aortic backward wave pressure and between time to the peak of the aortic forward wave and PPc were independently associated with LVMI (Table 2.3 and 2.5) and similar interactions were noted in both men and women (Table 2.7). However, no interaction between reflection time and aortic backward wave pressure was independently associated with LVMI (Table 2.3 and 2.4). An interaction between time to the peak of the aortic forward wave and aortic backward wave pressure and between time to the peak of the aortic forward wave and PPc was also independently associated with LVH (Table 2.6). Importantly, with adjustments for the same confounders given in Table 2.3, no significant interactions between HR and aortic backward wave pressure (partial r =0.03, P = 0.43) or between ejection duration and aortic backward wave pressure (partial r = 0.04, P = 0.31) were independently associated with LVMI. Moreover, no interaction between aortic forward wave pressure and time to the peak of the aortic forward wave (partial r = 0.04, P = 0.28), was independently associated with LVMI.

2.4.6 Time to the peak of the aortic forward wave determines the impact of backward waves on left ventricular mass and hypertrophy

The independent associations between the time to the peak of the aortic forward wave-aortic backward wave pressure or time to the peak of the aortic forward wave–PPc inter-actions and LVMI translated into a stepwise increase in the strength (Figures 2.5 and 2.6) and magnitude

Table 2.6. Multivariate adjusted relations between aortic haemodynamics and left ventricular hypertrophy (LVH).

		All participants (n=701)		Untreated participants (n=597)	
LVH versus	Adjustors	Odds ratio† (95% CI)	p-value	Odds ratio† (95% CI)	p-value
		Individual terms			
PPc	†	1.013 (0.995 to 1.032)	=0.16	1.022 (1.001 to 1.044)	<0.05
Pb	†	1.040 (1.001 to 1.079)	<0.05	1.069 (1.023 to 1.116)	<0.005
Pf	†	1.012 (0.986 to 1.039)	=0.37	1.026 (0.997 to 1.057)	=0.08
Ft	†	0.998 (0.993 to1.004)	=0.58	0.996 (0.989 to 1.004)	=0.30
Rt	†	0.995 (0.978 to 1.012)	=0.55	0.992 (0.974 to 1.011)	=0.42
		Interactive terms			
PPc*Ft	PPc, Ft, †	1.001 (1.000 to 1.001)	<0.01	1.001 (1.000 to 1.001)	=0.06
Pb*Ft	Pb, Ft, †	1.001 (1.000 to 1.002)	<0.01	1.001 (1.000 to 1.002)	<0.05
Pb*Rt	Pb, Rt, †	1.000 (0.998 to 1.002)	=0.77	1.001 (0.999 to 1.003)	=0.38

PPc, central aortic pulse pressure; Pb, backward wave pressure; Pf, forward wave pressure; Ft, forward peak pressure time; Rt, aortic pulse reflection time; PPc*Ft, Pb*Ft, Pb*Rt indicate interactions between these terms. †Adjustments are for age, sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and the individual terms in interactive analysis (as indicated).

Table 2.7. Sex-specific effects for the association of the interaction between the time to the peak of the forward wave (Ft) and aortic pressures versus left ventricular mass index (LVMI) or LV hypertrophy (LVH).

		Women (n=443)		Men (n=258)	
LVMI versus	Adjustors	Partial r† (95%CI)	p-value	Partial r† (95%CI)	p-value
PPc*Ft	PPc,Ft, †	0.115 (0.020 to 0.208)	<0.02	0.112 (-0.019 to 0.240)	=0.09
Pb*Ft	Pb, Ft, †	0.100 (0.005 to 0.193)	<0.05	0.143 (0.017 to 0.264)	<0.05
LVH versus	Adjustors	Odds ratio† (95% CI)	p-value	Odds ratio† (95% CI)	p-value
PPc*Ft	PPc, Ft,	1.001 (1.000 to 1.001)	<0.02	1.001 (1.000 to 1.001)	=0.10
Pb*Ft	Pb, Ft,	1.001 (1.000 to 1.002)	<0.05	1.001 (1.000 to 1.003)	=0.05

PPc, central aortic pulse pressure; Pb, backward wave pressure; PPc*Ft, Pb*Ft, indicate interactions between these terms. †Adjustments are for age, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and the individual terms in interactive analysis (as indicated).

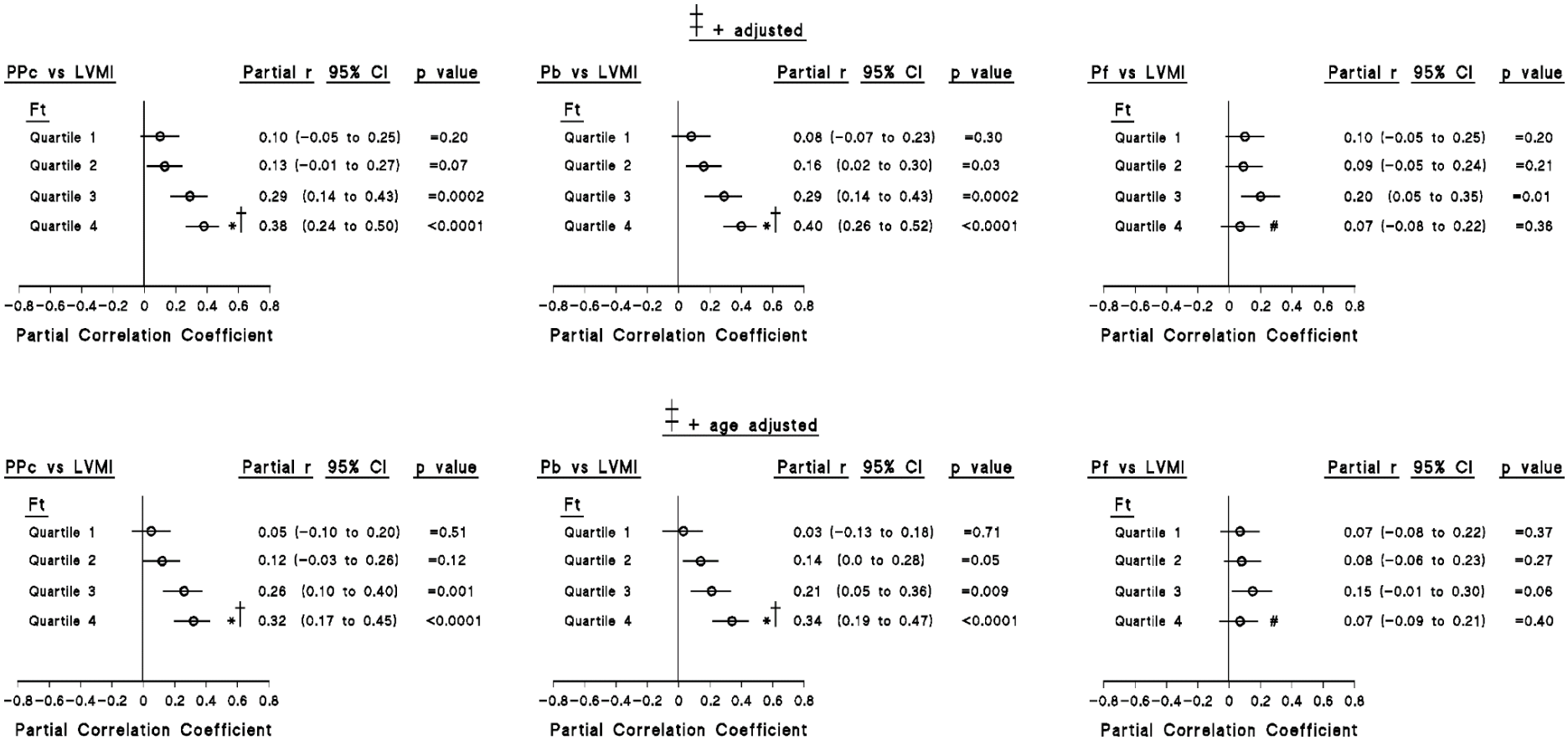

Figure 2.5. Multivariate adjusted relations (partial r) between central aortic pulse pressure (PPc), backward wave pressures (Pb), or forward wave pressures (Pf) and left ventricular mass index (LVMI) across quartiles of the Ft (n=701). Adjustments are for age as indicated and sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus, or an percentage glycated haemoglobin more than 6.1%. For comparison of slope: *p<0.05, p<0.01 versus quartile 1, †p<0.05 versus quartile 2. #P<0.0005 versus slope of aortic backward wave pressure–LVMI relationship in quartile 4. see Table 2.3 for abbreviations.

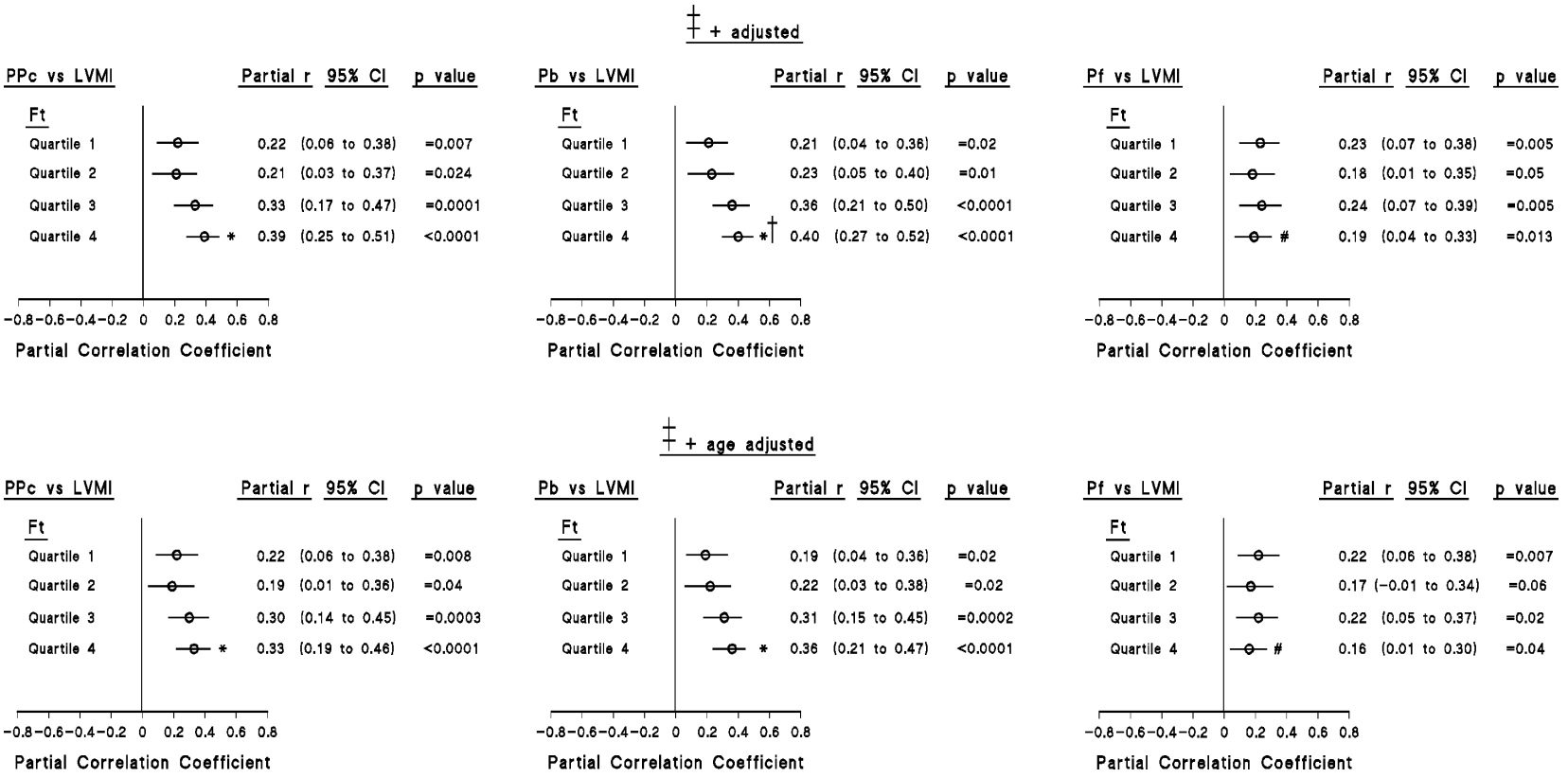

Figure 2.6. Multivariate adjusted relations (partial r) between central aortic pulse pressure (PPc), backward wave pressures (Pb), or forward wave pressures (Pf) and left ventricular mass index (LVMI) across quartiles of the time to the peak of the forward wave (Ft) in participants having never received antihypertensive therapy (n=597). The upper figures are not adjusted for age and lower figures are also adjusted for age. ‡Additional adjustments are for sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1%. For comparison of partial r value: *p<0.05 quartile 4 versus quartile 1, †p<0.05 quartile 4 versus quartile 2, #p=0.01 compared to partial r for Pb vs LVMI relationship in quartile 4.

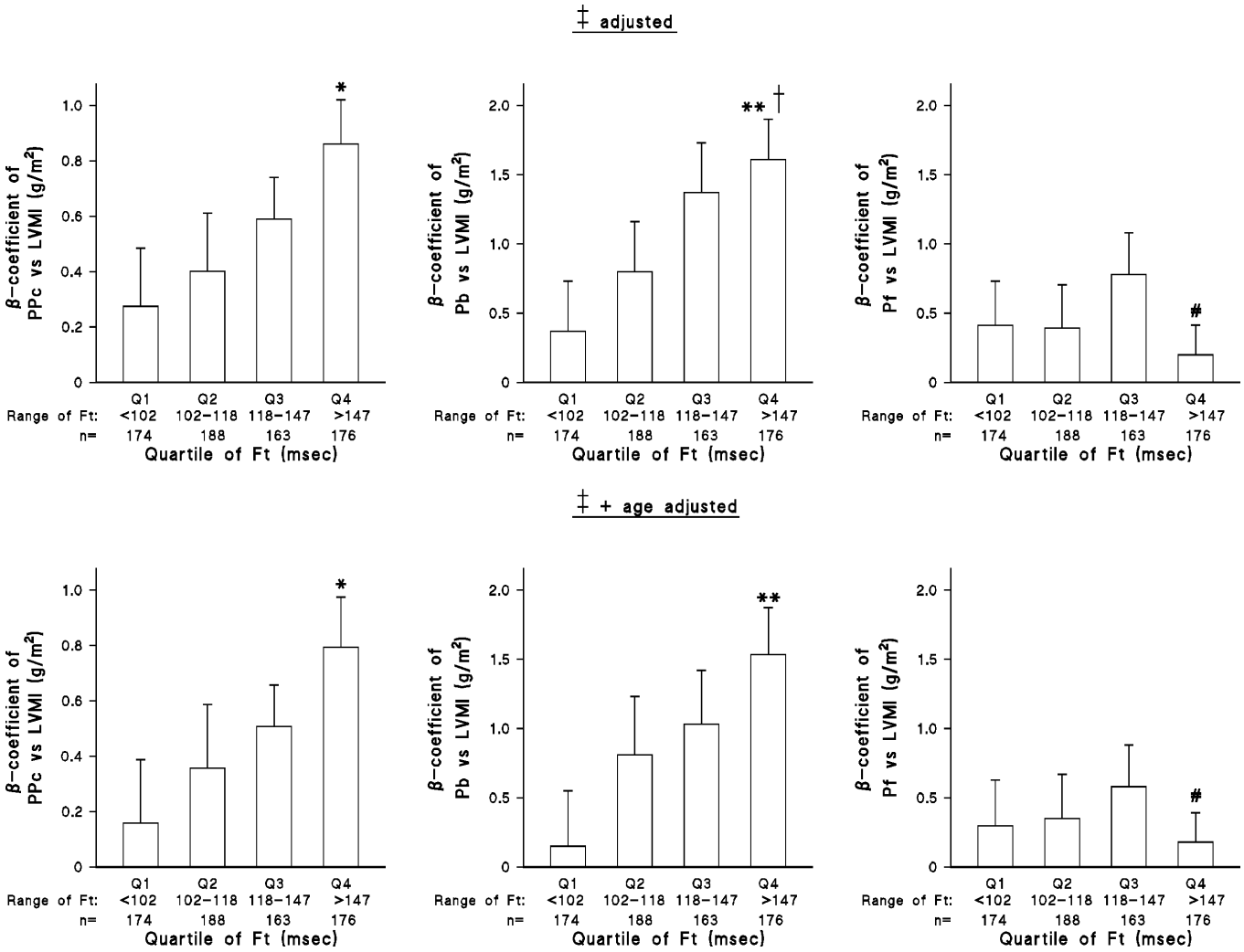

Figure 2.7. Slopes (β-coefficients) of the multivariate adjusted relations between central aortic pulse pressure (PPc), backward wave pressures (Pb), or forward wave pressures (Pf) and left ventricular mass index (LVMI) across quartiles of the time to the peak of the forward wave (Ft) (n=701). The upper panels do not include adjustments for age, whilst the lower panels are also adjusted for age. ‡Additional adjustments are for sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1%. For significance of relations see figure 2.5. For comparison of slope: *p<0.05, **p<0.01 vs quartile 1, †p<0.05 versus quartile 2. #p<0.0005 vs slope of Pb-LVMI relationship in quartile 4.

(Figure 2.7) of the associations between aortic backward wave pressure or PPc and LVMI across quartiles of time to the peak of the aortic forward wave, with the highest quartile of time to the peak of the aortic forward wave showing markedly stronger relations than the lowest two quartiles of time to the peak of the aortic forward wave. In stepwise regression models, although several factors were independently associated with LVMI across quartiles of time to the peak of the aortic forward wave (Table 2.8), the factor most strongly associated with LVMI in the highest quartile of time to the peak of the aortic forward wave was aortic backward wave pressure, whereas the contribution of aortic backward wave pressure in the lowest quartile of time to the peak of the aortic forward wave was negligible. Similar findings were noted for PPc (data not show).

The independent associations between the time to the peak of the aortic forward wave-aortic backward wave pressure or time to the peak of the aortic forward wave–PPc interactions and LVH also translated into an increase in the associations between aortic backward wave pressure or PPc and LVH in the highest as compared with the other three quartiles of time to the peak of the aortic forward wave (Table 2.9). In contrast, relations between aortic forward wave pressure and LVMI (Figure 2.6) or LVH (Table 2.9) did not differ across quartiles of time to the peak of the aortic forward wave. As compared with aortic forward wave pressure-LVMI or aortic forward wave pressure-LVH relations, markedly stronger relations with a greater magnitude of the effect of aortic backward wave pressure on LVMI or LVH was noted in the highest quartile of time to the peak of the aortic forward wave only (Figures 2.5-2.7, and Table 2.9). Aortic backward wave pressure was also more closely associated with LVMI in the highest versus the lowest quartile of ejection duration, but the relationship between aortic backward wave pressure was not more closely associated with LVMI in the lowest as compared to any of the other three quartiles of HR (Table 2.10). Adjustments for aortic augmented pressure only partially modified the incremental slope of the relations between aortic backward wave pressure and LVMI across quartiles of time to the peak of the aortic forward wave (Figure 2.8). No stepwise increases in the

Table 2.8. Stepwise regression analysis showing the contribution of aortic backward wave pressures (Pb) as compared to other factors to variations in left ventricular mass indexed to body surface area (LVMI) across quartiles of the time to the peak of the forward wave (Ft).

Quartiles of Ft	Quartile 1		Quartile 2		Quartile 3		Quartile 4	
Range of Ft	<102		102 to 118		118 to 147		>147	
LVMI versus	partial r^2	p value	partial r^2	p value	partial r^2	p value	partial r^2	p value
Pb	-	-	0.075	<0.0001	0.100	<0.0001	0.204	<0.0001
Mean arterial pressure	0.026	<0.05	-	-	-	-	-	-
Age	-	-	-	-	0.021	=0.05	-	-
Male gender	0.071	<0.001	0.019	=0.05	-	-	-	-
Regular alcohol	-	-	0.017	=0.07	0.019	=0.06	0.017	=0.06
DM or HbA1c>6.1%	0.016	=0.09	-	-	-	-	-	-

Also included in regression models that failed to independently associate with LVMI were body mass index and regular tobacco use.

Table 2.9. Multivariate adjusted relations between central aortic pulse pressure (PPc), backward wave pressures (Pb) or forward (Pf) wave pressures and left ventricular hypertrophy (LVH) across quartiles of the time to the peak of the aortic forward wave (Ft).

Quartiles of Ft→	1			2			3			4		
Range of Ft	<102			102 to 118			118 to 147			>147		
n= (% LVH)	174 (17)			188 (13)			163 (15)			176 (17)		
LVH versus	OR* (95% CI)	Wald X^2	p-value	OR* (95% CI)	Wald X^2	p-value	OR* (95% CI)	Wald X^2	p-value	OR* (95% CI)	Wald X^2	p-value
PPc	0.996 (0.947 to 1.045)	0.03	=0.86	0.978 (0.927 to 1.030)	0.70	=0.40	1.003 (0.964 to 1.036)	0.03	=0.86	1.062† (1.012 to 1.119)	5.60	<0.05
Pb	0.989 (0.908 to 1.074)	0.07	=0.80	0.976 (0.887 to 1.072)	0.25	=0.62	0.994 (0.915 to 1.070)	0.02	=0.88	1.144$^{\dagger\#}$ (1.045 to 1.264)	7.81	<0.01
Pf	1.010 (0.940 to 1.081)	0.08	=0.77	0.955 (0.881 to 1.029)	1.37	=0.24	1.042 (0.976 to 1.116)	1.59	=0.21	1.015$^{\$}$ (0.964 to 1.059)	0.40	=0.53

See tables 2.2 and 2.3 for abbreviations. OR, Odds ratio.* Adjustors included in logistic regression models are age, sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1%. †p<0.05 compared to quartiles 1 and 2; $^{\#}$p<0.05 compared to quartiles 1, 2 and 3; $^{\$}$ p<0.005 compared to OR for Pb vs LVH in quartile 4.

Table 2.10. Multivariate adjusted relationships between aortic backward wave pressures (Pb) and left ventricular mass index (LVMI) across quartiles of LV ejection time or heart rate.

Pb versus LVMI

LVMI versus	Partial r* (95% CI)	p-value	Partial r* (95% CI)	p-value	Partial r* (95% CI)	p-value	Partial r* (95% CI)	p-value
Quartiles of LV ejection time→	1		2		3		4	
Range of LV ejection time	<311 msec		311 to 326 msec		327 to 340 msec		≥341 msec	
n=	166		178		181		176	
Pb	0.16 (0.01 to 0.31)	<0.05	0.21 (0.06 to 0.36)	<0.01	0.14 (0.01 to 0.28)	p=0.07	0.31† (0.16 to 0.43)	<0.0001
Quartiles of heart rate→	1		2		3		4	
Range of heart rate	<58 beats/min		58 to 64 beats/min		65 to 71 beats/min		≥72 beats/min	
n=	170		177		166		188	
Pb	0.15 (-0.01 to 0.30)	=0.06	0.18 (0.03 to 0.32)	<0.02	0.20 (0.05 to 0.35)	<0.01	0.14 (-0.01 to 0.28)	=0.06

*Adjustors included in regression models are age, sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1%.†p<0.05 versus partial r for quartile 1 using z-statistics.

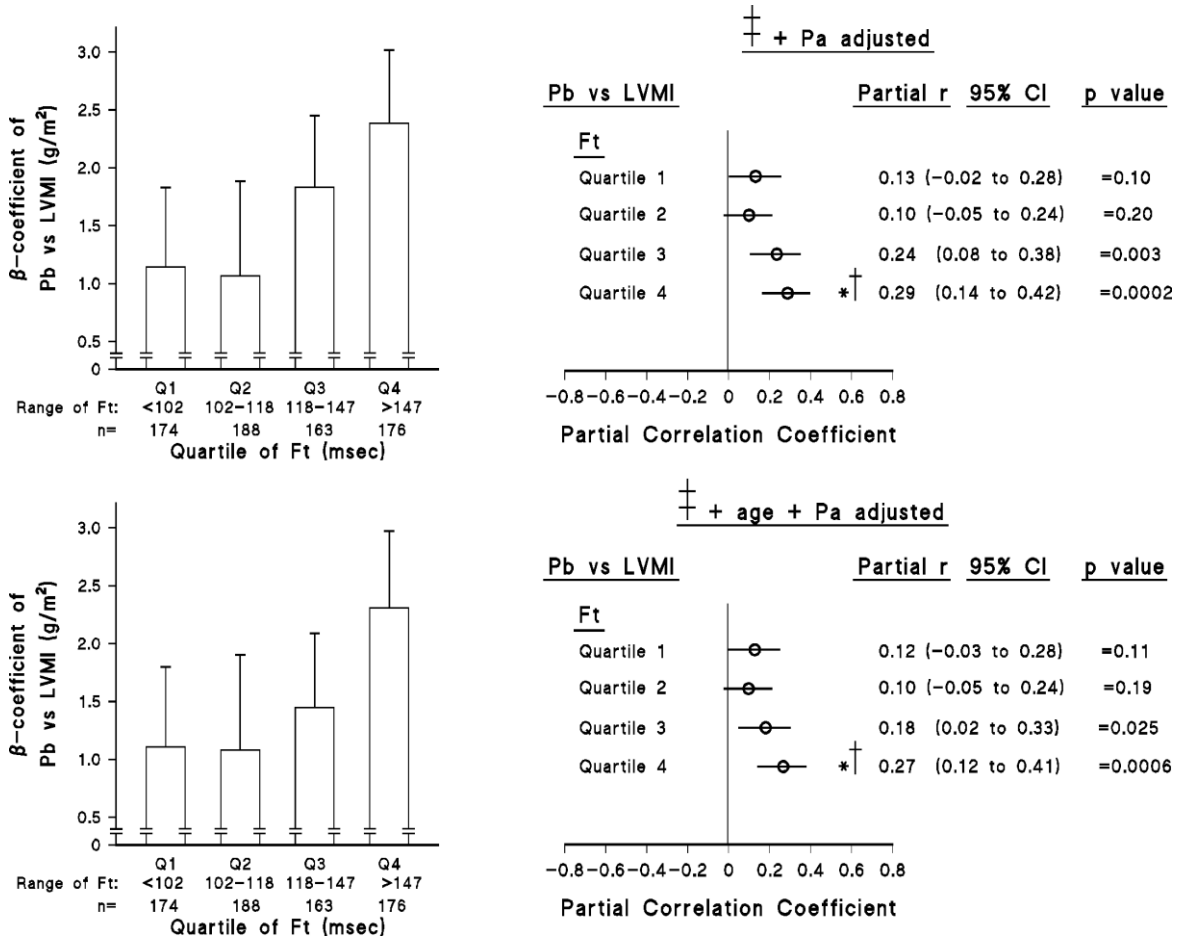

Figure 2.8. Impact of adjustments for augmented pressures (Pa) on the strength (partial r) and slope (β-coefficient) of the multivariate adjusted relations between aortic backward wave pressures (Pb) and left ventricular mass index (LVMI) across quartiles of the time to the peak of the forward wave (Ft). The upper figures are not adjusted for age and lower figures are also adjusted for age. ‡Additional adjustments are for sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1%. For comparison of partial r value: *p<0.05 versus quartile 1; †p<0.05 versus quartile 2.

strength and magnitude of the association between aortic backward wave pressure and LVMI across quartiles of reflection time were noted (data not shown).The independent association between the time to the peak of the aortic forward wave-aortic backward wave pressure or time to the peak of the aortic forward wave-PPc interactions and LVH translated into a greater performance of aortic backward wave pressure and PPc for LVH detection in the highest as compared to the first three quartiles of Time to the peak of the aortic forward wave (Figure 2.9). In contrast, the ability of aortic forward wave pressure to detect LVH did not differ across quartiles of time to the peak of the aortic forward wave (Figure 2.9).

2.4.7. Correlation between time to the peak of the aortic forward wave and systolic function

No correlation between and time to the peak of the aortic forward wave and LV ejection fraction was noted (r =0.0001, P =1.00).

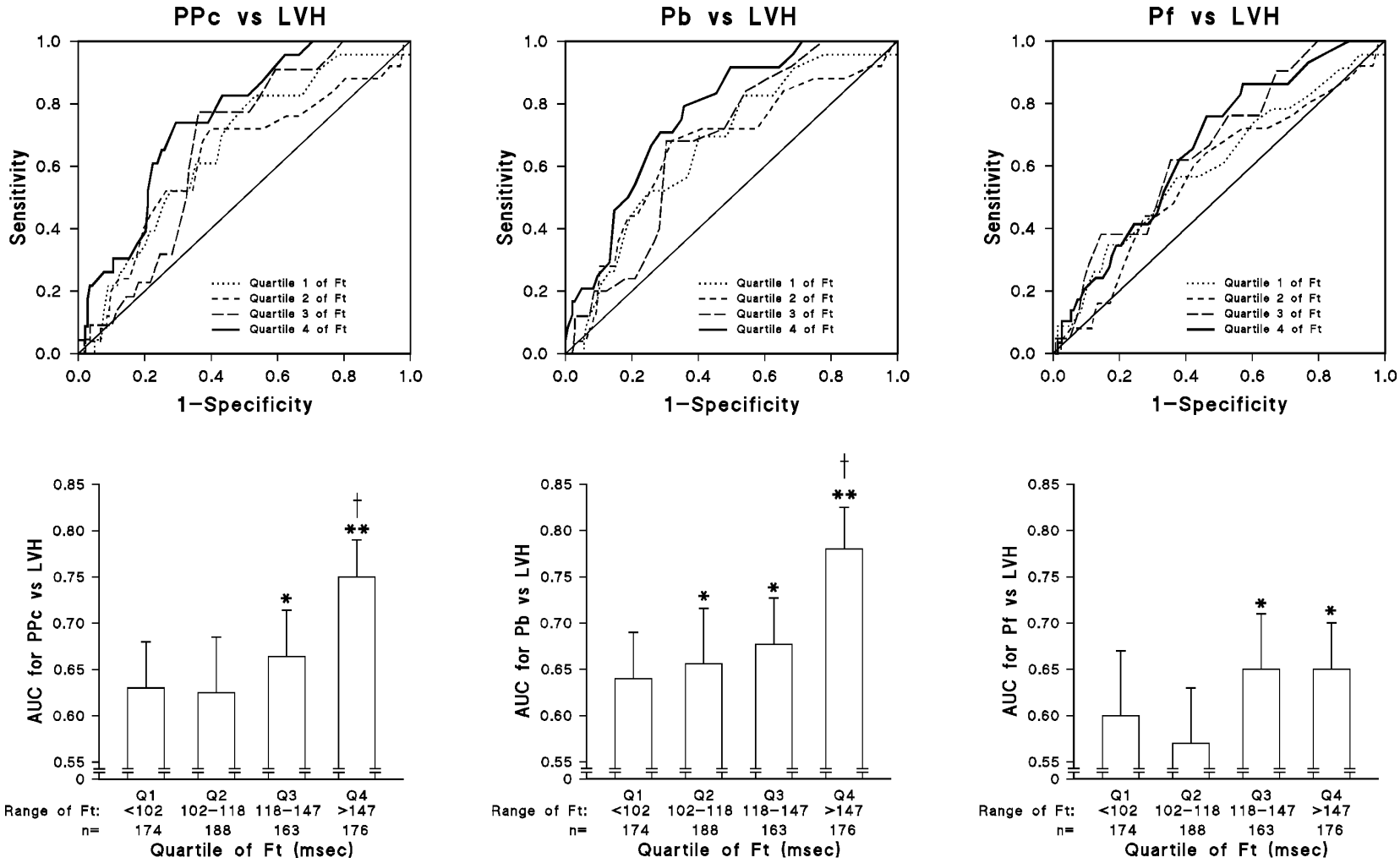

Figure 2.9. Receiver-operating characteristic (ROC) curves and the area under the curve (AUC±SEM) for the detection of left ventricular hypertrophy (LVH)(n=108 of 701 of central aortic pulse pressure (PPc), and backward (Pb) and forward (Pf) wave pressures across quartiles of the time to the peak of the forward wave (Ft). *p<0.05, **p<0.0005 for AUC of ROC curves; †p<0.05 vs quartiles 1 and 2.

2.5 Discussion

The main findings of the present study are as follows: in a relatively large community-based sample of adults either receiving no antihypertensive therapy or receiving stable low-dose thiazide therapy for at least a year, we show that the time to the peak of the aortic forward wave, but not the time of wave reflection, has a major impact on relations between backward wave pressures and aortic augmented or pulse pressures and between backward wave or aortic pulse pressures and LVMI or LVH. In this regard, across quartiles of an increasing duration of time to the peak of the forward wave an increase in relations between the magnitude of backward wave pressures and augmented pressures or PPc and between the magnitude of backward wave or aortic pulse pressures and LVMI or LVH were noted. Importantly, backward wave pressures and PPc were only independently associated with LVH in the highest quartile of the time to the peak of the aortic forward wave.

Aortic pressure augmentation is mediated by several factors including an increased magnitude of backward wave pressures, an enhanced speed of wave reflection and an extended time to the peak of the aortic forward wave. Although the magnitude of backward wave pressure is a well-established cause of adverse effects on the cardiovascular system (Booysen et al., 2015; Chirinos et al., 2012; Sibiya et al., 2015; Vlachopoulos et al., 2010; Wang et al., 2010; Weber et al., 2012), the exact role of the timing of forward and backward waves in contributing to the adverse effects in adults of backward wave pressure, is poorly documented. Although heart fail with a preserved ejection fraction is associated with prolonged mechanical systole (Weber et al., 2006), the extent to which the time to the peak of the forward wave contributes to producing cardiac changes in heart fail is unknown. In the present study, the time to peak of the forward wave determined the impact of backward wave pressures on LVMI and LVH, a cardiac change that is well accepted as progressing to heart fail with either a preserved or reduced ejection fraction. Hence, the present results provide a possible mechanism that may explain the

relationship between a prolonged mechanical systole and heart fail with a preserved ejection fraction (Weber et al., 2006).

Although β-adrenergic receptor blockade extends the systolic period and augments PPc (Williams and Lacy, 2009), the extent to which a greater impact of backward waves explains the reduced efficacy of β-adrenergic receptor blocking agents in improving outcomes compared with alternative antihypertensive agents has not been reported on. In this regard, prolonged mechanical systole may be associated with an increased stroke volume which could produce adverse effects through an enhanced forward wave pressure. In contrast, a prolonged mechanical systole may increase the chances of the backward wave overlapping with the for-ward wave, thus augmenting the deleterious impact of backward waves on cardiovascular end organs. The present study provides the first evidence to show that relations between aortic backward wave pressures (and hence PPc) and cardiac end-organ changes (LVMI and LVH) are strongly determined by the time to the peak of the aortic forward wave. In contrast, relations between forward wave pressures and LVMI or LVH did not depend on the time to the peak of the aortic forward wave. Hence, the present study suggests that prolonged mechanical systole mediates adverse effects on the heart by enhancing the deleterious impact of aortic backward wave and pulse pressures on cardiovascular end organs.

In the present study, the independent associations between aortic backward wave pressure and LVMI or LVH at higher time to the peak of the aortic forward wave values were only partially accounted for by augmented pressures. In this regard, indexes of aortic pressure augmentation should not be seen as an accurate composite of the adverse effects of the time to the peak of the forward wave and the magnitude of backward wave pressures. Indeed, augmented pressures also incorporate the impact of the speed of wave reflection which, as indicated in the present study, plays little role in contributing to the impact of backward wave pressures on LVMI and LVH. Moreover, there are now several lines of evidence to suggest that indexes of pressure augmentation are influenced by factors other than backward wave pressures

or the timing of pressure waves (Davies et al., 2010; Fok et al., 2014c; Hughes et al., 2013; Lim et al., 2012) and these factors may confound relations between backward wave pressures and end-organ measures. Indeed, augmented pressures and augmentation index underestimate the effect of backward waves on aortic pulse pressure and LVMI (Booysen et al., 2015).

In the present study, we were unable to show significant independent associations between ejection duration-aortic backward wave pressure or HR–aortic backward wave pressure interactions and LVMI or LVH. These findings may be explained by the weak correlations between the time to the peak of the forward wave and either ejection duration ($r^2 = 0.10$) or HR ($r^2 = 0.04$). The present data therefore indicate that when identifying potential adverse effects of backward waves on the heart, neither ejection duration (which may be determined from electrocardiography) nor HR may be employed as accurate surrogates for the time to the peak of the forward wave. Nevertheless, as with the stronger independent correlations noted between aortic backward wave pressure and LVMI in the highest as compared with the lowest quartile of time to the peak of the aortic forward wave, stronger independent correlations were also noted between aortic backward wave pressure and LVMI in the highest as compared with the lowest quartile of ejection duration. Hence, although wave separation analysis may be the most accurate method of identifying wave timing effects on backward wave and hence aortic pulse pressure end-organ relations, in the absence of wave separation analysis, ejection duration may be considered as a proxy for time to the peak of the forward wave. Whether ejection duration guides the effects of PPc calculated from simple clinical measures (imputed PPc), which has been demonstrated to predict outcomes beyond brachial pressures in several studies (Benetos et al., 2010; Bursztyn et al., 2016; Regnault et al., 2012) requires further evaluation. In this regard, imputed PPc and ejection duration (obtained from electrocardiography) may be acquired at no additional cost and hence, would be ideally suited for use in resource-limited settings.

As with previous reports (Booysen et al., 2015; Sibiya et al., 2015), the results of the present study suggest that aortic backward wave pressures are more important than forward wave

pressures in contributing to the adverse effect of PPc on the heart. The results of the present study nevertheless extend these findings by suggesting that the impact of backward wave pressure and hence PPc on the heart occurs mainly in those with an extended time to peak systole (highest quartile of time to peak systole). Indeed, only in the highest quartile of the time to peak systole was back-ward wave pressure and PPc associated with LVH independent of mean arterial pressure (distending pressures) and several additional confounders including age.

Although there is increasing evidence that aortic backward wave pressures are key elements in cardiovascular risk prediction or preclinical end-organ changes (Booysen et al., 2015; Chirinos et al., 2012; Sibiya et al., 2015; Wang et al., 2010; Weber et al., 2012), this finding has been inconsistent (Mitchell et al., 2010a; Vlachopoulos et al., 2010). The present results suggest that the impact of backward waves on LVMI or LVH will occur only in those with an extended time to the peak of the aortic forward wave. In those studies that failed to show an impact of aortic BP on risk beyond brachial BP (Mitchell et al., 2010a; Vlachopoulos et al., 2010) whether a significant proportion of participants had an extended systolic period comparable with that noted in the highest quartile of time to the peak of the forward wave in the present study, is unknown. The present results suggest that aortic backward wave and pulse pressure effects on cardiovascular end organs should always be interpreted in the context of time to peak forward wave pressures. In this respect, an extended time to peak forward wave pressure, such as may occur with several agents that influence HR (such as β-adrenergic receptor blockers), in conditions associated with long QT (electrocardiographic QT interval) syndrome, or in the elderly who have a prolonged mechanical systole, may increase the impact of backward waves on the heart. If confirmed in out-come-driven studies, under these circumstances it is possible that only in those with an extended time to the peak of the forward wave will aortic pressures be particularly useful in risk predicting beyond brachial BP.

One possible interpretation of our findings is that a prolonged time to the peak of the aortic forward wave may reflect the impact of a reduced systolic function (which results in an

increased LVMI), which attenuates the ratio of the pre-ejection period (isovolumic contraction time)/ejection time. In this case, the impact of time to the peak of the aortic forward wave would be an epiphenomenon rather than being causally related to the impact of aortic backward wave pressure on LVMI. However, in the present study no correlation between time to the peak of the aortic forward wave and LV ejection fraction was noted. Hence, at present the only explanation for our data is that a prolonged time to the peak of the aortic forward wave increases the chances that the backward wave augments aortic pulse pressure and consequently LVMI.

There are several limitations to the present study that warrant consideration. First, the present study was a cross-sectional design. Hence, we cannot determine whether associations reported on are indeed cause and effect. Further longitudinal studies are therefore required. Second, the assumptions intrinsic to the use of the 'triangulation method' of aortic wave separation may not be ideal (Kips et al., 2009). However, in 392 participants of the present sample with aortic velocity measurements, we were able to show a correlation (r^2) between aortic backward wave pressure derived from the 'triangulation method' versus 'actual aortic flow' methods of wave separation of 0.82. Moreover, we show similar relations (as compared with when using the triangular waveform approach to wave separation analysis) between aortic haemodynamic parameters derived from wave separation analysis using the 'actual aortic flow' method and LVMI. Hence, at least in the present study, the use of the 'triangulation method' is unlikely to have significantly affected our results. 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 are likely to have been underestimated using the current approach. However, aortic forward wave pressure would have been affected as much by this limitation as aortic backward wave pressure, and time to the peak of the aortic forward wave-aortic backward wave pressure, but not time to the peak of the aortic forward wave–aortic forward wave pressure interactions were independently associated with LVMI and LVH. Fourth, we were not statistically powered to compare slopes (magnitude) of

aortic pressure versus LVMI relations in only untreated participants and hence our results may have been affected by the impact of therapy. However, by excluding participants on agents other than low-dose thiazide diuretic therapy, more than a single agent, or receiving therapy for less than a year, we have to some degree controlled for variations in the type and duration of therapy. In addition, in sensitivity analysis conducted only in untreated participants, we show essentially the same results for interactive analysis and when comparing the strength of relations between aortic function and LVMI across quartiles of time to the peak of the aortic forward wave.

In conclusion, in the present study we show that the impact of aortic backward wave pressures on LVMI and LVH, are strongly influenced by the time to the peak of the aortic forward wave. Only in the highest quartile of time to the peak of the aortic forward wave, did backward wave pressures show strong independent associations with both LVMI and LVH. Moreover, only in the highest quartile of time to the peak of the aortic forward wave, did backward wave pressures show stronger independent associations with both LVMI and LVH than forward wave pressures. Thus, the adverse effects of aortic backward wave pressures on cardiovascular end organs may be strongly determined by the time to the peak of the aortic forward wave.

Chapter 3

Enhanced Aortic Reflected Wave Magnitude Accounts for the Impact of Female Gender on Aortic Pressure Augmentation in a Group of African Ancestry

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3.0 Abstract

Aortic reflected wave magnitude (RM) may not account for sex-specific differences in aortic pressure augmentation in Caucasians. However, aortic reflected waves are greater in groups of African descent than other ethnic groups. We determined whether RM or alternative factors explain the impact of sex on aortic augmented pressure (Pa) in participants of African ancestry. We assessed aortic function (radial applanation tonometry, SphygmoCor) in 1,197 randomly recruited community participants of African ancestry (age ≥ 16 years). Aortic forward (Pf) and backward (Pb) wave separation was performed assuming an aortic triangular flow wave validated against aortic velocity measurements. Across the adult lifespan, women had greater multivariate-adjusted augmentation index (AIx) and Pa. This was associated with multivariate-adjusted age-related increases in Pb, RM (Pb/Pf), and time to the peak of Pf and decreases in backward wave foot time; but not increases in Pf. With adjustors, Pa was associated with female gender (β-coefficient = 3.81 ± 0.34), a relationship which was markedly attenuated by adjustments for RM (β-coefficient = 1.78 ± 0.31, $P < 0.0001$ vs. without adjustments for RM), and Pb (β-coefficient = 2.05 ± 0.19, $P < 0.0001$ vs. without adjustments for Pb), but not by adjustments for Pf, time to the peak of Pf, or backward wave foot time. Similarly, AIx was associated with female gender, a relationship which was markedly attenuated by adjustments for RM, Pb, and backward wave foot time, but not alternative factors. In conclusion, in contrast to reports in alternative populations, the relationship between aortic pressure augmentation and female gender in participants of African descent is accounted for mainly by increases in RM.

Keywords: Aortic augmentation index; aortic reflected waves; blood pressure; hypertension; sex.

3.1 Introduction

Pulse pressure (PP) is a strong determinant of cardiovascular damage beyond steady-state pressures and as such is incorporated in guidelines for risk prediction (Mancia et al., 2013). Several aspects of aortic function regulate central arterial PP including aortic stiffness and backward wave pressures (Agabiti-Rosei et al., 2007, 2007; Avolio et al., 2009; Nichols et al., 2011). The relative contribution of backward traveling waves to central arterial PP is identified as augmentation index (AIx) and AIx (or augmented pressure [Pa]) is in some studies, an independent predictor of cardiovascular outcomes (Vlachopoulos et al., 2010). Whilst increases in AIx have traditionally been attributed to increases in aortic stiffness which enhance pulse wave velocity and cause pre-mature arrival of backward waves in systole, several alternative factors have been identified as contributing more to variations in AIx or Pa than aortic stiffness. In this regard, factors that influence the forward traveling wave amplitude (Cheng et al., 2012; Davies et al., 2010; Fok et al., 2014c; Hughes et al., 2013), the magnitude of the forward wave (Torjesen et al., 2014), the magnitude of the backward wave (Torjesen et al., 2014), forward wave peak width (Torjesen et al., 2014), and the slope of the backward wave upstroke (Torjesen et al., 2014), all contribute to variations in AIx. Although a shorter stature, and hence an earlier return of reflected waves has generally been the explanation for an increased AIx in women, more recently an increased forward wave peak width, slope of the backward pressure wave, and forward wave amplitude, but not backward wave amplitude have been suggested to be the main determinants of an increased AIx in women of the Framingham Heart study (Torjesen et al., 2014). Whether the same factors explain sex differences in AIx or Pa in alternative population groups is nevertheless uncertain.

Whilst increases in the magnitude of the forward wave exceed that of the backward wave in explaining age-related increases in aortic PP in the Framingham study (Mitchell et al., 2010b), the magnitude of backward waves exceeds that of the forward wave in contributing to age-

related variations in aortic PP in a community of African ancestry (Booysen et al., 2015). Furthermore, in groups of African ancestry backward waves contribute more to variations in measures of cardiovascular end-organ damage than forward waves (Booysen et al., 2015; Sibiya et al., 2015). However, whether increases in backward wave magnitude account for sex differences in AIx or Pa in groups of African descent has not been determined. As the factors that determine sex-related differences in AIx may assist in identifying targets for therapeutic interventions, it is important to assess whether backward wave magnitude explains sex differences in AIx in groups of African descent. Hence, in the present study, we assessed the relative contribution of aortic forward and backward wave magnitude and timing to the relationship between sex and AIx or Pa in a relatively large community-based sample of African ancestry.

3.2 Methods

3.2.1 Study group

The present study was conducted according to the principles outlined in the Helsinki declaration. The Human Research Ethics Committee of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The present study design has previously been described (Woodiwiss et al., 2009; Redelinghuys et al., 2010; Norton et al., 2012). Briefly, 1,197 participants from randomly recruited (from the population census Figure s of 2001) families of Black African descent (Nguni and Sotho chiefdoms) from the South West Township (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years with central aortic hemodynamic measurements were evaluated.

3.2.2 Clinical, demographic, and anthropometric measurements

A questionnaire was administered to obtain demographic and clinical data (Norton et al., 2012, 2008; Redelinghuys et al., 2010; Woodiwiss et al., 2009). Height and weight were measured using standard approaches and participants were considered to be overweight if their body mass index (BMI) was ≥25 kg/ m^2 and obese if their BMI was ≥30 kg/m^2. High-quality brachial blood pressure (BP) measurements were obtained as previously described (Woodiwiss et al., 2009) in the seated position after 5 minutes of rest by a trained nurse-technician using a standard mercury sphygmomanometer 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 BP ≥140/90 mm Hg or the use of antihypertensive medication. Laboratory blood tests of renal function, liver function, blood glucose, hematological parameters, and percentage glycated hemoglobin (HbA_{1c}) were performed. Diabetes mellitus (DM) or an abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA_{1c} value greater than 6.1%.

3.2.3 Pulse wave analysis

Central aortic haemodynamics were estimated using pulse wave and wave separation analysis as previously described (Booysen et al., 2015; Norton et al., 2012; Redelinghuys et al., 2010; Sibiya et al., 2015). 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, Houston, TX) inter-faced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty, West Ryde, New South Wales, Australia). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The

peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software. Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded. Central aortic PP (PPc) was determined as the difference between aortic systolic BP and diastolic BP. Mean arterial pressure was determined using SphygmoCor software. Aortic Pa was determined as the difference between the pressures at the second and the first systolic peaks of the aortic pulse wave. To avoid obtaining negative aortic AIx values in young participants, AIx was determined as the pressure at the second systolic peak of the aortic pulse wave/the pressure at the first systolic peak of the aortic pulse wave expressed as a per-centage. Aortic backward (Pb) and forward (Pf) wave pressures were determined from wave separation analysis of the SphygmoCor-derived aortic waveform using a "triangular flow wave" (also from SphygmoCor software) (Westerhof et al., 2006). The reflection magnitude (RM) (sometimes referred to as the reflection coefficient or reflection factor) was calculated as Pb/Pf. Backward wave foot time, and time to the peak of the forward wave, were also determined from SphygmoCor software.

3.2.4 Data analysis

For database management and statistical analysis, SAS software, version 9.3 (SAS Institute, Cary, NC) was employed. To compare aortic hemodynamics between women and men at similar ages and accounting for confounders within each age range, aortic hemodynamic variables were compared at 10-year increments in age from 20 years of age upward with adjustments for variations in age, age^2, mean arterial pressure, pulse rate, BMI, regular smoking, regular alcohol intake, and the presence of DM or an HbA_{1c} >6.1% within each 10-year age category. Stepwise multiple linear regression analysis was performed to determine the independent relations between aortic hemodynamic parameters. Also included in the models

were age, age^2, sex, mean arterial pressure, pulse rate, BMI, regular smoking, regular alcohol intake, the presence of DM or an HbA_{1c} >6.1%, and treatment for hypertension. To determine probability values, further adjustments for nonindependence of family members was performed using nonlinear regression analysis (mixed procedure as defined in the SAS package). To ens that the results were not influenced by the effects of antihypertensive therapy, which can markedly modify aortic hemodynamics, sensitivity analysis was conducted in participants not receiving antihypertensive therapy. Moreover, to ensure that the use of the "triangular flow wave" to separate the forward and backward waves did not affect the interpretation of the data, sensitivity analysis was also conducted in only those participants in whom the actual aortic flow wave was employed to separate the forward and backward waves.

3.3 Results

3.3.1 Characteristics of the participants

The clinical and demographic characteristics of the female and male participants are shown in Table 3.1. Two percent of participants had a history of cardiovascular disease. Importantly, a high proportion of participants had hypertension, and 37.7% of hypertensive women and 63.6% of hypertensive men were not receiving antihypertensive therapy. Moreover, 60.5% of women and 63.4% of men receiving therapy had an uncontrolled BP. Women were markedly more obese.

3.3.2 Sex differences in aortic haemodynamic variables

With adjustments for age, age^2, mean arterial pressure, BMI, pulse rate, regular smoking, regular alcohol, DM or an HbA_{1c} >6.1%, and treatment for hypertension, females were noted to

Table 3.1. Characteristics of women and men in the study sample.

	Women	Men	p value
Sample size	781	416	
Age (years)	44.7±17.7	43.4±19.5	=0.25
Body mass index (kg/m^2)	32.0±8.2	24.9±5.2	<0.0001
% Obese	58.0	16.4	<0.0001
Regular tobacco (% subjects)	6.2	32.6	<0.0001
Regular alcohol (% subjects)	13.4	34.1	<0.0001
% with DM or HbA_{1c}>6.1%	28.4	20.5	<0.005
% Hypertensive	45.2	45.9	=0.81
% Treated for hypertension	28.2	17.3	<0.0001
Pulse rate (beats/min)	68±11	62±11	<0.0001
Brachial SBP/DBP (mm Hg)	127±23/83±13	131±23/85±12	<0.005/<0.005
Mean arterial pressure (mm Hg)	99±16	101±15	<0.05
Brachial pulse pressure (PP)(mm Hg)	44±15	46±18	=0.10

Data are expressed as mean ± SD or proportions. DM, diabetes mellitus; HbA_{1c}, glycosylated haemoglobin; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

have an increased PPc, Pa, AIx, Pb, RM, and time to the peak of the forward wave and decreased backward wave foot time as compared to men (Tables 3.2, 3.3 and 3.4). However, women and men had a similar Pf (Tables 3.2, 3.3 and 3.4).

3.3.3 Sex differences in aortic haemodynamics across the adult age range

With adjustments within each age category for age, age^2, mean arterial pressure, BMI, pulse rate, regular smoking, regular alcohol, DM or and HbA_{1c} >6.1%, and treatment for hypertension, females were noted to have an increased Pa and AIx as compared to males across the adult lifespan (Figures 3.1 and 3.2). Whilst in men, Pa and AIx increased across the adult life span, in women, increases in Pa and AIx were noted until 40–50 years of age and thereafter they tended to remain similar or decrease (Figures 3.1 and 3.2). Whilst with adjustments, no marked sex differences in Pf were noted across the adult age range (Figures 3.1 and 3.2), multivariate-adjusted RM (Figures 3.1 and 3.2), Pb (Figure 3.3), and time to the peak of the forward wave (Figures 3.1 and 3.2) were greater and backward wave foot time was lower in women as compared to men, with the biggest differences noted for RM (Figures 3.1 and 3.2) and Pb (Figure 3.3).

3.3.4 Correlates of augmentation index and augmented pressure

In stepwise regression models, Pa (Tables 3.5 and 3.6) and AIx (Tables 3.7, 3.8 and 3.9) were independently associated with female gender. The inclusion of RM markedly reduced the slope (β-coefficient) of the relation between female gender and AIx or Pa (Tables 3.4 - 3.7). However, the inclusion of neither Pf nor the time to the peak of the forward wave, produced significant decreases in the slope (β-coefficient) of the relation between female gender and Pa or

Table 3.2. Multivariate-adjusted aortic haemodynamic factors in women and men of a community of African ancestry.

	Women	Men	p value
Sample size	781	416	
Central aortic SBP (mm Hg)	121±7	119±7	=0.005
Central aortic PP (PPc) (mm Hg)	36±11	34±11	=0.001
Aortic forward wave pressure (Pf) (mm Hg)	24±8	25±9	=0.10
Aortic backward (reflected) wave pressure (Pb)(mm Hg)	18±6	16±6	<0.0001
Aortic reflected wave magnitude (RM=Pb/Pf)	76±17	63±18	<0.0001
Aortic augmented pressure (Pa) (mm Hg)	12±5	8±6	<0.0001
Aortic augmentation index (AIx) (%)	148±19	129±20	<0.0001
Time to peak forward wave (Ft) (msecs)	132±36	120±38	<0.0001
Backward wave foot time (Bt) (msecs)	115±12	121±13	<0.0001

Data are expressed as mean ± SD. Adjustments are for age, age^2, mean arterial pressure, pulse rate, body mass index, regular smoking, regular alcohol intake, the presence of diabetes mellitus or an HbA_{1c}>6.1%, and treatment for hypertension. Probability values were further adjusted for non-independence of family members using non-linear regression analysis (mixed procedure).

Table 3.3. Multivariate-adjusted aortic haemodynamic factors in women and men not receiving antihypertensive medication from a community of African ancestry.

	Women	Men	p value
Sample size	561	344	
Central aortic SBP (mm Hg)	117±6	115±7	<0.0005
Central aortic PP (PPc) (mm Hg)	34±10	31±11	<0.0001
Aortic forward wave pressure (Pf) (mm Hg)	23±7	23±8	=0.24
Aortic backward (reflected) wave pressure (Pb)(mm Hg)	17±5	15±5	<0.0001
Aortic reflected wave magnitude (RM=Pb/Pf)	74±16	62±17	<0.0001
Aortic augmented pressure (Pa) (mm Hg)	11±5	7±5	<0.0001
Aortic augmentation index (AIx) (%)	146±20	127±20	<0.0001
Time to peak forward wave (Ft) (msecs)	130±35	116±36	<0.0001
Backward wave foot time (Bt) (msecs)	117±12	123±13	<0.0001

Data are expressed as mean ± SD. Adjustments are for age, age^2, mean arterial pressure, pulse rate, body mass index, regular smoking, regular alcohol intake, and the presence of diabetes mellitus or an HbA_{1c}>6.1%. Probability values were further adjusted for non-independence of family members using non-linear regression analysis (mixed procedure).

Table 3.4. Multivariate-adjusted aortic haemodynamic factors in women and men in whom aortic forward and backward waves were separated using an actual aortic flow wave.

	Women	Men	p value
Sample size	250	142	
Central aortic SBP (mm Hg)	119±6	117±7	=0.005
Central aortic PP (PPc) (mm Hg)	36±10	32±11	<0.01
Aortic forward wave pressure (Pf) (mm Hg)	24±7	24±8	=0.28
Aortic backward (reflected) wave pressure (Pb)(mm Hg)	18±5	15±6	<0.0005
Aortic reflected wave magnitude (RM=Pb/Pf)	75±15	62±16	<0.0001
Aortic augmented pressure (Pa) (mm Hg)	12±5	8±5	<0.0001
Aortic augmentation index (AIx) (%)	149±21	130±23	<0.0001
Time to peak forward wave (Ft) (msecs)	126±32	117±35	<0.05
Backward wave foot time (Bt) (msecs)	116±11	122±11	<0.0001

Data are expressed as mean ± SD. Adjustments are for age, age^2, mean arterial pressure, pulse rate, body mass index, regular smoking, regular alcohol intake, the presence of diabetes mellitus or an HbA_{1c}>6.1%, and treatment for hypertension. Probability values were further adjusted for non-independence of family members using non-linear regression analysis (mixed procedure).

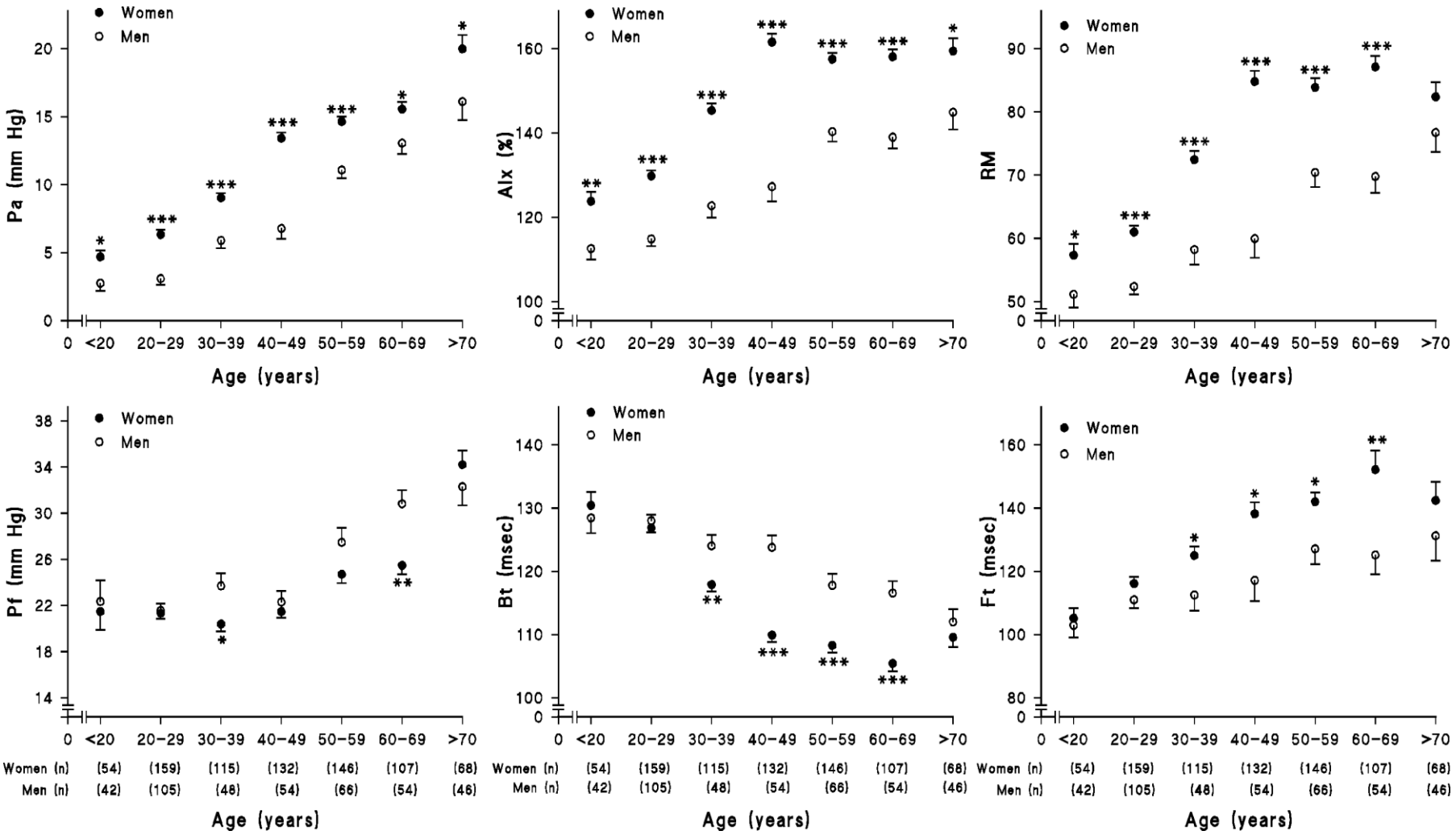

Figure 3.1. Differences in multivariate adjusted aortic haemodynamic variables between women and men of a community sample of African ancestry across the adult age-range. See Table 3.2 for abbreviations. *p<0.05, **p<0.005, ***p<0.0001 vs men in that age range. Adjustments are for age, age^2, mean arterial pressure, pulse rate, BMI, regular smoking, regular alcohol intake and the presence of diabetes mellitus or an HbA_{1c}>6.1% and antihypertensive therapy within each 10-year age category. Probability values were further adjusted for non-independence of family members using non-linear regression analysis (mixed procedure).

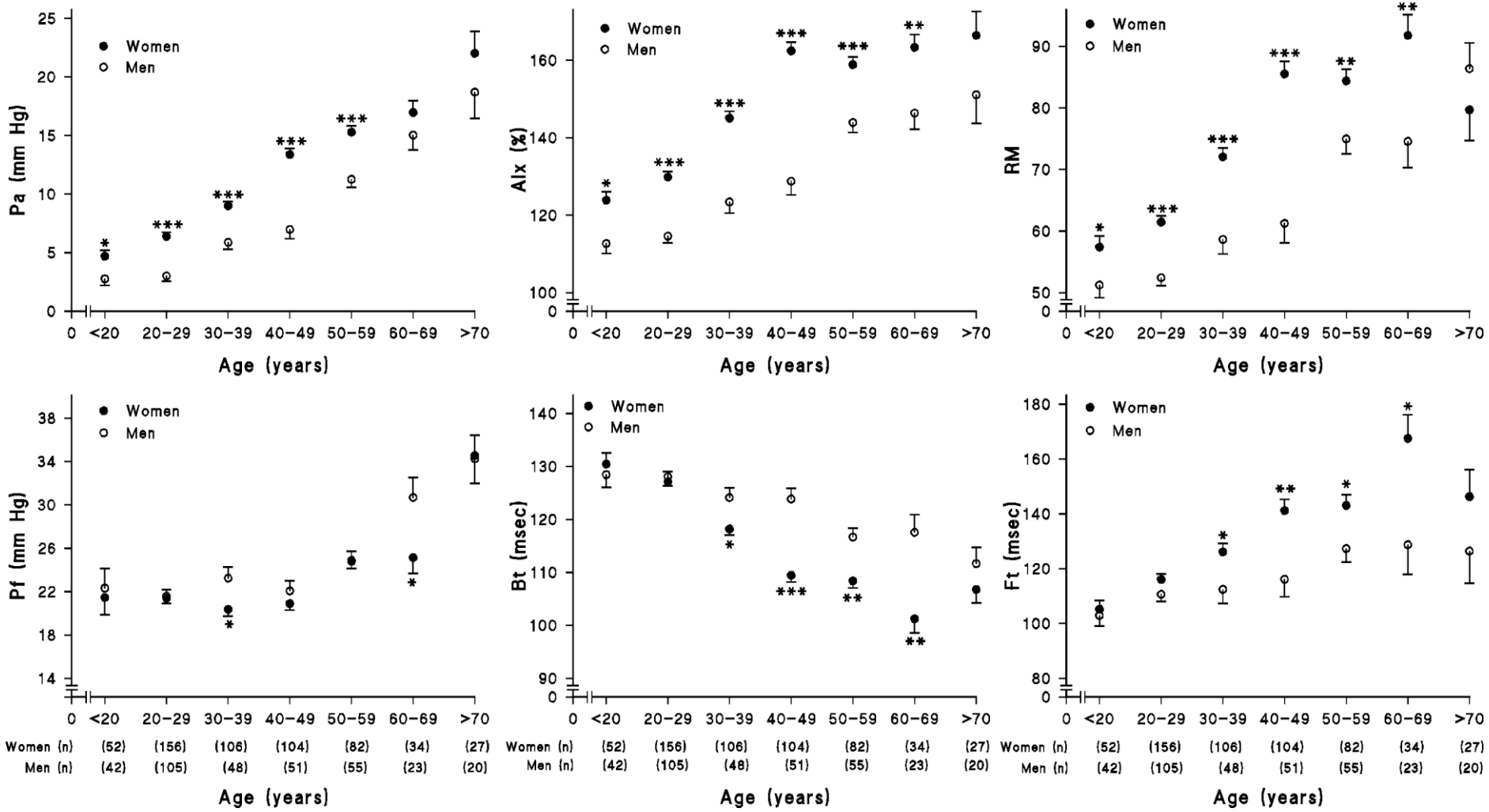

Figure 3.2. Differences in multivariate adjusted aortic haemodynamic variables between women and men not receiving antihypertensive therapy from a community sample of African ancestry across the adult age-range. See Table 2 for abbreviations. *p<0.05, **p<0.005, ***p<0.0001 vs men in that age range. Adjustments are as indicated in figure 3.1, but without adjustments for antihypertensive therapy. Probability values were further adjusted for non-independence of family members using non-linear regression analysis (mixed procedure).

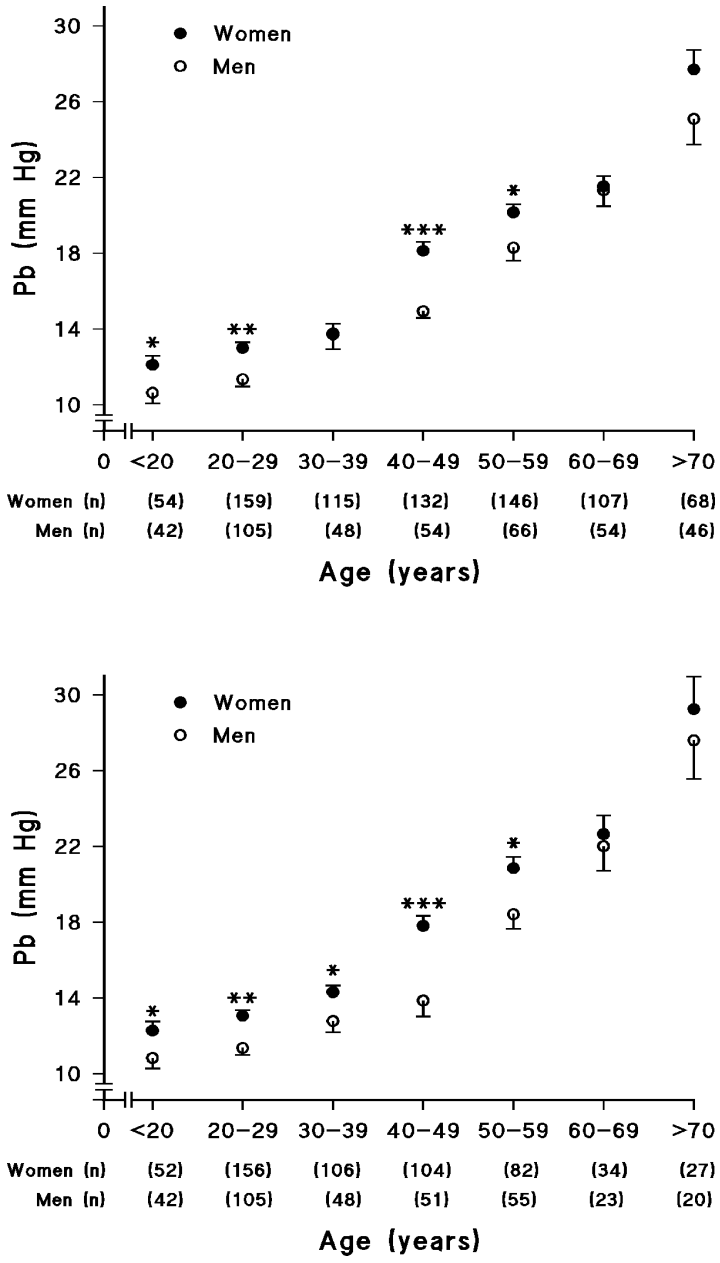

Figure 3.3. Differences in multivariate adjusted aortic backward wave pressures (Pb) between women and men of a community sample of African ancestry across the adult age-range. The upper panel shows data in all participants and the lower panel data obtained in those not receiving antihypertensive therapy. See Table 3.2 for abbreviations. *p<0.05, **p<0.005, ***p<0.0001 vs men in that age range. Adjustments are for age, age^2, mean arterial pressure, pulse rate, BMI, regular smoking, regular alcohol intake and the presence of diabetes mellitus or an HbA_{1c}>6.1% and antihypertensive therapy (in all participants) within each 10-year age category. Probability values were further adjusted for non-independence of family members using non-linear regression analysis (mixed procedure).

Table 3.5. Correlates of aortic augmented pressures in a community of African ancestry (n=1197).

	Model 1		Model 2		Model 3		Model 4		Model 5	
Variable	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value
Age	0.05±0.04	=0.25	-0.17±0.04	<0.0001	0.15±0.04	=0.0005	-0.02±0.04	=0.70	-0.12±0.04	<0.01
Age^2	0.0017±0.0005	<0.0005	0.0031±0.0004	<0.0001	0.0004±0.0004	=0.37	0.0021±0.0004	<0.0001	0.0029±0.0004	<0.0001
Female	**3.81±0.34**	**<0.0001**	**1.78±0.31***	**<0.0001**	**4.12±0.32†**	**<0.0001**	**3.41±0.34†**	**<0.0001**	**2.96±0.33†**	**<0.0001**
BMI	-0.06±0.02	<0.005	-0.01±0.02	=0.81	-0.08±0.02	<0.0001	-0.05±0.02	=0.01	-0.05±0.02	<0.05
Heart rate	-0.22±0.01	<0.0001	-0.09±0.01	<0.0001	-0.24±0.01	<0.0001	-0.20±0.01	<0.0001	-0.18±0.01	<0.0001
MAP	0.23±0.01	<0.0001	0.18±0.01	<0.0001	0.19±0.01	<0.0001	0.23±0.01	<0.0001	0.22±0.01	<0.0001
Smoking	1.42±0.42	<0.001	1.15±0.35	<0.005	1.21±0.38	<0.005	1.32±0.40	=0.001	1.40±0.38	<0.0005
Treatment#	-1.43±0.39	<0.0005	-1.06±0.33	<0.005	-1.48±0.36	<0.0001	-1.34±0.38	<0.0005	-1.39±0.36	=0.0001
RM	-	-	**0.15±0.01**	**<0.0001**	-	-	-	-	-	-
Pf	-	-	-	-	**0.21±0.02**	**<0.0001**	-	-	-	-
Ft	-	-	-	-	-	-	**0.028±0.004**	**<0.0001**	-	-
Bt	-	-	-	-	-	-	-	-	**-0.147±0.011**	**<0.0001**
Model r^2	0.636	<0.0001	0.736	<0.0001	0.685	<0.0001	0.658	<0.0001	0.689	<0.0001

BMI, body mass index; MAP, mean arterial pressure. For additional abbreviations see table 2. Also included in the models were regular alcohol intake and diabetes mellitus or an HbA_{1c}>6.1%. # indicates treatment for hypertension. Probability values were further adjusted for non-independence of

family members using non-linear regression analysis (mixed procedure). *$p<0.0001$ versus model 1 (without RM). †indicates no difference from model 1 ($p>0.07$).

Table 3.6. Correlates of aortic augmented pressures in participants not receiving antihypertensive medication from a community of African ancestry (n=905).

	Model 1		Model 2		Model 3		Model 4		Model 5	
Variable	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value
Age	0.039±0.051	=0.44	-0.20±0.04	<0.0001	0.14±0.05	<0.005	-0.049±0.049	=0.32	-0.127±0.045	=0.005
Age^2	0.0020±0.0006	<0.001	0.0035±0.0004	<0.0001	0.0006±0.0005	=0.28	0.0026±0.0005	<0.0001	0.0030±0.0005	<0.0001
Female	**3.97±0.37**	**<0.0001**	**1.94±0.31***	**<0.0001**	**4.10±0.35†**	**<0.0001**	**3.52±0.36†**	**<0.0001**	**3.05±0.33†**	**<0.0001**
BMI	-0.11±0.03	<0.0001	-0.04±0.02	<0.05	-0.11±0.02	<0.0001	-0.08±0.02	<0.001	-0.08±0.02	<0.0005
Heart rate	-0.19±0.02	<0.0001	-0.07±0.01	<0.0001	-0.21±0.01	<0.0001	-0.18±0.01	<0.0001	-0.16±0.01	<0.0001
MAP	0.24±0.01	<0.0001	0.18±0.01	<0.0001	0.20±0.01	<0.0001	0.24±0.01	<0.0001	0.21±0.01	<0.0001
Smoking	1.30±0.44	<0.0001	1.14±0.35	<0.005	1.02±0.41	<0.05	1.23±0.41	<0.005	1.45±0.38	<0.0005
RM	-	-	**0.17±0.01**	**<0.0001**	-	-	-	-	-	-
Pf	-	-	-	-	**0.196±0.031**	**<0.0001**	-	-	-	-
Ft	-	-	-	-	-	-	**0.032±0.004**	**<0.0001**	-	-
Bt	-	-	-	-	-	-	-	-	**-0.163±0.012**	**<0.0001**
Model r^2	0.638	<0.0001	0.764	<0.0001	0.677	<0.0001	0.668	<0.0001	0.708	<0.0001

BMI, body mass index; MAP, mean arterial pressure. For additional abbreviations see table S1. Also included in the models were regular alcohol intake and diabetes mellitus or an HbA_{1c}>6.1%. Probability values were further adjusted for non-independence of family members using non-linear regression analysis (mixed procedure). *p<0.0001 versus model 1 (without RM). †indicates no difference from model 1 (p>0.07).

Table 3.7. Correlates of aortic augmentation index in a community of African ancestry (n=1197).

	Model 1		Model 2		Model 3		Model 4		Model 5	
Variable	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value
Age	1.76±0.17	<0.0001	0.59±0.11	<0.0001	1.37±0.17	<0.0001	1.41±0.16	<0.0001	0.89±0.15	<0.0001
Age^2	-0.012±0.002	<0.0001	-0.005±0.001	<0.0001	-0.008±0.002	<0.0001	-0.0098±0.0016	<0.0001	-0.0063±0.0015	<0.0001
Female	**18.45±1.30**	**<0.0001**	**7.40±0.90****	**<0.0001**	**17.76±1.26†**	**<0.0001**	**16.28±1.24†**	**<0.0001**	**13.55±1.11***	**<0.0001**
BMI	-0.41±0.08	<0.0001	-0.099±0.056	=0.08	-0.35±0.08	<0.0001	-0.37±0.08	<0.0001	-0.32±0.07	<0.0001
Heart rate	-0.77±0.05	<0.0001	-0.024±0.037	=0.53	-0.76±0.05	<0.0001	-0.64±0.05	<0.0001	-0.55±0.04	<0.0001
MAP	0.48±0.04	<0.0001	0.19±0.03	<0.0001	0.59±0.04	<0.0001	0.45±0.04	<0.0001	0.38±0.03	<0.0001
Smoking	5.36±1.57	<0.001	3.92±1.03	<0.0005	5.77±1.52	<0.0005	4.85±1.47	=0.001	5.02±1.31	<0.0005
Treatment#	-4.58±1.48	<0.005	-2.62±0.97	<0.01	-4.32±1.42	<0.005	-4.09±1.38	<0.005	-4.65±1.23	<0.0005
RM	-	-	**0.86±0.02**	**<0.001**	-	-	-	-	-	-
Pf	-	-	-	-	**-0.55±0.07**	**<0.0001**	-	-	-	-
Ft	-	-	-	-	-	-	**0.17±0.01**	**<0.0001**	-	-
Bt	-	-	-	-	-	-	-	-	**-0.82±0.04**	**<0.0001**
Model r^2	0.494	<0.0001	0.778	<0.0001	0.523	<0.0001	0.550	<0.0001	0.638	<0.0001

BMI, body mass index; MAP, mean arterial pressure. For additional abbreviations see table 2. Also included in the models were regular alcohol intake and diabetes mellitus or an HbA_{1c}>6.1%. # indicates treatment for hypertension. Probability values were further adjusted for non-independence of

family members using non-linear regression analysis (mixed procedure). *$p<0.005$, **$p<0.0001$ versus model 1 (without RM). †indicates no difference from model 1 ($p>0.22$).

Table 3.8. Correlates of aortic augmentation index in participants not receiving antihypertensive medication from a community of African ancestry (n=905).

	Model 1		Model 2		Model 3		Model 4		Model 5	
Variable	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value
Age	1.83±0.20	<0.0001	0.55±0.13	<0.0001	1.33±0.20	<0.0001	1.35±0.19	<0.0001	0.94±0.17	<0.0001
Age^2	-0.013±0.002	<0.0001	-0.004±0.001	=0.001	-0.007±0.002	<0.005	-0.009±0.002	<0.0001	-0.007±0.002	<0.0001
Female	**18.40±1.48**	**<0.0001**	**7.33±0.93****	**<0.0001**	**18.18±1.43**†	**<0.0001**	**15.78±1.38**†	**<0.0001**	**13.42±1.21***	**<0.0001**
BMI	-0.53±0.10	<0.0001	-0.21±0.06	<0.001	-0.49±0.10	<0.0001	-0.42±0.09	<0.0001	-0.45±0.08	<0.0001
Heart rate	-0.71±0.06	<0.0001	0.014±0.040	=0.73	-0.72±0.06	<0.0001	-0.58±0.06	<0.0001	-0.50±0.05	<0.0001
MAP	0.53±0.05	<0.0001	0.20±0.03	<0.0001	0.66±0.05	<0.0001	0.49±0.04	<0.0001	0.39±0.04	<0.0001
Smoking	3.75±1.73	<0.05	2.89±1.04	<0.01	4.50±1.67	<0.01	3.35±1.59	<0.05	4.05±1.38	<0.005
RM	-	-	**0.95±0.02**	**<0.0001**	-	-	-	-	-	-
Pf	-	-	-	-	**-0.63±0.09**	**<0.0001**	-	-	-	-
Ft	-	-	-	-	-	-	**0.21±0.02**	**<0.0001**	-	-
Bt	-	-	-	-	-	-	-	-	**-0.92±0.04**	**<0.0001**
Model r^2	0.499	<0.0001	0.817	<0.0001	0.530	<0.0001	0.571	<0.0001	0.669	<0.0001

BMI, body mass index; MAP, mean arterial pressure. For additional abbreviations see table S1. Also included in the models were regular alcohol intake and diabetes mellitus or an HbA_{1c}>6.1%. Probability values were further adjusted for non-independence of family members using non-linear regression analysis (mixed procedure). *p<0.01, **p<0.0001 versus model 1 (without RM). †indicates no difference from model 1 (p>0.20).

Table 3.9. Correlates of aortic augmentation index in participants in whom the aortic forward and backward waves were separated using actual aortic flow waves (n=392).

	Model 1		Model 2		Model 3		Model 4		Model 5	
Variable	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value	β-coefficient (±SEM)	p-value
Age	1.05±0.18	<0.0001	0.32±0.11	<0.005	1.00±0.18	<0.0001	0.97±0.18	<0.0001	0.65±0.15	<0.0001
Age^2	-0.006±0.002	<0.005	-0.002±0.001	=0.14	-0.004±0.002	<0.05	-0.006±0.002	<0.005	-0.005±0.002	<0.005
Female	**18.05±2.25**	**<0.0001**	**5.41±1.41****	**<0.0005**	**17.53±2.18†**	**<0.0001**	**16.99±2.22†**	**<0.0001**	**11.48±1.89***	**<0.0001**
BMI	-0.22±0.14	=0.14	-0.04±0.08	=0.63	-0.17±0.14	=0.24	-0.20±0.14	=0.16	-0.33±0.12	<0.005
Heart rate	-0.76±0.08	<0.0001	0.20±0.06	<0.001	-0.75±0.08	<0.0001	-0.69±0.08	<0.0001	-0.43±0.07	<0.0001
MAP	0.45±0.06	<0.0001	0.12±0.04	<0.005	0.56±0.06	<0.0001	0.43±0.06	<0.0001	0.36±0.05	<0.0001
Smoking	2.13±2.67	=0.42	1.37±1.58	=0.39	2.76±2.59	=0.29	1.95±2.62	=0.46	1.92±2.17	=0.38
Treatment	-1.24±1.63	=0.45	-2.39±0.96	<0.02	-1.61±1.58	=0.31	-1.04±1.60	=0.52	-0.17±1.32	=0.90
RM	-	-	**1.02±0.04**	**<0.0001**	-	-	-	-	-	-
Pf	-	-	-	-	**-0.63±0.12**	**<0.0001**	-	-	-	-
Ft	-	-	-	-	-	-	**0.12±0.03**	**<0.0001**	-	-
Bt	-	-	-	-	-	-	-	-	**1.06±0.08**	**<0.0001**
Model r^2	0.455	<0.0001	0.810	<0.0001	0.490	<0.0001	0.477	<0.0001	0.640	<0.0001

BMI, body mass index; MAP, mean arterial pressure. For additional abbreviations see table S1. Also included in the models were regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and treatment for hypertension. Probability values were further adjusted for non-independence of family

members using non-linear regression analysis (mixed procedure). *$p<0.01$, **$p<0.0001$ versus model 1 (without RM). †indicates no difference from model 1 ($p>0.20$).

AIx (Tables 3.7 - 3.9). Although the inclusion of time to the foot of backward wave did attenuate relations between female gender and AIx (Tables 3.7 - 3.9), the inclusion of time to the foot of the backward wave did not significantly attenuate relations between female gender and Pa (Tables 3.5 and 3.6).

3.4 Discussion

The main findings of the present study are as follows: In a randomly selected community sample of African ancestry, augmented pressures and AIx were independently related to female gender and these gender-specific effects were attributed largely to the impact of backward wave pressures and magnitude, with little or inconsistent contributions from forward wave pressures, time to the peak of the forward wave, or backward wave foot time. Several recent studies have underscored the potential importance of aortic backward (reflected) waves, as described from wave separation analysis, as a possible independent determinant of cardiovascular end-organ damage (Booysen et al., 2015; Sibiya et al., 2015) and cardiovascular outcomes (Chirinos et al., 2012; Wang et al., 2010; Weber et al., 2012; Zamani et al., 2014). Although the backward wave, as indexed by the reflection factor (backward wave/forward wave pressures), has been demonstrated to play little role in the increases in augmented pressures and AIx in women as compared to men of the Framingham Heart Study (Torjesen et al., 2014), whether the same holds true for alternative population groups is uncertain. In this regard, groups of African ancestry have a particularly high AIx as compared to Caucasian, American-Indian, and Chinese populations (Chirinos et al., 2011). Moreover, in contrast to the Framingham Heart Study, where increases in the magnitude of the forward wave exceeds that of the backward wave in explaining age-related increases in aortic PP (Mitchell et al., 2010b), the magnitude of backward waves exceeds that of the forward wave in contributing to age-related variations in aortic PP in a community of African ancestry (Booysen et al., 2015). Hence, the question arises as to whether

forward wave pressures and alternative factors contribute more than reflected wave magnitude or pressures to sex differences in AIx in groups of African ancestry. In the present study, we show a marked contribution of the RM and backward wave pressures with little contribution of the forward wave to the relationship between augmented pressures and AIx and female gender in a group of African ancestry. The present study therefore highlights the importance of targeting aortic backward rather than forward waves in females of African ancestry.

In the present study, the greater impact of adjustments for RM rather than Pb on the relationship between gender and either Pa or AIx is attributed to the fact that Pb is strongly influenced by Pf and hence that relations between Pb and AIx are in part attributed to the inverse relationship between Pf and AIx. Indeed, the correlation (r^2) between Pb and Pf in the present study sample was equal to 0.56 ($P < 0.0001$). To exclude forward wave effects on Pb, Pb was expressed as a proportion or percentage of Pf (RM). Hence, RM (Pb/Pf) was used as a simple approach to identifying the forward wave-independent effects of backward wave pressures.

Although the reasons for possible differences in the impact of backward vs. forward waves on the relationship between AIx and female gender in the present vs. prior (Torjesen et al., 2014) studies are unclear, several possibilities may be considered. In this regard, groups of African ancestry are well-recognized as being salt-sensitive and we have previously demonstrated that relations between salt-intake and BP in groups of African ancestry are mainly with PP, including aortic PP and AIx, rather than with steady-state pressure (Redelinghuys et al., 2010). Thus, salt-intake may produce a pronounced effect on aortic backward wave pressures in groups of African descent and this may manifest in a sex-specific manner. Second, other than ethnic differences between the Framingham Heart Study (Torjesen et al., 2014) and the present study there are several demographic differences between these studies including a greater prevalence of hypertension and a higher BMI in women as compared to men in the present study. These factors were nevertheless in-part controlled for in multivariate adjustments (by adjusting for MAP and BMI in the models) and are therefore unlikely to explain differences between

studies. In addition, in the present study sample, BMI was inversely associated with backward wave magnitude and pressure and as women were more obese than men, this is more likely to have attenuated RM and Pb in women. Third, there is some evidence that human immunodeficiency virus (HIV) infection is associated with an increased aortic AIx (Kuilder et al., 2017). As the prevalence of HIV infection is estimated to be over 10% of the population in South Africa (GBD 2015 HIV Collaborators, 2016), it is possible that HIV infection may contribute to AIx in the current study sample. In this regard, because we did not have access to pre-and post-test counselling, we could not perform routine HIV testing. However, there are no marked sex differences in HIV prevalence rates in adults (GBD 2015 HIV Collaborators, 2016). Therefore, it is unlikely that HIV infection can explain sex-specific effects on AIx in the present study. Fourth, an important consideration is that in the prior study conducted in an alternative ethnic group (Torjesen et al., 2014), the contribution of factors to the aortic pressure wave was assessed by modifying the forward wave while keeping the reflected wave constant. This analysis (Torjesen et al., 2014) was conducted without first assessing whether the modified forward flow wave, when summed to the backward flow wave, provided a realistic flow. Hence, whether different approaches to assessing hemodynamic factors explain the differences between studies in those factors that contribute to sex-specific differences in AIx, is uncertain. Similar to the Framingham Heart Study which demonstrated that women have a wider forward wave peak than men (Torjesen et al., 2014), in the present study, we show that women have a longer time to the peak of the forward wave than men. This is likely to be explained by a longer systolic ejection time (Gatzka et al., 2001) and the well described increase in left ventricular systolic function reported to occur in women as compared to men. This could in-part mediate deleterious effects on end-organs by increasing the chance of the backward wave merging with the forward wave. However, in contrast to Caucasian community samples (Torjesen et al., 2014), where a wider peak of the forward wave accounts for a significant proportion of the enhanced AIx in women as compared to men, in the present study, adjustments for the time to the peak of the

forward wave produced only a modest (not significant) attenuating effect on relations between Pa or AIx and female gender. As SphygmoCor software does not assess the width of the forward wave peak, we were unable to evaluate the role of the width of the forward wave peak. Hence, we may have underestimated the impact of a longer systolic ejection time and left ventricular systolic function in women on AIx or Pa. As the present study was conducted in the SOWETO community of Johannesburg, South Africa, the question arises as to whether this community represents groups of African ancestry in general. In this regard, people living in this community are largely derived from Nguni and Sotho chiefdoms and based on linguistic and genetic studies these chiefdoms originate from west and east Africa during the expansion of Bantu-speaking peoples (Li et al., 2014). Hence, at least from a genetic perspective, the present data is likely to represent groups of African descent in Africa as a whole and in other areas of the world. However, we cannot exclude the possibility of differences in environmental influences modifying the impact of aortic backward waves in women as opposed to men in the present community sample. In the present study, because of the absence of this measure in SphygmoCor software, we also did not assess the contribution of the maximal slope (time derivative) of the upstroke of the backward wave to sex-specific effects on AIx or Pa. In this regard, an increased slope of the upstroke of the backward wave has also been demonstrated to contribute to sex differences in AIx or Pa (Torjesen et al., 2014). However, it is unclear whether an increased slope of the upstroke of the backward wave contributes to end-organ changes independent of backward wave magnitude. Hence, further studies are required to evaluate whether the upstroke of the backward wave contributes to cardiovascular damage independent of backward wave magnitude and whether these effects are sex-dependent.

There are several limitations to the present study that warrant consideration. First, to determine aortic pressure, the radial pulse wave was converted into an aortic pressure wave using an algorithm (generalized transfer function) which is incorporated into the manufacturer's software. Because of an inability to externally validate this algorithm, this approach may be

limited. Second, the assumptions intrinsic to the use of the "triangulation method" of aortic wave separation may not be ideal (Kips et al., 2009). In this regard, as compared to a triangular flow wave, where the leading edge is assumed to be linear from the inflection point to the dicrotic notch, the aortic flow wave may have a leading edge which is best represented by a shoulder. As a consequence, reflected wave magnitude derived from wave separation analysis employing a "triangular flow wave" may not correlate that well with reflected wave magnitude derived from wave separation analysis employing an actual flow wave (Kips et al., 2009). However, in 392 participants of the present sample with aortic velocity measurements, we were able to show a correlation (r^2) between backward wave pressure derived from the "triangulation method" vs. "actual aortic flow" methods of wave separation of 0.82 and between forward wave pressure derived from the "triangulation method" vs. "actual aortic flow" methods of wave separation of 0.88. Moreover, in sensitivity analysis conducted only in those participants who had wave separation analysis performed using an actual aortic flow wave, we were able to reproduce the essential findings of the present study. Hence, at least in the present study, the use of the "triangulation method" is unlikely to have significantly affected our results. 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 are likely to have been underestimated using the current approach. However, neither AIx nor RM (which are ratios of 2 pressures, both affected to a similar extent by calibration errors) and hence the sex-specific effects of RM on AIx reported on in the present study are likely to have been affected by errors in calibration.

In conclusion, in the present study, we show in a large randomly recruited community-based sample of African descent in South Africa, that the relationship between aortic augmented pressures or AIx and female gender is to a large extent attributed to an increased backward wave magnitude rather than forward wave pressure. This is in contrast to what has been noted in largely Caucasian population samples (Torjesen et al., 2014) and highlights the need to

therapeutically target aortic backward wave magnitude rather than forward wave pressure specifically in women of African descent.

Chapter 4

The Second Systolic Peak of the Peripheral Pulse More Closely Indexes the Adverse Effects of Reflected Waves than Central Aortic Augmentation Pressures.

This chapter will be submitted for consideration of publication as follows;

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4.0 Abstract

Although central aortic augmentation pressure (Pa) is employed as an index of aortic reflected wave function, it underestimates the adverse effects of backward wave pressure (Pb). To what degree the peak of the second systolic shoulder of the peripheral pulse (minus diastolic blood pressure) (pP_2), which may not be confounded by the same factors as Pa, indexes the adverse effects of Pb, is unknown. In 1367 participants from a community sample we compared the relationships between pP_2 versus Pa and Pb, central aortic pulse pressure (PPc) (SphygmoCor software) or left ventricular mass index (LVMI)(echocardiography)(n=986). pP_2 was more strongly correlated with Pb than was Pa ($p<0.0001$ for comparison of relations) and the greatest difference in relations between Pa and Pb versus pP_2 and Pb was at the highest tertile of time to the foot of the backward wave. Neither the time to the inflection point nor the presence of Murgo type C waveforms influenced relations between Pa and Pb. The relations between pP_2 and PPc ($r^2=0.96$) were also stronger than those of Pa versus PPc ($r^2=0.86$, $p<0.0001$ for comparison of relations), but similar to Pb-PPc relations ($r^2=0.96$) and the differences in relations between Pa and PPc as compared to pP_2 or Pb versus PPc, were greatest at the highest tertile of time to the foot of the backward wave. These differences in effects of pP_2 and Pa on Pb and PPc translated into significant brachial systolic BP-independent relations between pP_2 (β-coefficient=0.180±0.060 $p<0.005$) or Pb (β-coefficient=0.179±0.052 $p<0.001$), but not Pa (p β-coefficient=0.089±0.047, $p=0.06$) and LVMI. In conclusion, the height of the second systolic shoulder of the peripheral pulse provides an index of aortic wave reflection that largely avoids the limitations of the use of central aortic augmentation pressures.

Key words: Aortic pressure, backward waves, augmentation index, left ventricular mass.

4.1 Introduction

The adverse haemodynamic effects of blood pressure (BP) are mediated by both a steady-state as well as a pulsatile component of BP (Nichols et al., 2011). A key determinant of central aortic pulse pressure (PPc) is wave reflection, which generates a backward pressure wave (Pb) and augments aortic systolic pressure if returning to the ascending aorta sufficiently early (Nichols et al., 2011). Several studies have demonstrated that Pb, or the indexes thereof, are associated with cardiovascular damage beyond conventional risk factors including brachial BP (Chirinos et al., 2012; Sibiya et al., 2015; Wang et al., 2010; Weber et al., 2012). However, to accurately determine Pb effects, wave separation analysis performed from the generation of both an aortic pressure and flow waveform, is required (Nichols et al., 2011). An alternative approach is to assess the extent to which the aortic pulse is augmented by the reflected wave (augmented pressures [Pa]) (Nichols et al., 2011). However, the role of Pa as an index of reflected waves is uncertain and whether alternative indexes of aortic wave reflection, which are simple to derive, offer an appropriate alternative is unknown.

Although Pa or Pa/PPc (augmentation index [AIx]) may predict cardiovascular outcomes (Chirinos et al., 2005; London et al., 2001; Ueda et al., 2004; Weber et al., 2005; Williams et al., 2006) and associate with end-organ changes (Hashimoto et al., 2007; Sibiya et al., 2014; Vlachopoulos et al., 2010; Weber et al., 2006; Westerbacka et al., 2005) independent of brachial BP, the use of Pa or AIx as an index of wave reflection in risk prediction has been challenged. In this regard, because of the impact of various confounding elements, Pa and AIx have been demonstrated to underestimate the contribution of Pb to PPc and end organ effects (Booysen et al., 2015). Moreover, the ability of Pb or the reflected wave magnitude (derived from wave separation analysis) to predict outcomes is often not closely reproduced by the use of Pa or AIx (Chirinos et al., 2012; Wang et al., 2010). However, the height of the second systolic shoulder of the peripheral pulse (pP_2) (minus diastolic blood pressure), which represents that component of

the peripheral waveform generated by Pb, is an attractive alternative to the use of Pa. Indeed, pP_2 being derived from a peripheral pulse waveform alone, does not require the generation of an aortic pulse waveform and may not be subject to the same confounding effects as Pa. In this regard, there is some evidence that pP_2 may offer a more reliable index of the adverse effects of reflected waves than Pa. Indeed, whilst Pa may not predict on-treatment changes in left ventricular mass index (LVMI) beyond brachial BP, pP_2 may (Hashimoto et al., 2006). However, the extent to which pP_2 reflects the adverse effects of Pb and whether pP_2 is a better index of the adverse effects of Pb than Pa, is unknown. Consequently, in the present study conducted in a large community-based sample we assessed the extent to which pP_2 reflects the adverse effects of Pb on PPc and LVMI and whether pP_2 is a better index of the adverse effects of Pb on PPc and LVMI than Pa.

4.2 Methods

4.2.1 Study groups.

The present study was conducted according to the principles outlined in the Helsinki declaration. The Human Research Ethics Committee 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 (Norton et al., 2012; Woodiwiss et al., 2009). In the present study 1367 participants from randomly recruited (from the population census Figures of 2001) families of black African descent (Nguni and Sotho chiefdoms) from the South West Township (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years of age, were evaluated. In a sub-study, 986 participants had echocardiographic measurements.

4.2.2 Clinical, demographic, and anthropometric measurements.

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

4.2.3 Pulse wave analysis.

Central aortic haemodynamics were estimated using pulse wave and wave separation analysis as previously described (Booysen et al., 2015; Norton et al., 2012; Sibiya et al., 2014; Sibiya et al., 2015). After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm) pulse were recorded by applanation tonometry during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software.

Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded. Central aortic PP (PPc) was determined as the difference between aortic systolic (SBP) and diastolic (DBP) BP. Indexes of aortic reflected waves (Pa, pP_2, and Pb) were determined using SphygmoCor software. Aortic forward (Pf) and backward wave (Pb) pressures were determined from wave separation analysis of the SphygmoCor-derived aortic pressure waveform using a 'triangular flow wave' (also from SphygmoCor software) (Booysen et al., 2015; Sibiya et al., 2015). Those factors that influence the degree of overlap between the backward and forward wave pressures, including the time to the peak of the forward wave (Ft), and the time to the foot of the reflected wave (Rt foot), were also determined from SphygmoCor software.

4.2.4 Left ventricular mass.

Echocardiographic measurements were recorded and analysed off-line by experienced investigators who were unaware of the clinical data of the participants and who had a low degree of inter- and intra-observer variability (Woodiwiss et al., 2009). Left ventricular mass index (LVMI) was determined from transthoracic two-dimensional targeted M-mode echocardiography obtained in the long axis parasternal view. Variables were analysed according to the American Society of Echocardiography convention (Sahn et al., 1978). Left ventricular mass was determined using a standard formula (Devereux et al., 1986) and indexed (LVMI) to body surface area. Left ventricular hypertrophy (LVH) was identified as an LVMI $\geq$ 95 g/m^2 for women and $\geq$ 115 g/m^2 for men.

4.2.5 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 percentages. Multiple linear regression analysis was performed to determine the independent relations between aortic function variables or between aortic function and LVMI. Relationships were compared using z statistics. As aortic function changes considerably at age 50 years, sensitivity analysis was conducted in those < and those ≥ 50 years of age.

4.3 Results

4.3.1 Participant characteristics.

The participant characteristics are given in Table 4.1. Apart from those without echocardiography being modestly younger and fewer being treated for hypertension, no differences were noted in the characteristics of participants with and without echocardiography (Table 4.2). More women than men volunteered for the study. A striking prevalence of participants were obese or hypertensive. A high proportion of hypertensives had uncontrolled BP values despite a significant proportion of these individuals receiving antihypertensive medication. A high proportion of participants had LVH.

4.3.2. Relations between Pa or pP2 and aortic backward wave pressures.

Although Pa and pP_2 were both strongly correlated with Pb, the relationship between Pa and Pb was weaker than that of pP_2 and Pb (Figure 4.1). These differences in relations between Pb versus Pa and pP_2 were noted in both women and in men and in both younger (<50 years) and older (≥50 years) age groups (Figure 4.2). Importantly, although the relations between Pa and Pb

Table 4.1. Participant characteristics.

	All	Echocardiography
n=	1367	986
Age (years)	45.0±18.3	45.9±18.1
Sex (% male)	33.4	33.0
Body mass index (kg/m^2)	29.6±7.9	29.7±7.7
% Overweight/obese	22.7/44.1	23.4/45.2
% Hypertensive	46.3	47.7
% Uncontrolled hypertension	14.0	14.2
% Treated for hypertension	24.6	26.8
% Diabetes mellitus	8.1	8.4
% Regular smokers	15.4	15.0
% Regular alcohol intake	21.1	20.5
SBP/DBP (mm Hg)	128±22/83±13	128±22/83±13
Pulse pressure (PP) (mm Hg)	45±15	45±15
Aortic SBP (mm Hg)	120±22	120±22
Aortic PP (mmHg)	35±14	36±14
Forward wave pressure (Pf) (mm Hg)	24±9	25±9
Backward wave pressure (Pb) (mm Hg)	17±8	17±8
Augmented pressures (Pa) (mm Hg)	11±8	11±7
Augmentation index (AIx)	141±25	141±25
Peripheral P_2 (mm Hg)	37±16	37±16
Time to inflection point (T_1)(msec)	105±13	105±13
Ft (msecs)	126±36	126±35
Reflected wave foot time (msec)	118±14	118±13
Left ventricular mass index (g/m^2)	-	75.5±28.7
Left ventricular hypertrophy (%)	-	15.2

SBP, systolic blood pressure; DBP, diastolic BP; Ft, time to the peak of the forward wave.

Table 4.2. Characteristics of participants without echocardiography.

		p value versus those with echocardiography
n=	381	
Age (years)	42.7±18.7	<0.01
Sex (% male)	34.4	=0.62
Body mass index (kg/m^2)	29.3±8.5	=0.34
% Overweight/obese	20.7/41.2	=0.06
% Hypertensive	42.8	=0.10
% Uncontrolled hypertension	13.4	=0.70
% Treated for hypertension	18.9	<0.005
% Diabetes mellitus	7.4	=0.52
% Regular smokers	16.3	=0.56
% Regular alcohol intake	22.6	=0.40
SBP/DBP (mm Hg)	129±23/84±12	=0.75/0.15
Pulse pressure (PP) (mm Hg)	45±17	=0.66
Aortic SBP (mm Hg)	120±22	=0.73
Aortic PP (mmHg)	35±15	=0.72
Forward wave pressure (Pf) (mm Hg)	24±8	=0.18
Backward wave pressure (Pb) (mm Hg)	17±8	=0.86
Augmented pressures (Pa) (mm Hg)	11±8	=0.93
Augmentation index (AIx)	142±25	=0.85
Peripheral P_2 (mm Hg)	37±18	=0.66
Time to inflection point (T_1)(msec)	105±11	=0.73
Ft (msecs)	128±38	=0.26
Reflected wave foot time (msec)	118±14	=0.81

See table 4.1 for abbreviations.

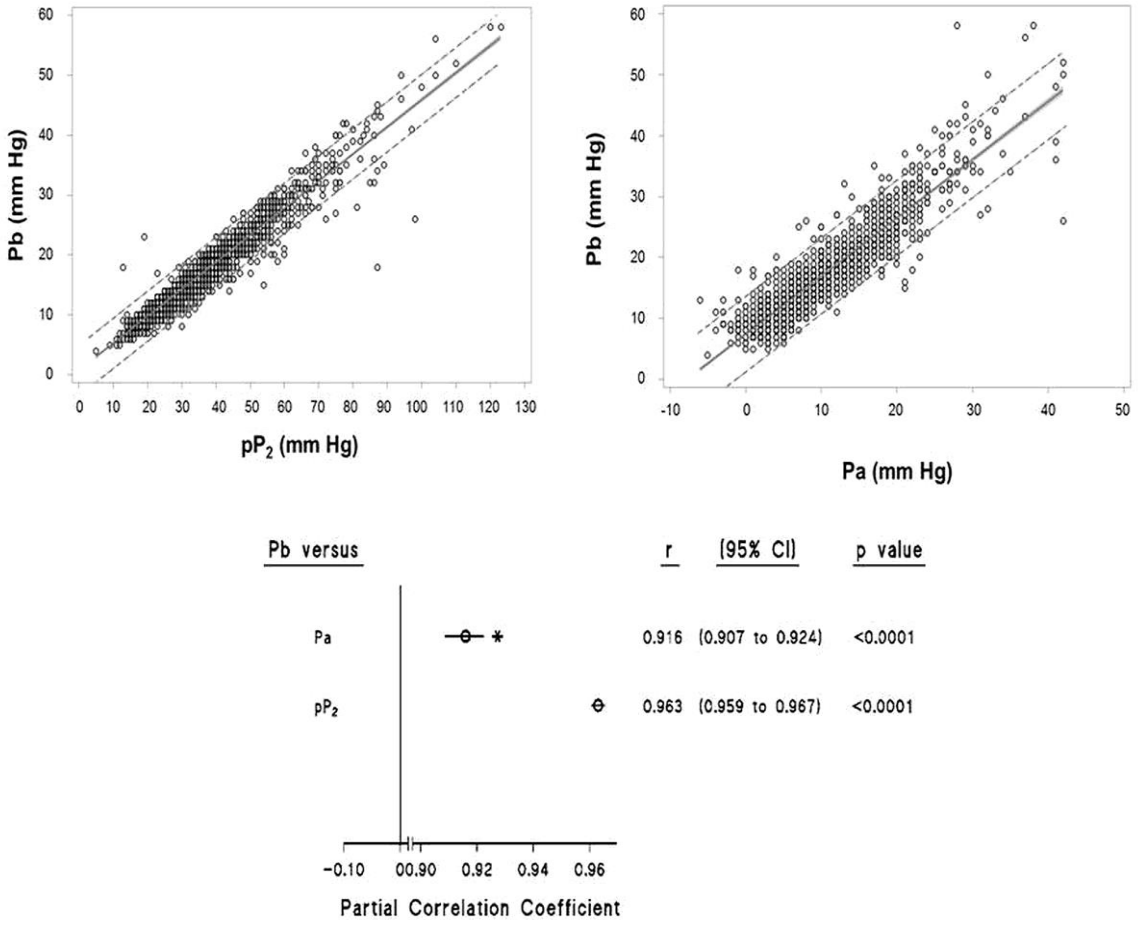

Figure 4.1. Relationships between aortic backward wave pressures (Pb) and either central aortic augmentation pressure (Pa) or the height of the second systolic shoulder of the peripheral pulse (pP_2). *$p<0.0001$ versus relationship between pP_2 and Pb.

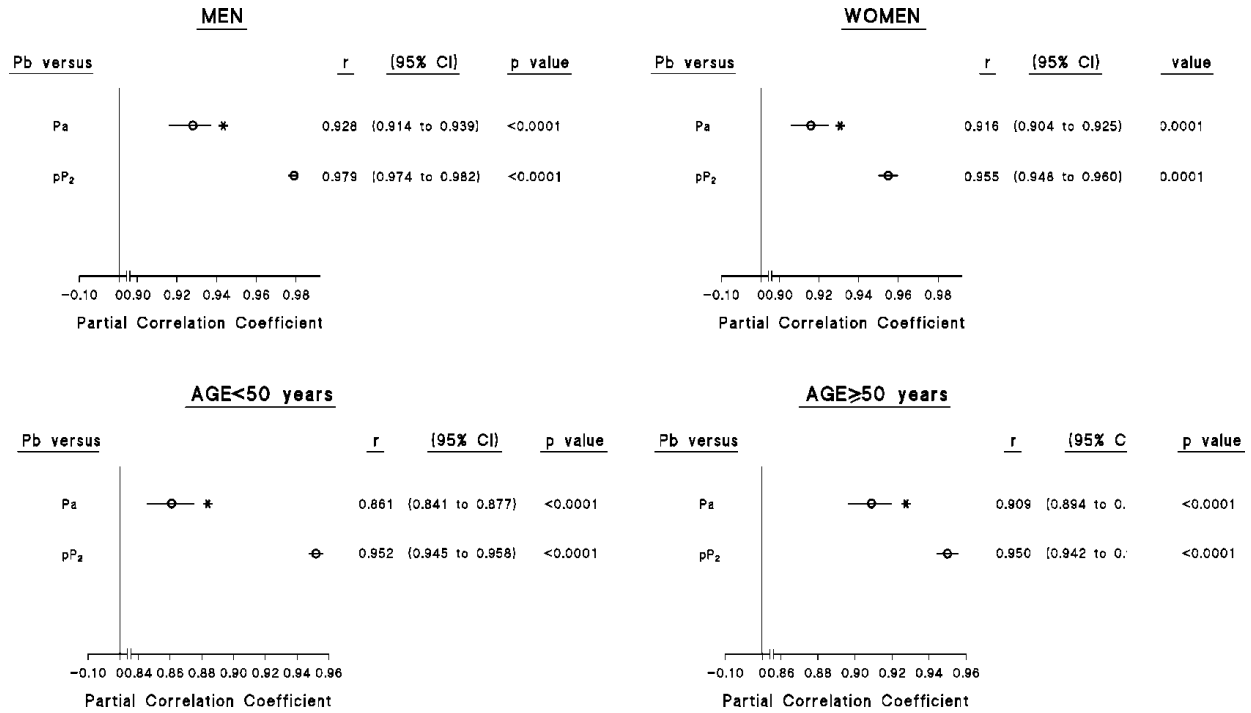

Figure 4.2. Relationships between aortic backward wave pressures (Pb) and either central aortic augmentation pressure (Pa) or the height of the second systolic shoulder of the peripheral pulse (pP_2) in women and in men ore in those < or ≥50 years of age. See table 4.1 for abbreviations. *p<0.0001 versus relations between pP_2 and Pb.

versus pP_2 and Pb differed across tertiles of the time to the foot of the backward wave, the greatest differences in the relationships were at the highest tertile, where the least overlap between forward and backward pressure waves occurs (Table 4.3). These differences in relations were attributed to a graded decrease in the strength of the Pa versus Pb relationship at increasing tertiles of the time to the foot of the backward wave (Table 4.3). In contrast however, consistent differences were noted in the relations between Pa and Pb versus pP_2 and Pb across all tertiles of the time to the peak of the forward wave and the time to the inflection point of the first systolic shoulder (Table 4.3). Only 29 participants had Murgo type C waveforms and censoring of these data failed to modify relations between Pa and Pb (data not shown). In contrast to the variable relations between Pa and Pb across tertiles of time to the foot of the backward wave, similar relations were noted between pP_2 and Pb across tertiles of time to the foot of the backward wave (Table 4.3).

4.3.3. Impact of time to the foot of the backward wave on proportionate differences between Pa and Pb.

Aortic Pa gave lower values than Pb (Table 4.1). However, the proportionate differences ([Pb-Pa]/Pb x 100) between Pa and Pb were greatest at the highest tertiles of time to the foot of the backward wave (Figure 4.3). Thus Pa underestimated Pb effects more markedly at higher than at lower tertiles of time to the foot of the backward wave (Figure 4.3). In keeping with amplification of pressure waves from the aorta to the periphery, pP_2 values were greater than Pb values (Table 4.1). In contrast to the variable proportionate differences between Pa and Pb across tertiles of time to the foot of the backward wave, the proportionate differences between pP_2 and Pb ([Pb-P_2]/Pb x 100) were consistent across tertiles of the time to the foot of the backward wave (Figure 4.3). The systematic nature of the differences between pP_2 and Pb allowed for the derivation of an equation that corrected for these differences and when imputed Pb was derived

Table 4.3. Relationships between aortic backward wave pressures (Pb) and alternative indexes of aortic reflected waves in a community sample.

Pb vs →	Aortic Pa r^2 (95% CI)	Peripheral P_2 r^2 (95% CI)	p values (comparison of r^2)
Tertiles of time to the foot of the backward wave			
Tertile 1 (n=352)	0.905 (0.884 to 0.922)	0.967 (0.959 to 0.973)	<0.0001
Tertile 2 (n=480)	0.919 (0.903 to 0.931)	0.943 (0.932 to 0.952)	<0.005
Tertile 3 (n=535)	0.856* (0.832 to 0.877)	0.952 (0.943 to 0.959)	<0.0001
Tertiles of time to the peak of the forward wave			
Tertile 1 (n=450)	0.910 (0.893 to 0.925)	0.971 (0.966 to 0.976)	<0.0001
Tertile 2 (n=464)	0.918 (0.902 to 0.931)	0.959 (0.951 to 0.966)	<0.0001
Tertile 3 (n=453)	0.932 (0.918 to 0.943)	0.965 (0.958 to 0.971)	<0.0001
Tertiles of time to the inflection point of the first systolic shoulder			
Tertile 1 (n=449)	0.916 (0.900 to 0.930)	0.962 (0.954 to 0.968)	<0.0001
Tertile 2 (n=433)	0.930 (0.916 to 0.942)	0.965 (0.957 to 0.971)	<0.0001
Tertile 3 (n=485)	0.910 (0.893 to 0.924)	0.962 (0.955 to 0.968)	<0.0001

*p<0.001 versus relations for tertiles 1 and 2. All r^2 values are significant at p<0.0001.

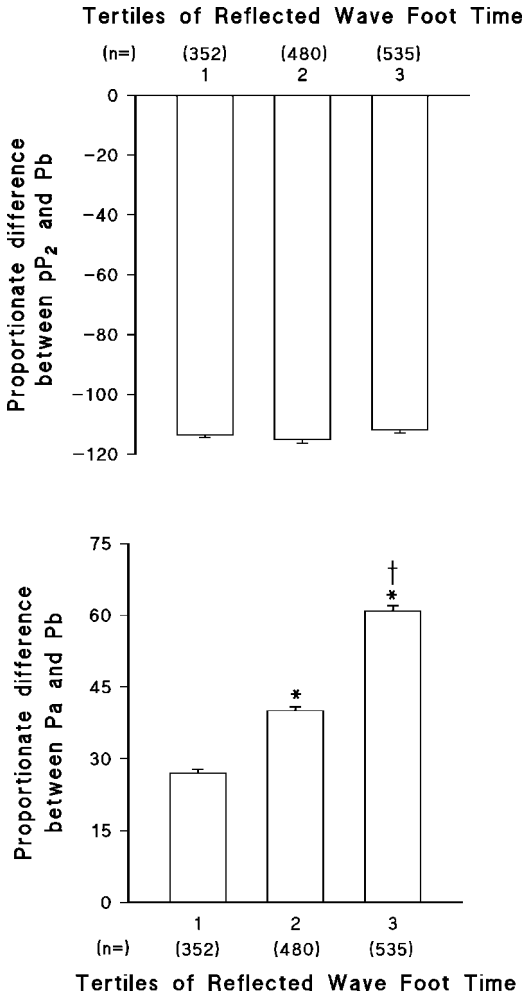

Figure 4.3. Determinants of the relations between indexes of aortic reflected waves and central aortic pulse pressure (PPc). Data shown are relations across tertiles of factors that influence the overlap between the aortic forward and backward waves and the time to the inflection point of the first systolic shoulder. *$p<0.05$, **$p<0.0005$ versus relations in tertile 1, $^{\dagger}p<0.0001$ versus relations between pP_2 and PPc. $^{\#}p<0.0001$ versus relations between Pb and PPc.

from pP_2, this resulted in striking similarities between actual and imputed Pb and limited SD effects in Bland Altman analysis (Figure 4.4). In contrast, derivation of an equation that corrected for differences between Pa and Pb resulted in imputed Pb values, which although similar between actual and imputed Pb values, showed much wider SD effects in Bland Altman analysis (Figure 4.4).

4.3.4 Relationships between indices of aortic wave reflection and aortic PP.

Although independent of Pf, all indexes of wave reflection (Pb, Pa and pP_2) were strongly associated with PPc, Pa showed the weakest contribution to variations of PPc (r^2=0.864 95% CI=0.850-0.877), but pP_2 (r^2=0.958, 95% CI=0.953-0.967) showed similar relations with PPc as Pb (r^2=0.959, 95% CI=0.954-0.963, $p<0.0001$ versus r^2 value for Pa-Pb relationship). Consistent with a greater impact of Pb on PPc as the speed of wave reflection increases, the relationship between Pb and PPc showed modest stepwise decreases in strength across an increasing time to the foot of the backward wave (Figure 4.5). Importantly, although the relationship between Pa and PPc versus pP_2 or Pb and PPc differed across all tertiles of the time to the foot of the backward wave, relationships between Pa and Pb were weakest at the highest tertile, where the least overlap between forward and backward waves occur (Figure 4.5). However, consistent differences were noted in the relations between Pa and PPc versus pP_2 or Pb versus PPc across all tertiles of the time to the peak of the forward wave and the time to the inflection point of the first systolic shoulder (Table 4.4). In contrast to the variable differences in relations between Pa and PPc as opposed to Pb and PPc across tertiles of time to the foot of the backward wave, pP_2 and PPc showed similar relations as Pb and PPc across tertiles of time to the foot of the backward wave (Figure 4.5).

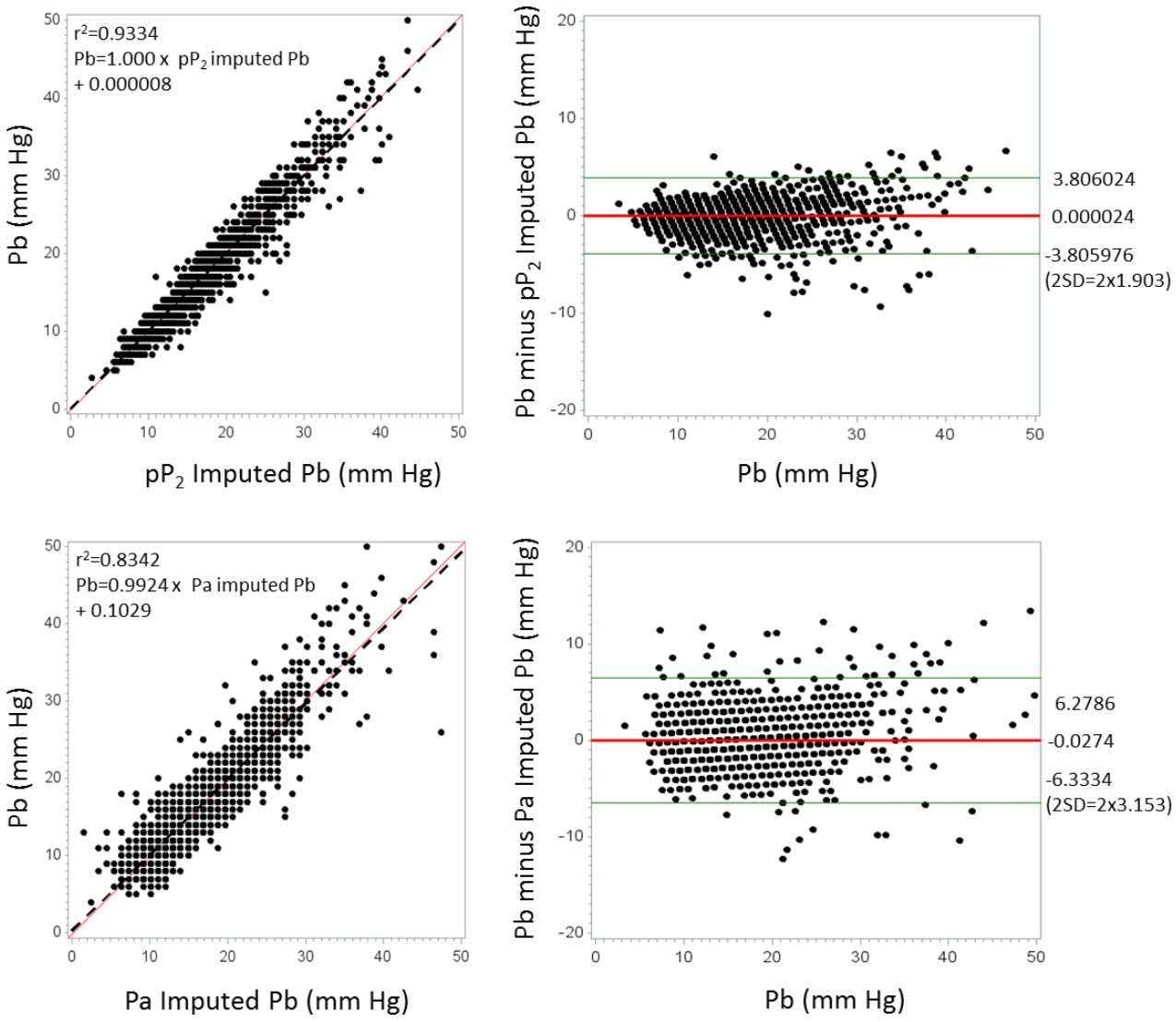

Figure 4.4. Correlations (left panels) and differences (right panels) between Pb and imputed Pb calculated from pP_2 (upper panels) or Pa (lower panels). Imputed Pb was calculated from the slopes and intercepts of the Pb versus pP_2 relationship or the Pb versus Pa relations given in figure 1. Imputed Pb=([0.456 x pP_2] + 0.483) and imputed Pb=([0.954 x Pa] + 7.29).

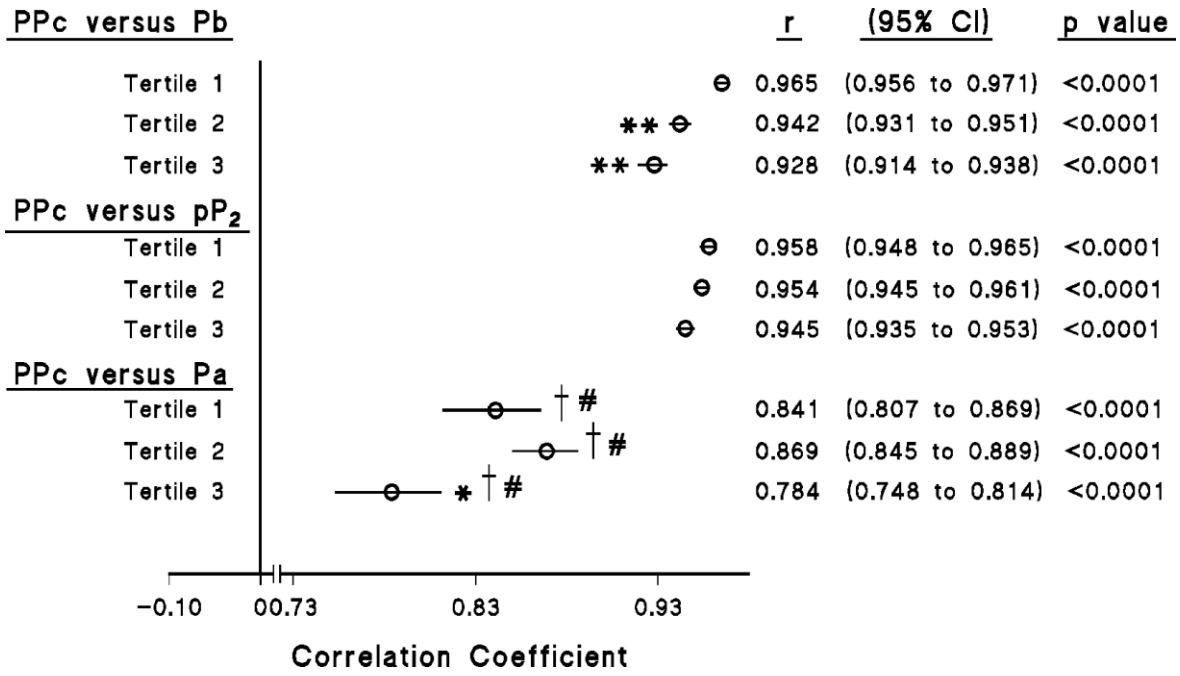

Figure 4.5. Determinants of the relations between indexes of aortic reflected waves and central aortic pulse pressure (PPc). Data shown are relations across tertiles of factors that influence the overlap between the aortic forward and backward waves and the time to the inflection point of the first systolic shoulder. *$p<0.05$, **$p<0.0005$ versus relations in tertile 1, $^{\dagger}p<0.0001$ versus relations between pP_2 and PPc. $^{\#}p<0.0001$ versus relations between Pb and PPc.

Table 4.4. Relationships between indices of aortic reflected waves and central aortic pulse pressure (PPc) in a community sample.

PPc vs	Aortic Pa	Peripheral P_2	Aortic Pb
	r^2 (95% CI)	r^2 (95% CI)	r^2 (95% CI)
Tertiles of time to the peak of the forward wave			
Tertile 1 (n=450)	0.847* (0.818 to 0.871)	0.947 (0.937 to 0.956)	0.957 (0.949 to 0.964)
Tertile 2 (n=464)	0.839* (0.809 to 0.864)	0.943 (0.931 to 0.952)	0.950 (0.940 to 0.958)
Tertile 3 (n=453)	0.884* (0.861 to 0.902)	0.971 (0.965 to 0.976)	0.970 (0.964 to 0.975)
Tertiles of time to the inflection point of the first systolic shoulder			
Tertile 1 (n=449)	0.866* (0.841 to 0.887)	0.955 (0.946 to 0.963)	0.956 (0.948 to 0.964)
Tertile 2 (n=433)	0.889* (0.867 to 0.907)	0.961 (0.953 to 0.968)	0.961 (0.953 to 0.968)
Tertile 3 (n=485)	0.847* (0.820 to 0.871)	0.957 (0.949 to 0.964)	0.960 (0.952 to 0.966)

*$p<0.0001$ versus relations between peripheral P_2 or Pb and PPc. All r^2 values are significant at $p<0.0001$.

4.3.5 Relationships between indexes of aortic wave reflection and LVMI.

Independent of brachial SBP or PP and additional confounders, Pb and pP_2, but not Pa were independently associated with LVMI (Table 4.5). Importantly, both Pb and pP_2 contributed as much (standardized β-coefficient) to variations in LVMI as did brachial SBP or PP (Table 4.5). The independent relationship between Pb or pP_2 and LVMI translated into consistent increases in multivariate adjusted (including adjustments for brachial SBP) LVMI values across quintiles of Pb or pP_2, with little difference in LVMI at each quintile of Pb versus pP_2 (Figure 4.6).

4.4 Discussion

The main findings of the present study conducted in a large, randomly recruited, community-based sample are as follows; First, we show stronger relations between the height of the second systolic peak of the peripheral pulse (pP_2) and backward wave pressures (Pb) as compared to between aortic augmented pressures (Pa) and Pb. These differences in relations were most evident in the highest tertile of the time to the foot of the backward wave, but they were not affected by the time to the peak of the forward wave or the time to the inflection point of the first systolic shoulder. The presence of Murgo type C waveforms could not explain differences in these relations. Second, we show that whilst Pa showed markedly weaker relations with central aortic PP (PPc) than Pb, pP_2 showed a similar strength of the relationship with PPc as did Pb. Again these differences in relations between Pa and PPc were noted in the highest tertiles of the time to the foot of the backward wave, but not in the time to the peak of the forward wave or the time to the inflection point of the first systolic shoulder. Third, we show that independent of confounders including brachial SBP or PP, that Pb and pP_2, but not Pa were

Table 4.5. Relative contribution (standardized β-coefficient) of indices of aortic reflected waves as compared to alternative risk factors to variations in left ventricular mass indexed to body surface area (LVMI) in a community sample.

Model with→	Aortic Pa		Peripheral P_2		Aortic Pb	
LVMI vs	β-coeff.±SEM*	p value	β-coeff.±SEM*	p value	β-coeff.±SEM*	p value
			Brachial SBP in model			
Age	0.116±0.039	<0.005	0.097±0.039	<0.02	0.100±0.038	<0.01
Sex (male)	0.190±0.033	<0.0001	0.193±0.033	<0.0001	0.186±0.033	<0.0001
Brachial SBP	0.173±0.044	<0.0001	0.097±0.055	=0.08	0.102±0.050	<0.05
Reflected wave	0.089±0.047	=0.06	0.180±0.060	<0.005	0.179±0.052	<0.001
			Brachial PP in model			
Age	0.112±0.039	<0.005	0.103±0.039	<0.01	0.107±0.038	<0.01
Sex (male)	0.193±0.033	<0.0001	0.193±0.033	<0.0001	0.188±0.033	<0.0001
Brachial PP	0.187±0.042	<0.0001	0.118±0.060	=0.05	0.111±0.058	=0.06
Reflected wave	0.088±0.046	=0.06	0.155±0.067	<0.05	0.161±0.061	<0.01

*Also included in the models were regular smoking, regular alcohol intake and diabetes mellitus. See table 1 for abbreviations.

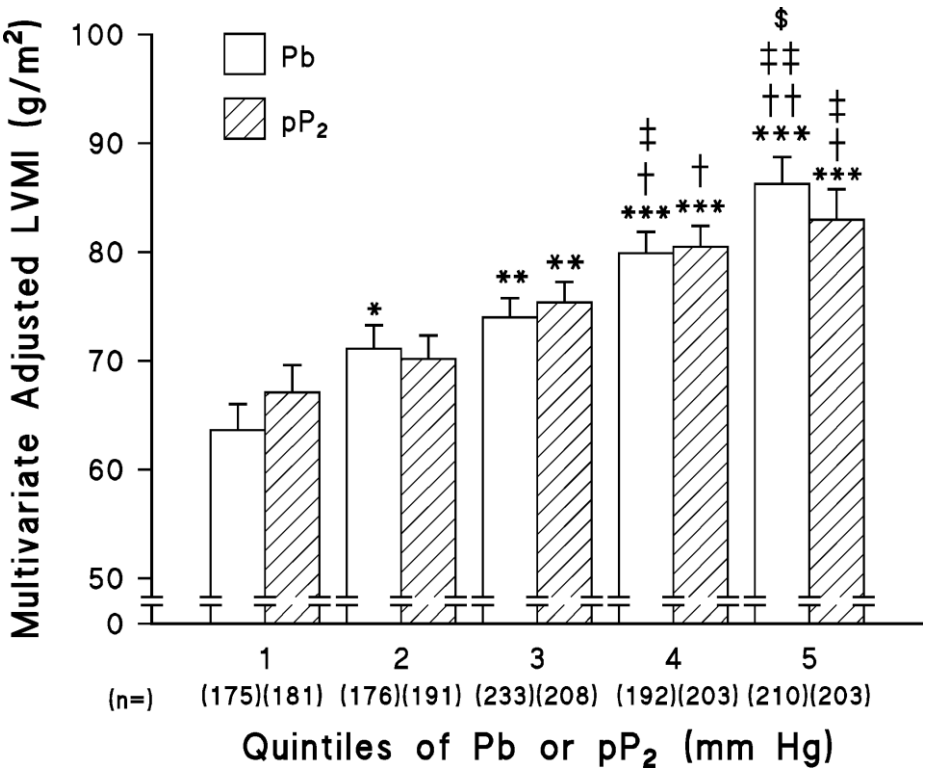

Figure 4.6. Multivariate adjusted left ventricular mass index (LVMI) values across quintiles of aortic backward wave pressures (Pb) or the second systolic shoulder of the peripheral pulse (pP_2). See table 1 for abbreviations. Adjustments are for age, sex, brachial systolic blood pressure, regular smoking, regular alcohol intake and diabetes mellitus. *p<0.05, **p<0.005, ***p<0.0005 versus quintile 1. †p<0.005, ††p<0.0001 versus quintile 2, ‡p<0.05, ‡‡p<0.0005 versus quintile 3, $^{\$}$p<0.05 versus quintile 4.

independently associated with LVMI and that multivariate adjusted LVMI showed consistent and similar increases across quintiles of Pb and pP_2.

The use of augmented pressures as a measure of wave reflection is complicated by several factors. In this regard, one possible confounding effect is an increased speed of wave reflection producing an earlier return of the reflected wave, thus potentiating Pa and AIx in the absence of any changes in Pb (Nichols et al., 2011). However, in the present study we show that an earlier time to the foot of the backward wave, which suggests a greater speed of wave reflection, was associated with stronger relations between Pb and PPc. Consequently, a greater speed of wave reflection should not be viewed as a factor that diminishes relations between Pb and PPc, but rather enhances them. In contrast, the relationship between Pa and either Pb or PPc in the present study was more markedly attenuated by an increased time to the foot of the backward wave than that between pP_2 or Pb and PPc. Therefore, any factor that decreases the speed of wave reflection is likely to reduce the ability of Pa to index the adverse effects of Pb. These changes are likely to occur for several reasons, First, with less of an overlap of Pf and Pb, Pa tends to underestimate the impact of the actual Pb required to produce effects on PPc. Second, the impact of Pb on PPc is determined by both pressure augmentation and a re-reflected wave effect which in-part accounts for forward wave pressure effects on PPc (Phan et al., 2016). The presence of a re-reflected waves thus markedly overestimates the contribution of Pf to variations in PPc and possibly accounts for double the relative impact of Pb as compared to Pf on PPc (Booysen et al., 2015). Importantly, pP2 is a late pressure wave derived from wave reflection from the lower part of the body, thus providing a measure of backward travelling waves that is not confounded by overlap with forward travelling pressure waves. Thus, pP2 provides an index of the absolute effects of backward waves. Hence the proportionate difference between pP_2 and Pb is consistent across tertiles of time to the foot of the backward wave and thus, although providing absolute values that are amplified in comparison to Pb, do not suffer the same limitations as an index of wave reflection as Pa when assessing relations with PPc and end-organ

measures. Importantly, although absolute pP_2 values are amplified in comparison to Pb values, these effects are systematic and hence allow for the imputation of Pb values that closely approximate actual Pb values.

There are several alternative explanations for the limited ability of Pa (or AIx) to index aortic backward wave effects. In this regard, the determination of Pa is dependent on the time to the inflection point of the first systolic shoulder, a change that may be influenced by left ventricular systolic function (Cheng et al., 2012). Under these circumstances, if the time of the reflected wave occurs early in systole, there may be difficulty in identifying this shoulder, thus generating inaccuracies in Pa values. However, this possibility is unlikely in the present study, as the strength of relationships between Pa and either Pb, or PPc were similar across tertiles of the time to the inflection point. Left ventricular function may also modify the shape and height of the pulse pressure waveform, thus confounding the ability to identify reflected wave effects (Fok et al., 2014a; Karamanoglu and Feneley, 1999). This is nevertheless also an unlikely possibility in the present study, as this would create the same inaccuracies in the use of the second systolic shoulder at the peripheral pulse as an index of backward wave function. Murgo type C waveforms may also limit the ability to assess Pa (Hughes et al., 2013). In these waveforms the presence of a forward travelling wave causes a late shoulder in the pressure waveform that gives rise to negative values of Pa. However, in the present study only 29 participants had Murgo type C waveforms and the weaker relations between Pa and Pb as compared to pP_2 and Pb were retained when censoring data from participants with Murgo type C waveforms. The possibility that augmented pressures are determined largely by aortic reservoir function, whilst reflected waves contribute very little (Davies et al., 2010), also requires consideration. However, if Pa is determined largely by reservoir function, then again it is difficult to explain the closer relations between pP_2 and Pb as compared to Pa and Pb in the present study. In this regard, if reservoir function explains Pa, then it should also explain pP_2 and pP_2 should suffer the same limitations as Pa as an index of wave reflection.

There are several implications to the data in the present study. First, the present study suggests that Pa and hence augmentation index, is indeed determined by aortic reflected waves, but that the relationship between Pa and Pb is attenuated for several reasons, In this regard, Pa fails to account for the impact of Pb on wave re-reflection and is determined by the degree of overlap between the forward and backward travelling waves, thus underestimating the actual impact of Pb on aortic PP. These circumstances render Pa a poor index of reflected wave effects. In contrast pP2 is not subject to the same limitations. Importantly, the ability of pP_2 to index the adverse effects of Pb also suggests that aortic backward wave effects can be estimated from peripheral rather than central aortic pulse wave analysis. This together with the finding that the second systolic shoulder of the peripheral pulse closely represents the impact of aortic systolic BP (Norton et al., 2012), suggests that devices to determine the impact of several parameters related to aortic BP do not require the generation of an aortic pulse wave. In this regard, there are uncertainties as to the calibration of aortic BP. Rather, these devices simply require a determination of the peripheral pulse wave, which can be reliably calibrated. This does not however replace the greater value of the more precise determination of aortic backward wave pressures using wave separation analysis, which requires the generation of aortic pressure and flow waves.

In conclusion, in the present study conducted in a large community-based sample, we note that Pa markedly underestimates the adverse effects of aortic reflected waves. In contrast, because it is not subject to the same confounding effects as Pa, pP_2 is more closely associated with backward wave pressures and aortic PP than Pa and is equally as well associated with LVMI as aortic backward wave pressures. Hence, in contrast to Pa, which has a limited ability to index the adverse effects of reflected waves beyond brachial BP, pP_2 derived from a peripheral pulse wave alone, may be employed as a reasonable index of the adverse effects of aortic reflected waves independent of brachial BP.

Chapter 5

Mechanisms of Atenolol-Induced Increases in Aortic Augmentation Index in Spontaneously Hypertensive Rats.

Grace Tade, Gavin R Norton, Glenda Norman, Adamu Bamaiyi, Keneilwe Mmope, Daniel Fernandes, Mohlabani Masiu, Angela Woodiwiss.

5.0 Abstract

In the treatment of hypertension, atenolol-based therapy does not reduce events as well as other agents, an effect attributed to an adverse impact on central pressure augmentation. Whether the central pressure effect represents an impact on heart rate (class effect) or is specific to the vascular effects of atenolol on wave reflection, is unknown. We assessed the impact of atenolol on central arterial function (carotid pressure and aortic velocity and flow measurements) in spontaneously hypertensive rats (SHR) over a range of distending pressures (mean arterial pressure [MAP])(phenylephrine administration). As compared to normotensive Wistar controls, SHR showed an increased MAP, forward wave pressures (Pf), backward wave pressures (Pb), augmentation index (AIx) and reflected wave magnitude (RM=Pb/Pf). However, at comparable MAP values (including in the presence of nifedipine treatment), SHR showed similar AIx and RM values as normotensive Wistar rats. Atenolol given to SHR for 3 months produced marked antihypertensive effects (tail cuff blood pressures, $p<0.0001$) in association with a reduced heart rate ($p<0.0001$). Across a range of MAP values however, AIx was increased by atenolol therapy and these changes were associated with both an increased time to peak forward wave pressure and an enhanced RM, but not with changes in aortic flow. Hence, atenolol therapy enhances AIx through an impact on both class-dependent effects (increased time to peak forward wave pressures) as well as through an impact on wave reflection independent of arteriolar function. In conclusion, atenolol-based antihypertensive therapy produces adverse effects on aortic pressures through heart rate-dependent and independent mechanisms.

Key words: β-adrenoreceptor blockers, aortic pulse pressure, aortic forward waves, aortic backward wave pressures, spontaneously hypertensive rats.

5.1 Introduction

Several large studies comparing the effects of alternative antihypertensive agents with that of therapy that includes the β-adrenoreceptor blocker atenolol, have established superiority of alternative agents over atenolol-based therapy (Lindholm et al., 2005; Reboussin et al., 2018; Wiysonge and Opie, 2013). Consequently, although atenolol is an effective antihypertensive agent, contemporary guidelines do not recommend atenolol as first-line therapy. One explanation for the attenuated efficacy of atenolol at reducing mortality is that for a given decrease in brachial blood pressure (BP), atenolol-based therapy is unable to reduce central arterial pulse pressure (PP) as effectively as alternative agents (Boutouyrie et al., 2010; Sluyter et al., 2016; Williams et al., 2006). In this regard, it is well-accepted that the pulsatile component of BP is as important in mediating the adverse effects of BP on the cardiovascular system as steady-state pressures (Benetos et al., 1997; Celis et al., 2001; Darne et al., 1989; Madhavan et al., 1994; Mitchell et al., 1997), and that aortic PP may be more important than brachial PP in producing these effects (Kollias et al., 2016; Roman and Devereux, 2014; Vlachopoulos et al., 2010; Williams et al., 2006). However, the impact of atenolol on central arterial pressure is poorly understood. Whether this is a class effect of all β-adrenoreceptor blockers or an impact specific to atenolol or similar agents with limited direct vasodilator properties, is unknown. As there are compelling arguments for the use of this class of agents for the treatment of hypertension in several cardiovascular conditions including heart failure, coronary artery disease and arrhythmias, a better understanding of the impact of atenolol in hypertension is required.

The limited impact of atenolol on aortic PP is associated with an inability to reduce aortic augmentation index (AIx) as effectively as alternative agents (Chen et al., 1995; Manisty et al., 2009; Manisty and Hughes, 2013; Sluyter et al., 2016; Williams et al., 2006). In this regard, AIx is determined by several factors (Nichols et al., 2011) including backward wave pressure (Pb)

produced by wave reflection; forward wave pressures (Pf) determined by ventricular ejection and aortic impedance; or time to the peak of the forward wave or speed of wave reflection, either of which influence the chances of Pb augmenting systolic pressure. The impact of atenolol on AIx may be explained by a decreased heart rate, and hence either an increased stroke volume (force-frequency relationship) and Pf, or an increased ejection duration and prolonged time to the peak of Pf (Williams and Lacy, 2009), effects that are shared by all β-adrenoreceptor blockers. Alternatively, the lack of vasodilator effects of atenolol may influence the magnitude of Pb through effects that are independent of Pf and arteriolar function (i.e. medium-sized or small arteries). However, the impact of atenolol on reflected wave function, independent of alternative agents and at comparable steady-state pressures (mean arterial pressure-MAP) using wave separation analysis has not been determined. Consequently, using simultaneous carotid pressure and aortic velocity and diameter measurements, we developed an approach to assessing aortic function in rats over a range of steady-state pressures. In the present study we aimed to determine the impact of atenolol-based therapy on aortic function in spontaneously hypertensive rats (SHR).

5.2 Methods

5.2.1 Study groups

The Animal Ethics Screening Committee of the University of the Witwatersrand approved the protocols (approval numbers: 2015/07/29/A and 2016/03/08/A). We treated 7 month old male SHR with either the β-adrenergic-receptor blocker, atenolol (25 mg/kg/day) (n=8), the calcium channel blocker, nifedipine (40 mg/kg/day) (n=9) or the vehicle (n=8) by gavage for 3 months. Data obtained were compared with age-matched normotensive Wistar control rats (n=6). Awake restrained rats had tail cuff blood pressures (BP) determined using

standard techniques (BIOPAC systems, Inc. NIBP250 blood pressure amplifier) on several occasions throughout the study. Rats were habituated to these measurements by placing them in restrainers for 10 minutes at a time on 3 separate occasions and allowing them to undergo the BP measurement procedure before BP values were recorded.

5.2.2 Aortic and systemic haemodynamic assessments.

Anaesthesia was induced with isoflurane (5%) in oxygen and maintained with 2% isoflurane. Carotid pressures (assumed to be only 1 mm Hg higher than aortic pressure and hence employed as a surrogate thereof) were recorded using a 5-10 cm long PP50 heparinised saline-filled polyethylene catheter, coupled to a pressure transducer attached to a Powerlab System (4/30 model ML866, Adinstruments system, Australia). The catheter was inserted into the left common carotid artery, advanced 1.5 to 2.0 cm until the tip of the catheter was close to the origin of the carotid artery from the aorta, and subsequently the catheter was tied in place. The amplitude-frequency response of the catheter-transducer dome combination employed was uniform to 10 Hz. Only recordings from rats where the carotid pressure wave showed a clearly discernible first systolic shoulder were evaluated (Figure 5.1, upper panel) and the point of inflection was identified from the second derivative of the differential of the pressure change over time. Whilst carotid pressures were recorded echocardiographic measurements were obtained by an experienced observer (AJW) with the rats (anterior chest hair shaved) in the left lateral decubitus position using an Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.) equipped with a 10 MHz linear array transducer and electrocardiogram (ECG). Velocity waveforms and aortic diameter

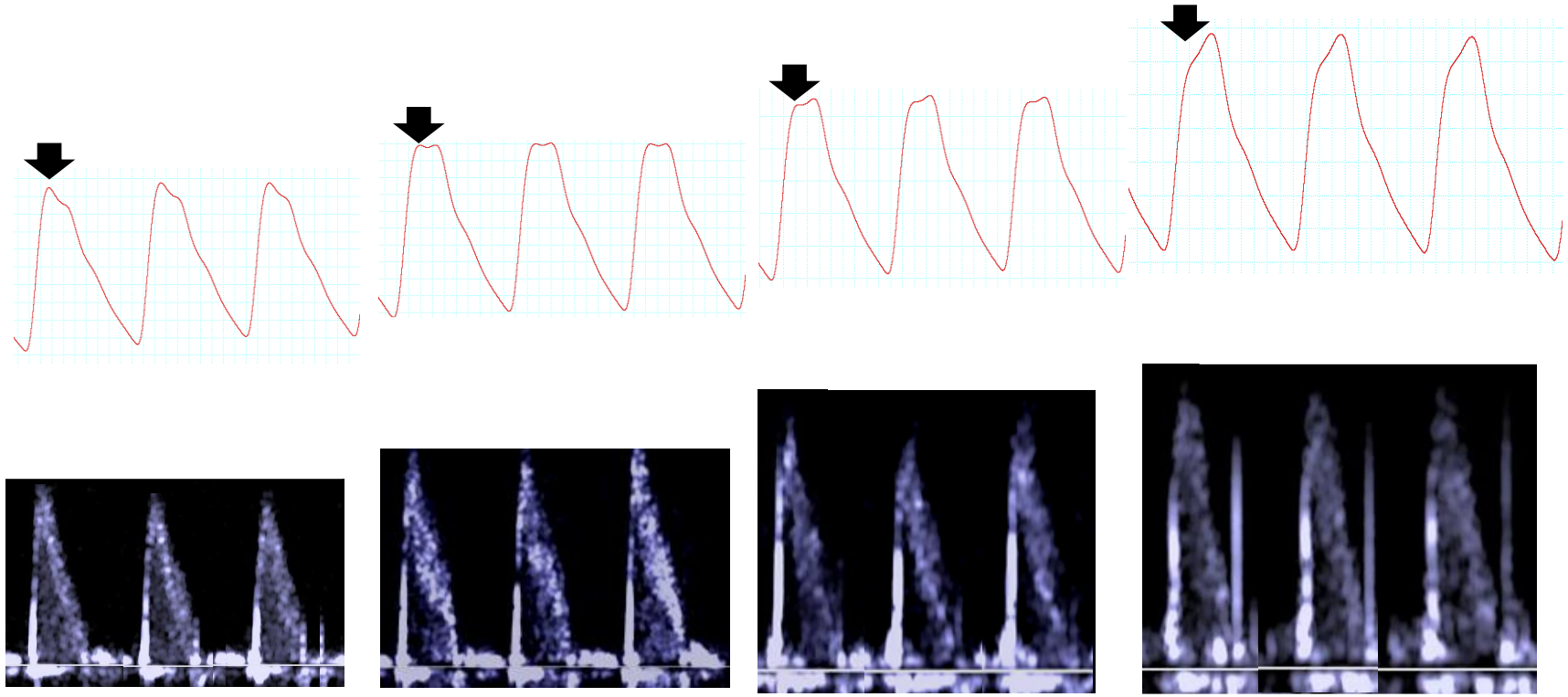

Figure 5.1. Carotid artery pressures generated at incremental distending pressures produced by the administration of the vasoconstrictor phenylephrine (upper panel) and corresponding aortic velocity measurements obtained in the aortic outlet tract (lower panel). Arrows point to the first systolic shoulder.

measurements were obtained in the outflow tract (just beyond the aortic root at the tip of the aortic valves when in the open position) in the long-axis parasternal view (Figure 5.1, lower panel). A frame rate of >110 frames per second was employed for all images.

5.2.3. Aortic pressure wave components.

Aortic flow waveforms were generated from aortic velocity and diameter measurements as described in chapter 1 (Figure 1.3). To perform wave separation analysis using an aortic flow waveform, 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 1.3). The foot of the flow waveform was aligned with the foot of the pressure 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). Using Zc values and the flow and pressure waveforms, wave separation analysis was performed as illustrated in Figure 1.3 and based on the following formulae: Forward wave pressure=(PPc + QxZc)/2 and Backward wave pressure=(PPc – QxZc)/2 (Murgo et al., 1980; Westerhof et al., 2006), where Q is flow. Wave separation analysis was done in the time domain based upon the consensus that this analysis can be done in either the time or the frequency domain (Segers et al., 2017). Reflected wave magnitude (RM), a Pf-independent measure of reflected wave function, was calculated as Pb/Pf. Augmented pressure (Pa) was determined as the difference between aortic pulse pressure (PP) and the pressure at the inflection point of the first systolic shoulder-diastolic BP. Augmentation index (AIx) was determined as Pa/PP an expressed as a percentage.

Systemic haemodynamic values were determined from systemic vascular resistance (SVR), stroke volume (SV), cardiac output (CO), heart rate (HR) and mean arterial pressure

(MAP) assessments, where MAP was determined from the electronic mean of pulsatile pressures and SVR from MAP/CO, assuming that right atrial pressure=0 mm Hg. Cardiac output was determined from the product of SV and HR where SV was determined from aortic velocity and diameter measurements in the aortic outflow tract using a standard formula.

To compare haemodynamic values in SHR at similar hypertensive MAP values, aortic function was assessed at baseline and following repeated doses of phenylephrine (0.5 mg/kg intraperitoneal) (average of 7.5 mg/rat over 7 doses) given to anesthetised normotensive Wistar rats, untreated SHR or SHR receiving atenolol therapy until MAP values achieved at least 180 mm Hg. This approach was employed to simulate the vasoconstrictor effects of hypertension and thus to compare aortic function in treated and untreated SHR at similar steady-state pressures. This approach could not be employed in nifedipine-treated SHR as the presence of vasodilator therapy prevented comparable BP values from being achieved in response to phenylephrine.

5.2.4. Left ventricular systolic function.

Time to peak left ventricular (LV) ejection and LV ejection duration were determined from aortic velocity measurements. Left ventricular systolic function was determined from LV end systolic (LVESD) and end diastolic (LVEDD) internal diameters (leading edge method) obtained from two-dimensional directed (long axis parasternal view) M-Mode images obtained in the short axis of the heart as close to the tip of the mitral valves as possible in all rats. Left ventricular endocardial fractional shortening (FSend) was calculated from LV (EDD-ESD)/EDD expressed as a percentage.

5.2.5 Data analysis.

For comparisons of tail cuff BP over time, and aortic function over a range of incremental MAP values, a two-way repeated measures ANOVA with a Tukey *post hoc* test was performed.

5.3. Results

5.3.1. Baseline BP and heart rate.

Table 5.1 shows BP and heart rate values in awake restrained and in anaesthetised SHR as compared to normotensive Wistar rats and the impact of antihypertensive therapy. SHR had increased tail-cuff systolic BP and an increased invasive systolic, diastolic and mean arterial BP and a lower heart rate as compared to normotensive Wistar controls. Both atenolol and nifedipine therapy reduced tail-cuff or invasive systolic, diastolic and mean arterial pressure, but nifedipine therapy produced a markedly greater benefit. In addition, atenolol therapy further reduced heart rate.

5.3.2. Baseline haemodynamics.

Tables 5.1 and 5.2 show pulsatile and systemic haemodynamics and the determinants thereof in anaesthetised SHR as compared to normotensive rats and the impact of antihypertensive therapy thereon. As compared to age-matched normotensive rats, under anaesthetic conditions, untreated SHR showed a markedly increased MAP and systemic vascular resistance (SVR), and an increased PP. Increases in PP were associated with an enhanced Pf, Pb,

Pa, AIx and reflected wave magnitude (RM=Pb/Pf) as well an increased time to peak Pf, time to peak flow and time to the foot of Pb. Nifedipine therapy produced reductions in SVR, Pa and

Table 5.1. Impact of antihypertensive therapy on blood pressure (BP) and heart rate in spontaneously hypertensive rats (SHR).

Rat group→	NT	SHR		
Treatment	Untreated	Untreated	Atenolol	Nifedipine
n=	6	8	8	9
Tail cuff in awake, restrained rats				
Systolic BP (mm Hg)	145±7	220±7***	187±5***†††	134±9**†††
Heart rate (beats/minute)	420±10	397±20*	312±6***†††	413±17†
Central arterial in anaesthetized rats				
Systolic BP (mm Hg)	126±9.4	176±2***	135±6*†††	127±5†††
Diastolic BP (mm Hg)	87±6.2	124±1***	93±5*†††	82±6†††
Mean arterial P (mm Hg)	102±7	141±1***	107±5†††	97±5†††
Pulse pressure (mm Hg)	39±5	52±2***	42±2†††	45±3**†††
Heart rate (beats/minute)	342±12	273±10***	225±6***†††	282±11***

NT, normotensive; SHR, spontaneously hypertensive rats; BP, blood pressure; P, pressure. *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus NT; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$ versus untreated SHR.

Table 5.2. Impact of antihypertensive therapy on baseline haemodynamics in anaesthetized spontaneously hypertensive rats (SHR).

Rat group→	NT	SHR		
Treatment	Untreated	Untreated	Atenolol	Nifedipine
n=	6	8	8	9
SVR (mm Hg/ml.min)	1.68±0.29	2.79±0.30*	2.66±0.25*	1.61±0.10†
Cardiac output (mls/min)	66.3±7.5	54.5±5.0*	45.9±4.6*	63.1±6.5†
Stroke volume (mls/beat)	0.191±0.016	0.198±0.015	0.204±0.019	0.222±0.17
Augmented pressure (mm Hg)	10.4±3.4	18.6±2.1*	12.1±1.9†	11.3±1.1†
AIx (%)	22.8±3.8	35.5±2.8*	27.5±3.3†	24.5±1.3†
Zc (dyne x cm/cm^5)	7696.4±627.3	8871.9±823.2	8773.5±699.9	7707.8±529.8
Pf (mm Hg)	31.2±2.2	38.2±1.3*	30.7±1.1†	36.2±2.3
Pb (mm Hg)	13.0±2.1	19.8±0.6*	17.2±1.0*	17.9±1.2*
RM (%)	40.7±3.8	55.0±3.0*	55.9±2.9*	49.9±2.6*
Time to Pf (msecs)	34.3±6.1	50.3±7.3*	31.5±1.7†	30.7±1.2†
Time to peak flow (msecs)	25.2±0.7	31.6±1.1*	30.4±0.9*	24.3±1.5†
Ejection duration (msecs)	69.2±1.1	72.9±2.7	80.8±2.0*†	70.7±3.6
Time to foot of Pb (msecs)	24.0±2.7	33.0±2.3*	31.1±1.1	25.6±1.6†
Time to peak Pb (msecs)	64.3±3.6	74.5±2.9	83.5±5.2	74.2±6.2
Duration to peak Pb (msecs)	36.4±5.3	42.3±2.6	52.4±4.8	51.9±5.2
LV FSend (%)	38±3	40±6	36±2	42±2

SHR, spontaneously hypertensive rats; SVR, systemic vascular resistance; AIx, augmentation index; Zc, aortic characteristic impedance; Pf, peak forward wave pressure; Pb, peak backward wave pressure; RM, reflected wave magnitude (Pb/Pf x 100); LV FSend, left ventricular fractional shortening. NT, normotensive. *$p<0.05$versus NT, . $^{\dagger}p<0.05$ versus untreated SHR.

AIx, but not Pf or Pb or RM and decreased time to peak Pf, time to peak flow, and time to foot of Pb. Atenolol therapy decreased Pa, AIx and Pf, but not SVR, Pb or RM. Atenolol therapy also decreased time to peak Pf and prolonged ejection duration. No differences in stroke volume, the aortic velocity time integral or LV endocardial fractional shortening were noted between SHR and normotensive rats and treatment did not affect these parameters.

5.3.3. Central haemodynamics in hypertensives at comparable steady-state pressures.

The central arterial pressure waveform in SHR was similar in shape to that of the pressure waveform obtained in normotensive rats after phenylephrine administration but was of a longer duration (Figure 5.2). However, at comparable steady-state pressures (MAP), as compared to NT rats, SHR had an increased Pf (Figure 5.3), an effect attributed to a lower heart rate and hence a higher stroke volume (force-frequency relationship) (Figure 5.3), but not to changes in Zc (Figure 5.3). Importantly, the lower heart rate in SHR was not associated with an extended ejection duration or longer time to the peak of the forward wave pressure (Pf), data not shown). Moreover, these differences in SHR as compared to NT rats did not translate into alterations in PP (data not shown). No consistent differences in reflected wave function (Pb, AIx or RM) were noted between SHR and NT rats (Figure 5.4).

5.3.4. Impact of atenolol therapy on central haemodynamics at comparable steady-state pressures.

At comparable, but higher steady state pressures in SHR, atenolol therapy produced central arterial pressure waveform which showed a first systolic shoulder at a lower point on the upstroke of the pressure wave (Figure 5.5). Atenolol therapy caused a further reduction in heart

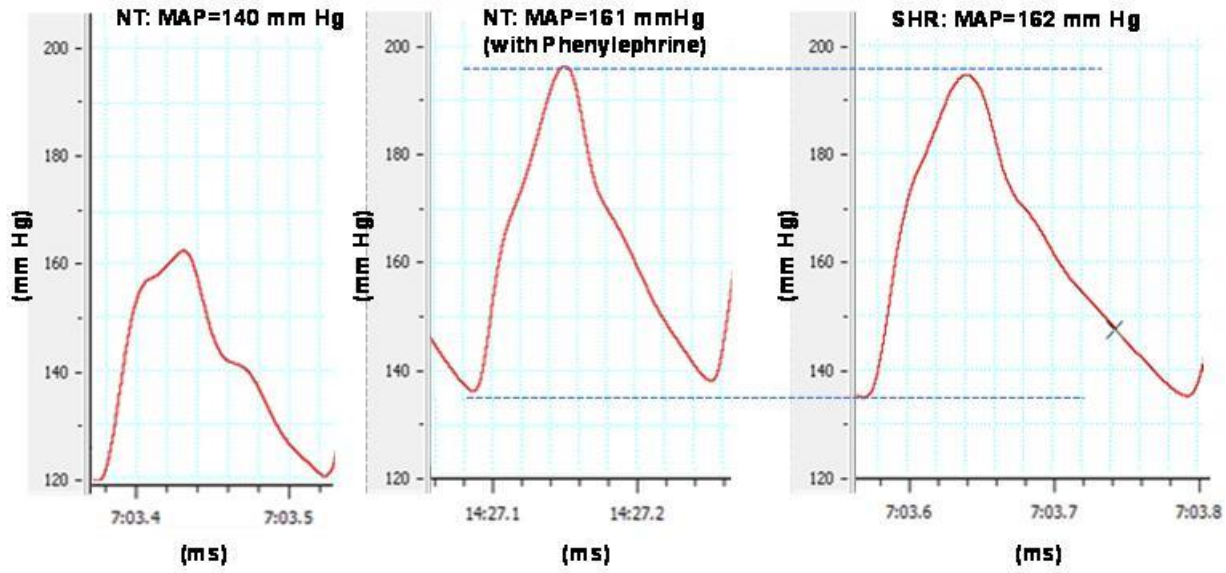

Figure 5.2. Comparison of central arterial pressure waveforms in spontaneously hypertensive rats (SHR) at baseline versus normotensive (NT) Wistar rat at baseline and after the administration of the vasoconstrictor phenylephrine (PE).

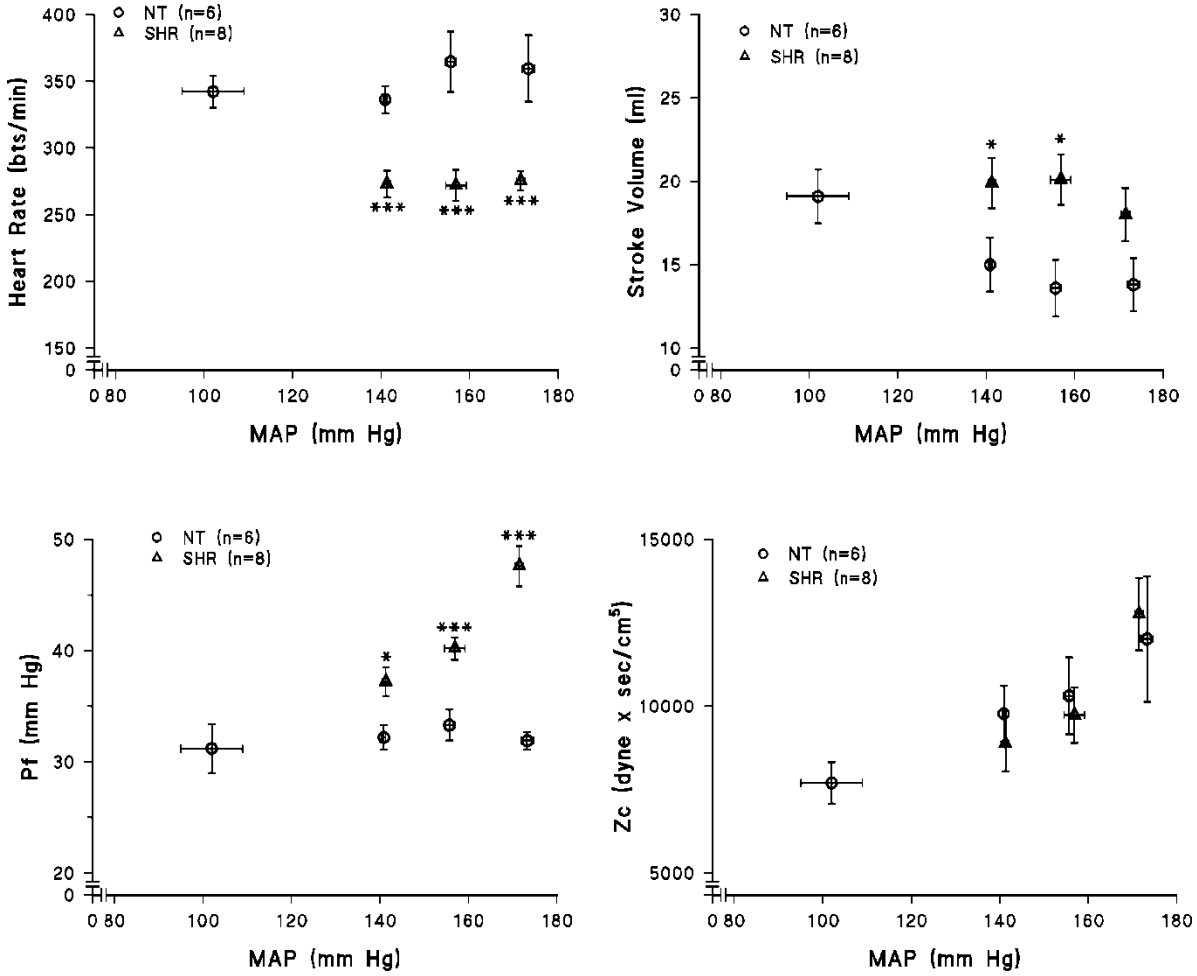

Figure 5.3. Major differences in central aortic haemodynamics at comparable steady-state pressures (mean arterial pressure [MAP] in spontaneously hypertensive rats (SHR) and normotensive (NT) Wistar rats. Pf, peak forward wave pressure; Zc, characteristic impedance.

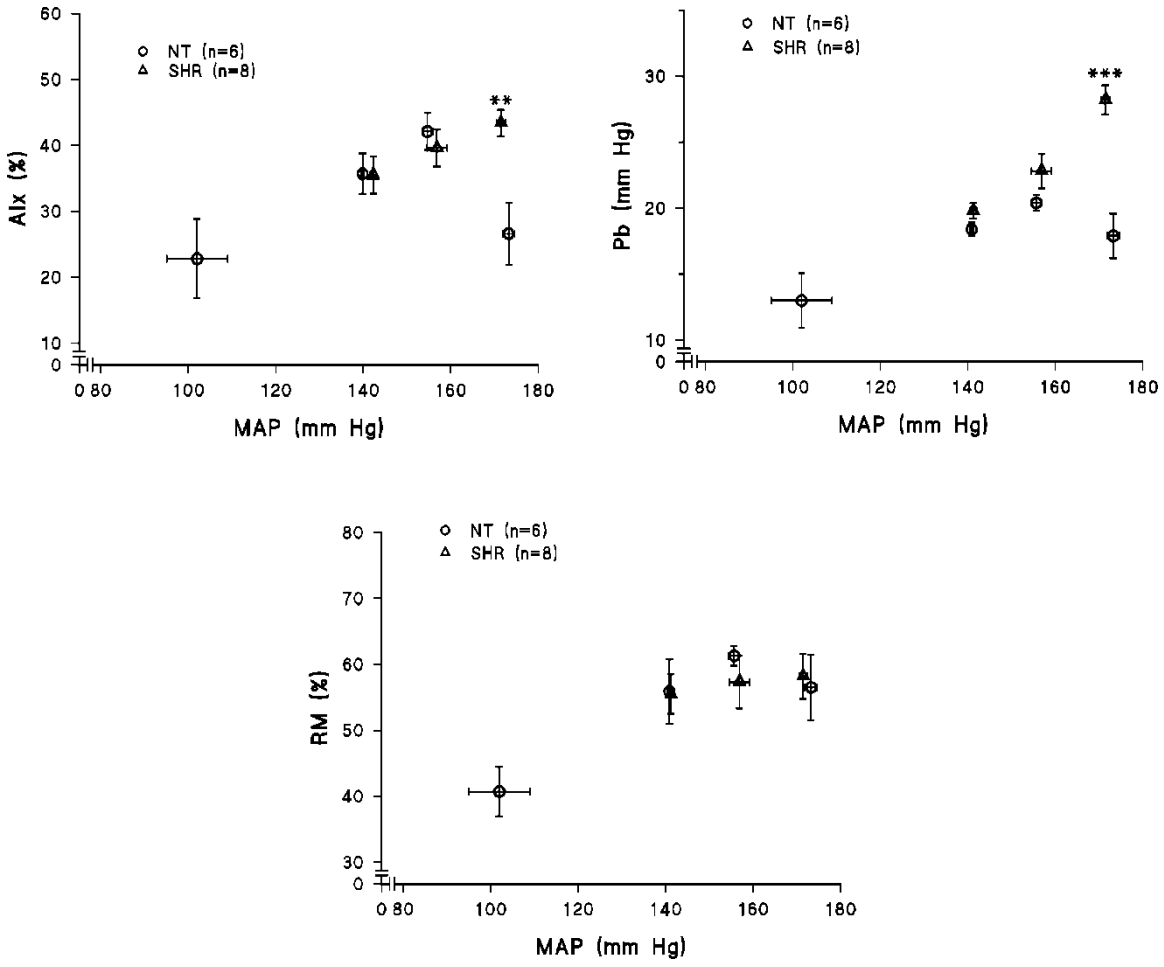

Figure 5.4. Aortic reflected wave function at comparable steady-state pressures (mean arterial pressure [MAP] in spontaneously hypertensive rats (SHR) and normotensive (NT) Wistar rats. Pb, peak backward wave pressure; AIx, augmentation index; RM, reflected wave magnitude (RM=Pb/Pf x 100).

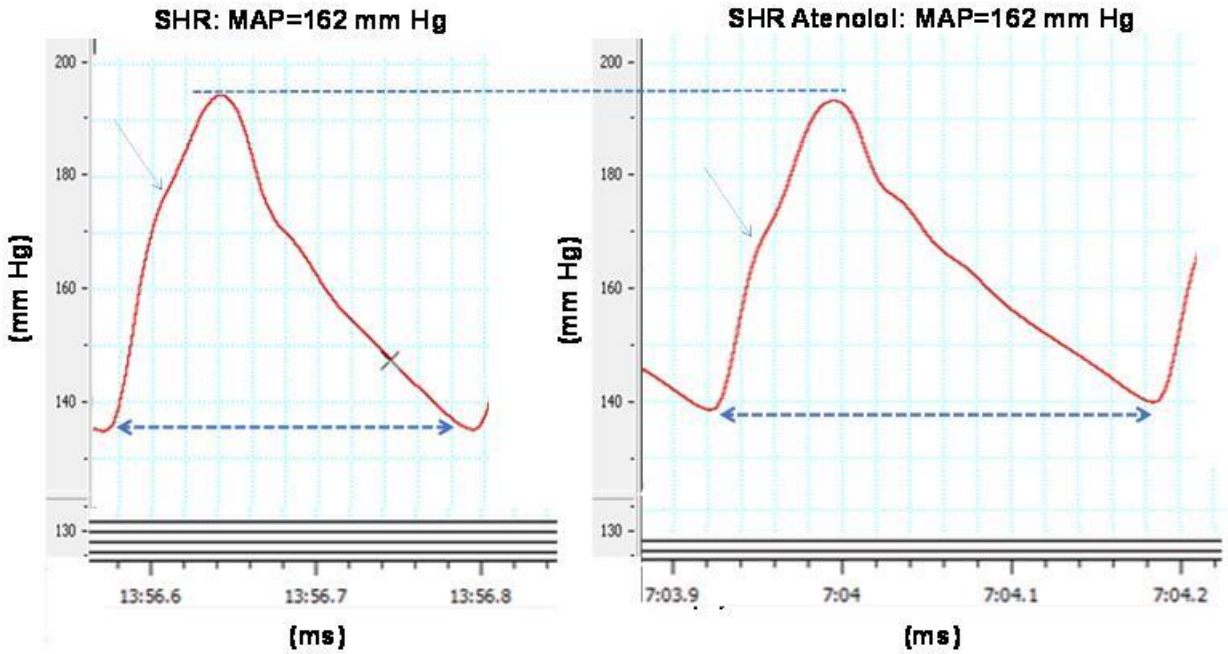

Figure 5.5. Comparison of central arterial pressure waveforms in spontaneously hypertensive rats (SHR) either receiving atenolol or the vehicle.

rate in SHR, but this failed to result in a further increase in stroke volume and hence Pf (Figure 5.6). Although atenolol therapy failed to increase stroke volume, the same volume of blood was ejected over a longer duration extending the time to the peak of Pf and reducing Pf (Figure 5.6). The lower Pf produced by atenolol therapy did not however translate into a decreased Pb and hence, AIx and RM were increased by atenolol therapy (Figure 5.7).

5.4. Discussion

The main findings of the present study are as follows: Over a range of steady-state pressures, as compared to normotensive rats, SHR are characterized by an enhanced forward wave pressure (Pf), associated with a reduced heart rate and increased stroke volume. Importantly, although atenolol therapy reduced BP, at comparable steady-state pressures and over a hypertensive BP range, atenolol therapy given to SHR, although decreasing Pf, did not modify Pb, and this was associated with an increased RM. Together with a prolonged time to the peak of the forward pressure wave associated with an extended ejection duration, (a modification required to maintain stroke volume), which may increase the overlap between Pb and Pf, atenolol-induced increases in RM translated into an enhanced AIx. Thus, through vascular effects that are unlikely to be arteriolar in nature (data compared at similar MAP values), atenolol enhances Pb for a given impact of Pf.

Several factors contribute to an enhanced PP, of which an increase in steady-state pressures (as indexed by MAP) is an important determinant thereof. In this regard, an increased MAP produces aortic distension and consequently augments aortic stiffness (without changing the structure of the aorta), Zc and hence Pf and PP (Nichols et al., 2011). Increases in aortic stiffness also produce a greater speed of wave reflection, an earlier return of the reflected wave and hence an increased systolic pressure augmentation and PP (Nichols et al., 2011). Moreover,

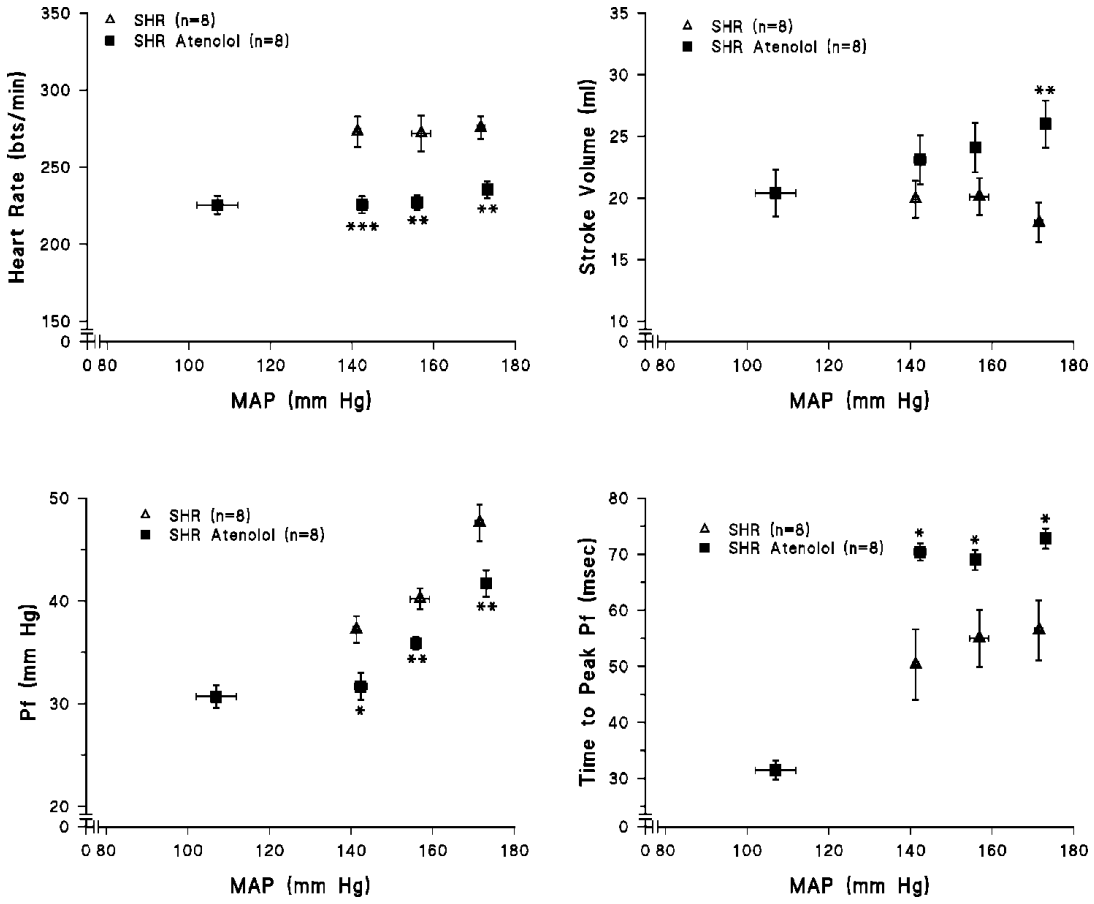

Figure 5.6. Impact of atenolol therapy on central aortic haemodynamics at comparable steady-state pressures (mean arterial pressure [MAP] in spontaneously hypertensive rats (SHR) and normotensive (NT) Wistar rats. Pf, peak forward wave pressure.

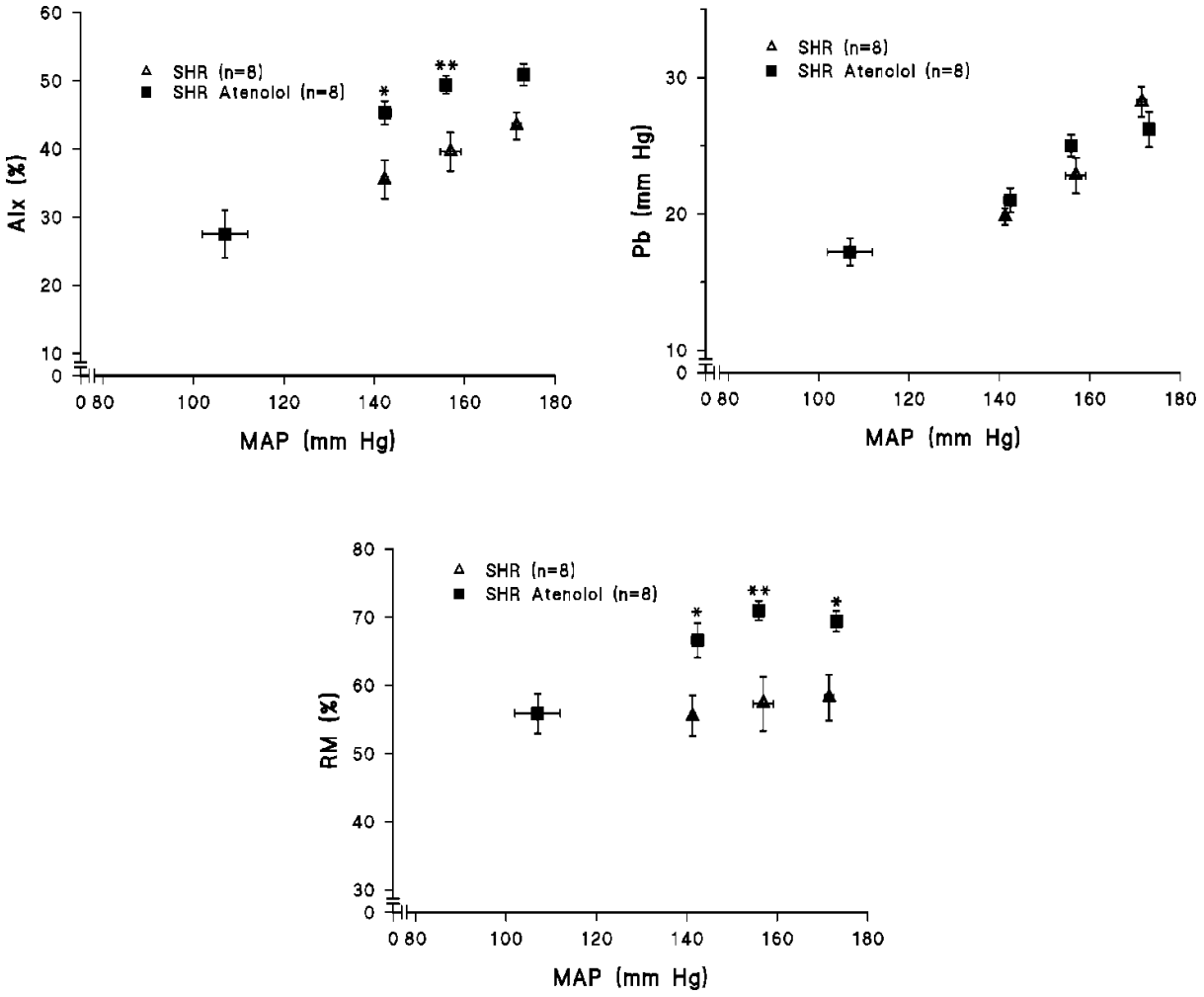

Figure 5.7. Impact of atenolol therapy on aortic reflected wave function at comparable steady-state pressures (mean arterial pressure [MAP] in spontaneously hypertensive rats (SHR) and normotensive (NT) Wistar rats. Pb, peak backward wave pressure; AIx, augmentation index; RM, reflected wave magnitude (RM=Pb/Pf x 100).

increases in MAP are determined largely by arteriolar constriction, which increase Pb and hence PP (Nichols et al., 2011). Hence, in combined systolic and diastolic hypertension, increases in PP are largely associated with an enhanced MAP and decreases in PP with antihypertensive therapy with reductions in MAP. Importantly however, we show for the first time that for a given distending pressure (MAP), that despite a lower Pf produced by atenolol administration to SHR, that Pb does not decrease commensurate with the expected reduction in inertial energy (produced by an attenuated Pf). This effect of atenolol was associated with an increased reflected wave magnitude (RM), and hence it is likely that atenolol increases backward wave pressures through effects that cannot be attributed to arteriolar vasoconstriction (detected at similar MAP values).

Although several prior studies suggest that atenolol is relatively ineffective at reducing aortic reflected wave function, these studies employed aortic augmentation index or augmented pressures as an index of backward wave function. In this regard, it is now well accepted that these indices of wave reflection may be determined by several factors including ventricular function and hence forward wave pressures, the speed of wave reflection, ejection duration and vascular tone (Cheng et al., 2012; Davies et al., 2010; Fok et al., 2014; Karamanoglu and Feneley, 1999). Indeed, the impact of atenolol on augmented pressures has been attributed to a reduced heart rate, and hence a prolonged ejection duration (Williams and Lacy, 2009), rather than to a direct effect on the magnitude of the backward wave *per se*. Consequently, the exact mechanism responsible for the limited beneficial effect of atenolol on PP is uncertain. In contrast, using wave separation analysis in the present study we have demonstrated that independent of Pf and arteriolar function (as indexed by MAP), atenolol given to SHR increases the reflected wave magnitude. Hence, the present results suggest that the limited benefit of atenolol on aortic PP may not only be attributed to an extended ejection duration and hence time to the peak of the forward wave as previously suggested (Williams and Lacy, 2009), but also to a direct effect on arteries to modify the magnitude of wave reflection.

The factors that influence the magnitude of aortic Pb beyond the inertial forces generated

by Pf or arteriolar constriction have been sought for several decades. In this regard, reflected wave magnitude (RM=Pb/Pf) or index increases with age independent of MAP (Mitchell et al., 2010; Namasivayam et al., 2016, 2009) but the factors that determine this relationship remain elusive. The present study suggests that beyond arteriolar function, a vascular effect of atenolol on arteries distal to the aorta influences RM. In this regard, it is well recognised that β_1-adrenoreceptor blockers have some β_2-adrenoreceptor blocking properties. Whether a vasoconstrictor effect of atenolol on β_2-adrenoreceptors in arteries distal to the aorta is sufficient to account for an increased RM is unknown. In this regard, alternative studies are required to evaluate the relative impact of several adrenergic receptor blockers with varying β_1 versus β_2-adrenoreceptor blocking properties on RM at similar MAP values in SHR. Although the present study does not identify factors that explain age-related increases in RM beyond MAP, the present study nevertheless provides further evidence that vascular factors other than those which influence arteriolar function may be important in controlling reflected wave function.

Importantly, there is presently little evidence to suggest that even vasodilator antihypertensive agents reduce aortic reflected waves beyond reductions in either MAP or the inertial effects of Pf. Indeed, a meta-analysis of several studies and a more contemporary study suggest that current therapy has little effect on AIx (Agnoletti et al., 2013; Manisty and Hughes, 2013). Hence, it is unlikely that even vasodilator agents offer the advantage of reducing Pb independent of arteriolar effects or the inertial effects of Pf. However, the present study suggests that beyond arteriolar function, atenolol has a disadvantage in that relative to Pf it increases Pb, which is wasted energy as it opposes flow, relative to Pf, which is useful energy, as it drives flow. Whether alternative β-adrenoreceptor blockers with vasodilator properties (for example those with α-adrenoreceptor properties) have similar deleterious actions, warrants further study. This question is of major clinical significance as there are compelling indications for the use of β-adrenoreceptor blockers in hypertensives with ischemic heart disease or heart failure. In this regard, RM is an independent predictor of outcomes for these events (Chirinos et al., 2012;

Zamani et al., 2014) and increases in RM produced by β-blockers could therefore have deleterious effects on the heart.

In the present study we show that for a given MAP, atenolol administration to SHR decreased Pf and maintained Pb, but that ultimately the decreased Pf failed to translate into a reduced PP. As PP is determined by both Pf and Pb, why a reduction in Pf did not decrease PP when Pb remained unchanged, is unclear. In this regard however, several factors may account for Pf effects on PP including the shape and overall area (duration x magnitude) of the forward wave, which is likely to have been altered by atenolol therapy as it resulted in a prolonged time to the peak of ejection. Alternatively, despite being a lower magnitude than Pf, Pb may contribute more to variations in PP. Indeed, when Pf and Pb are included in the same regression models when assessing relations with aortic PP, Pb contributes substantially more than Pf to variations in aortic PP and this occurs across the human lifespan (Booysen et al., 2015). However, alternative studies suggest that increases in Pf in response to adrenergic inotropic stimulation have a much greater impact on PP than increases in Pb produced by adrenergic-induced vasoconstriction (Fok et al., 2014a). Further studies are therefore warranted to identify the factors that determine the extent of the contribution of Pf to PP.

An important limitation of the present study is the use of rats to assess the effects of hypertension and the treatment thereof on aortic function. In this regard, there are ethical considerations in humans to assessing aortic function across a range of mean pressures, including in the hypertensive range, and in the absence of vasodilator therapy. Moreover, the present study was performed under anaesthetic conditions that are well-recognised as altering cardiovascular function. However, to exclude anaesthetic effects would require the development of specialised technology not currently available to determine Pf and Pb in awake, ambulatory genetic models of hypertension.

In conclusion, in the present study we show that in SHR, for a given MAP, atenolol-based antihypertensive therapy reduces Pf, but that through an increase in reflected wave

magnitude, and prolonged time to the peak of the forward wave enhances augmented pressures. These effects of atenolol therapy on reflected wave magnitude are likely to occur through an impact on arteries distal to the aorta and independent of arteriolar function. This effect could in-part explain the limited impact of atenolol-based therapy on PP in hypertension.

Chapter 6

Contextual Narrative and Conclusions

6.1 Introduction

Although it is well accepted that hypertension is a major cause of cardiovascular events, the exact contribution of the component pressures of BP that cause cardiovascular damage, remains uncertain. This has resulted in a considerable uncertainty and debate as to which BP measures best predict events and which measures should be targeted therapeutically. The adverse effects of hypertension are recognised as being mediated by increases in both steady-state (mean arterial pressure [MAP]) and pulsatile pressure loads (pulse pressure [PP]). Whilst for convenience diastolic blood pressure (BP) is employed as the index of steady-state pressure load, systolic BP is the clinical measure of pulsatile pressure load. The significance of PP and hence systolic BP as a cause of cardiovascular damage is highlighted by the benefits of antihypertensive agents on events in isolated systolic hypertension, where diastolic BP is often lower than normal. Importantly, increases in PP are not unique to isolated systolic hypertension. Most patients with uncontrolled hypertension have increases in PP and the impact of PP on events in these patients is at least as important as the impact of MAP.

Although increases in PP are in-part dependent on MAP, the mechanisms responsible for increments in steady-state and pulsatile pressure loads are fundamentally different. Of importance and not well recognized in basic physiology or medical texts is that PP is determined by the summation at points of overlap of two pressure waves. These two pressure waves consist of a forward travelling pressure wave generated by ventricular ejection and a backward travelling pressure wave produced by reflection of the forward wave off points of arterial narrowing. Whilst the forward pressure wave is determined by ventricular ejection and aortic stiffness, which is the principal determinant of resistance to flow in a pulsatile system (impedance), the backward travelling pressure wave is determined by the forward wave through Newton's Laws of Motion and through vascular tone. As the relative contribution of the forward and backward travelling pressure waves to cardiovascular end organ changes and events has produced

conflicting data, in the present thesis I evaluated the impact of several factors that could influence whether reflected pressure waves determine cardiovascular damage and assuming that reflected pressure waves can enhance risk prediction, whether the measurement of backward wave pressures can be simplified. The controversy of whether the forward or backward pressure waves contribute more to cardiovascular changes, and the issue of how best to assess reflected wave effects has been extensively reviewed in a critical manner in chapter 1 of the present thesis. In the present chapter of this thesis, I will highlight the main findings of the studies conducted as part of the present thesis and place them in context with what is presently known.

6.2 Does the timing of pressure waves influence the impact of wave reflection?

It is well-recognized that when the speed of both forward and backward wave travel increases or the reflection points where backward waves are generated move closer to the heart (which theoretically can occur with vasoconstriction or in someone with a shorter as opposed to taller stat) that the reflected wave will return to the proximal aorta earlier. The consequence will be that the overlap of the backward with the forward pressure wave will increase (the backward wave will return at a time point where the forward pressure wave is in a pressure antinode rather than pressure node), summation of the two pressure waves will be greater and for a given reflected wave magnitude, and the impact of the reflected wave pressures on aortic PP will increase (Figure 6.1). In addition, a prolonged ejection period or a prolonged time to peak aortic flow, will extend the time to the peak of the aortic forward pressure wave. Once again, if the time of the reflected wave return remains unchanged, a greater overlap between the backward with the forward pressure wave will occur (the backward wave will return at a time point where the forward pressure wave is more in a pressure antinode rather than a pressure node).

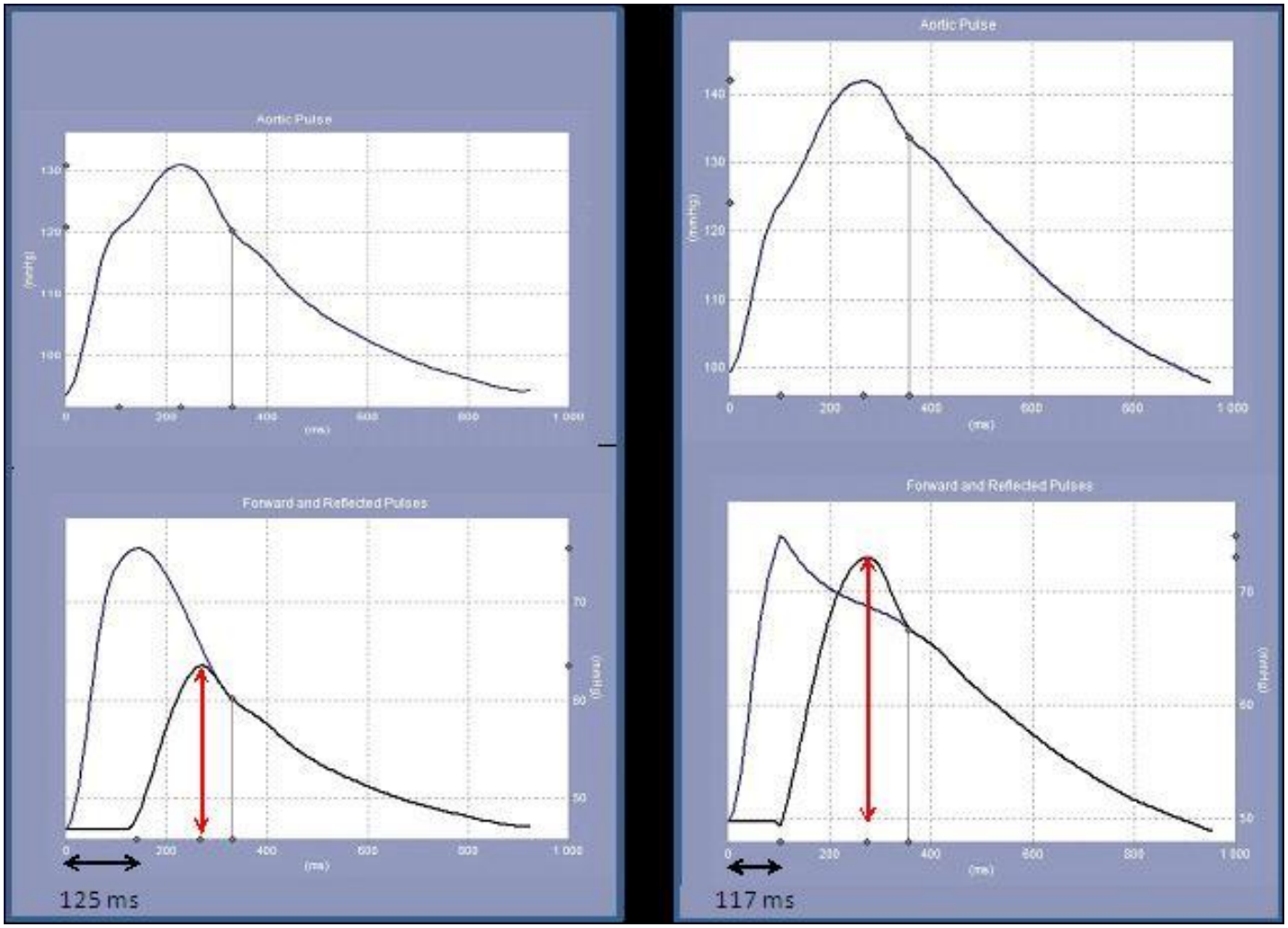

Figure 6.1. Impact of an earlier return of the backward pressure wave on aortic pulse pressure. Left panels show the effect of a late return and right panels the effect of an early return of the backward pressure wave. Upper panels show aortic pulse and lower panels show forward and backward wave pressures.

Thus, summation of the two pressure waves will once again be greater and for a given reflected wave magnitude, the impact of the reflected wave pressure on aortic PP will increase (Figure 6.2). These concepts have been well described, but the extent to which the timing of wave reflection or the duration of the time to peak aortic flow influence the impact of aortic backward wave pressures on end organ changes in the general population has never been described. In the present thesis I therefore examined the extent to which variations in either of these two effects could determine the impact of backward waves on LVMI in a large random community-based sample. Importantly, in this study sample we had previously demonstrated that beyond steady-state pressures, backward pressure waves contribute far more than forward pressure waves to variations in aortic PP (Booysen et al., 2015) or end-organ measures including LVMI (Booysen et al., 2015; Sibiya et al., 2015)).

The findings of the impact of pressure wave timing on the effects of backward wave pressures on LVMI have been thoroughly described in chapter 2 of the present thesis and published in the *Journal of Hypertension* (Tade et al., 2016). In brief (however), I demonstrated in a relatively large community-based sample of adults either receiving no antihypertensive therapy or receiving stable low-dose thiazide diuretic therapy for at least a year, that the time to the peak of the aortic forward wave, but not the time of wave reflection, has a major impact on relations between backward wave pressures and aortic augmented or pulse pressures and between backward wave or aortic pulse pressures and LVMI or LVH. How do these results contribute toward our understanding of the role of wave reflection in cardiovascular disease?

The contribution of backward wave pressures toward cardiovascular damage is controversial. In this regard, whilst several studies have demonstrated that aortic reflected waves contribute more or at least the same to variations in end-organ measures (Booysen et al., 2015; Chirinos et al., 2012; Sibiya et al., 2015; Vlachopoulos et al., 2010; Wang et al., 2010; Weber et al., 2012; Zamani et al., 2015), the Framingham Heart Study failed to show a contribution of reflected waves to several end organ measures (Cooper et al., 2015). Moreover, whilst the Multi-

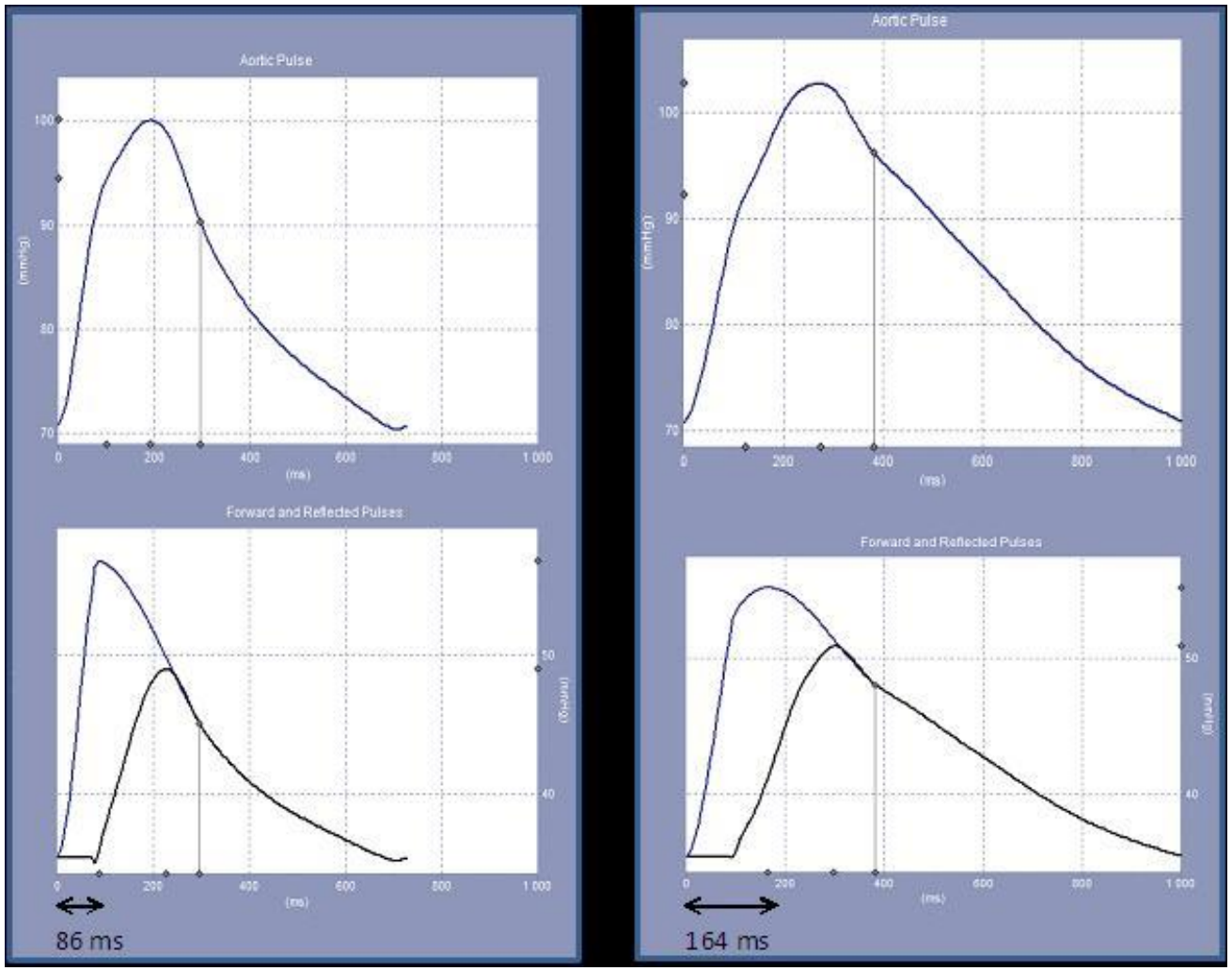

Figure 6.2. Impact of an extended time to the peak of the forward pressure wave on aortic pulse pressure. Left panels show the effect of a short duration and right panels the effect of a long duration of the time to the peak of the forward pressure wave. Upper panels show aortic pulse and lower panels show forward and backward wave pressures.

Ethnic Study in Atherosclerosis demonstrated a more important contribution of wave reflection to cardiovascular events than the forward pressure wave (Chirinos et al., 2012; Zamani et al., 2015), the Framingham Heart Study showed no contribution of wave reflection to cardiovascular events (Cooper et al., 2015; Mitchell et al., 2010b). Consequently, these conflicting results suggest that, if wave reflection is an important determinant of the adverse effects of pulsatile load on the cardiovascular system, that alternative factors influence whether these effects occur. In this regard, the present study suggests that variations in the time to the peak forward wave pressure may have a major influence on backward wave pressure effects on end-organ measures. Indeed, it was only at the highest quartile of time to the peak of the forward wave pressure that striking relations between backward wave pressures and hence aortic PP and LVH were noted. Consequently, the question arises as to whether in those studies that failed to show an impact of aortic BP on risk beyond brachial BP (Mitchell et al., 2010a; Vlachopoulos et al., 2010), a significant proportion of participants had an extended systolic period comparable with that noted in the highest quartile of time to the peak of the forward wave in the present study? The present results suggest that aortic backward wave and PP effects on cardiovascular end organs should always be interpreted in the context of time to peak forward wave pressures.

The results of the present study not only provide possible explanations for diverse findings on the role of wave reflection in mediating cardiovascular damage, but there are several clinical implications. In this regard, reflection wave magnitude predicts the development of heart fail beyond conventional risk factors (Chirinos et al., 2012). Importantly, heart failure with a preserved ejection fraction is associated with prolonged mechanical systole (Weber et al., 2006). The results of the present study therefore raise the question of whether a prolonged mechanical systole results in heart fail by extending the time to the peak of the forward wave, thus enhancing the impact of backward waves on cardiac load. In addition, the results of the present study provide insights into why, in the treatment of hypertension, β-adrenergic receptor blockers are not as effective as alternative agents at preventing events. In this regard, β-adrenergic receptor

blockade extends the systolic period and consequently augments aortic PP (Williams and Lacy, 2009). However, whether an extended systolic period enhances the impact of aortic reflected waves on cardiovascular damage has never been demonstrated. The present study therefore provides the first evidence to show that relations between aortic backward wave pressures (and hence PPc) and cardiac end-organ changes (LVMI and LVH) are strongly determined by the time to the peak of the aortic forward wave. Hence, the present study provides evidence to support the notion that a prolonged mechanical systole mediates adverse effects on the heart by enhancing the deleterious impact of aortic backward wave and pulse pressure. The present study also provides important evidence to show that age-related increases in the ejection period and hence the time to the peak of the forward pressure wave have a major effect on the adverse impact of aortic backward wave pressures on the heart. These data therefore provide an important mechanism that may explain why aging effects on the cardiovascular system are enhanced and also indicate that in future studies, the impact of wave reflection on cardiovascular events needs to be considered in the context of the time to the peak of flow or at least the ejection period.

6.3 Clinical and demographic factors that determine backward wave pressures and their impact on AIx.

An important consideration that may explain the conflicting results of the role of reflected waves in mediating cardiovascular damage is the studies evaluating these effects, studied patients with very different clinical and demographic features. The question therefore arises as to what clinical or demographic factors influence reflected waves or their impact on cardiovascular end organs. One well described factor that impacts on PP is gender. As highlighted in the introduction to this thesis, women have a higher PP than men and several factors may explain these differences. An important and well-described alteration that explains

sex differences is changes in PP augmentation (AIx). As highlighted in the introductory chapter and again in chapter 3, in the Framingham Heart Study sex differences in AIx were attributed largely to an increased forward wave peak width, slope of the backward pressure wave, and forward wave amplitude, but not backward wave amplitude (Torjesen et al., 2014). As the participants of the Framingham Heart Study are largely of European ancestry and we previously demonstrated marked ethnic differences in AIx between groups of African and European ancestry (Chirinos et al., 2011), in chapter 3 of the present thesis I explored whether similar factors could account for sex differences in AIx in a large community-based sample of African ancestry.

As described in chapter 3 of the present thesis and published in the *American Journal of Hypertension* (Tade et al., 2017), I demonstrated that AIx was independently related to female gender and these gender-specific effects were attributed largely to the impact of backward wave pressures and magnitude, with little or inconsistent contributions from forward wave pressures, time to the peak of the forward wave, or backward wave foot time. Importantly, although time to the peak of the forward wave failed to contribute to any large extent to sex-differences in AIx, I also demonstrated that females have marked age-related increases in the time to the peak of the forward wave, whilst men fail to show similar changes. Thus, time to the peak of the forward wave could account for age-related sex differences in PP.

In contrast to the Framingham Heart Study, where increases in the magnitude of the forward wave exceeds that of the backward wave in explaining age-related increases in aortic PP (Mitchell et al., 2010b), the magnitude of backward waves exceeds that of the forward wave in contributing to variations in aortic PP in communities of African ancestry (Booysen et al., 2015). Also, in contrast to the Framingham Heart Study which failed to show relations between aortic backward wave pressures and LVMI (Cooper et al., 2015), in groups of African ancestry, aortic backward wave pressures contribute more than forward wave pressures to several end organ measures including LVMI (Booysen et al., 2015; Sibiya et al., 2015). These previous findings

(Booysen et al., 2015; Sibiya et al., 2015)) suggest ethnic effects of aortic backward waves may occur and these ethnic differences may explain the markedly higher AIx values in groups of African ancestry as compared to alternative ethnic groups (Chirinos et al., 2011). The results of the present study further suggest that the main impact of reflected waves on the cardiovascular system in groups of African descent is likely to be in women as compared to men and hence that women are at a greater risk of BP-related adverse effects than men for a given level of brachial BP control. The present study therefore highlights the importance of targeting aortic backward rather than forward waves in females of African ancestry.

Similar to the Framingham Heart Study which demonstrated that women have a wider forward wave peak than men (Torjesen et al., 2014), in the present study, we show that women have a longer time to the peak of the forward wave than men. This is likely to be explained by a longer systolic ejection time (Gatzka et al., 2001) and the well described increase in left ventricular systolic function reported to occur in women as compared to men. This could in-part mediate deleterious effects on end-organs by increasing the chance of the backward wave merging with the forward wave. Indeed, as indicated in the findings described in chapter 2, the time to the peak of the forward pressure wave markedly influences whether the reflected wave is independently associated with LVMI (Tade et al., 2016). In contrast to Caucasian community samples (Torjesen et al., 2014), where a wider peak of the forward wave accounted for a significant proportion of the enhanced AIx in women as compared to men, in the present study, adjustments for the time to the peak of the forward wave produced only a modest (not significant) attenuating effect on relations between Pa or AIx and female gender. Thus, it may be argued that sex-differences in the time to the peak of the forward wave pressure may not translate into a significantly enhanced impact of the reflected wave on cardiovascular end organs. However, as previously described by our group, AIx is not a good index of the impact of wave reflection on LVMI, particularly in women (Booysen et al., 2015; Sibiya et al., 2015). Thus, further work is required to evaluate whether the impact of wave reflection on end-organ is

enhanced in women through an increased time to the peak of the forward travelling pressure wave.

6.4 Mechanisms that explain the impact of β-adrenergic receptor blockers on aortic augmentation index

As underscored in several sections throughout the present thesis, the impact of wave reflection on aortic pressures and hence cardiovascular damage is determined by several factors. As highlighted in chapter 2, and discussed above, a prolonged time to the peak of the forward pressure wave has a marked impact on the adverse effects of reflected waves on cardiovascular end organs (Tade et al., 2016). This has been proposed as one of the mechanisms responsible for the reduced efficacy as compared to alternative antihypertensive agents of β-adrenoreceptor blockers on central aortic PP and hence cardiovascular events despite similar reductions in peripheral BP (Lindholm et al., 2005; Reboussin et al., 2018; Wiysonge and Opie, 2013). In this regard, β-adrenoreceptor blockers extend the duration of systole. Indeed, for the same reduction in brachial BP as compared to alternative agents, a marked increase in AIx is noted with β-adrenoreceptor blocker-based therapy (Williams et al., 2006) and this is strongly driven by heart rate (Williams and Lacy, 2009).

Although these effects have been demonstrated for atenolol, whether this is a class-specific effect or a general effect related to all β-adrenoreceptor blockers is unknown. If the impact of atenolol on AIx is attributed to heart rate changes and an increased time to the peak of the forward wave, then this is indeed a general effect that will accompany all β-adrenoreceptor blockers. This has implications for the use of β-adrenoreceptor blockers when there are compelling reasons to use them in the treatment of hypertension (such as in heart failure or ischaemic heart disease). However, being an agent without direct vasodilator properties, atenolol may not reduce backward wave pressures through vascular effects. Thus, the adverse impact of

atenolol on AIx may in-part be through effects unrelated to heart rate and thus would not be class specific. Under these circumstances, the use of β-adrenoreceptor blockers may not produce as marked an adverse effect on aortic function. However, because β-adrenoreceptor blockers are no longer advocated as first line therapy in the treatment of hypertension, there are presently no studies where the impact of atenolol on AIx has been determined in the absence of vasodilator therapy. Moreover, all studies assessing the impact of atenolol on aortic function have employed AIx as the only index of wave reflection, with no studies assessing the impact on forward and backward wave pressures derived from wave separation analysis. Although this question cannot be answered in human studies because of the ethical dilemma of using β-adrenoreceptor blockers as monotherapy, we developed an approach to assessing aortic function using invasive techniques and Doppler imaging in the outflow tract in rats. We subsequently assessed the impact of atenolol on AIx and associated alterations over a range of MAP values in a well-recognised animal model of hypertension, the spontaneously hypertensive rat (SHR). The results of this study are described and discussed in chapter 5. What were the main findings and their implications?

As discussed in chapter 5, we demonstrated that as assessed over a range of steady-state pressures induced by the administration of the vasoconstrictor phenylephrine, atenolol given to SHRs did indeed cause an increased AIx. In addition, consistent with the proposed mechanism of this effect (Williams and Lacy, 2009), atenolol resulted in a well-recognised class-specific effect of all β-adrenoreceptor blockers, and that is an extended ejection duration and hence increased time to the peak of the forward pressure wave. Through an increased overlap between forward and backward wave pressures, this effect could have, in-part, accounted for the impact of atenolol on AIx. Importantly however, atenolol did not increases stroke volume through a force-frequency effect, but rather maintained stroke volume by ejecting the same volume of blood over a longer duration, the consequence being a reduced forward wave pressure. Although this should have, through inertial effects and Newton's 3rd Law of Motion, resulted in a reduced backward

wave pressure, this did not occur. This we attribute to the adverse effects of atenolol on wave reflection as relative to forward wave pressures, backward wave pressures were increased (reflected wave magnitude was enhanced). These data provide the first direct evidence that atenolol produces adverse effects on central arterial function not only through class effects (by extending the time to the peak of the forward pressure wave), but also through effects specific to non-vasodilator β-adrenoreceptor blockers (through vascular effects on wave reflection).

In the study described in chapter 5 we have not as, yet, evaluated the relative impact of the class-specific effect versus the adverse effect on reflected wave function produced by atenolol on AIx. Hence, at present we are unable to draw any certain conclusions as to whether the adverse effect is class- or drug-specific. In this regard, further studies are required to assess the impact of vasodilator β-adrenoreceptor blockers such as carvedilol on AIx in SHR. However, we are unlikely to be able to assess this effect over a range of comparable distending pressures as we have noted that vasodilation (such as with the calcium channel blocker, nifedipine) limits the ability to increase steady-state pressures with vasoconstrictors. We are nevertheless at present modelling the pressure wave generated by the product of aortic flow and Zc (Phan et al., 2016) as this will allow us to determine the extent to which overlap between this pressure wave and the backward pressure wave and the impact on wave re-reflection contribute to changes in AIx on atenolol therapy.

6.5 Are there alternative approaches to wave separation analysis to assessing reflected wave effects?

As underscored in the introduction to the present thesis there are several approaches to assessing reflected wave effects. Traditionally, reflected wave effects were determined by employing AIx as a measure of the ability of backward pressure waves to augment aortic pulse pressure, an entirely logical and well-established approach. However, as extensively discussed in

the introduction to the present thesis and described by our group (Booysen et al., 2015), for several reasons this approach may nevertheless underestimate the impact of wave reflection. Consequently, to determine the impact of wave reflection more recently several studies have employed formal wave separation analysis. These approaches nevertheless require the generation of a simultaneous aortic pressure and flow wave, which limits the ability to assess reflected wave effects. Whilst a triangular flow waveform can be assumed, which requires no additional analysis and can be embedded in software thus automating the assessment, more accurate assessments require a measured or "physiological" flow waveform which are difficult to embed in software and hence limit automation. However, in addition, the second systolic shoulder of the peripheral pulse (pP_2), although amplified in the upper limb, is thought to be generated by the transmission of reflected waves derived from the lower half of the body that are transmitted into the upper limbs (Nichols et al., 2011). Moreover, aortic reflected waves are thought to occur mainly from wave return from the lower limbs (Nichols et al., 2011). Thus, although reflected waves derived from the triangular flow wave may be sufficient to assess backward wave effects, an alternative evaluation that could be considered to complement the assessment of reflected wave effects from the triangular waveform may be pP_2. As described in chapter 4 of the present thesis I therefore assessed the ability of pP_2 to reflect the impact of backward wave pressures as determined using a triangular flow wave. What were the findings and what are the implications thereof?

Importantly, in a large community-based sample we were able to show that pP_2 was more strongly correlated with Pb than was Pa. In this regard, the greatest difference in relations between Pa and Pb versus pP_2 and Pb was at the highest tertile of time to the foot of the backward wave. Neither the time to the inflection point nor the presence of Murgo type C waveforms influenced relations between Pa and Pb. The relations between pP_2 and aortic PP were also stronger than those of Pa versus aortic PP, but similar to Pb-aortic PP relations and the differences in relations between Pa and aortic PP as compared to pP_2 or Pb versus aortic PP, were greatest at the highest tertile of time to the foot of the backward wave. These differences in

effects of pP_2 and Pa on Pb and aortic PP translated into significant brachial systolic BP-independent relations between pP_2 or Pb, but not Pa and LVMI. Consequently, these data show that pP_2, although amplified during transmission outward to the arm, closely reflects the impact of peak aortic backward wave pressures derived from wave separation analysis performed employing a triangular waveform. At present we are performing wave separation analysis using an actual aortic flow waveform in a much larger sample than that employed in the present thesis for validation purposes. In this regard, in this larger study sample we intend to determine how closely pP_2 and backward wave pressures obtained from wave separation analysis obtained from the triangular or "physiological" flow wave reflect backward wave pressure effects determined from an actual aortic flow wave. Only once these analyses are complete will we be able to determine with some confidence the accuracy of pP_2 or alternative surrogate approaches to assessing the impact of backward wave pressure effects.

6.6 Limitations

There are several limitations to the studies conducted in the present thesis and these have largely been addressed in the discussion sections of each of the data chapters or discussed in aforementioned sections of the present chapter. However, there are several general limitations that warrant highlighting. When initiating the present study some years ago, our group did not perform assessments of aortic velocity in the outflow tract in the initial few years. Hence, we are only now completing analyses on a larger cohort of participants where wave separation analysis is being conducted using an actual aortic flow waveform and the role of wave re-reflection (Phan et al., 2016) is being evaluated. Second, for several reasons we are unable to follow-up participants in the community-based study and hence longitudinal analyses or analyses where events are assessed cannot be performed. Thus, whether relationships noted with end-organ measures are cause and effect and translate into events is unknown. Third, for ethical reasons it

is difficult to assess the impact of atenolol monotherapy on aortic function at similar steady-state pressures in human studies. Hence, these studies were conducted in animal models and under anaesthetic conditions. However, we have noted similar differenced in aortic function between refractory hypertensives either receiving or not receiving atenolol therapy (Masiu et al., unpublished data). Thus, it is possible that the impact of atenolol on aortic function noted in SHR reflect similar changes noted in humans.

6.7 Conclusions

In conclusion, in the present thesis I show that an extended time to the peak of the forward wave pressure determines the impact of backward wave pressures on LVM; that atenolol-based antihypertensive therapy not only produced adverse effects on aortic function by extending the time to the peak of the forward wave through heart rate-related effects (class effect), but also increases wave reflection (specific to non-vasodilator β-adrenergic receptor blockers); that the adverse effects of female gender on aortic function in groups of African ancestry are through enhanced backward pressure waves and that backward wave pressure effects, although inadequately indexed by aortic augmented pressure, can be appropriately indexed by the second systolic shoulder of the peripheral pulse. These data provide further insights into the adverse effects and appropriate measures of central arterial wave reflection.

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Appendix 1

Ethics clearance certificates.

Appendix 2

"Turnitin" Plagiarism report

Appendix 3

Grace Tade, Gavin R. Norton, Hendrik L. Booysen, Moekanyi J. Sibiya, Imraan Ballim, Pinhas Sareli, Elena Libhaber, Olebogeng H.I. Majane, and Angela J. Woodiwiss. Time to peak of the aortic forward wave determines the impact of aortic backward wave and pulse pressure on left ventricular mass. *Journal of Hypertension* 2017; 22(6): 410-415.

Appendix 4

Grace Tade, Gavin R. Norton,1 Hendrik L. Booysen,1 Moekanyi J. Sibiya,1 Imraan Ballim,1 Pinhas Sareli,1 and Angela J. Woodiwiss. Enhanced Aortic Reflected Wave Magnitude Accounts for the Impact of Female Gender on Aortic Pressure Augmentation in a Group of African Ancestry. *American Journal of Hypertension* 2017; 30(8); 781-790.

Ms

by Oluwatosin Grace Tade

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Publication

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5 Bryan Hodson, Gavin R. Norton, Imraan Ballim, Pinhas Sareli, Angela J. Woodiwiss. "Contribution of backward and forward wave pressures to age-related increases in aortic pressure in a community sample not receiving antihypertensive therapy", Journal of the American Society of Hypertension, 2017 — 1%
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6 Adamu J Bamaiyi, Gavin R Norton, Vernice Peterson, Glenda Norman, Frank B Mojiminiyi, Angela J Woodiwiss. "Limited Impact of β-Adrenergic Receptor Activation on Left Ventricular Diastolic Function in Rat Models of Hypertensive Heart Disease", Journal of Cardiovascular Pharmacology, 2018 — 1%
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R14/49 Oluwatosin Grace Tade and Prof Angela Woodiwiss

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170763

NAME: (Principal Investigator)	Oluwatosin Grace Tade and Prof Angela Woodiwiss
DEPARTMENT:	Physiology Cardiovascular Pathophysiology and Genomics Research Unit University of the Witwatersrand
PROJECT TITLE:	Effect of Hypertension and Arteriolar Function on Central Waveforms
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M170401
SUPERVISOR:	Prof Gavin Norton
APPROVED BY:	Professor P. Cleaton-Jones Chairperson, HREC (Medical)
DATE OF APPROVAL:	16/08/2017

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 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand 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 July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 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

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Woodiwiss/Norton

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER MO70469**
PROJECT	Gene Candidates As Determinants of Blood Pressure and Intermediary Phenotypes in Pathogenesis of Hypertension in Black S Africans
INVESTIGATORS	Profs A/G Woodiwiss/Norton
DEPARTMENT	School of Physiology
DATE CONSIDERED	07.05.09
DECISION OF THE COMMITTEE*	Approved unconditionally (refer M020472)

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.05.09 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Woodiwiss A Prof

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Woodiwiss/Norton et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-04-72

PROJECT Gene Candidates As Determinants of Blood Pressure And Intermediary Phenotypes In Pathogenesis of Hypertension In Black South Africans

INVESTIGATORS Prof's AJ/G et al Woodiwiss/Norton et al

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2007 -05- 09
JOHANNESBURG

DECISION OF THE COMMITTEE *

Approved unconditionally

This clearance is valid and within the Wits 5 year validity

DATE 02-05-14 **CHAIRMAN** .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof AJ Woodiwiss
Dept of School of Physiology, Wits Medical School

Works2\lain0015\HumEth97.wdb\M 02-04-72

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2015/07/29/A

APPLICANT: Ms O Tade

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: The effect of acute and chronic anti-hypertensive therapy on carotid-femoral pulse wave velocity in SHRs rats

Number and Species

111 SHR Rats

Approval was given for the use of animals for the project described above at an AESC meeting held on 2015/07/28. This approval remains valid until 2017/08/13.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

None

Signed: _____ Date: 21st August 2015
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ Date: 21. August 2015
(Registered Veterinarian)

cc: Supervisor: Dr F Michel
Director: CAS

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AESC 2012 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE

MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Oluwatosin Tade

b. Department: Physiology

c. Experiment to be modified / extended

		AESC NO	
Original AESC number	2015	07	29/A
Other M&Es :			

d. Project Title: The effect of acute and chronic anti-hypertensive therapy on carotid-femoral pulse wave velocity in SHRs.

		No.	Species
e.	Number and species of animals originally approved:	111	SH Rats
f.	Number of additional animals previously allocated on M&Es:	-	-
g.	Total number of animals allocated to the experiment to date:	58	SH Rats
h.	Number of animals used to date:	6	SH Rats

i. Specific modification / extension requested:

a.)To administer antihypertensive drugs by gavage for both acute and chronic phases.

b.)To administered drugs 7days prior to measurement for the acute phase.

c.)To add one chronic (Labetolol) and two acute (labetalol and carvedilol) groups

d.) 36 extra rats will be required.

j. Motivation for modification / extension:

a and b.) The vehicle (alcohol) of the antihypertensives reduces blood pressure when administered intra-arterially. Furthermore, the anti-hypertensives even if dissolved in alcohol first, before added to drinking water, come out of solution when added to drinking water.

C and d.) We are presently assessing the effect of atenolol which is a beta adrenergic selective blocker. We think it is important to also evaluate the effects of other beta blockers (selective and non-selective) hence the need for additional groups; carvedilol and labetalol. Although both are non-selective beta blockers, carvedilol has a calcium channel blocking property and labetalol is used in treating heart attack. We would therefore like to compare these to the effect of a selective beta

adrenergic blocker; atenolol.

Date: 19 Oct, 2015 Signature:

RECOMMENDATIONS: Approved.

i. Administration of antihypertensive drugs by gavage for both acute and chronic phases.

ii. Administration of drugs 7days prior to measurement for the acute phase.

iii. Inclusion of one chronic (Labetolol) and two acute (labetalol and carvedilol) treatment groups

iv. 36 extra rats.

Date: 28th October 2015 Signature:

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2016/03/08/A

APPLICANT: Mr B Adamu

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: Impact of habitual exercise training on central aortic pressures in spontaneously hypertensive rats (SHRs)

Number and Species

60 male SHR rats

Approval was given for the use of animals for the project described above at an AESC meeting held on 2016/03/29. This approval remains valid until 2018/04/10.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

Signed: _____ Date: 15th April 2016
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ Date: 14 April 2016
(Registered Veterinarian)

cc: Supervisor: Professor G Norton
Director: CAS

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AESC 2012 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: **ADAMU BAMAIYI**

b. School and email address: **PHYSIOLOGY, adamu.jibril@udusok.edu.ng**

c. Experiment to be modified / extended **AESC NO**

Original AESC number	**2016**	**03**	**08/A**
Other M&Es :			

d. Project Title: **IMPACT OF EXERCISE-TRAINING ON CENTRAL AORTIC PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHRs)**

		No.	**Species**
e.	Number and species of animals originally approved:	60	*SHRs*
f.	Number of additional animals previously allocated on M&Es:	-	-
g.	Total number of animals allocated to the experiment to date:	60	*SHRs*
h.	Number of animals used to date:	41	*SHRs*

i. Specific modification / extension requested:

- 16 additional male Wistar rats (8 five months old, 4 four months old and 4 three months old) will be used to carry out same terminal procedures as the exercise SHRs
- Grace Tade to be added to the study

j. Motivation for modification / extension:

-The exercise SHRs (five months old) have been compared with non-exercise SHRs (five months old) and exercise reduces the central haemodynamic parameters to values that are similar to those in younger SHRs (three months old). However, it is important to know whether the central haemodynamic values in the younger SHRs (three months old) and exercise SHRs (five months old) are similar (or not) to those in normotensive Wistar rats. The data in normotensive Wistar rats is important to help explain that chronic exercise in the older SHRs is responsible for the improvement in the central haemodynamic parameters.
-Professor Gavin Norton will carry out the carotid/aortic cannulation while Professor Angela Woodiwiss will carry out the echocardiography during the termination of the rats.
-Grace and I will be involved in data collection and assist in the data analysis.

Date:15/5/2017 Signature:

RECOMMENDATIONS: Approved. i. 16 additional Wistar rats as detailed above. ii. Inclusion of Grace Tade as a co-worker.

Date: 9 June 2017 Signature:

Chairman, AESC

Original Article

Time to the peak of the aortic forward wave determines the impact of aortic backward wave and pulse pressure on left ventricular mass

Grace Tade*, Gavin R. Norton*, Hendrik L. Booysen, Moekanyi J. Sibiya, Imraan Ballim, Pinhas Sareli, Elena Libhaber, Olebogeng H.I. Majane, and Angela J. Woodiwiss*

Aim: To determine the degree to which an extended time to the peak of the aortic forward wave or early wave reflection time enhance associations between aortic backward wave pressure and hence central aortic pulse pressure (PPc) and left ventricular mass index (LVMI).

Methods: In 701 adult participants from a community sample either receiving no antihypertensive therapy or receiving low-dose thiazide diuretic monotherapy for at least a year (the major therapy employed), we assessed aortic haemodynamics (SphygmoCor software and wave separation analysis; AtCor Medical, West Ryder, New South Wales, Australia) and LVMI (echocardiography).

Results: An interaction between time to the peak of the aortic forward wave and aortic backward wave pressure was independently associated with aortic augmented pressure ($P < 0.01$), PPc ($P < 0.005$), LVMI ($P < 0.01$), and LV hypertrophy (LVH; $P = 0.01$). The time to the peak of the aortic forward wave–aortic backward wave pressure interaction translated into a stepwise increase in the independent association between aortic backward wave pressure and aortic augmented pressure or PPc across quartiles of time to the peak of the aortic forward wave ($P < 0.05$ to < 0.0001 for comparison of slopes of relations). Furthermore, the time to the peak of the aortic forward wave–aortic backward wave pressure interaction translated into an increase in the independent association between PPc or aortic backward wave pressure and LVMI ($P < 0.05$ to < 0.001 for comparison of slopes and strength of relations) or LVH ($P < 0.05$ for comparisons of odds ratios), but not between forward wave pressures and LVMI or LVH across quartiles of time to the peak of the aortic forward wave. A markedly better ability of aortic backward wave pressure and PPc, but not forward wave pressures to detect LVH was noted in the highest as compared with the first three quartiles of time to the peak of the aortic forward wave ($P < 0.05$). In contrast, reflection time failed to influence the impact of aortic backward wave pressure or PPc on LVMI.

Conclusions: Time to the peak of the aortic forward wave, but not early wave reflection markedly influences the impact of aortic backward wave pressure and hence aortic pulse pressure on LVMI and LVH in adults.

Keywords: aortic pulse pressure, augmentation index, central blood pressure, left ventricular mass index, reflected waves

Abbreviations: AIx, aortic augmentation index; BP, blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; Ft, time to the peak of the aortic forward wave; HbA1c, percentage glycated haemoglobin; LVH, left ventricular hypertrophy; LVM, left ventricular mass; LVMI, left ventricular mass index

INTRODUCTION

Pulse pressure is generally accepted as a strong determinant of cardiovascular damage beyond steady-state pressures. Central aortic pulse pressure (PPc) may be considerably lower than brachial pulse pressure; controlled by different mechanisms to brachial pulse pressure; and possibly more closely associated with end-organ changes than brachial pulse pressure [1–3]. Although the fundamental determinants of PPc are well described [1–3], the contribution of each of these elements to cardiovascular damage is a topic of continuous debate. Despite several studies addressing the relative contribution of the component aortic forward and reflected (backward) waves of PPc to variations in end-organ changes [4–9], the contribution of the timing of these waveforms has received less attention.

Although reflected waves enhance coronary flow when returning in diastole, in adulthood early reflected waves arrive in systole and augment PPc [3]. Even in adulthood advancing age is associated with a gradual increase in the

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*Grace Tade, Gavin R. Norton and Angela J. Woodiwiss contributed equally to this work.

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speed of wave reflection, a change which contributes to age-related increases in augmented pressures. Variations in the time of wave reflection may therefore contribute to cardiovascular damage produced by backward waves in adulthood [10]. β-adrenergic receptor blockers, that are not as effective at reducing cardiovascular events as other antihypertensive agents, extend the time to the peak of the forward wave [11] by slowing ventricular ejection. Assuming that reflection time remains unchanged, an extended time to the peak of the aortic forward wave may therefore increase the chances of the backward wave overlapping with the forward wave, thus enhancing the possibility of early backward waves augmenting PPc. Hence, an increased time to the peak of the aortic forward wave may also determine the adverse effects of backward wave pressures on cardiovascular end organs. Indeed, heart failure with a preserved ejection fraction, a form of heart failure that may be determined in part by increases in aortic backward wave pressures, is associated with prolonged mechanical systole [12]. Although indices of aortic pressure augmentation are independently associated with cardiovascular outcomes [4], these indices reflect a composite of backward wave pressures and the timing of aortic pressure waves. The exact role of time to the peak of the aortic forward wave or reflection time in determining backward wave pressure effects on cardiovascular outcomes or cardiovascular end organs is unknown. In the present study, we therefore aimed to test the hypothesis that either reflection time or time to the peak of the aortic forward wave significantly determine the contribution of aortic backward wave pressures and hence PPc to variations in left ventricular mass (LVM) and LV hypertrophy (LVH) in a large, community-based sample.

METHODS

Study group

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 and M12–04–108). Participants gave informed, written consent. The present study design has previously been described [13–16]. Briefly, 1192 participants from families of black African descent (Nguni and Sotho chiefdoms) with siblings older than 16 years with central haemodynamic measurements were randomly recruited (based on the census figures of 2001) from the South West Township (SOWETO) of Johannesburg, South Africa. In a substudy, 812 participants had LVMI determined using echocardiography. Of these participants, 701 were either not receiving antihypertensive therapy ($n = 597$) or were receiving low-dose thiazide diuretic monotherapy for at least a year (the recommended first line agent in South Africa).

Clinical, demographic, and anthropometric measurements

A standardized questionnaire was administered to obtain demographic and clinical data [13–16]. Height and weight were measured using standard approaches and participants were identified as being overweight if their BMI was at least 25 kg/m^2 and obese if their BMI was at least 30 kg/m^2. High-quality blood pressure (BP) measurements were obtained by a trained nurse technician using a standard mercury sphygmomanometer according to guidelines and as previously described [14]. Adult (24–32 cm), large adult (32–42 cm), and extralarge adult (38–50 cm) cuffs were available for use and the cuff size employed was determined by arm circumference. Korotkov phases I and V were employed to identify SBP and DBP, respectively and care was taken to avoid auscultatory gaps. BP was taken as the mean of five measurements obtained at least 30 s apart in the seated position after 10 min of rest. Hypertension was defined as a mean BP at least140 and/or 90 mmHg or the use of antihypertensive medication. Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin were performed. Diabetes mellitus (DM) or an abnormal blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or a percentage glycated haemoglobin value greater than 6.1%.

Pulse wave analysis

Central aortic haemodynamics were estimated using radial applanation tonometry and SphygmoCor software as previously described [8,9,15,16]. Central aortic pulse pressure (PPc) was determined as the difference between central aortic systolic BP and DBP and pulse pressure amplification was determined as brachial pulse pressure /PPc. Aortic backward and forward wave pressures, and time to the peak of the aortic forward wave and reflection time were determined using SphygmoCor software which separates the aortic waveform using a triangular flow wave [17] (Figure S1, http://links.lww.com/HJH/A689). Importantly, the use of a 'triangular flow waveform' to perform wave separation analysis assumes that the peak of the flow wave occurs at the same time as the point of inflection on the upstroke of the aortic pressure wave (P_1 time in the upper panel of Figure S1, http://links.lww.com/HJH/A689) (or at one-third ejection duration). However, this assumption is not always true as the peak of the flow wave may precede the pressure wave and hence, may occur earlier. We therefore assessed the extent to which a hypothetical earlier peak of the aortic flow wave would alter the ability to accurately evaluate the time to the peak of the forward pressure wave (supplemental methods, http://links.lww.com/HJH/A689). In this regard, a hypothetical assumption that the peak of the aortic flow wave occurs at a point half the time to the inflection point produced little effect on the ability to identify the time to the peak of the aortic forward wave (supplemental methods and Figures S2 and S3, http://links.lww.com/HJH/A689).

Because the "triangular flow waveform" approach generates a hypothetical waveform, in 392 participants we also separated the forward and backward waves using aortic flow waveforms derived from aortic velocity and diameter measurements obtained using echocardiography (five-chamber view). In this cohort, we validated the independent and interactive relations between aortic haemodynamic parameters generated using the 'triangular' flow waveform and LVMI.

Aortic augmented pressure was determined using SphygmoCor software and identified as the difference

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between central aortic systolic BP and pressure at the first systolic peak of the aortic pulse wave (Figure S1, http://links.lww.com/HJH/A689). To avoid obtaining negative aortic augmentation indexes (AIx) values in young participants, AIx was determined as the pressure at the second systolic peak (P_2) of the aortic pulse wave/the pressure at the first systolic peak (P_1) of the aortic pulse wave expressed as a percentage (Figure S1, http://links.lww.com/HJH/A689). LV ejection duration was also determined from SphygmoCor software (time from the beginning of the forward wave to end systole identified from the dicrotic notch).

Echocardiography

LVM was determined from transthoracic two-dimensional targeted M-mode echocardiographic images obtained in the parasternal long-axis view as previously described [8,9,13,14,16]. Variables were analysed according to the American Society of Echocardiography convention [18]. LVM was determined using a standard formula [19] and indexed (LVMI) to body surface area. LV hypertrophy (LVH) was identified as an LVMI more than 95 g/m^2 for women and more than 115 g/m^2 for men. LV ejection fraction was calculated from [(end diastolic−end systolic volume)/end diastolic volume] × 100, where LV volumes were determined from the biplane Simpson rule as applied to LV internal dimensions assessed from a four-chamber view.

Statistical analysis

For database management and statistical analysis, SAS software, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) was employed. To in part control for variations in the type and duration of antihypertensive therapy in the primary analysis we included untreated participants together with participants receiving low-dose thiazide diuretic monotherapy for at least a year (the recommended first line agent in South Africa), which of those being treated, 48.4% were receiving. To ensure that relationships occurred independent of the use of antihypertensive therapy, sensitivity analysis was conducted in participants not receiving antihypertensive therapy. To determine relationships, multivariate linear (continuous analysis LVMI) or logistic (dichotomous LVH) regression analysis was performed with appropriate adjustments. Adjustments included in multivariate models were those correlated with central haemodynamic variables or LVMI in bivariate analysis. To determine probability values, further adjustments for nonindependence of family members was performed using nonlinear regression analysis (mixed procedure as defined in the SAS package). Regression coefficients were compared with Z statistics. To compare the performance of aortic haemodynamic variables for LVH detection, the area under the receiver operating curve (AUC) was evaluated.

RESULTS

Characteristics of participants

The clinical and demographic characteristics of the participants are shown in Table 1. A total of 1.9% of participants had a history of cardiovascular disease. Importantly, a high proportion (38.7%) of participants had hypertension, and 61.6% of hypertensive participants were not receiving therapy. Moreover, 32.5% of all participants and 58.7% of participants receiving antihypertensive therapy had uncontrolled hypertension. Of the participants with echocardiography 15.4% had LVH. Other than moderately greater proportions of participants treated for hypertension and hypertensive participants controlled to target BP, no differences in the clinical and demographic characteristics of the participants included in the echocardiographic substudy were noted as compared with those not included in the substudy (Table S1, http://links.lww.com/HJH/A689).

TABLE 1. Characteristics of the study sample

Characteristics	
Sample size (% women)	701 (63.2)
Age (years)	42.5 ± 17.6
BMI (kg/m^2)	28.9 ± 7.5
% obese	41.4
Regular tobacco (% study participants)	16.0
Regular alcohol (% study participants)	20.7
% with diabetes mellitus or HbA_{1c}>6.1%	22.2
% Hypertensive	38.7
% Treated for hypertension	14.8
% Hypertensives controlled to target BP	15.9
% of all with uncontrolled BP	32.5
Pulse rate (beats/min)	65.7 ± 11.3
Brachial SBP/DBP (mmHg)	128 ± 22/83 ± 12
Brachial pulse pressure (mmHg)	43 ± 15
Brachial mean arterial pressure (mmHg)	99 ± 15
Central aortic pulse pressure (mmHg)	34 ± 14
Aortic forward wave pressure (mmHg)	23.5 ± 8.4
Aortic reflected wave pressure (mmHg)	16.6 ± 7.6
Aortic augmented pressure	9.9 ± 7.3
Aortic augmentation index (%)	140.6 ± 25.0
Forward peak pressure time (ms)	125.6 ± 36.9
Aortic pulse reflection time (ms)	118.4 ± 14.1
Pulse pressure amplification	1.31 ± 0.18
Left ventricular mass index (g/m^2)	75.0 ± 29.0

Data expressed as mean ± SD or proportions.
BP, blood pressure; HbA_{1C}, glycosylated hemoglobin.

Factors associated with time to the peak of the aortic forward wave and reflection time

In stepwise regression models, the independent determinants of time to the peak of the aortic forward wave were age (partial $r = 0.31$, $P < 0.0001$), heart rate (HR) (partial $r = -0.20$, $P < 0.0001$) and female sex (partial $r = 0.13$, $P < 0.0001$). Moreover, the independent determinants of reflection time were age (partial $r = -0.50$, $P < 0.0001$), male sex (partial $r = 0.16$, $P < 0.0001$), HR (partial $r = 0.18$, $P < 0.0001$), and mean arterial pressure (partial $r = -0.14$, $P < 0.0001$).

Associations of time to the peak of the aortic forward wave or reflection time with aortic haemodynamics

In descending order of importance (comparison of partial r values using Z statistics), in a multivariate model, aortic backward wave pressure (partial $r^2 = 0.85$, $P < 0.0001$), forward wave pressure (partial $r^2 = 0.02$, $P < 0.0001$), time to the peak of the aortic forward wave (partial $r^2 = 0.003$,

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$P < 0.0005$) and reflection time (partial $r^2 = 0.0008$, $P < 0.05$) were independently associated with PPc. With [or without (data not shown)] adjustments for age, aortic augmented pressure was associated with time to the peak of the aortic forward wave (partial $r = 0.25$ $P < 0.0001$), reflection time (partial $r = -0.43$, $P < 0.0001$), and aortic backward wave pressure (partial $r = 0.85$, $P < 0.0001$). Moreover, with [or without (data not shown)] adjustments for age, AIx was associated with time to the peak of the aortic forward wave (partial $r = 0.41$, $P < 0.0001$), reflection time (partial $r = -0.63$, $P < 0.0001$), and aortic backward wave pressure (partial $r = 0.41$, $P < 0.0001$).

With adjustments for reflection time and the individual terms, an interaction between time to the peak of the aortic forward wave and aortic backward wave pressure was independently associated with aortic augmented pressure ($P < 0.01$). However, no interaction between reflection time and aortic backward wave pressure was independently associated with aortic augmented pressure ($P = 0.42$). Moreover, with adjustments for reflection time and aortic forward wave pressure and the individual terms, an interaction between time to the peak of the aortic forward wave and aortic backward wave pressure was independently associated with PPc ($P < 0.005$). In analysis conducted in 392 participants, where aortic backward wave pressure was calculated, with adjustments for the individual terms and aortic forward wave pressure, no interactions between reflection time and aortic backward wave pressure were independently associated with aortic augmented pressure or PPc.

Time to the peak of the aortic forward wave determines relations between backward waves and aortic pressures

Although time to the peak of the aortic forward wave contributed to only a small proportion of the variation in aortic augmented pressure or PPc, with adjustments for reflection time (for aortic augmented pressure) or reflection time and aortic forward wave pressure (for PPc), the independent association of the time to the peak of the aortic forward wave–aortic backward wave pressure interaction and aortic augmented pressure or PPc translated into incremental increases in the slope of the relationship between aortic backward wave pressure and aortic augmented pressure or between aortic backward wave pressure and PPc across quartiles of time to the peak of the aortic forward wave (Fig. 1). In this regard, the highest quartile of time to the peak of the aortic forward wave showed a greater slope of the aortic backward wave pressure-aortic augmented pressure or aortic backward wave pressure–PPc relations than the lowest two quartiles of time to the peak of the aortic forward wave (Fig. 1).

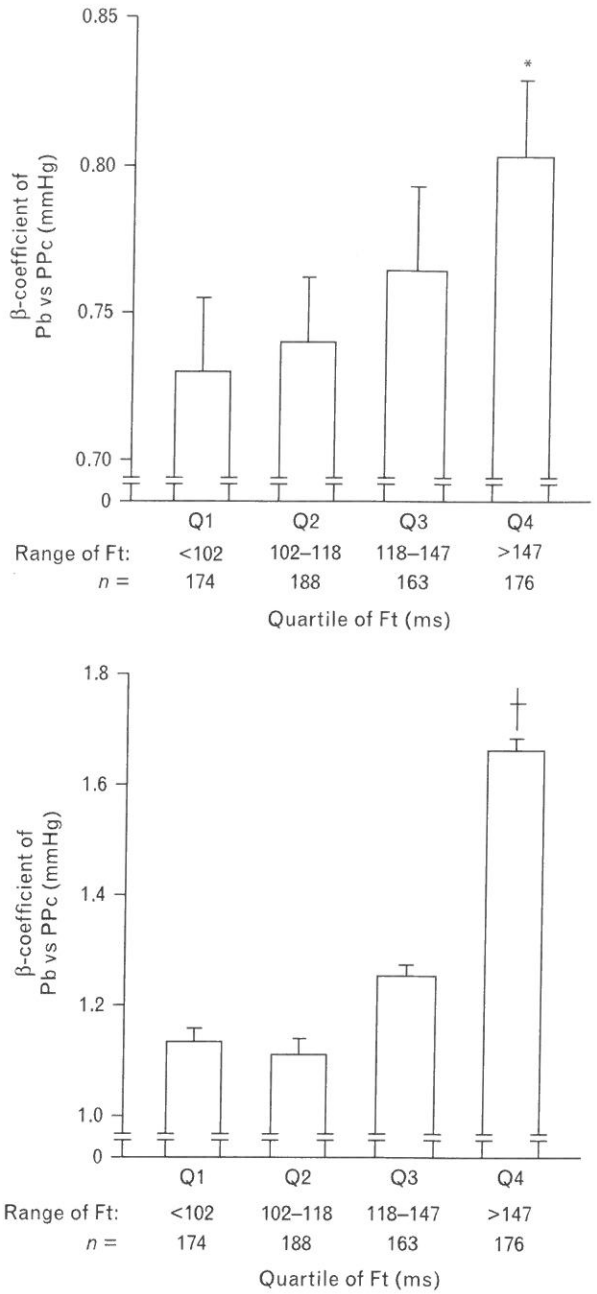

FIGURE 1 Slopes (β-coefficients) of the multivariate adjusted relations between Pb and Pa or central aortic pulse pressures (PPc) across quartiles of the time to the peak of the forward wave ($n = 701$). Adjustments are for the time of wave reflection in relations with Pa and both the time of wave reflection and aortic forward wave pressures in relations with PPc. All relations are significant at $P < 0.0001$. For comparison of slope: *$P < 0.05$ versus quartiles 1 and 2, †$P < 0.0001$ versus quartiles 1, 2 and 3. Ft, time to the peak of the aortic forward wave; Pa, aortic augmented pressures; Pb, aortic backward wave pressure.

Associations of time to the peak of the aortic forward wave or reflection time with left ventricular mass and hypertrophy

In multivariate models, PPc, aortic backward wave pressure, aortic forward wave pressure, but not reflection time or time to the peak of the aortic forward wave were independently associated with LVMI (Table 2, S2 and S3, http://links.lww.com/HJH/A689), with aortic backward wave pressure demonstrating stronger relations with LVMI than aortic forward wave pressure (Table 2 and S3, http://links.lww.com/HJH/A689). Importantly, similar relations were noted between aortic forward wave pressure or aortic backward wave pressure and LVMI when aortic backward wave pressure or aortic forward wave pressure were derived from wave separation analysis employing actual

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TABLE 2. Multivariate adjusted relations between aortic haemodynamics and left ventricular mass index

LVMI versus	Adjustors	All participants ($n=701$) Partial r^2 (95% CI)	*P* value	Untreated participants ($n=597$) Partial r^2 (95% CI)	*P* value
Individual terms					
PPc	[a]	0.20 (0.13–0.27)	<0.0001	0.25 (0.17–0.32)	<0.0001
Pb	[a]	0.20 (0.12–0.27)[b]	<0.0001	0.26 (0.18–0.33)[b]	<0.0001
Pf	[a]	0.11 (0.03–0.18)	<0.005	0.16 (0.08–0.24)	<0.0005
Ft	[a]	−0.01 (−0.08 to 0.06)	=0.79	−0.01 (−0.08 to 0.08)	=0.93
Rt	[a]	−0.01 (−0.08 to 0.06)	=0.78	−0.01 (−0.09 to 0.07)	=0.82
Interactive terms					
PPc*Ft	PPc, Ft[a]	0.09 (0.02–0.16)	=0.01	0.10 (0.01–0.18)	=0.02
Pb*Ft	Pb, Ft[a]	0.11 (0.03–0.18)	<0.01	0.09 (0.01–0.17)	=0.02
Pb*Rt	Pb, Rt[a]	−0.03 (−0.11 to 0.04)	=0.41	−0.02 (−0.10 to 0.07)	=0.70

PPc*Ft, Pb*Ft, Pb*Rt indicate interactions between these terms.
CI, confidence interval; Ft, forward peak pressure time; Pb, backward wave pressure; Pf, forward wave pressure; PPc, central aortic pulse pressure; Rt, aortic pulse reflection time.
[a]Adjustments are for age, sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an $HbA_{1c} > 6.1\%$, and the individual terms in interactive analysis (as indicated).
[b]$P < 0.05$ versus Pf.

flow measurements versus aortic backward wave pressure or aortic forward wave pressure derived from the triangular waveform approach to wave separation analysis (Table S3, http://links.lww.com/HJH/A689 versus Table 2). Although aortic backward wave pressure was independently associated with LVH, neither PPc, aortic forward wave pressure, reflection time nor time to the peak of the aortic forward wave were independently associated with LVH (Table 3). Although neither reflection time nor time to the peak of the aortic forward wave were independently associated with LVMI or LVH, independent of the individual terms and confounders, an interaction between time to the peak of the aortic forward wave and aortic backward wave pressure and between time to the peak of the aortic forward wave and PPc were independently associated with LVMI (Table 2 and S3, http://links.lww.com/HJH/A689) and similar interactions were noted in both men and women (Table S4, http://links.lww.com/HJH/A689). However, no interaction between reflection time and aortic backward wave pressure was independently associated with LVMI (Table 2 and S3, http://links.lww.com/HJH/A689). An interaction between time to the peak of the aortic forward wave and aortic backward wave pressure and between time to the peak of the aortic forward wave and PPc was also independently associated with LVH (Table 3). Importantly, with adjustments for the same confounders given in Table 2, no significant interactions between HR and aortic backward wave pressure (partial $r=0.03$, $P=0.43$) or between ejection duration and aortic backward wave pressure (partial $r=-0.04$, $P=0.31$) were independently associated with LVMI. Moreover, no interaction between aortic forward wave pressure and time to the peak of the aortic forward wave (partial $r=0.04$, $P=0.28$), was independently associated with LVMI.

Time to the peak of the aortic forward wave determines the impact of backward waves on left ventricular mass and hypertrophy

The independent associations between the time to the peak of the aortic forward wave-aortic backward wave pressure or time to the peak of the aortic forward wave–PPc interactions and LVMI translated into a stepwise increase in the strength (Figure S4, http://links.lww.com/HJH/A689) and magnitude (Fig. 2) of the associations between aortic backward wave pressure or PPc and LVMI across quartiles of time to the peak of the aortic forward wave, with the highest quartile of time to the peak of the aortic forward wave showing markedly stronger relations than the lowest two quartiles of time to the peak of the aortic forward wave. In stepwise regression models, although several factors were independently associated with LVMI across quartiles of time to the peak of the aortic forward wave

TABLE 3. Multivariate adjusted relations between aortic haemodynamics and left ventricular hypertrophy

LVH versus	Adjustors	All participants ($n=701$) Odds ratio[a] (95% CI)	*P* value	Untreated participants ($n=597$) Odds ratio[a] (95% CI)	*P* value
Individual terms					
PPc	[a]	1.013 (0.995–1.032)	=0.16	1.022 (1.001–1.044)	<0.05
Pb	[a]	1.040 (1.001–1.079)	<0.05	1.069 (1.023–1.116)	<0.005
Pf	[a]	1.012 (0.986–1.039)	=0.37	1.026 (0.997–1.057)	=0.08
Ft	[a]	0.998 (0.993–1.004)	=0.58	0.996 (0.989–1.004)	=0.30
Rt	[a]	0.995 (0.978–1.012)	=0.55	0.992 (0.974–1.011)	=0.42
Interactive terms					
PPc*Ft	PPc, Ft[a]	1.001 (1.000–1.001)	<0.01	1.001 (1.000–1.001)	=0.06
Pb*Ft	Pb, Ft[a]	1.001 (1.000–1.002)	<0.01	1.001 (1.000–1.002)	<0.05
Pb*Rt	Pb, Rt[a]	1.000 (0.998–1.002)	=0.77	1.001 (0.999–1.003)	=0.38

PPc*Ft, Pb*Ft, Pb*Rt indicate interactions between these terms.
CI, confidence interval; Ft, forward peak pressure time; Pb, backward wave pressure; Pf, forward wave pressure; PPc, central aortic pulse pressure; Rt, aortic pulse reflection time.
[a]Adjustments are for age, sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an $HbA_{1c} > 6.1\%$, and the individual terms in interactive analysis (as indicated).

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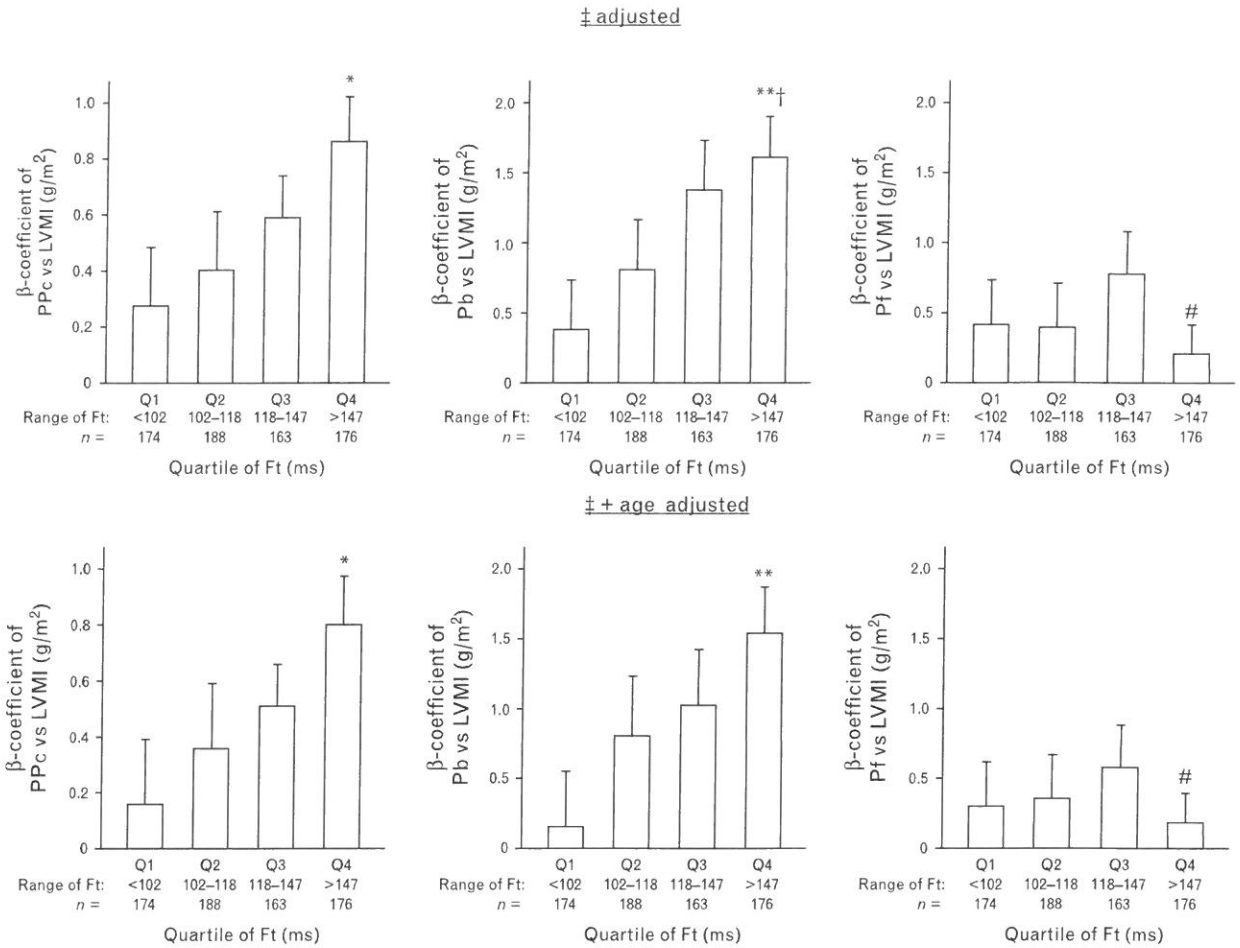

FIGURE 2 Slopes (β-coefficients) of the multivariate adjusted relations between PPc, Pb, or Pf and LVMI across quartiles of the Ft (n = 701). ‡Adjustments are for age as indicated and sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus, or an percentage glycated haemoglobin more than 6.1%. For significance of relations see figure S2. For comparison of slope: $^{*}P < 0.05$, $^{**}P < 0.01$ versus quartile 1, $^{\dagger}P < 0.05$ versus quartile 2. $^{\#}P < 0.0005$ versus slope of aortic backward wave pressure–LVMI relationship in quartile 4. see Table 2 for abbreviations.

(Table S5, http://links.lww.com/HJH/A689), the factor most strongly associated with LVMI in the highest quartile of time to the peak of the aortic forward wave was aortic backward wave pressure, whereas the contribution of aortic backward wave pressure in the lowest quartile of time to the peak of the aortic forward wave was negligible. Similar findings were noted for PPc (data not show). The independent associations between the time to the peak of the aortic forward wave-aortic backward wave pressure or time to the peak of the aortic forward wave–PPc interactions and LVH also translated into an increase in the associations between aortic backward wave pressure or PPc and LVH in the highest as compared with the other three quartiles of time to the peak of the aortic forward wave (Table 4). In contrast, relations between aortic forward wave pressure and LVMI (Fig. 2) or LVH (Table 4) did not differ across quartiles of time to the peak of the aortic forward wave. As compared with aortic forward wave pressure-LVMI or aortic forward wave pressure-LVH relations, markedly stronger relations with a greater magnitude of the effect of aortic backward wave pressure on LVMI or LVH was noted in the highest quartile of time to the peak of the aortic forward wave only (Fig. 2, S5, S6, http://links.lww.com/HJH/A689 and Table 4). Aortic backward wave pressure was also more closely associated with LVMI in the highest versus the lowest quartile of ejection duration, but the relationship between aortic backward wave pressure was not more closely associated with LVMI in the lowest as compared to any of the other three quartiles of HR (Table S6, http://links.lww.com/HJH/A689). Adjustments for aortic augmented pressure only partially modified the incremental slope of the relations between aortic backward wave pressure and LVMI across quartiles of time to the peak of the aortic forward wave (Figure S6, http://links.lww.com/HJH/A689). No stepwise increases in the strength and magnitude of the association between aortic backward wave pressure and LVMI across quartiles of reflection time were noted (data not shown).

The independent association between the time to the peak of the aortic forward wave-aortic backward wave pressure or time to the peak of the aortic forward wave-PPc interactions and LVH translated into a greater performance of aortic backward wave pressure and PPc for LVH detection in the highest as compared to the first three quartiles of Time to the peak of the aortic forward wave (Fig. 3). In contrast, the ability of aortic forward wave

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TABLE 4. Multivariate adjusted relations between central aortic pulse pressure, backward wave pressures or forward wave pressures and left ventricular hypertrophy across quartiles of the time to the peak of the aortic forward wave

Quartiles of Ft→	1			2			3			4		
Range of Ft	<102			102 to 118			118 to 147			>147		
N = (% LVH)	174 (17)			188 (13)			163 (15)			176 (17)		
LVH versus	OR (95% CI)[a]	Wald X^2	*P* value	OR (95% CI)[a]	Wald X^2	*P* value	OR (95% CI)[a]	Wald X^2	*P* value	OR (95% CI)[a]	Wald X^2	*P* value
PPc	0.996 (0.947–1.045)	0.03	=0.86	0.978 (0.927–1.030)	0.70	=0.40	1.003 (0.964–1.036)	0.03	=0.86	1.062 (1.012–1.119)[b]	5.60	<0.05
Pb	0.989 (0.908–1.074)	0.07	=0.80	0.976 (0.887–1.072)	0.25	=0.62	0.994 (0.915–1.070)	0.02	=0.88	1.144 (1.045–1.264)[b,c]	7.81	<0.01
Pf	1.010 (0.940–1.081)	0.08	=0.77	0.955 (0.881–1.029)	1.37	=0.24	1.042 (0.976–1.116)	1.59	=0.21	1.015 (0.964–1.059)[d]	0.40	=0.53

CI, confidence interval; Ft, forward peak pressure time; OR, Odds ratio; Pb, backward wave pressure; Pf, forward wave pressure; PPc, central aortic pulse pressure.
[a]Adjustors included in logistic regression models are age, sex, mean arterial pressure, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1%.
[b]$P<0.05$ compared with quartiles 1 and 2.
[c]$P<0.05$ compared with quartiles 1, 2 and 3.
[d]$P<0.005$ compared with OR for Pb versus LVH in quartile 4.

pressure to detect LVH did not differ across quartiles of time to the peak of the aortic forward wave (Fig. 3).

Correlation between time to the peak of the aortic forward wave and systolic function

No correlation between and time to the peak of the aortic forward wave and LV ejection fraction was noted ($r=-0.0001$, $P=1.00$).

DISCUSSION

The main findings of the present study are as follows: in a relatively large community-based sample of adults either receiving no antihypertensive therapy or receiving stable low-dose thiazide diuretic therapy for at least a year, we show that the time to the peak of the aortic forward wave, but not the time of wave reflection, has a major impact on relations between backward wave pressures and aortic augmented or pulse pressures and between backward wave or aortic pulse pressures and LVMI or LVH. In this regard, across quartiles of an increasing duration of time to the peak of the forward wave an increase in relations between the magnitude of backward wave pressures and augmented pressures or PPc and between the magnitude of backward wave or aortic pulse pressures and LVMI or LVH were noted. Importantly, backward wave pressures and PPc were only independently associated with LVH in the highest quartile of the time to the peak of the aortic forward wave.

Aortic pressure augmentation is mediated by several factors including an increased magnitude of backward wave pressures, an enhanced speed of wave reflection and an extended time to the peak of the aortic forward wave. Although the magnitude of backward wave pressure is a well established cause of adverse effects on the cardiovascular system [4–9], the exact role of the timing of forward and backward waves in contributing to the adverse effects in adults of backward wave pressure, is poorly documented. Although heart failure with a preserved ejection fraction is associated with prolonged mechanical systole [12], the extent to which the time to the peak of the forward wave contributes to producing cardiac changes in heart failure is unknown. In the present study, the time to peak of the forward wave determined the impact of backward wave pressures on LVMI and LVH, a cardiac change that is well accepted as progressing to heart failure with either a preserved or reduced ejection fraction. Hence, the present results provide a possible mechanism that may explain the relationship between a prolonged mechanical systole and heart failure with a preserved ejection fraction [12].

Although β-adrenergic receptor blockade extends the systolic period and augments PPc [11], the extent to which a greater impact of backward waves explains the reduced efficacy of β-adrenergic receptor blocking agents in improving outcomes compared with alternative antihypertensive agents has not been reported on. In this regard, prolonged mechanical systole may be associated with an increased stroke volume which could produce adverse effects through an enhanced forward wave pressure. In contrast, a prolonged mechanical systole may increase the

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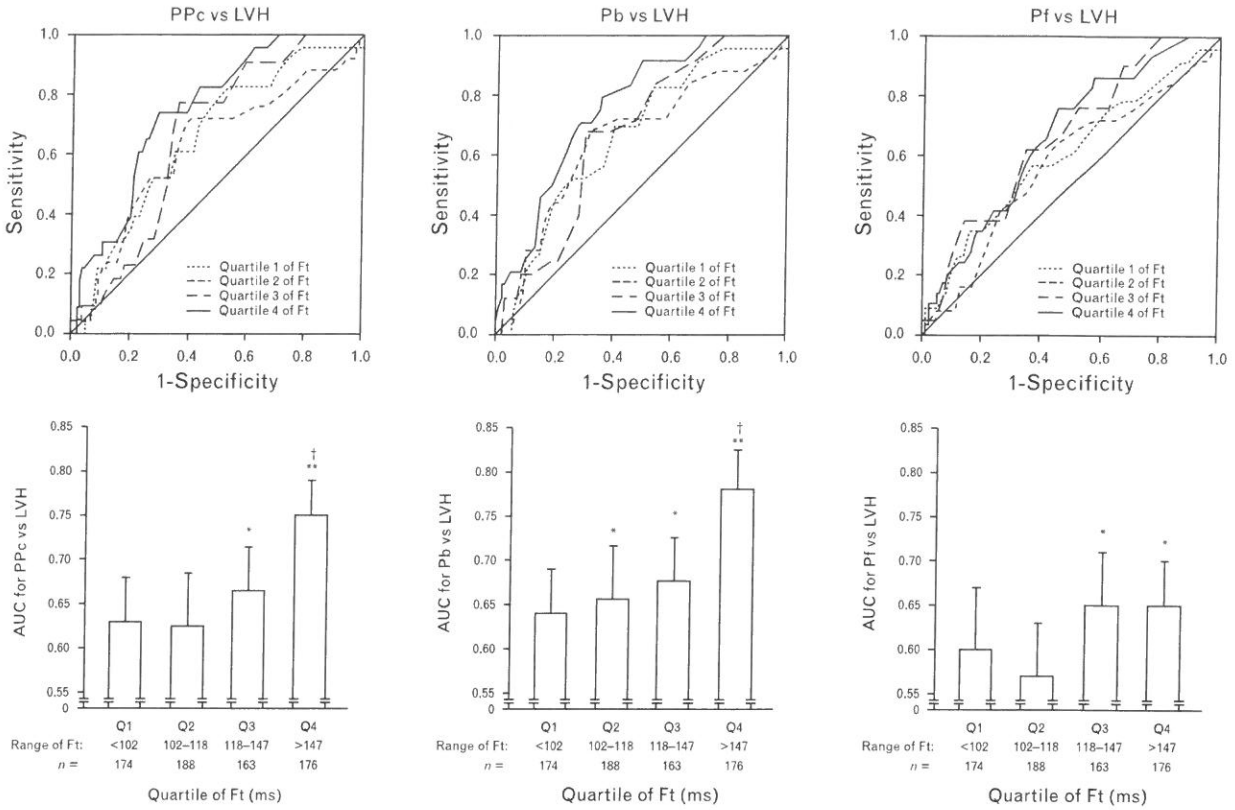

FIGURE 3 Receiver-operating characteristic curves and the area under the curve (AUC ± SEM) for the detection of LVH ($n = 108$ of 701) of PPc Pb and Pf wave pressures across quartiles of the Ft. $^{*}P < 0.05$, $^{**}P < 0.0005$ for AUC of receiver-operating characteristic curves; $^{\dagger}P < 0.05$ versus quartiles 1 and 2. Ft, time to the peak of the aortic forward wave; LVMI, left ventricular mass index; Pb, aortic backward wave pressure; Pf, forward wave pressure, PPc, central aortic pulse pressures.

chances of the backward wave overlapping with the forward wave, thus augmenting the deleterious impact of backward waves on cardiovascular end organs. The present study provides the first evidence to show that relations between aortic backward wave pressures (and hence PPc) and cardiac end-organ changes (LVMI and LVH) are strongly determined by the time to the peak of the aortic forward wave. In contrast, relations between forward wave pressures and LVMI or LVH did not depend on the time to the peak of the aortic forward wave. Hence, the present study suggests that prolonged mechanical systole mediates adverse effects on the heart by enhancing the deleterious impact of aortic backward wave and pulse pressures on cardiovascular end organs.

In the present study, the independent associations between aortic backward wave pressure and LVMI or LVH at higher time to the peak of the aortic forward wave values were only partially accounted for by augmented pressures. In this regard, indexes of aortic pressure augmentation should not be seen as an accurate composite of the adverse effects of the time to the peak of the forward wave and the magnitude of backward wave pressures. Indeed, augmented pressures also incorporate the impact of the speed of wave reflection which, as indicated in the present study, plays little role in contributing to the impact of backward wave pressures on LVMI and LVH. Moreover, there are now several lines of evidence to suggest that indexes of pressure augmentation are influenced by factors other than backward wave pressures or the timing of pressure waves [20–23] and these factors may confound relations between backward wave pressures and end-organ measures. Indeed, augmented pressures and augmentation index underestimate the effect of backward waves on aortic pulse pressure and LVMI [8].

In the present study, we were unable to show significant independent associations between ejection duration-aortic backward wave pressure or HR–aortic backward wave pressure interactions and LVMI or LVH. These findings may be explained by the weak correlations between the time to the peak of the forward wave and either ejection duration ($r^2 = 0.10$) or HR ($r^2 = -0.04$). The present data therefore indicate that when identifying potential adverse effects of backward waves on the heart, neither ejection duration (which may be determined from electrocardiography) nor HR may be employed as accurate surrogates for the time to the peak of the forward wave. Nevertheless, as with the stronger independent correlations noted between aortic backward wave pressure and LVMI in the highest as compared with the lowest quartile of time to the peak of the aortic forward wave, stronger independent correlations were also noted between aortic backward wave pressure and LVMI in the highest as compared with the lowest quartile of ejection duration. Hence, although wave separation analysis may be the most accurate method of identifying wave timing effects on backward wave and hence aortic pulse pressure end-organ relations, in the absence of wave separation analysis, ejection duration may be considered as a proxy for time to the peak of the forward wave.

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Whether ejection duration guides the effects of PPc calculated from simple clinical measures (imputed PPc), which has been demonstrated to predict outcomes beyond brachial pressures in several studies [24–26] requires further evaluation. In this regard, imputed PPc and ejection duration (obtained from electrocardiography) may be acquired at no additional cost and hence, would be ideally suited for use in resource-limited settings.

As with previous reports, [8,9], the results of the present study suggest that aortic backward wave pressures are more important than forward wave pressures in contributing to the adverse effect of PPc on the heart. The results of the present study nevertheless extend these findings by suggesting that the impact of backward wave pressure and hence PPc on the heart occurs mainly in those with an extended time to peak systole (highest quartile of time to peak systole). Indeed, only in the highest quartile of the time to peak systole was backward wave pressure and PPc associated with LVH independent of mean arterial pressure (distending pressures) and several additional confounders including age.

Although there is increasing evidence that aortic backward wave pressures are key elements in cardiovascular risk prediction or preclinical end-organ changes [5–9], this finding has been inconsistent [4,27]. The present results suggest that the impact of backward waves on LVMI or LVH will occur only in those with an extended time to the peak of the aortic forward wave. In those studies that failed to show an impact of aortic BP on risk beyond brachial BP [4,27] whether a significant proportion of participants had an extended systolic period comparable with that noted in the highest quartile of time to the peak of the forward wave in the present study, is unknown. The present results suggest that aortic backward wave and pulse pressure effects on cardiovascular end organs should always be interpreted in the context of time to peak forward wave pressures. In this respect, an extended time to peak forward wave pressure, such as may occur with several agents that influence HR (such as β-adrenergic receptor blockers), in conditions associated with long QT (electrocardiographic QT interval) syndrome, or in the elderly who have a prolonged mechanical systole, may increase the impact of backward waves on the heart. If confirmed in outcome-driven studies, under these circumstances it is possible that only in those with an extended time to the peak of the forward wave will aortic pressures be particularly useful in risk predicting beyond brachial BP.

One possible interpretation of our findings is that a prolonged time to the peak of the aortic forward wave may reflect the impact of a reduced systolic function (which results in an increased LVMI), which attenuates the ratio of the preejection period (isovolumic contraction time)/ejection time. In this case, the impact of time to the peak of the aortic forward wave would be an epiphenomenon rather than being causally related to the impact of aortic backward wave pressure on LVMI. However, in the present study no correlation between time to the peak of the aortic forward wave and LV ejection fraction was noted. Hence, at present the only explanation for our data is that a prolonged time to the peak of the aortic forward wave increases the chances that the backward wave augments aortic pulse pressure and consequently LVMI.

There are several limitations to the present study that warrant consideration. First, the present study was a cross-sectional design. Hence, we cannot determine whether associations reported on are indeed cause and effect. Further longitudinal studies are therefore required. Second, the assumptions intrinsic to the use of the 'triangulation method' of aortic wave separation may not be ideal [28]. However, in 392 participants of the present sample with aortic velocity measurements, we were able to show a correlation (r^2) between aortic backward wave pressure derived from the 'triangulation method' versus 'actual aortic flow' methods of wave separation of 0.82. Moreover, we show similar relations (as compared with when using the triangular waveform approach to wave separation analysis) between aortic haemodynamic parameters derived from wave separation analysis using the 'actual aortic flow' method and LVMI. Hence, at least in the present study, the use of the 'triangulation method' is unlikely to have significantly affected our results. Third, in the present study, calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries [29]. Hence, aortic pressures are likely to have been underestimated using the current approach. However, aortic forward wave pressure would have been affected as much by this limitation as aortic backward wave pressure, and time to the peak of the aortic forward wave-aortic backward wave pressure, but not time to the peak of the aortic forward wave–aortic forward wave pressure interactions were independently associated with LVMI and LVH. Fourth, we were not statistically powered to compare slopes (magnitude) of aortic pressure versus LVMI relations in only untreated participants and hence our results may have been affected by the impact of therapy. However, by excluding participants on agents other than low-dose thiazide diuretic therapy, more than a single agent, or receiving therapy for less than a year, we have to some degree controlled for variations in the type and duration of therapy. In addition, in sensitivity analysis conducted only in untreated participants, we show essentially the same results for interactive analysis and when comparing the strength of relations between aortic function and LVMI across quartiles of time to the peak of the aortic forward wave.

In conclusion, in the present study we show that the impact of aortic backward wave pressures on LVMI and LVH, are strongly influenced by the time to the peak of the aortic forward wave. Only in the highest quartile of time to the peak of the aortic forward wave, did backward wave pressures show strong independent associations with both LVMI and LVH. Moreover, only in the highest quartile of time to the peak of the aortic forward wave, did backward wave pressures show stronger independent associations with both LVMI and LVH than forward wave pressures. Thus, the adverse effects of aortic backward wave pressures on cardiovascular end organs may be strongly determined by the time to the peak of the aortic forward wave.

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Conflicts of Interest

There are no conflicts of interest.

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Reviewer's Summary Evaluation

Referee 2

The results of this study showed that the relation between aortic backward wave pressures and left ventricular mass index or left ventricular hypertrophy is strongly affected by the time to the peak of the aortic forward wave. Further studies will have to exclude the hypothesis that a prolonged time to the peak of the aortic forward wave is an epiphenomenon secondary to a prolonged isovolumic contraction time associated with early left ventricular dysfunction.

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Enhanced Aortic Reflected Wave Magnitude Accounts for the Impact of Female Gender on Aortic Pressure Augmentation in a Group of African Ancestry

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BACKGROUND

Aortic reflected wave magnitude (RM) may not account for sex-specific differences in aortic pressure augmentation in Caucasians. However, aortic reflected waves are greater in groups of African descent than other ethnic groups. We determined whether RM or alternative factors explain the impact of sex on aortic augmented pressure (Pa) in participants of African ancestry.

METHODS

We assessed aortic function (radial applanation tonometry, SphygmoCor) in 1,197 randomly recruited community participants of African ancestry (age ≥ 16 years). Aortic forward (Pf) and backward (Pb) wave separation was performed assuming an aortic triangular flow wave validated against aortic velocity measurements.

RESULTS

Across the adult lifespan, women had greater multivariate-adjusted augmentation index (AIx) and Pa. This was associated with multivariate-adjusted age-related increases in Pb, RM (Pb/Pf), and time to the peak of Pf and decreases in backward wave foot time; but not increases in Pf. With adjustors, Pa was associated with female gender (β-coefficient = 3.81 ± 0.34), a relationship which was markedly attenuated by adjustments for RM (β-coefficient = 1.78 ± 0.31, $P < 0.0001$ vs. without adjustments for RM), and Pb (β-coefficient = 2.05 ± 0.19, $P < 0.0001$ vs. without adjustments for Pb), but not by adjustments for Pf, time to the peak of Pf, or backward wave foot time. Similarly, AIx was associated with female gender, a relationship which was markedly attenuated by adjustments for RM, Pb, and backward wave foot time, but not alternative factors.

CONCLUSIONS

In contrast to reports in alternative populations, the relationship between aortic pressure augmentation and female gender in participants of African descent is accounted for mainly by increases in RM.

Keywords: aortic augmentation index; aortic reflected waves; blood pressure; hypertension; sex.

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Pulse pressure (PP) is a strong determinant of cardiovascular damage beyond steady-state pressures and as such is incorporated in guidelines for risk prediction.[1] Several aspects of aortic function regulate central arterial PP including aortic stiffness and backward wave pressures.[2–4] The relative contribution of backward traveling waves to central arterial PP is identified as augmentation index (AIx) and AIx (or augmented pressure [Pa]) is in some studies, an independent predictor of cardiovascular outcomes.[5] Whilst increases in AIx have traditionally been attributed to increases in aortic stiffness which enhance pulse wave velocity and cause premature arrival of backward waves in systole, several alternative factors have been identified as contributing more to variations in AIx or Pa than aortic stiffness. In this regard, factors that influence the forward traveling wave amplitude,[6–9] the magnitude of the forward wave,[10] the magnitude of the backward wave,[10] forward wave peak width,[10] and the slope of the backward wave upstroke,[10] all contribute to variations in AIx. Although a shorter stature, and hence an earlier return of reflected waves has generally been the explanation for an increased AIx in women, more recently an increased forward wave peak width, slope of the backward pressure wave, and forward wave amplitude, but not backward wave amplitude have been suggested to be the main determinants of an increased AIx in women of the Framingham Heart study.[10] Whether the same factors explain sex differences in AIx or Pa in alternative population groups is nevertheless uncertain.

Whilst increases in the magnitude of the forward wave exceed that of the backward wave in explaining age-related increases in aortic PP in the Framingham study,[11] the magnitude of backward waves exceeds that of the forward wave in contributing to age-related variations in aortic PP in a community of African ancestry.[12] Furthermore, in groups of

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African ancestry backward waves contribute more to variations in measures of cardiovascular end-organ damage than forward waves.[12,13] However, whether increases in backward wave magnitude account for sex differences in AIx or Pa in groups of African descent has not been determined. As the factors that determine sex-related differences in AIx may assist in identifying targets for therapeutic interventions, it is important to assess whether backward wave magnitude explains sex differences in AIx in groups of African descent. Hence, in the present study, we assessed the relative contribution of aortic forward and backward wave magnitude and timing to the relationship between sex and AIx or Pa in a relatively large community-based sample of African ancestry.

METHODS

Study group

The present study was conducted according to the principles outlined in the Helsinki declaration. The Human Research Ethics Committee of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The present study design has previously been described.[14–17] Briefly, 1,197 participants from randomly recruited (from the population census figures of 2001) families of Black African descent (Nguni and Sotho chiefdoms) from the South West Township (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years with central aortic hemodynamic measurements were evaluated.

Clinical, demographic, and anthropometric measurements

A questionnaire was administered to obtain demographic and clinical data.[14–17] Height and weight were measured using standard approaches and participants were considered to be overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. High-quality brachial blood pressure (BP) measurements were obtained as previously described[15] in the seated position after 5 minutes of rest by a trained nurse-technician using a standard mercury sphygmomanometer 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 BP ≥140/90 mm Hg or the use of antihypertensive medication. Laboratory blood tests of renal function, liver function, blood glucose, hematological parameters, and percentage glycated hemoglobin (HbA_{1c}) were performed. Diabetes mellitus (DM) or an abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA_{1c} value greater than 6.1%.

Pulse wave analysis

Central aortic hemodynamics were estimated using pulse wave and wave separation analysis as previously described.[12,13,16,17] 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, Houston, TX) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty, West Ryde, New South Wales, Australia). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software. Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded. Central aortic PP (PPc) was determined as the difference between aortic systolic BP and diastolic BP. Mean arterial pressure was determined using SphygmoCor software. Aortic Pa was determined as the difference between the pressures at the second and the first systolic peaks of the aortic pulse wave. To avoid obtaining negative aortic AIx values in young participants, AIx was determined as the pressure at the second systolic peak of the aortic pulse wave/the pressure at the first systolic peak of the aortic pulse wave expressed as a percentage. Aortic backward (Pb) and forward (Pf) wave pressures were determined from wave separation analysis of the SphygmoCor-derived aortic waveform using a "triangular flow wave" (also from SphygmoCor software).[18] The reflection magnitude (RM) (sometimes referred to as the reflection coefficient or reflection factor) was calculated as Pb/Pf. Backward wave foot time, and time to the peak of the forward wave, were also determined from SphygmoCor software.

Data analysis

For database management and statistical analysis, SAS software, version 9.3 (SAS Institute, Cary, NC) was employed. To compare aortic hemodynamics between women and men at similar ages and accounting for confounders within each age range, aortic hemodynamic variables were compared at 10-year increments in age from 20 years of age upward with adjustments for variations in age, age^2, mean arterial pressure, pulse rate, BMI, regular smoking, regular alcohol intake, and the presence of DM or an HbA_{1c} >6.1% within each 10-year age category. Stepwise multiple linear regression analysis was performed to determine the independent relations between aortic hemodynamic parameters. Also included in the models were age, age^2, sex, mean arterial pressure, pulse rate, BMI, regular smoking, regular alcohol intake, the presence of DM or an HbA_{1c} >6.1%, and treatment for hypertension. To determine probability values, further adjustments for nonindependence of family members was performed using nonlinear regression analysis (mixed procedure as defined in the SAS package). To ensure that the results were not influenced by the effects of antihypertensive therapy, which can markedly modify aortic hemodynamics, sensitivity analysis was conducted in participants not receiving antihypertensive therapy. Moreover, to ensure that the use of the "triangular flow wave" to separate the forward and backward waves did not affect the interpretation of the data, sensitivity analysis was also conducted in only those participants in whom the actual aortic flow wave was employed to separate the forward and backward waves.

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RESULTS

Characteristics of the participants

The clinical and demographic characteristics of the female and male participants are shown in Table 1. Two percent of participants had a history of cardiovascular disease. Importantly, a high proportion of participants had hypertension, and 37.7% of hypertensive women and 63.6% of hypertensive men were not receiving antihypertensive therapy. Moreover, 60.5% of women and 63.4% of men receiving therapy had an uncontrolled BP. Women were markedly more obese.

Sex differences in aortic hemodynamic variables

With adjustments for age, age^2, mean arterial pressure, BMI, pulse rate, regular smoking, regular alcohol, DM or an HbA_{1c} >6.1%, and treatment for hypertension, females were noted to have an increased PPc, Pa, AIx, Pb, RM, and time to the peak of the forward wave and decreased backward wave foot time as compared to men (Tables 2, Supplementary Tables S1 and S2). However, women and men had a similar Pf (Tables 2, Supplementary Tables S1 and S2).

Sex differences in aortic hemodynamics across the adult age range

With adjustments within each age category for age, age^2, mean arterial pressure, BMI, pulse rate, regular smoking, regular alcohol, DM or and HbA_{1c} >6.1%, and treatment for hypertension, females were noted to have an increased Pa and AIx as compared to males across the adult lifespan (Figures 1 and 2). Whilst in men, Pa and AIx increased across the adult life span, in women, increases in Pa and AIx were noted until 40–50 years of age and thereafter they tended to remain similar or decrease (Figures 1 and 2). Whilst with adjustments, no marked sex differences in Pf were noted across the adult age range (Figures 1 and 2), multivariate-adjusted RM (Figures 1 and 2), Pb (Supplementary Figure S1), and time to the peak of the forward wave (Figures 1 and 2) were greater and backward wave foot time was lower in women as compared to men, with the biggest differences noted for RM (Figures 1 and 2) and Pb (Supplementary Figure S1).

Correlates of AIx and Pa

In stepwise regression models, Pa (Tables 3 and Supplementary Table S3) and AIx (Tables 4, Supplementary Tables S4 and S5) were independently associated with female gender. The inclusion of RM markedly reduced the slope (β-coefficient) of the relation between female gender and AIx or Pa (Tables 3 and 4, Supplementary Tables S3–S5). Similarly, the inclusion of Pb reduced the slope (β-coefficient ± SEM) of the relation between female gender and AIx (18.5 ± 1.3 to 15.1 ± 1.2) or Pa (3.81 ± 0.34 to 2.05 ± 0.19), whilst in these models Pb showed independent relations with AIx (β-coefficient ± SEM = 0.97 ± 0.09, $P < 0.0001$) or Pa (β-coefficient ± SEM = 0.73 ± 0.02, $P < 0.0001$). However, the inclusion of neither Pf nor the time to the peak of the forward wave, produced significant decreases in the slope (β-coefficient) of the relation between female gender and Pa or AIx and indeed Pf was inversely associated with AIx (Tables 4, Supplementary Tables S4 and S5). Although the inclusion of time to the foot of backward wave did attenuate relations between female gender and AIx (Tables 4 and Supplementary Tables S4 and S5), the inclusion of time to the foot of the backward wave did not significantly attenuate relations between female gender and Pa (Tables 3 and Supplementary Table S3).

DISCUSSION

The main findings of the present study are as follows: In a randomly selected community sample of African ancestry,

Table 1. Characteristics of women and men in the study sample

	Women	Men	*P* value
Sample size	781	416	
Age (years)	44.7 ± 17.7	43.4 ± 19.5	0.25
Body mass index (kg/m^2)	32.0 ± 8.2	24.9 ± 5.2	<0.0001
% Obese	58.0	16.4	<0.0001
Regular tobacco (% subjects)	6.2	32.6	<0.0001
Regular alcohol (% subjects)	13.4	34.1	<0.0001
% with DM or HbA_{1c} > 6.1%	28.4	20.5	<0.005
% Hypertensive	45.2	45.9	0.81
% Treated for hypertension	28.2	17.3	<0.0001
Pulse rate (beats/min)	68 ± 11	62 ± 11	<0.0001
Brachial SBP/DBP (mm Hg)	127 ± 23/83 ± 13	131 ± 23/85 ± 12	<0.005/<0.005
Mean arterial pressure (mm Hg)	99 ± 16	101 ± 15	<0.05
Brachial pulse pressure (PP) (mm Hg)	44 ± 15	46 ± 18	0.10

Data are expressed as mean ± SD or proportions. Abbreviations: BP, blood pressure; DBP, diastolic BP; DM, diabetes mellitus; HbA_{1c}, glycosylated hemoglobin; SBP, systolic BP.

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Table 2. Multivariate-adjusted aortic hemodynamic factors in women and men of a community of African ancestry

	Women	Men	*P* value
Sample size	781	416	
Central aortic SBP (mm Hg)	121 ± 7	119 ± 7	0.005
Central aortic PP (PPc) (mm Hg)	36 ± 11	34 ± 11	0.001
Aortic forward wave pressure (Pf) (mm Hg)	24 ± 8	25 ± 9	0.10
Aortic backward (reflected) wave pressure (Pb) (mm Hg)	18 ± 6	16 ± 6	<0.0001
Aortic reflected wave magnitude (RM = Pb/Pf)	76 ± 17	63 ± 18	<0.0001
Aortic augmented pressure (Pa) (mm Hg)	12 ± 5	8 ± 6	<0.0001
Aortic augmentation index (AIx) (%)	148 ± 19	129 ± 20	<0.0001
Time to peak forward wave (Ft) (ms)	132 ± 36	120 ± 38	<0.0001
Backward wave foot time (Bt) (ms)	115 ± 12	121 ± 13	<0.0001

Data are expressed as mean ± SD. Adjustments are for age, age^2, mean arterial pressure, pulse rate, body mass index, regular smoking, regular alcohol intake, the presence of diabetes mellitus or an HbA_{1c} > 6.1%, and treatment for hypertension. Probability values were further adjusted for nonindependence of family members using nonlinear regression analysis (mixed procedure).

augmented pressures and AIx were independently related to female gender and these gender-specific effects were attributed largely to the impact of backward wave pressures and magnitude, with little or inconsistent contributions from forward wave pressures, time to the peak of the forward wave, or backward wave foot time.

Several recent studies have underscored the potential importance of aortic backward (reflected) waves, as described from wave separation analysis, as a possible independent determinant of cardiovascular end-organ damage[12,13] and cardiovascular outcomes.[19–22] Although the backward wave, as indexed by the reflection factor (backward wave/forward wave pressures), has been demonstrated to play little role in the increases in augmented pressures and AIx in women as compared to men of the Framingham Heart Study,[10] whether the same holds true for alternative population groups is uncertain. In this regard, groups of African ancestry have a particularly high AIx as compared to Caucasian, American-Indian, and Chinese populations.[23] Moreover, in contrast to the Framingham Heart Study, where increases in the magnitude of the forward wave exceeds that of the backward wave in explaining age-related increases in aortic PP,[11] the magnitude of backward waves exceeds that of the forward wave in contributing to age-related variations in aortic PP in a community of African ancestry.[12] Hence, the question arises as to whether forward wave pressures and alternative factors contribute more than reflected wave magnitude or pressures to sex differences in AIx in groups of African ancestry. In the present study, we show a marked contribution of the RM and backward wave pressures with little contribution of the forward wave to the relationship between augmented pressures and AIx and female gender in a group of African ancestry. The present study therefore highlights the importance of targeting aortic backward rather than forward waves in females of African ancestry.

In the present study, the greater impact of adjustments for RM rather than Pb on the relationship between gender and either Pa or AIx is attributed to the fact that Pb is strongly influenced by Pf and hence that relations between Pb and AIx are in part attributed to the inverse relationship between Pf and AIx. Indeed, the correlation (r^2) between Pb and Pf in the present study sample was equal to 0.56 ($P < 0.0001$). To exclude forward wave effects on Pb, Pb was expressed as a proportion or percentage of Pf (RM). Hence, RM (Pb/Pf) was used as a simple approach to identifying the forward wave-independent effects of backward wave pressures.

Although the reasons for possible differences in the impact of backward vs. forward waves on the relationship between AIx and female gender in the present vs. prior[10] studies are unclear, several possibilities may be considered. In this regard, groups of African ancestry are well-recognized as being salt-sensitive and we have previously demonstrated that relations between salt-intake and BP in groups of African ancestry are mainly with PP, including aortic PP and AIx, rather than with steady-state pressure.[16] Thus, salt-intake may produce a pronounced effect on aortic backward wave pressures in groups of African descent and this may manifest in a sex-specific manner. Second, other than ethnic differences between the Framingham Heart Study[10] and the present study there are several demographic differences between these studies including a greater prevalence of hypertension and a higher BMI in women as compared to men in the present study. These factors were nevertheless in-part controlled for in multivariate adjustments (by adjusting for MAP and BMI in the models) and are therefore unlikely to explain differences between studies. In addition, in the present study sample, BMI was inversely associated with backward wave magnitude and pressure and as women were more obese than men, this is more likely to have attenuated RM and Pb in women. Third, there is some evidence that human immunodeficiency virus (HIV) infection is associated with an increased aortic AIx.[24] As the prevalence of HIV infection is estimated to be over 10% of the population in South Africa,[25] it is possible that HIV infection may contribute to AIx in the current study sample. In this regard, because we did not have access to pre- and post-test counselling, we could not perform routine HIV testing. However, there are no marked sex differences in HIV prevalence rates in adults.[25] Therefore, it is unlikely that HIV infection can explain sex-specific effects on AIx in the present study. Fourth, an important consideration is that in the prior study conducted in an alternative ethnic group,[10] the contribution of factors to the aortic pressure wave was assessed by modifying the forward wave while keeping the reflected wave constant. This analysis[10] was conducted without first assessing whether the modified forward flow wave, when summed to the backward flow wave, provided a realistic flow. Hence,

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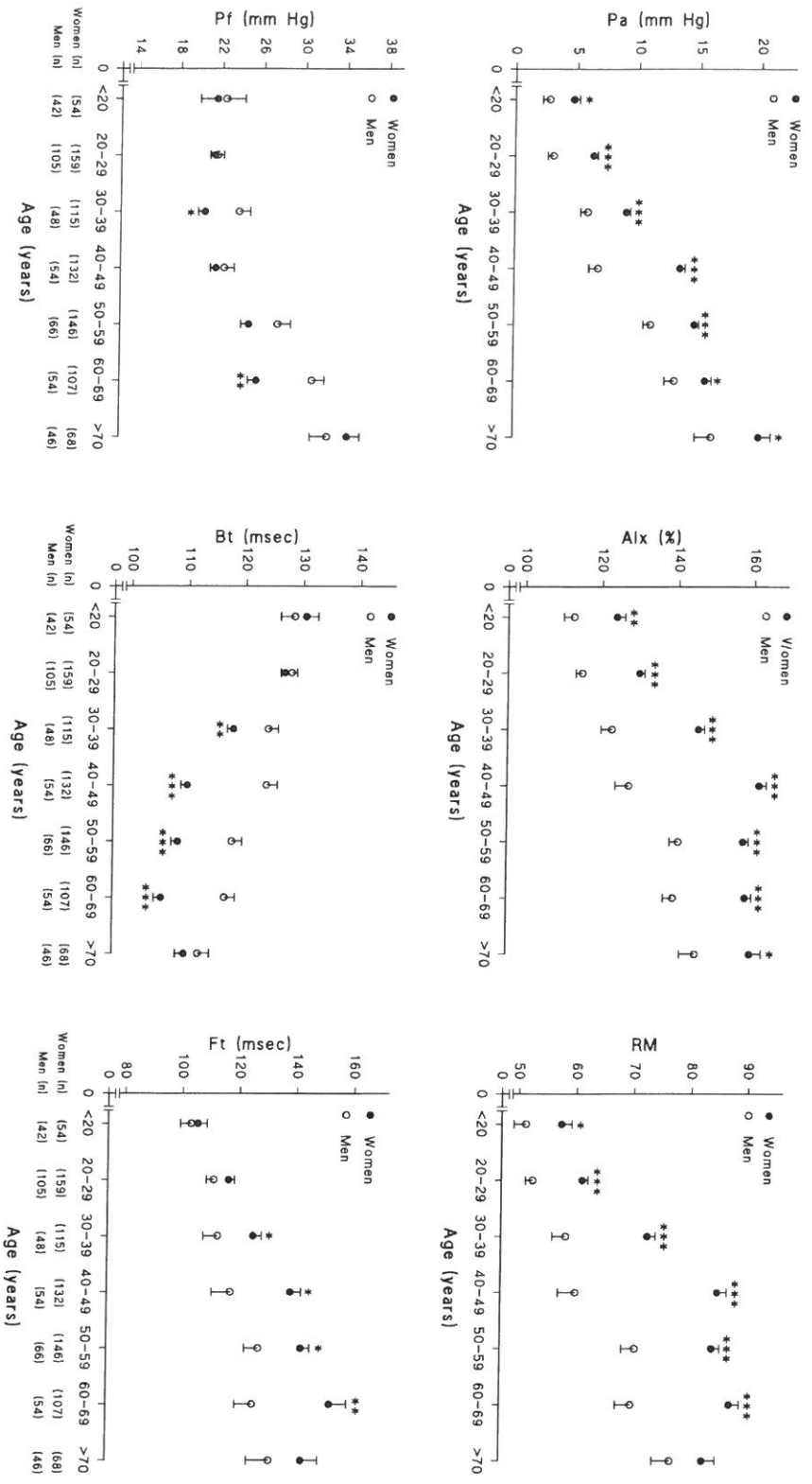

Figure 1. Differences in multivariate-adjusted aortic hemodynamic variables between women and men of a community sample of African ancestry across the adult age range. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0001$ vs. men in that age range. Adjustments are for age, age^2, mean arterial pressure, pulse rate, BMI, regular smoking, regular alcohol intake, and the presence of diabetes mellitus or an $HbA_{1c} > 6.1\%$ and antihypertensive therapy within each 10-year age category. Probability values were further adjusted for nonindependence of family members using nonlinear regression analysis (mixed procedure). Abbreviations: AIx, aortic augmentation index; BMI, body mass index; Bt, backward wave foot time; Ft, time to peak forward wave; Pa, aortic augmented pressure; Pf, aortic forward wave pressure; RM, reflection magnitude.

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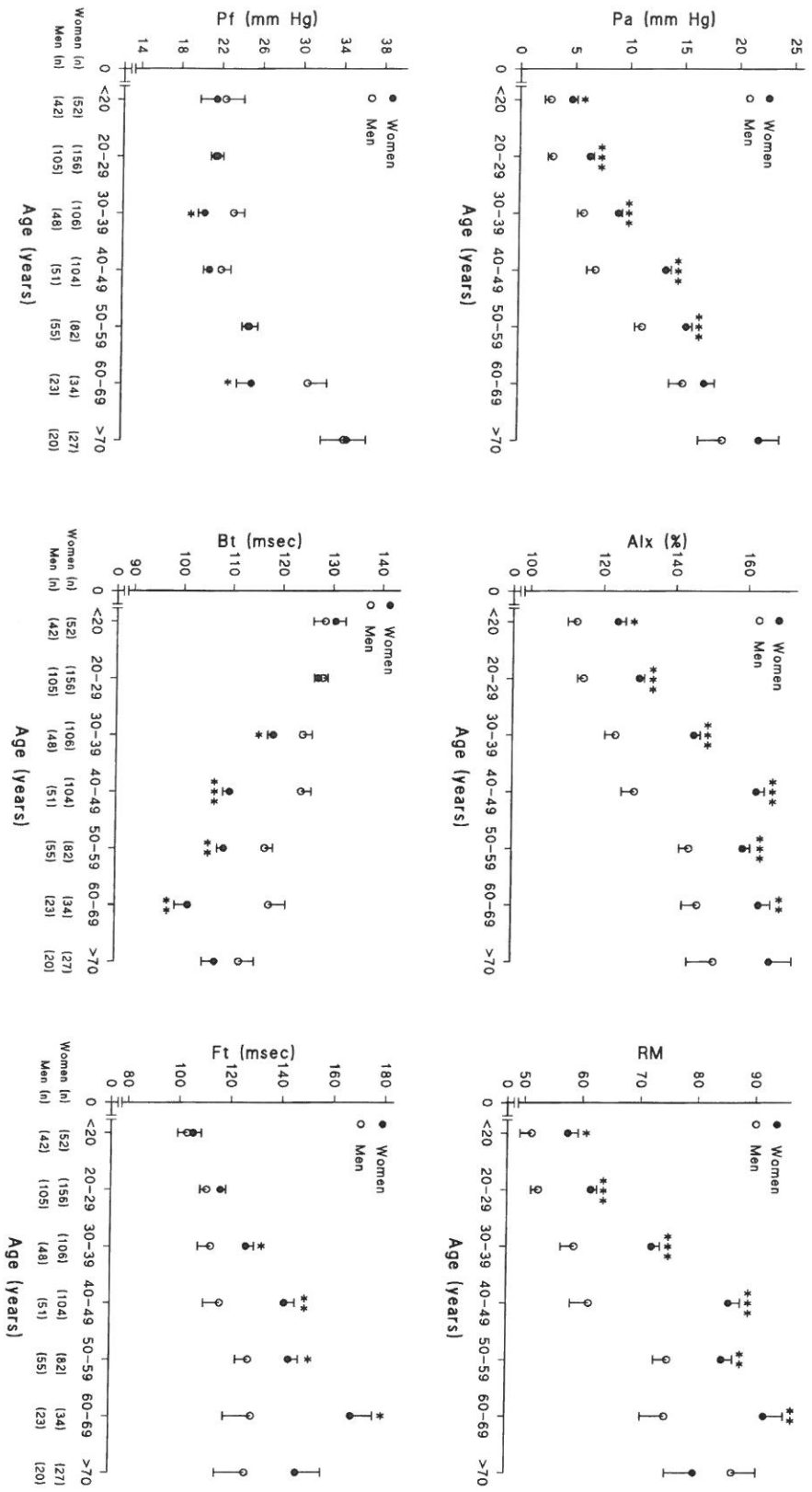

Figure 2. Differences in multivariate-adjusted aortic hemodynamic variables between women and men not receiving antihypertensive therapy from a community sample of African ancestry across the adult age range. *$P < 0.05$, **$P < 0.005$, ***$P < 0.0001$ vs. men in that age range. Adjustments are as indicated in Figure 1, but without adjustments for antihypertensive therapy. Probability values were further adjusted for nonindependence of family members using nonlinear regression analysis (mixed procedure). Abbreviations: AIx, aortic augmentation index; Bt, backward wave foot time; Ft, time to peak forward wave; Pa, aortic augmented pressure; Pf, aortic forward wave pressure; RM, reflection magnitude.

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Table 3. Correlates of aortic augmented pressures in a community of African ancestry (n = 1,197)

Variable	Model 1		Model 2		Model 3		Model 4		Model 5	
	β coefficient (±SEM)	*P* value	β coefficient (±SEM)	*P* value	β coefficient (±SEM)	*P* value	β coefficient (±SEM)	*P* value	β coefficient (±SEM)	*P* value
Age	0.05 ± 0.04	0.25	−0.17 ± 0.04	<0.0001	0.15 ± 0.04	0.0005	−0.02 ± 0.04	0.70	−0.12 ± 0.04	<0.01
Age^2	0.0017 ± 0.0005	<0.0005	0.0031 ± 0.0004	<0.0001	0.0004 ± 0.0004	0.37	0.0021 ± 0.0004	<0.0001	0.0029 ± 0.0004	<0.0001
Female	3.81 ± 0.34	<0.0001	1.78 ± 0.31*	<0.0001	4.12 ± 0.32[a]	<0.0001	3.41 ± 0.34[a]	<0.0001	2.96 ± 0.33[a]	<0.0001
BMI	−0.06 ± 0.02	<0.005	−0.01 ± 0.02	0.81	−0.08 ± 0.02	<0.0001	−0.05 ± 0.02	0.01	−0.05 ± 0.02	<0.05
Heart rate	−0.22 ± 0.01	<0.0001	−0.09 ± 0.01	<0.0001	−0.24 ± 0.01	<0.0001	−0.20 ± 0.01	<0.0001	−0.18 ± 0.01	<0.0001
MAP	0.23 ± 0.01	<0.0001	0.18 ± 0.01	<0.0001	0.19 ± 0.01	<0.0001	0.23 ± 0.01	<0.0001	0.22 ± 0.01	<0.0001
Smoking	1.42 ± 0.42	<0.001	1.15 ± 0.35	<0.005	1.21 ± 0.38	<0.005	1.32 ± 0.40	0.001	1.40 ± 0.38	<0.0005
Treatment[b]	−1.43 ± 0.39	<0.0005	−1.06 ± 0.33	<0.005	−1.48 ± 0.36	<0.0001	−1.34 ± 0.38	<0.0005	−1.39 ± 0.36	0.0001
RM	–	–	0.15 ± 0.01	<0.0001	–	–	–	–	–	–
Pf	–	–	–	–	0.21 ± 0.02	<0.0001	–	–	–	–
Ft	–	–	–	–	–	–	0.028 ± 0.004	<0.0001	–	–
Bt	–	–	–	–	–	–	–	–	−0.147 ± 0.011	<0.0001
Model r^2	0.636	<0.0001	0.736	<0.0001	0.685	<0.0001	0.658	<0.0001	0.689	<0.0001

Included in the models were regular alcohol intake and diabetes mellitus or an HbA_{1c} > 6.1%. Probability values were further adjusted for nonindependence of family members using nonlinear regression analysis (mixed procedure). *P < 0.0001 vs. model 1 (without RM). Abbreviations: BMI, body mass index; Bt, backward wave foot time; Ft, time to peak forward wave; MAP, mean arterial pressure; Pf, aortic forward wave pressure; RM, reflection magnitude.

[a]Indicates no difference from model 1 (P > 0.07).

[b]Indicates treatment for hypertension.

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Table 4. Correlates of aortic augmentation index in a community of African ancestry (n = 1,197)

Variable	Model 1		Model 2		Model 3		Model 4		Model 5	
	β coefficient (±SEM)	*P* value	β coefficient (±SEM)	*P* value	β coefficient (±SEM)	*P* value	β coefficient (±SEM)	*P* value	β coefficient (±SEM)	*P* value
Age	1.76 ± 0.17	<0.0001	0.59 ± 0.11	<0.0001	1.37 ± 0.17	<0.0001	1.41 ± 0.16	<0.0001	0.89 ± 0.15	<0.0001
Age^2	−0.012 ± 0.002	<0.0001	−0.005 ± 0.001	<0.0001	−0.008 ± 0.002	<0.0001	−0.0098 ± 0.0016	<0.0001	−0.0063 ± 0.0015	<0.0001
Female	18.45 ± 1.30	<0.0001	7.40 ± 0.90**	<0.0001	17.76 ± 1.26[a]	<0.0001	16.28 ± 1.24[a]	<0.0001	13.55 ± 1.11*	<0.0001
BMI	−0.41 ± 0.08	<0.0001	−0.099 ± 0.056	0.08	−0.35 ± 0.08	<0.0001	−0.37 ± 0.08	<0.0001	−0.32 ± 0.07	<0.0001
Heart rate	−0.77 ± 0.05	<0.0001	−0.024 ± 0.037	0.53	−0.76 ± 0.05	<0.0001	−0.64 ± 0.05	<0.0001	−0.55 ± 0.04	<0.0001
MAP	0.48 ± 0.04	<0.0001	0.19 ± 0.03	<0.0001	0.59 ± 0.04	<0.0001	0.45 ± 0.04	<0.0001	0.38 ± 0.03	<0.0001
Smoking	5.36 ± 1.57	<0.001	3.92 ± 1.03	<0.0005	5.77 ± 1.52	<0.0005	4.85 ± 1.47	0.001	5.02 ± 1.31	<0.0005
Treatment[b]	−4.58 ± 1.48	<0.005	−2.62 ± 0.97	<0.01	−4.32 ± 1.42	<0.005	−4.09 ± 1.38	<0.005	−4.65 ± 1.23	<0.0005
RM	–	–	0.86 ± 0.02	<0.001	–	–	–	–	–	–
Pf	–	–	–	–	−0.55 ± 0.07	<0.0001	–	–	–	–
Ft	–	–	–	–	–	–	0.17 ± 0.01	<0.0001	–	–
Bt	–	–	–	–	–	–	–	–	−0.82 ± 0.04	<0.0001
Model r^2	0.494	<0.0001	0.778	<0.0001	0.523	<0.0001	0.550	<0.0001	0.638	<0.0001

Included in the models were regular alcohol intake and diabetes mellitus or an HbA_{1c} > 6.1%. Probability values were further adjusted for nonindependence of family members using nonlinear regression analysis (mixed procedure). *P < 0.005, **P < 0.0001 vs. model 1 (without RM). Abbreviations: BMI, body mass index; Bt, backward wave foot time; Ft, time to peak forward wave; MAP, mean arterial pressure; Pf, aortic forward wave pressure; RM, reflection magnitude.

[a]Indicates no difference from model 1 (P > 0.22).

[b]Indicates treatment for hypertension.

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whether different approaches to assessing hemodynamic factors explain the differences between studies in those factors that contribute to sex-specific differences in AIx, is uncertain.

Similar to the Framingham Heart Study which demonstrated that women have a wider forward wave peak than men,[10] in the present study, we show that women have a longer time to the peak of the forward wave than men. This is likely to be explained by a longer systolic ejection time[26] and the well described increase in left ventricular systolic function reported to occur in women as compared to men. This could in-part mediate deleterious effects on end-organs by increasing the chance of the backward wave merging with the forward wave. However, in contrast to Caucasian community samples,[10] where a wider peak of the forward wave accounts for a significant proportion of the enhanced AIx in women as compared to men, in the present study, adjustments for the time to the peak of the forward wave produced only a modest (not significant) attenuating effect on relations between Pa or AIx and female gender. As SphygmoCor software does not assess the width of the forward wave peak, we were unable to evaluate the role of the width of the forward wave peak. Hence, we may have underestimated the impact of a longer systolic ejection time and left ventricular systolic function in women on AIx or Pa.

As the present study was conducted in the SOWETO community of Johannesburg, South Africa, the question arises as to whether this community represents groups of African ancestry in general. In this regard, people living in this community are largely derived from Nguni and Sotho chiefdoms and based on linguistic and genetic studies these chiefdoms originate from west and east Africa during the expansion of Bantu-speaking peoples.[27] Hence, at least from a genetic perspective, the present data is likely to represent groups of African descent in Africa as a whole and in other areas of the world. However, we cannot exclude the possibility of differences in environmental influences modifying the impact of aortic backward waves in women as opposed to men in the present community sample.

In the present study, because of the absence of this measure in SphygmoCor software, we also did not assess the contribution of the maximal slope (time derivative) of the upstroke of the backward wave to sex-specific effects on AIx or Pa. In this regard, an increased slope of the upstroke of the backward wave has also been demonstrated to contribute to sex differences in AIx or Pa.[10] However, it is unclear whether an increased slope of the upstroke of the backward wave contributes to end-organ changes independent of backward wave magnitude. Hence, further studies are required to evaluate whether the upstroke of the backward wave contributes to cardiovascular damage independent of backward wave magnitude and whether these effects are sex-dependent.

There are several limitations to the present study that warrant consideration. First, to determine aortic pressure, the radial pulse wave was converted into an aortic pressure wave using an algorithm (generalized transfer function) which is incorporated into the manufacturer's software. Because of an inability to externally validate this algorithm, this approach may be limited. Second, the assumptions intrinsic to the use of the "triangulation method" of aortic wave separation may not be ideal.[28] In this regard, as compared to a triangular flow wave, where the leading edge is assumed to be linear from the inflection point to the dicrotic notch, the aortic flow wave may have a leading edge which is best represented by a shoulder. As a consequence, reflected wave magnitude derived from wave separation analysis employing a "triangular flow wave" may not correlate that well with reflected wave magnitude derived from wave separation analysis employing an actual flow wave.[28] However, in 392 participants of the present sample with aortic velocity measurements, we were able to show a correlation (r^2) between backward wave pressure derived from the "triangulation method" vs. "actual aortic flow" methods of wave separation of 0.82 and between forward wave pressure derived from the "triangulation method" vs. "actual aortic flow" methods of wave separation of 0.88. Moreover, in sensitivity analysis conducted only in those participants who had wave separation analysis performed using an actual aortic flow wave, we were able to reproduce the essential findings of the present study. Hence, at least in the present study, the use of the "triangulation method" is unlikely to have significantly affected our results. Third, in the present study, calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries.[29] Hence, aortic pressures are likely to have been underestimated using the current approach. However, neither AIx nor RM (which are ratios of 2 pressures, both affected to a similar extent by calibration errors) and hence the sex-specific effects of RM on AIx reported on in the present study are likely to have been affected by errors in calibration.

In conclusion, in the present study, we show in a large randomly recruited community-based sample of African descent in South Africa, that the relationship between aortic augmented pressures or AIx and female gender is to a large extent attributed to an increased backward wave magnitude rather than forward wave pressure. This is in contrast to what has been noted in largely Caucasian population samples[10] and highlights the need to therapeutically target aortic backward wave magnitude rather than forward wave pressure specifically in women of African descent.

SUPPLEMENTARY DATA

Supplementary data are available at *American Journal of Hypertension* online.

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DISCLOSURE

The authors declared no conflict of interest.

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