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Relationship between Aortic pulse wave velocity and left ventricular mass in a group of African ancestry is not accounted for by Aortic pressures

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Relationship Between Aortic Pulse Wave Velocity and Left Ventricular Mass in a Group of African Ancestry is Not Accounted for by Aortic Pressures.

Hamza Bello

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, for the degree of Master of Science in Medicine.

Johannesburg, 2017

Abstract

Aortic pulse wave velocity (PWV) and backward waves, as determined from wave separation analysis, predict cardiovascular events beyond brachial blood pressure (BP). However, the extent to which these aortic hemodynamic variables contribute independent of each other is uncertain. In 749 randomly selected participants of African ancestry we therefore assessed the extent to which relationships between aortic PWV or backward wave pressures (Pb)(and hence central aortic pulse pressure [PPc]) and left ventricular mass index (LVMI) occur independent of each other. Aortic PWV, PPc, forward wave pressure (Pf) and Pb were determined using radial applanation tonometry and SphygmoCor software and LVMI using echocardiography. 44.5% of participants had an increased LVMI-$ht^{1.7}$. With adjustments for age, brachial systolic BP or PP and additional confounders, PPc and Pb, but not Pf was independently related to LVMI and LV hypertrophy (LVH) in both men and women. However, PWV was independently associated with LVMI in women (partial $r=0.16$, $p<0.001$), but not in men (partial $r=0.03$) and PWV was independently associated with LVH in women ($p<0.05$), but not in men ($p=0.07$). With PWV and Pb included in the same multivariate regression models, PWV (partial $r=0.14$, $p<0.005$) and Pb (partial $r=0.10$, $p<0.05$) contributed to a similar extent to variations in LVMI in women. In addition, with PWV and Pb included in the same multivariate regression models, PWV ($p<0.05$) and Pb ($p<0.02$) contributed to LVH in women. In conclusion, aortic PWV and backward wave pressure (and hence pulse pressure) although both associated with LVMI and LVH, produce effects which are independent of each other.

DECLARATION

I declare that this dissertation is my own work. It is being submitted for the Degree of Masters in Science in Medicine in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. The work in this thesis has not been submitted for any Degree or examination in this University or any other University.

Hamza Bello, on this…….....day of…….2017

I certify that the studies contained in this dissertation have the approval of the Committee for Research in Human Participants of the University of the Witwatersrand, Johannesburg. The ethics approval number is M02-04-72 and renewed as M07-04-69 and M12-04-108.

……...	……..
Gavin R. Norton (Supervisor1)	Angela J. Woodiwiss (Supervisor2)
Date……	Date……

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Dedication:

I dedicate this work to my late Parents, Bello Danladi and Aishatu Bello, for struggling to make me a better person. To my family, Habiba, Khadijah, and Aliyu.

Conference Presentations and Publications.

Conference presentation

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Paper under-review

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List of abbreviations and acronyms.

AIx	Augmentation index.
BP	Blood pressure.
BMI	Body Mass Index
CV	Cardiovascular.
CVD	Cardiovascular disease.
CRP	C-reactive protein.
CPGRU	Cardiovascular Pathophysiology and Genomics Research Unit.
Cm	Centimetres.
DM	Diabetes Mellitus.
E	Young`s modulus
ECG	Electrocardiography.
Etc.	et cetera.
ESRD	End-stage renal disease.
Ft	Time to the peak of forward wave.
g	grams
GFR	Glomerular filtration rate.
h	Wall thickness of the vessel.
HDL	High density lipoprotein
IVS	Interventricular septum.
HbA_{1C}	Glycosylated haemoglobin
l	Litre.
Kg	Kilograms
LV	Left ventricle.

LVH	Left ventricular hypertrophy.
LVM	Left Ventricular Mass.
LVMI	Left Ventricular Mass Index.
LDL	Low density lipoprotein.
M	Mass.
MI	Myocardial infarction
Mol	Mole.
MAP	Mean arterial pressure.
MCI	Mild cognitive impairment.
MmHg	Milliliters of Mercury.
NHSL	National Health Service Laboratory.
Pa	Augmented pressure.
PPc	Central pulse pressure
PP	Pulse pressure.
PWT	Posterior wall thickness
PWV	Pulse Wave Velocity.
Pb	Backward wave pressure.
PPc	Central pulse pressure
Pf	Forward wave pressure.
Rm	Reflected wave magnitude.
Rt	Time to the foot of backward wave
s	Seconds.
SBP	Systolic blood pressure.
SOWETO	South Western Townships
t	Time.
TPR	Total Peripheral Resistance.

2D	Two-Dimensional.
≥	Greater than or equals to.
>	Greater than.
µ	Micro

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Preface.

Cardiovascular disease (CVD) has reached epidemic proportions globally. In Sub Saharan Africa, including South Africa, the mortality could be as high as 350/100,000 population. Hypertension is one of the leading causes of CVD, and is often associated with an increase in large artery stiffness, as indexed using the easy to measure carotid-femoral (aortic) pulse wave velocity (PWV). Aortic PWV predicts stroke, and myocardial infarction, beyond conventional cardiovascular risk factors, including brachial blood pressure. It is estimated that an increase in aortic PWV by 1 m/s corresponds to a 14-15% increased risk of a cardiovascular event beyond conventional risk factors. However, the exact mechanisms responsible for the ability of aortic PWV to predict cardiovascular events is still uncertain.

In the present dissertation, I aimed at evaluating the contribution of aortic haemodynamics to brachial BP-independent relations between PWV and LVMI in a community sample of black African ancestry. In the first chapter, I review the current evidence for a role of aortic PWV and other haemodynamic factors to mediating cardiovascular damage. In this same chapter I argue in favor of performing the study conducted. In chapter 2, I describe the methodology employed to perform the present study, and include a description of participant recruitment, and the methods for assessing aortic PWV, aortic blood pressure and left ventricular mass as well as the approaches that I employed for data analysis. In chapter 3, I describe the results of the study, and finally in chapter 4, I discuss my data in the context of existing literature on this topic and pose potential clinical implications as well as highlight the limitations of the study.

Chapter 1

Aortic stiffness: Current understanding of the contribution to cardiovascular disease.

1.1 Introduction

Cardiovascular disease (CVD) is a broad term that refers to several disorders of the heart and vessels which have a common aetiology. These disorders include cerebrovascular accidents or stroke, heart failure, myocardial infarction, peripheral arterial disease, and renal failure. All of these disorders contribute significantly to morbidity and mortality. Globally, it is estimated that approximately 8 million premature deaths occur per year that can be attributed to CVD (WHO, 2017, Lozano et al., 2013, and Cappuccio and Miller, 2016), and CVD is one of the leading causes of death in developing countries, including Africa (Boutayeb, 2006; Ebrahim and Smith, 2001; Kahn *et al.*, 2000; Reddy, 2002). It is imperative, to accurately predict and hence prevent the occurrence of CVD, and in this regard several modifiable and non-modifiable risk factors associate with CVD, such as high blood pressure (BP) (hypertension), high plasma low density lipoprotein (LDL) cholesterol concentrations, low plasma high density lipoprotein (HDL) cholesterol concentrations, diabetes mellitus, smoking, male gender, and advancing age.

It is well recognized that hypertension is one of the most important risk factors for CVD. Worldwide, hypertension contributes to about 50% of stroke and heart failure (Campbell *et al.*, 2016). In developing countries, hypertension is the leading cause of heart failure and coronary artery disease (Irazola *et al.*, 2016), as well as stroke (Akpalu *et al.*, 2015; Connor *et al.*, 2009; Mensah, 2008). Within the last 10 years, the risk of death from hypertension has increased by over 25% (Ibrahim and Damasceno, 2012). In South Africa, hypertension affects nearly a quarter of the entire population (Steyn *et al.*, 2008). However, in urban settings this could be as high as 50% (Malhotra *et al.*, 2008; Maseko *et al.*, 2011). There is substantial evidence that antihypertensive therapy reduces the risk of cardiovascular events (Clarke *et al.*, 2002). However, there is ongoing debate as to whom should receive therapy. In this regard, a continuous relationship exists between blood pressure (BP) and CVD, from BP values as low as 120/80 mmHg (Chobanian *et al.*, 2003; Vasan *et al.*, 2001), and more recently evidence is

beginning to emerge that treating those to BP values well below currently accepted thresholds for treatment (140/90 mm Hg), that is to achieve target BP values of less than 120/80 mm Hg, further reduces cardiovascular events (SPRINT Study, 2015). However, it may not be cost-effective to initiate therapy in persons with a BP below 140/90 mmHg. Hence there is a need to identify those at risk with BP values which would not traditionally be considered to require therapy. Although traditional risk factors including an increased BP measured at the brachial artery are well established causes of CVD (Menotti *et al.*, 2009), epidemiological studies have shown that these risk factors do not necessarily always account for all cardiovascular events (Harald *et al.*, 2008). Importantly, Individuals on medical therapy for a particular risk factor may sometimes have a worse cardiovascular outcome (Kohro *et al.*, 2008; Szecsenyi *et al.*, 2008). Therefore, risk assessment using traditional approaches alone, including the use of brachial BP, may not be sufficient to predict cardiovascular events.

1.2 Aortic blood pressure.

Aortic BP is often lower than brachial BP and because of the proximity of the aorta to end-organs, the hypothesis exists that aortic BP may be a more appropriate measure than brachial BP for predicting cardiovascular damage. Indeed, several lines of evidence suggest the superiority of aortic over brachial BP in predicting cardiovascular events or associating with end-organ measures (Jankowski *et al.*, 2008; Norton *et al.*, 2012; Roman *et al.*, 2007; Wang *et al.*, 2010). Indeed, in comparison with brachial BP, aortic BP may associate better with or independent of carotid intima-media thickness (Boutouyrie *et al.*, 1999; Norton *et al.*, 2012), left ventricular mass (LVM) (Boutouyrie *et al.*, 1995; Chen *et al.*, 1998; Deague *et al.*, 2001; Lekakis *et al.*, 2004; Norton *et al.*, 2012; Roman *et al.*, 2000), renal disease (Cohen and Townsend, 2011; Safar *et al.*, 2002; Safar *et al.*, 2004), myocardial infarction (Hansen *et al.*, 2006; Hirai *et al.*, 1989), and coronary artery disease in aged individuals and in the general population

(Roman *et al.*, 2007). However, in a meta-analyses of the data from several studies, aortic BP (central BP) produced only a trend effect (p=0.057) for predicting cardiovascular outcomes better than the brachial BP (Vlachopoulos *et al.*, 2010) and aortic BP did not add to risk prediction beyond brachial BP in the Framingham Heart Study (Reddy, 2002). Nevertheless, a review by (Roman *et al.*, 2007) and a meta-analysis (Ben-Shlomo *et al.*, 2014) provide clear evidence for a stronger association between aortic as compared to brachial BP and end-organ measures. Hence, it is important to understand the potential differences in the effects of aortic as compared to brachial BP. There are several differences in the determinants of aortic as opposed to brachial BP, and these may in-part explain why aortic BP is more closely associated with cardiovascular end-organ changes than brachial BP.

Arteries may be divided into two separate compartments based on their function. In this regard, “the conduit arterial system” consists mostly of branches distal to the aorta, and these arteries deliver blood to organs. On the other hand, the “pulsatile component” of the arterial tree, which consists mainly of the aorta and its immediate branches accommodate stroke volume ejected into vessels at a high pressure. Whilst the conduit system delivers blood at a high pressure, the pulsatile component serves a “cushioning effect” that is designed to reduce (dampen) pressure fluctuations arriving at the conduit system by way of the “windkessel” effect (Nichols *et al.*, 2011; Safar, 1989; Safar *et al.*, 2003). The “windkessel” effect is an effect named after early fire-engines which converted pulsatile flow (water was sucked into the conduit using a hand-held intermittent pump) into steady-state flow by dampening the flow pulsatility with the use of an air chamber (windkessel) and by creating resistance to flow in the fire hose. Hence, as with the “”windkessel system, the aorta is designed to dampen or reduce pressures, whilst more peripheral arteries are not.

In essence the aorta serves less as a conduit vessel than other arteries and more as a capacitance (obviously not nearly to the same extent as the venous system) or rather “cushioning” vessel (it accommodates stroke volume while dampening pressure pulsatility). In

order for it to act as a “cushioning” vessel, it has a low degree of stiffness and a high degree of elasticity driven largely by a greater quantity of elastin in the wall of the aorta as compared to other vessels. In essence the aorta employs the capacity to stretch to dampen pulsatility just as the “windkessel” system used air to dampen pulsatility. In contrast, distal or more peripheral arteries such as brachial and femoral arteries, exhibit a higher stiffness, a lower degree of elasticity and less of an ability to stretch as compared to the aorta (Mitchell, 2009; Mitchell *et al.*, 2004; Nichols *et al.*, 2011), a change which is accounted for by the gradual replacement of elastic tissue by smooth muscle as one moves from the aorta to the periphery. Peripheral arteries are stiffer not only because of a reduced elastin content, but also through a greater deposition of collagen as compared to the aorta (Nichols *et al.*, 2011). As a consequence of a higher peripheral artery as compared to aortic stiffness (as well as to a small degree because of the narrower radius of peripheral arteries as compared to the aorta), peripheral arteries create a higher resistance to blood flow than the aorta or more central arteries. Peripheral arterial pressure is therefore higher than central arterial pressure (Nichols *et al.*, 2011). However, this is generally the case for young adults, but with ageing, these differences in the physical characteristics of the aorta compared to more distal arteries changes. How does age alter the physical characteristics of the aorta compared to more distal arteries?

As will be discussed in greater detail in subsequent sections, with the advancement in age (>50 years) (Mitchell *et al.*, 2004; Oliver and Webb, 2003), there is fracture of elastin fibres in the aorta and a gradual replacement of elastic tissue with collagen. This then leads to stiffening of the aorta. However, because more distal arteries are not as affected by these changes as the aorta, with advanced aging aortic stiffness begins to approximate peripheral artery stiffness and this leads to pressures in the aorta increasing to similar values as those in peripheral arteries (Mceniery *et al.*, 2005; Nichols *et al.*, 2011; Townsend *et al.*, 2015). How therefore does the magnitude and shape of the pulse wave in the aorta differ from the brachial artery and how is this affected by age?

Following left ventricular (LV) contraction, a pressure waveform (forward wave pressure or Pf) is generated by blood being ejected into the proximal aorta. The wave generated exhibits characteristics that can be analyzed in real time (Cohen and Townsend, 2011). As the wave travels along the arterial tree, its magnitude and shape changes (Avolio *et al.*, 2009; García-Espinosa *et al.*, 2016; Nichols *et al.*, 2011), i.e. the peak of the waveform increases in amplitude and becomes more visible and narrows (**Figure 1.1)**, a change consequent to the previously mentioned differences in the physical characteristics of the vessels along the arterial tree (mainly due to an increased stiffness from the aorta to more peripheral arteries). Whilst diastolic BP remains unchanged from the aorta to peripheral arteries, the increased amplitude of the pulsatile pressure wave from the aorta to peripheral arteries results in increases in systolic BP. Hence, the difference between systolic and diastolic BP (pulse pressure [PP]) is amplified from the aorta to peripheral arteries. This is therefore called PP amplification. In this regard PP amplification is often striking in younger individuals who have a compliant aorta. However, as central aortic stiffness increases due to aging and several other factors as listed in **Table 1.1**, as previously indicated there is an increase in central aortic PP, while diastolic BP and mean arterial pressure (MAP) remain constant and brachial PP does not increase to the same degree (Cohen and Townsend, 2011; Mceniery *et al.*, 2014). The consequence is that PP amplification decreases with ageing and the risk factors listed in **Table 1.1**. Hence, ageing and risk factor-related increases in aortic stiffness reduce the difference between aortic and brachial PP. In other words, PP amplification is reduced as a consequence of increases in aortic stiffness mediated by ageing and a number of cardiovascular risk factors listed in **Table 1.1.** Moreover, brachial BP may not be a true predictor of end organ damage and this may be particularly evident in younger individuals (Cohen and Townsend, 2011). However, differences in stiffness

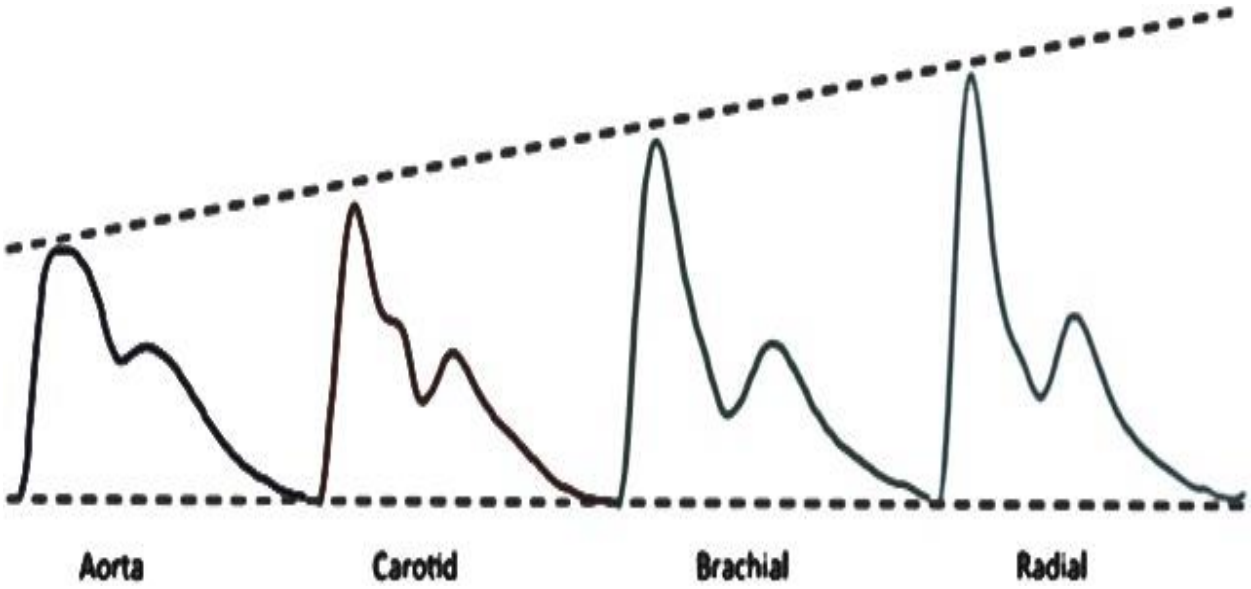


Figure 1.1. A diagrammatic representation of pressure amplification as it travels from the aorta to the peripheral arteries (Mceniery *et al.*, 2014).

Table 1.1. Clinical and demographic conditions associated with increases in aortic stiffness

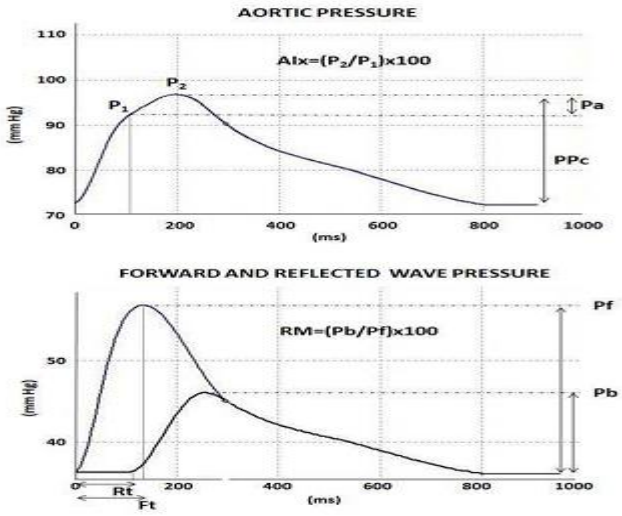
Demographic features	CV risk factor	CV disease
Aging	Hypertension	Coronary heart
Low birth weight	Obesity	Disease
Menopausal status	Smoking	Congestive heart failure
Lack of physical activity	Hypercholesterolemia	Fatal stroke
Genetic	Impaired glucose tolerance	Primary non-CV diseases
Background	Metabolic syndrome	ESRD
Parental history of hypertension	Type 1 diabetes	Moderate chronic kidney disease
Parental history of myocardial infraction	Type 2 diabetes	Rheumatoid arthritis
Genetic polymorphisms	Hyperhomocysteinaemia	Systemic Vasculitis
	High CRP level	Systemic lupus erythromatosus

CV; Cardiovascular, ESRD; end stage renal disease, CRP; C-reactive protein.

between the aorta and the peripheral arteries is not the only factor that determines variations in PP amplification. What else contributes toward age-related changes in PP amplification?

The pressure wave measured at any region of the arterial tree is not driven only by the forward (or incident) wave pressure (Pf), generated by LV ejection into the aorta, and hence by the extent of the stroke volume (determined by contractility and LV filling-Frank-Starling effect) and the magnitude of aortic impedance, (determined by aortic stiffness). Rather, the pressure wave measured at any region of the arterial tree is the sum of the forward wave as it meets a reflected or backward wave which generates a backward wave pressure (Pb). In this regard, forward waves are reflected at any point in the arterial tree where an impedance mismatch exists, such as arterial bifurcations. These reflected waves generate pressure waves that return to the LV (Avolio *et al.*, 2009). In young healthy individuals, reflected waves return early in diastole, and enhance diastolic pressure, thereby increasing coronary blood flow. However, from young adulthood to old age, reflected waves return sufficiently early that they augment PP and systolic BP (SBP), thus placing an excessive load on the cardiovascular system (Avolio *et al.*, 2009; Nichols *et al.*, 2011) **(Figure 1.2a).** Wave reflections are dependent on distal arteries rather than the aorta, as distal arteries are more muscular than central arteries. The characteristics of these arteries such as their geometry and their vasoactive properties affect wave reflection (Safar *et al.*, 2003). Vascular constriction, and a reduction in arterial cross-sectional area at the site of arterial bifurcations, increases wave reflections and these changes cause the early return of the reflected wave, thereby augmenting PP and systolic BP (Nichols *et al.*, 2011). Furthermore, vascular hypertrophy and remodelling (Mulvany and Aalkjaer, 1990), anatomical variations in the vascular network (Levy *et al.*, 2001), chemical factors (sodium and nitric oxide level), genetic make-up of an individual, and ageing, tend to have effects on distal arterial function and hence modify either backward wave magnitude or timing, thus also producing changes in aortic PP and systolic BP (Safar *et al.*, 2003). In essence, whilst Pf is

Figure 1.2a



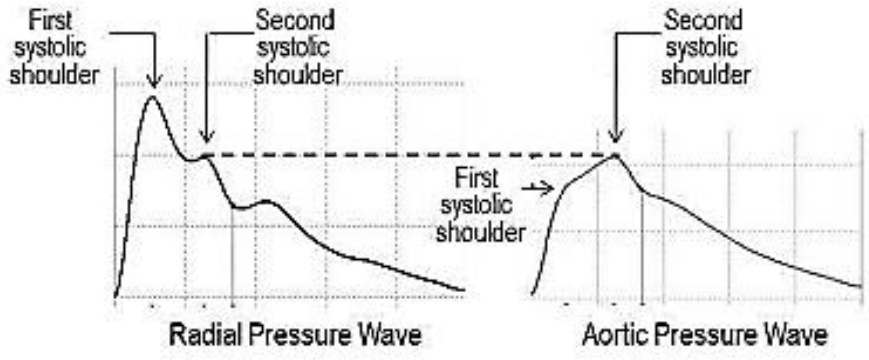


Figure 1.2b

Figure 1.2. (a and b). Original recordings of an aortic pressure wave and the component forward and backward (reflected) wave pressures after wave separation analysis (a) and a comparison of the radial and aortic pressure waves (b). Pb; backward wave, Pf; Forward wave, RM; reflected wave magnitude, AIx; aortic augmentation index, PPc; central pulse pressure, Pa; augmented pressure, Rt; time to the foot of Pb, Ft; time to the peak of Pf.

determined largely by stroke volume and the impedance of the aorta (a higher stiffness generates a greater impedance and an increased Pf), Pb is determined by total peripheral resistance (TPR) and the vascular tone of medium-sized or smaller arteries (Avolio *et al.*, 2009). The important question which arises from these facts is whether reflected waves are readily detected in brachial BP measurements?

Backward wave pressure is lower than the Pf, and appears in the brachial artery as a second systolic shoulder with a lower peak than the wave generated by the forward wave pressure **(Figure 1.2b)**. Hence, Pb is not as closely approximated by brachial artery BP measurements as is aortic BP. Nevertheless, in elderly populations there is an increase in the magnitude of Pb, and the peak of Pb as represented in the brachial pulse (second systolic shoulder) begins to approximate the peak of brachial BP (first systolic shoulder) (Nichols *et al.*, 2011). How are aortic backward wave effects determined in clinical or research practice?

Aortic augmentation index (AIx), which is determined as the pressure difference between the second systolic peak and the first systolic peak of the aortic pressure wave, expressed as a proportion or percentage of aortic PP **(Figure 1.2.a)**, is often used to evaluate the extent and magnitude of reflected wave pressures. Using this approach, some studies summarized in a meta-analysis have shown an association between aortic backward waves and cardiovascular events (Vlachopoulos *et al.*, 2010). However, in other studies, these findings, independent of brachial BP, have not being supported (Mitchell *et al.*, 2010). Importantly however, AIx is affected not only by the magnitude of Pb, but also by the magnitude of forward wave pressures, the duration of the forward wave (a longer duration increases the chances of the backward wave augmenting aortic PP), the time to wave reflection (a greater speed of wave reflection or a closer proximity to the LV of distal reflection sites, the greater the chance that the reflected wave returns early and augments aortic PP), heart rate (an increased heart rate increase AIx), body height (a shorter stature places reflection sites closer to the LV and hence produced earlier wave reflection) and other factors (Hope *et al.*, 2005; Mitchell, 2006). Hence, it is possible that

AIx is an inappropriate surrogate of actual aortic backward wave pressure. More recently, focus has been on obtaining the reflected wave magnitude (RM) from wave separation analysis using the triangular flow wave (Kips *et al.*, 2009; Westerhof *et al.*, 2006), as depicted in **Figure 1.2.a**. Although there is an ongoing debate on this topic, several studies have demonstrated that although reflected wave pressures are smaller than the forward wave pressures, reflected wave pressures associate more strongly with end-organ changes and the development of CVD than forward waves (Booysen *et al.*, 2015; Sibiya *et al.*, 2015).

In the present dissertation I evaluated whether relationships between aortic stiffness and an end organ measure (left ventricular mass) are explained by an impact of aortic stiffness on aortic forward wave or backward wave pressure and hence aortic PP. Hence, it is important that I elaborate more on the determinants of aortic stiffness; summarize the evidence to indicate that measures of aortic stiffness predict cardiovascular events; and review the possible explanations for the adverse effects of aortic stiffness on the cardiovascular system.

1.3 Large artery (aortic) stiffness.

An increase in large artery stiffness is a progressive, diffuse, and age-related process that takes place in all vascular beds (Izzo Jr, 2004), and the earliest detectable manifestation of structural and functional changes in the vascular wall (Cavalcante *et al.*, 2011). The changes in large arteries that lead to increases in arterial stiffness are in essence arteriosclerotic changes. The mechanisms that govern the development of arterial stiffness are complex. However, as previously mentioned it is postulated that gradual fragmentation of elastin occurs from a young age through to adulthood (O'rourke and Hashimoto, 2007; Sun, 2015), and that this is as a result of constant exposure to the change in magnitude and frequency of the pulsatile pressures (Mceniery *et al.*, 2010). In early childhood (<7 years), the medial layer of the arteries (including the aorta), develops elastic lamellae, and when this is completed, the gene responsible for this

is switched-off (Ott *et al.*, 2011; Wagenseil and Mecham, 2009). However, due to body growth, obesity or weight gain, there is an increase in the aortic diameter, which results in thinning and remodelling of the elastic lamellae, the consequence of which is an increase in wall stress, tension and strain, thereby leading to the elastin lamellae being replaced with collagen tissue (Lam *et al.*, 2010; Mitchell, 2015; Wagenseil, 2011). The arterial wall is progressively infiltrated with collagen which contributes to further stiffening and this collagen may become more cross-linked such as occurs with the formation of advanced glycosylation end product formation in diabetes mellitus or an enhanced lysyl oxidase activity such as occurs in conditions such as hypertension (Kovacic *et al.*, 2011). The increased collagen cross-linking results in a collagen molecule with an extremely high tensile strength which further stiffens the aorta. The fragmented elastin that occurs is believed to serve as a site for calcium deposition thereby leading to even further arterial wall stiffening (Dao *et al.*, 2005; Mceniery *et al.*, 2009). Importantly, clinically significant arteriosclerosis occurs in more than half of individuals aged over 60 years (Mceniery *et al.*, 2005).

Several factors may influence the degree of arterial stiffening including conventional cardiovascular risk factors (hypertension, diabetes mellitus, smoking, dyslipidemias, and obesity), the genetic make-up of an individual (Wilkinson *et al.*, 2009; Zieman *et al.*, 2005), and inflammation of the vascular wall, and many of these factors associated with urbanization (Wilkinson and Mceniery, 2012). These factors have been listed in **Table 1.1**. However, it is important to note that of all these factors, ageing and hypertension are the two most important accounting for most of the inter-individual variation in measures of arterial stiffness (Cecelja and Chowienczyk, 2009). Nevertheless, increases in aortic stiffness have been consistently demonstrated in diabetes mellitus and chronic kidney disease (Townsend *et al.*, 2015). Increases in arterial stiffness may have several adverse effects on the cardiovascular system. These are in-part summarized in **Figure 1.3**. First, and as described in the aforementioned

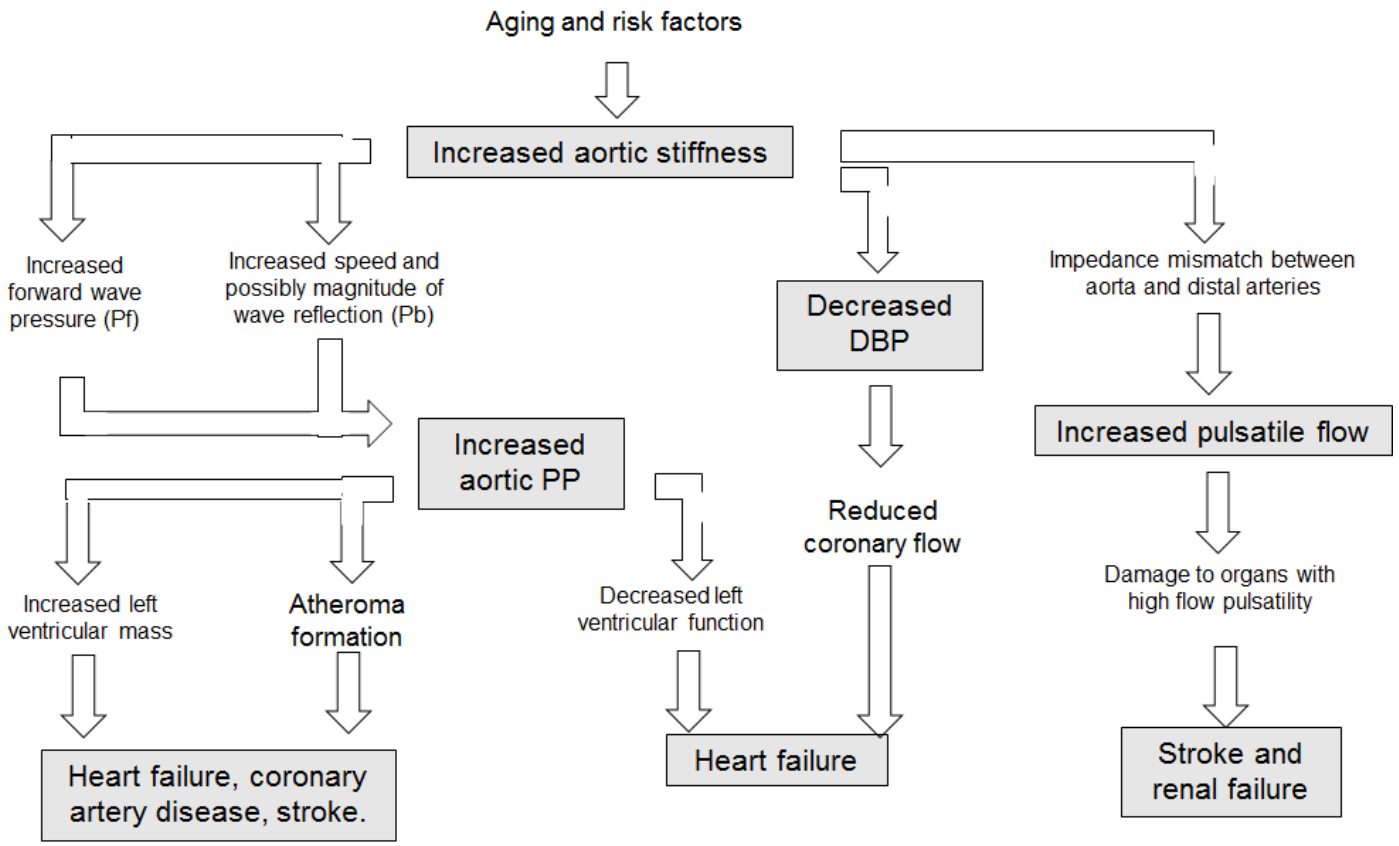


Figure 1.3. Mechanisms that may lead to the development of end organ damage with an elevated arterial stiffness.

section, increases in aortic stiffness enhance aortic impedance and subsequently increase aortic Pf and thus aortic PP and systolic BP (Nichols *et al.*, 2011). The consequence is an increased load (cardiac or vascular) or wall stress which produces several effects including damage to the heart and vessels, atheroma formation in vessels and cardiovascular hypertrophy with several detrimental results. Just as an increased aortic stiffness may enhance Pf, there is also no reason to believe that a similar effect does not occur for the returning (backward) wave and that aortic stiffness does not increase backward wave pressures and hence aortic PP as well. Simultaneously, because of the lack of ability of a stiff aorta to accommodate blood during systole, there is less elastic recoil during diastole and thus peripheral blood flow is reduced in diastole and diastolic BP decreases. The consequence of a reduced diastolic BP may be an attenuated myocardial blood flow as coronary flow mainly occurs in diastole and this is to a large extent driven by the pressure gradient across the coronary bed. The increased aortic PP and systolic BP may not be adequately detected in the brachial artery because of the aforementioned differences that exist between the aorta and the brachial BP. The higher aortic pressure will then increase load on the cardiovascular system or produce an increased pulsatile flow in those organs where microvascular flow is indeed pulsatile (kidneys and the brain). Pulsatile flow may itself have deleterious effects on these tissue beds (Mitchell, 2008). Second, with arterial stiffening, the impedance mismatch between the aorta and more distal vessels may be diminished, the consequence being that the pulse wave generated is not appropriately `buffered` and this, in-turn, leads to excess transmission of pulsatile energy into the microvasculature with further damage to these organs (Hashimoto and Ito, 2011; Mitchell *et al.*, 2011; O'rourke *et al.*, 2010; Smulyan *et al.*, 2016; Webb *et al.*, 2012). This transmission of pulsatile energy driven by increases in pulsatile flow may not be associated with increases in PP. Third, an enhanced aortic stiffness also increases the speed of wave reflection resulting in an earlier return of the reflected wave, thus augmenting aortic PP with further increases in loads on the cardiovascular system (Nichols *et al.*, 2011). Moreover, an

enhanced aortic stiffness may also result in an earlier return of the reflected wave independent of the speed of wave reflection. In this regard, it is proposed that the site of impedance mismatch is brought closer to the ascending aorta, thus shortening the distance that the backward wave has to travel. Although all three hypotheses are tenable, there is ongoing debate as to the validity or relative importance of one over the other hypothesis. In particular, the relative importance of an increased aortic stiffness on aortic PP as a cause of cardiovascular damage has been under scrutiny for some time. However, before addressing the issue of the evidence for and against this hypothesis, I will first discuss arterial stiffness as a cause or consequence of hypertension and then review the use of aortic pulse wave velocity as an index of aortic stiffness.

It was initially thought that an increased arterial stiffness is a complication (end-organ effect) of hypertension. However, several studies have demonstrated that increases in arterial stiffness antedate and hence contribute toward the development of hypertension and this may be particularly true of isolated systolic hypertension (Alghatrif *et al.*, 2013; Birru *et al.*, 2011). Indeed, in a prospective study that followed participants for a mean duration of 7 years, an increased in aortic stiffness, together with increases in Pf (possibly also due to increases in aortic stiffness), and AIx (which may or may not have been mediated by an increased aortic stiffness) were all demonstrated to be associated with an increased risk for the development of hypertension (Kaess *et al.*, 2012). In a mouse model of obesity, decreases in nitric oxide, increases in vascular wall matrix cross-linking and the production of inflammatory mediators were some of the proposed mechanisms that could lead to the development of increases in arterial stiffness and consequently hypertension. In this model arterial stiffness began to rise one month and systolic BP and PP began to increase 6 months after the diet was initiated, indicating arterial stiffness may precede hypertension. Moreover, weight loss was associated with reversal of the aforementioned changes (Weisbrod *et al.*, 2013).

1.4 Pulse wave velocity.

The velocity of pulse wave transmission along the aorta, which is estimated as carotid-femoral pulse wave velocity (PWV), is a non-invasive and gold standard measure of arterial stiffness (Laurent *et al.*, 2006; Mancia *et al.*, 2013; Van Bortel *et al.*, 2012). Mathematically it is expressed as PWV= L/Δt (m/s), where L is the distance in meters and Δt is the time interval in seconds and the relationship between PWV and arterial wall elasticity is expressed by the Moens-Koteweg equation: PWV=√E × h/2rρ, where E= Young`s modulus in the circumference direction, h= wall thickness of the vessel, r= inside radius of the tube, and ρ= density of the blood (approximately 1.05) (Mackenzie *et al.*, 2002; O'rourke *et al.*, 2002). Carotid-femoral PWV is the velocity of pulse wave travel along the aortic or aorto-iliac pathway and as inferred by the Moens-Koteweg equation, increases as aortic stiffness increases. In essence it is a measure of the velocity of the pulse waveform from the right common carotid artery to the right femoral artery (Van Bortel *et al.*, 2012). This approach employs the foot-to-foot method of assessing the transit time of the pulse wave where the foot of the wave is defined as the point at the end of diastole, and the transit time is the time of travel of the foot of the wave over a known distance. This method is independent of the effect of wave reflection. This approach to assessing arterial stiffness has been extensively validated and inaccuracies of velocity assessment are minimal. In this regard, the shorter the distance externally between the two recording sites, the greater the error in transit time (Laurent *et al.*, 2006), and hence velocity is best measured across the whole aortic bed. To determine the distance travelled either the use of the direct distance between the carotid and femoral sites is measured, or the distance from the carotid site to the sternal notch is subtracted from the distance from sternal notch to the femoral site. Presently there is agreement that the best approach to assessing the distance travelled by the pulse waveform is to take 80% of the direct distance between the carotid and femoral sites (Van Bortel *et al.*, 2012).

Although there are several alternative methods for the assessment of arterial stiffness apart from PWV (including carotid ultrasonography) (Mackenzie *et al.*, 2002), cardiovascular magnetic resonance imaging (Ibrahim *et al.*, 2010), cardio-ankle vascular index (Wang *et al.*, 2009) and echocardiography (Laurent *et al.*, 2006; Vlachopoulos *et al.*, 2015), none of these techniques are as easy to perform or as reproducible as carotid-femoral PWV and none of these assessments have as extensive evidence as aortic PWV for predicting risk beyond conventional risk factors. Moreover, the technology to measure aortic PWV is by no means as expensive as the technology required to perform magnetic resonance imaging or ultrasound approaches. With respect to the measurement of aortic PWV, there are several semi-automated devices available for the measurement including the Complior, SphygmoCor, Pulse pen, Periscope, and Vasera devices (Nichols *et al.*, 2011). The SphygmoCor and Pulse Pen devices calculate the time delay between two consecutive points in cardiac cycle using the R-wave of the Electrocardiogram (ECG) as a guide.

1.4.1 Age-related changes in aortic PWV

Several studies have described the age-related changes in aortic PWV and these findings have largely been consistent. However, there is some discrepancy as to how age-related increases in aortic PWV relate to aortic pressures. What are the consistencies and what are the discrepancies between studies? Although there is agreement that age is the strongest determinant of aortic PWV with PWV increasing by approximately 1 m/sec every 10 years, there is disagreement on the rate of change that occurs. Some studies have shown that the effect of age on PWV is a linear relationship (Mitchell *et al.*, 2004; Smulyan *et al.*, 2001). However, other studies evaluating the effect of age on PWV and aortic PP show a non-linear (closer to an exponential) relationship (Mitchell, 2015). In this regard, several studies show that aortic PWV increases only modestly between the ages of 45-65 years, while aortic PP begins to increase

significantly between the ages of 20-39 years (Hodson *et al.*, 2015; Mitchell *et al.*, 2010), and that this is driven to a large extent by aortic backward wave magnitude (Booysen *et al.*, 2015). However, after the age of 65 years, both PWV and aortic PP increase exponentially (Hodson *et al.*, 2015; Mitchell *et al.*, 2010). Although, as previously indicated aortic stiffness may influence aortic PP through an impact on aortic backward wave pressures and AIx (through an earlier return of the reflected wave), the limited effect of aortic PWV (the gold-standard measure of aortic stiffness) on AIx is nevertheless highlighted by the fact that AIx increases substantially between the ages of 45-65 years, which is several decades prior to when PWV begins to increase substantially and then AIx falls again after 65 years of age, when PWV is increasing rapidly.

1.4.2 Pulse wave velocity and cardiovascular risk

Carotid-femoral PWV is now a well-established predictor of cardiovascular events beyond conventional risk factors, including MAP (distending pressure which increases arterial stiffness through passive effects), and brachial systolic BP and PP (Baumann *et al.*, 2014; Ben-Shlomo *et al.*, 2014; Mitchell *et al.*, 2010; Russo *et al.*, 2012; Vlachopoulos *et al.*, 2010; Weber *et al.*, 2012; Woodard *et al.*, 2014), and these findings have been summarized in a meta-analysis of several studies (Vlachopoulos *et al.*, 2010), and an individual participant meta-analysis (Ben-Shlomo *et al.*, 2014). Carotid-femoral PWV predicts the majority of cardiovascular events including stroke and myocardial infarction (Ben-Shlomo *et al.*, 2014), and the ability of carotid-femoral PWV to predict events is noted across several co-morbidities (elderly and young-to-middle aged, the general population and clincial populations, smokers and non-smokers, diabetics and non-diabetics, hypertensives and normotensives and those with or without chronic kidney disease) (Ben-Shlomo *et al.*, 2014). For a 1 standard deviation increase in aortic PWV the risk (hazards ratio) is estimated to be 23% greater than normal for a coronary

event, 28% above normal for a stroke, and 30% above normal for a cardiovascular event (Ben-Shlomo *et al.*, 2014). Aortic PWV adds to the ability to detect risk beyond the Framingham Risk Score and other risk scoring systems (Laurent and Boutouyrie, 2013) and adds as much as other measures of end-organ damage including left ventricular hypertrophy, carotiod plaque and estimated glomerular filtration rate to risk prediction (Sehestedt *et al.*, 2010). Importantly, 21.8% of those at moderate risk are reclassified into a higher risk on the basis of aortic PWV and 14.3% at intermediate risk are reclassified into a higher risk, whilst only 1.4% are reclassified into a lower risk (Mitchell *et al.*, 2010; Sehestedt *et al.*, 2012). In a 51 month follow-up of patients with end-stage renal disease a change in aortic PWV predicted cardiovascular mortality independent of confounders including brachial BP (Guerin *et al.*, 2001). Hence, there is some evidence that interventions leading to an improved aortic PWV predict events. Because of the significant evidence in favour of aortic PWV predicting cardiovascular events beyond conventional risk factors, guidelines for the diagnosis and management of hypertension now recommend the measurement to predict risk (Mancia *et al.*, 2013). However, these guidelines list aortic PWV as an end-organ change (a composite measure of the complications of ageing, hypertension and several other risk factors) rather than a reflection of a haemodynanmic change (an increased aortic stiffness) with several possible adverse effects on the heart and vessels (as discussed in the section on aortic stiffness).

1.4.3 Could the brachial BP-independent ability of aortic stiffness to predict risk be mediated by an effect on aortic BP?

There are several reasons why aortic PWV, the gold-standard measurement of aortic stiffness, predicts risk independent of brachial BP and these have largely been described in previous discussion. To summarize, aortic BP may produce an increase in aortic Pf and hence PP, systolic BP and wall stress in the heart or vessels. However, because of PP amplification,

these effects are not readily detected at the brachial artery. Second, aortic stiffness may increase flow pulsatility (without necessarily increasing PP) by reducing the impedance mismatch that exists between the aorta and more distal vessels. Third, an increased aortic stiffness may produce earlier reflected waves and reflected waves of greater magnitude, and hence enhance aortic AIx and aortic PP. Fourth, increases in aortic PWV may reflect an end-organ change. What is the evidence for or against aortic PWV mediating effects on end-organs through increases in aortic PP?

Importantly, the Framingham Heart Study demonstrated that aortic PWV, but not aortic PP, PP amplification or AIx predicts risk beyond brachial PP (Mitchell *et al.*, 2010). Hence, there is question as to whether aortic PWV mediates cardiovascular damage through effects on Pf or Pb and AIx and hence aortic PP. Is there evidence for relations between aortic PWV and end-organ changes independent of brachial BP that may be mediated by increases in aortic Pf, Pb, AIx and PP? In this regard, one should not expect aortic PWV to modify renal or brain function through increases in PP (although this could happen). The adverse effects of aortic PWV on these organs is possibly through an increased flow pulsatility (unlike other organs flow in the kidneys and brain is pulsatile) independent of aortic PP. However, the target organ that is most likely affected by aortic stiffness-induced increases in aortic Pf and PP independent of brachial BP is left ventricle (LV) (see Figure 1.3). Indeed, as indicated in the above discussion PWV predicts not only stroke (or renal damage), but also coronary heart disease (Ben-Shlomo *et al.*, 2014; Vlachopoulos *et al.*, 2010). Hence the question arises as to whether aortic PWV is associated with LV mass (LVM) independent of brachial BP? In this regard, LV hypertrophy (LVH) is a well-recognized predictor of cardiovascular risk independent of conventional BP measurements (Casale *et al.*, 1986; Drazner *et al.*, 2004; Gardin *et al.*, 2001; Ghali *et al.*, 1998; Levy *et al.*, 1990; Levy *et al.*, 1994; Verdecchia *et al.*, 2001). Even in the absence of arterial hypertension, LVM is an independent risk factor for cardiovascular events (Gardin *et al.*, 2001). Importantly, irrespective of baseline conventional BP and treatment for hypertension, the

presence of LVH doubles the risk for cardiovascular events including myocardial infarction, and unstable angina (Verdecchia *et al.*, 2001). Left ventricular hypertrophy is also associated with the severity of coronary disease independent of conventional BP (Ghali et al., 1998). In addition, independent of conventional BP, LVH is associated with a greater relative risk of coronary multivessel disease (Liao *et al.*, 1995).

Many studies have shown that indices of arterial stiffness are associated with LVM (Baguet *et al.*, 2000; Bell *et al.*, 2015; Bouthier *et al.*, 1985; Boutouyrie *et al.*, 1995; Chen *et al.*, 1998; Chobanian *et al.*, 2003; Deague *et al.*, 2001; Gates *et al.*, 2003; Iketani *et al.*, 2000; Kobayashi *et al.*, 1996; Leoncini *et al.*, 2006; Libhaber *et al.*, 2008; Ohyama *et al.*, 2016; Roman *et al.*, 2000; Roman *et al.*, 1996; Tatchum-Talom *et al.*, 1995). In this regard however, a number of studies have indicated that the relationship between indices of arterial stiffness and LVM is not independent on conventional BP measured at the brachial artery (Baguet *et al.*, 2000; Bell *et al.*, 2015; Bouthier *et al.*, 1985; Chen *et al.*, 1998; Deague *et al.*, 2001; Roman *et al.*, 2000; Roman *et al.*, 1996). In contrast, three human studies suggest that arterial stiffness effects on LVM are independent of brachial artery BP (Lekakis *et al.*, 2004; Leoncini *et al.*, 2006; Libhaber *et al.*, 2008). One study was conducted in a very small study sample (Lekakis *et al.*, 2004). The second study related the less well recognized ambulatory arterial stiffness index with LVM (Leoncini *et al.*, 2006). Nevertheless the third study, performed by our group, showed strong independent relations between aortic PWV and LVM in women (Libhaber *et al.*, 2008). However, no study has assessed whether these brachial BP-independent relations between aortic PWV and LVM reflect an impact of aortic PWV on Pf, Pb, and hence a greater central aortic PP.

1.5 Problem statement

As indicated in the aforementioned discussion, aortic stiffness, as indexed using aortic PWV, is an established predictor of cardiovascular events beyond conventional risk factors, including brachial BP (Ben-Shlomo *et al.*, 2014; Vlachopoulos *et al.*, 2010), the measurement of aortic PWV is recommended by hypertension guidelines for risk prediction (Mancia *et al.*, 2013) In this regard, individual participant meta-analysis (Ben-Shlomo *et al.*, 2014), shows an ability of PWV to predict outcomes across a wide adult age range and in a variety of co-morbidities (Ben-Shlomo *et al.*, 2014). The reason why PWV predicts outcomes beyond conventional risk factors is nevertheless unclear with guidelines listing it as an end-organ change (Mancia *et al.*, 2013). However, there are several possible mechanisms that may explain the impact of PWV on risk prediction beyond brachial BP.

An increased aortic stiffness may reduce the impedance mismatch between the aorta and more distal vessels, resulting in a greater pulsatile flow and microvascular damage to the kidneys and brain (Chirinos *et al.*, 2012; Wang *et al.*, 2010; Weber *et al.*, 2012). However, PWV predicts not only stroke (or renal damage), but also coronary heart disease (Ben-Shlomo *et al.*, 2014; Vlachopoulos *et al.*, 2010), and hence alternative explanations for the ability of PWV to risk predict are required. In this regard, increases in aortic PWV reflect an enhanced aortic stiffness which amplifies central aortic PP (PPc). This occurs by creating an impedance to ventricular ejection and consequently producing increases in aortic forward (Pf) and possibly backward wave (Pb) pressures (Zamani *et al.*, 2014). An enhanced aortic PWV also increases the speed of wave reflection resulting in an earlier return of the reflected wave, thus augmenting PPc (Zamani *et al.*, 2014). Therefore, an increased PWV results in a greater Pf or Pb and PPc which amplifies LV and large vessel load which may, in turn, cause damage to these structures. In this regard, evidence to support this notion are reports that PWV is associated with LV mass (LVM) (Booysen *et al.*, 2015; Hashimoto and Ito, 2011; Sibiya *et al.*,

2015; Zamani *et al.*, 2015). However, no study has assessed whether these relations reflect an impact of aortic PWV on Pf, Pb and hence a greater PPc.

1.6 Aims

In the present study I therefore aimed to evaluate the contribution of Pf, Pb, and PPc to brachial BP-independent relations between PWV and LVMI in a relatively large randomly selected community sample of African ancestry.

Chapter 2

Materials and Methods

2.1 Study population.

The present study was conducted according to the guidelines outlined in the Helsinki Declaration. The study was approved by the University of the Witwatersrand Committee for Research on Human Subjects (approval number: M02-04-72 and renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The study design has been described previously (Norton *et al.*, 2012; Norton *et al.*, 2008; Redelinghuys *et al.*, 2010; Woodiwiss *et al.*, 2009). The study is cross-sectional. Nuclear families (either both parents and at least one sibling or one parent and at least two siblings) of black African descent, with siblings older than 16 years of age were randomly recruited from the South Western Townships (SOWETO) of Johannesburg, South Africa (from the population census figures of 2001). Participants were of the Nguni (Zulu, Xhosa, Ndebele, and Swati), Sotho (South, North Sotho and Tswana) or Venda chiefdoms. The sample largely consisted of Nguni and Sotho chiefdoms. The lack of representation from the Venda chiefdom reflects a lack of individuals of this chiefdom residing in these areas of Johannesburg. No subjects of mixed, Asian, or European ancestry were recruited and no Khoi-San subjects were recruited. A lower age limit of 16 years was included to avoid the impact of rapid growth on LV mass and BP. Random recruitment of community participants was based on the following approach: Street names and addresses of households were obtained from the department of home affairs, 2001 census. These households were allocated numbers, and numbers were selected from a random number generator. People residing in informal dwellings or institutions/ homes were not recruited. A photograph of an example of formal dwellings in SOWETO is given in **Figure 2.1**. Of the 1197 participants randomly recruited, in a sub-study, 749 had left ventricular mass index (LVMI) determined by echocardiography, and all aortic haemodynamic assessments**.**



Figure 2.1. Examples of formal dwellings in the suburban region where people were recruited for this study.

2.2 Site where measurements were conducted.

The measurements were carried out at the Cardiovascular Pathophysiology and Genomics Research Unit (CPGRU) facility, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg, South Africa. Participants were transported to the CPGRU on Wednesdays and Thursdays, which are the clinic days between 9.00 hours to 13.00 hours, during which time all the required measurements were carried out.

2.3 Clinical, demographic and anthropometric measurements.

A standardized questionnaire was administered, to obtain demographic and clinical data. The questionnaire was made available in English, but trained study assistants (a nursing sister and trained technician) familiar with the home (first) languages assisted with the completion of each questionnaire. Only same-sex assistants were used to assist each family member with the completion of the questionnaire. Assistance was only provided when requested. Nonetheless, the majority of participants were reasonably proficient in the English language (primary and secondary education is largely conducted in the English language). Study assistants first visited homes of subjects that agreed to participate in the study in order to develop a trusting relationship. The questionnaire was only completed at a subsequent clinic visit and then ambiguities checked by performing a follow-up home visit. If family members were absent at follow-up home visits, data was checked with them personally via telephonic conversations whenever possible. Ambiguities in answers to the questionnaire were detected by an independent observer prior to the second home visit. A pilot study was conducted in 20 subjects

to ensure that data obtained in the questionnaires were reproducible when obtained with the assistance of two separate study assistants.

The questionnaire sought specific answers to date of birth, gender (designated as either male of female), past medical history, including the presence or absence of hypertension, diabetes mellitus, renal disease, prior cardiovascular events, such as myocardial infarction, stroke, and angina pectoris. Past or current use of medications (analgesics), antihypertensive use (pills use to lower blood pressure) and pills used to lower blood sugar were also evaluated. Cigarette smoking history was assessed as either smoked in the past, or smoking presently. Previous and present history of the consumption of alcoholic beverages (beer, traditional beer, or any form of alcohol and daily quantity) was assessed. Caffeine consumption (number of cups of coffee), frequency of physical activity and family history of hypertension, diabetes mellitus, or heart disease was also assessed. For female participants, the history of monthly menstrual flow, previous or present use of contraceptive pills, and previous history of past pregnancy, were also evaluated. Most of these questions required a simple response of either “YES” or “NO” as answers. If there was any uncertainty as to the answers given to the questions asked at the initial visit, then this information, was requested during the next visit.

2.4 Blood pressure measurements.

Blood pressure (BP), was measured by a trained nurse-technician, according to guidelines of the American Heart Association and European Society of Hypertension recommendation using a standard mercury sphygmomanometer (O'brien *et al.*, 2003; Pickering *et al.*, 2005). The nurse was of the same ethnic origins (black African) as the participants and had previously lived in SOWETO. After being trained in the procedure, including being shown pitfalls of BP measurement (positioning of the cuff, positioning of the arm, first estimating systolic BP using a radial pulse measure in order to avoid increasing cuff pressures too high,

detecting auscultatory gaps, releasing valve pressure at the correct speed, using the correct cuff size, etc.), the assistant had to demonstrate an ability to perform the procedure on 20 subjects. The study assistant was then tested on her ability to measure BP in two ways. First she was asked to measure BP on a separate group of 20 subjects including patients with hypertension and their readings had to be within 4 mm Hg of a doctors/nursing sister's readings obtained with a stethoscope with two ear pieces. Second, the study assistant was asked to watch a video showing a simulated mercury column with Korotkoff sounds where observers were tested on their ability to detect phase I and V sounds under different circumstances including in the presence of a wide auscultatory gap and where phase V Korotkoff was taken as a "muffling" rather than a "disappearance" of sounds (Blood Pressure Measurement, British Medical Journal, BMA House London). To qualify as an observers all of her readings (n = 20) had to be within 4 mm Hg of the reference standard.

Blood pressure was recorded to the nearest 2 mm Hg. Korotkoff phases I and V were employed to identify systolic and diastolic BP respectively, and care was taken to avoid auscultatory gaps. Five consecutive BP readings were obtained, at least 30 seconds apart in a sitting position after 10 minutes of rest using an appropriately sized cuff for each of the participants, and the average of the five readings was subsequently employed for statistical analysis. Standard cuffs were used with an inflatable bladder with a length of 22 cm and a width of 12 cm, except when arm circumference exceeded 31 cm larger cuffs with a 31 x 15 cm bladder were employed. Participants were considered to be hypertensive if they had a systolic BP of ≥ 140 mmHg and/or a diastolic BP of ≥ 90 mmHg, or were receiving antihypertensive medication.

2.5 Anthropometry.

Body height, body weight, and waist circumference (WC) were measured. Body weight was measured using an adult weighing scale, and approximated to the nearest 0.5 kilograms. Height was measured using a Stadiometre and expressed in metres. Body mass index (BMI) was then derived from weight and height as weight in kg, divided by height squared (kg/m^2), and this was employed as a marker of adiposity. Waist circumference was assessed using a standard tape measure at a point midway between the last rib and the iliac crest during normal breathing with the arms outstretched and this was used a measure of abdominal obesity. Waist circumference was measured to the nearest 0.1 centimetres. Obesity was defined as a BMI of ≥30 kg/m^2 and overweight as a BMI ≥25 kg/m^2. In addition abdominal obesity was defined as a WC ≥88 cm in women and ≥102 cm in men (Williams, 2002).

2.6 Blood analysis.

After an overnight fasting period of 12 hours in all participants, a trained nurse-technician obtained venous blood samples from the participants. Appropriate blood samples were then centrifuged and serum or plasma separated from the samples. Standard laboratory blood tests for renal function (creatinine, sodium and potassium concentrations), liver function (alanine and aspartate transaminases, alkaline phosphatase, total bilirubin, and conjugated and unconjugated bilirubin concentrations), haematological parameters (full blood count), lipid profiles (total, LDL and HDL cholesterol concentrations) and percentage glycated haemoglobin were analysed. These data were used to identify medical conditions and syndromes. A “spot” urine analysis was also performed to screen for major clinical conditions, such as diabetes mellitus and renal pathology. The National Health Service Laboratory (NHSL) was utilised (on contract) for blood measurements to ensure reproducibility and reliability as these laboratories have been accredited as fulfilling all criteria of “good laboratory practice”**.** Serum cholesterol concentrations were analyzed with a cholesterol reagent on the Advia chemistry instrument

(Siemens, South Africa) (Urbina *et al.*, 2002). Serum HDL cholesterol concentration was analyzed by applying both heparin-calcium precipitation and agarose gel electrophoresis (Urbina *et al.*, 2009). Diabetes mellitus or an abnormal blood glucose control was defined as use of insulin or oral anti-hyperglycaemic medications, or a glycated haemoglobin value > 6.1% (Bennett *et al.*, 2007). Menopausal status was confirmed with measures of plasma follicle stimulating hormone concentrations. Dyslipidaemia was defined as serum levels of total cholesterol >6.5 mmol/l, LDL cholesterol >4.0 mmol/l, HDL cholesterol <1.2 mmol/l in females, and HDL cholesterol <1.0 mmol/l in males. An elevated serum creatinine concentration was defined as ≥107 µmol/l in females, and ≥115 µmol/l in males (Williams, 2002).

2.7 Echocardiography.

Echocardiographic measurements were performed by two experience observers (Professor Carlos Libhaber a trained cardiologist and Professor Angela Woodiwiss and experienced echocardiographer) who were unaware of the clinical data of the participants. All measurements were recoded and stored offline. I performed echocardiography on a number of patients (n=160) under the guidance of Professor Woodiwiss to ensure that I was able to obtain comparable images and that I understood the pitfalls of the measurement. However, the data employed for analysis was that obtained by Professors Libhaber and Woodiwiss as they have demonstrated a low degree of inter and intra-observer variability (Norton *et al.*, 2008; Woodiwiss *et al.*, 2009). Echocardiography was performed with the participants placed in the partial left decubitus position, with the head of the bed elevated to the 30 degrees position. A standard 12-lead electrocardiogram was used to identify diastolic and systolic periods of the cardiac cycle. Participants were first assessed for mitral and aortic valve abnormalities using two-dimensional (2D) guided colour Doppler imaging. To determine LVM, the transducer probe (2.5 MHz) was placed over the left intercostal space at the mid-clavicular line, with the probe

marker pointing to the right shoulder in order to obtain a long axis parasternal view window. Left ventricular (LV) dimensions were obtained from 2D guided (long axis parasternal view) M-mode echocardiography in the short axis of the LV, according to guidelines (Sahn *et al.*, 1978). To obtain M-Mode images in the short axis of the LV, a sample bar was placed at right angles to the LV posterior wall, and as close to the mitral valve as possible without the images of the mitral valve appearing in the M-Mode recording. The interventricular septal wall thickness (IVS) at end diastole and end systole, the posterior wall thickness (PWT) at end diastole and end systole and the end diastolic and end systolic internal dimensions of the LV were measured only when appropriate visualization of both the right and the left septal surfaces occurred and where the endocardial surfaces of both the septal and posterior wall were clearly visible (as depicted in **Figure 2.2**). Left ventricular mass was calculated using an anatomically validated standard formula (Devereux *et al.*, 1986), and indexed to height$^{1.7}$ (LVMI). Left ventricular hypertrophy (LVH) was defined as an LVMI-height$^{1.7}$ >80 g/m$^{1.7}$ for men, and 60 g/m$^{1.7}$ for females (Chirinos *et al.*, 2010).

2.8 Aortic haemodynamics.

After participants had rested for 15 minutes in the supine position, the waveform at the radial (dominant arm) pulse was recorded by applanation tonometry during an 8-seconds period using high-fidelity SPC-301 micromanometer (Miller Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, Version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) (see **Figures 2.3**). The pulse wave was calibrated by manual measurement (auscultation) of the 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 SphymoCor software (see **Figures 2.4**). Recordings where the systolic or diastolic variability of consecutive waveforms

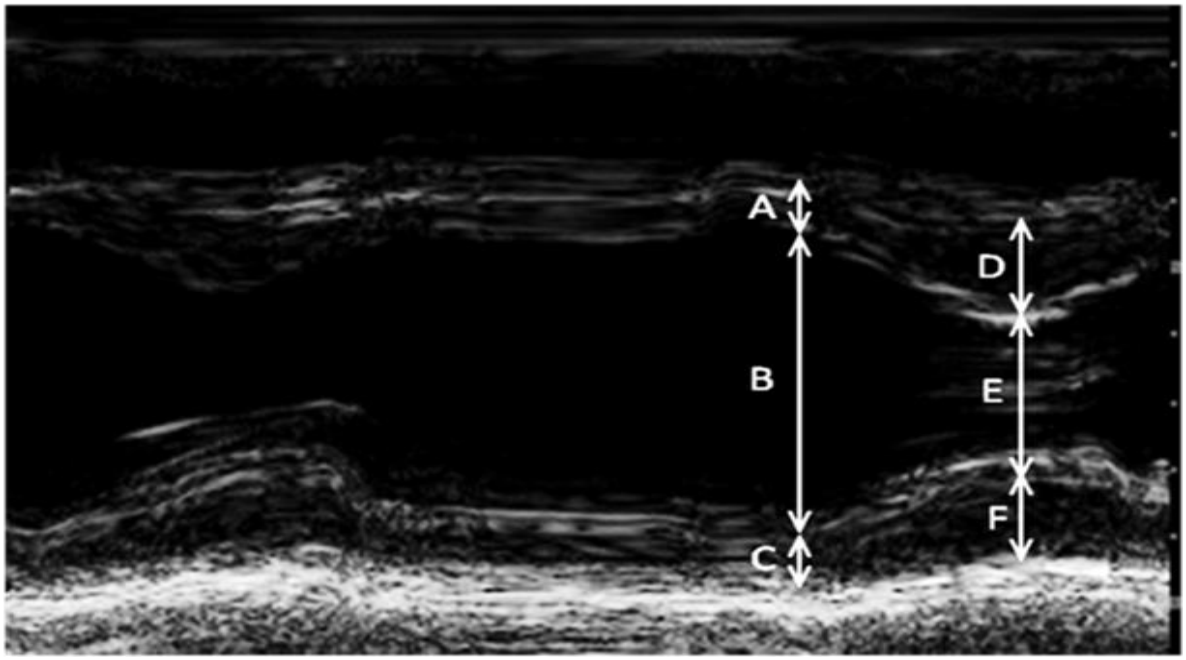


Figure 2.2 Two Dimensional M-mode guided echocardiographic image to assess LV dimensions. A is interventricular septal wall thickness in diastole, B is left ventricular end diastolic diameter, C is posterior wall thickness in diastole, D is interventricular septal wall in systole, E is left ventricular internal diameter in systole, and F is posterior wall thickness in systole

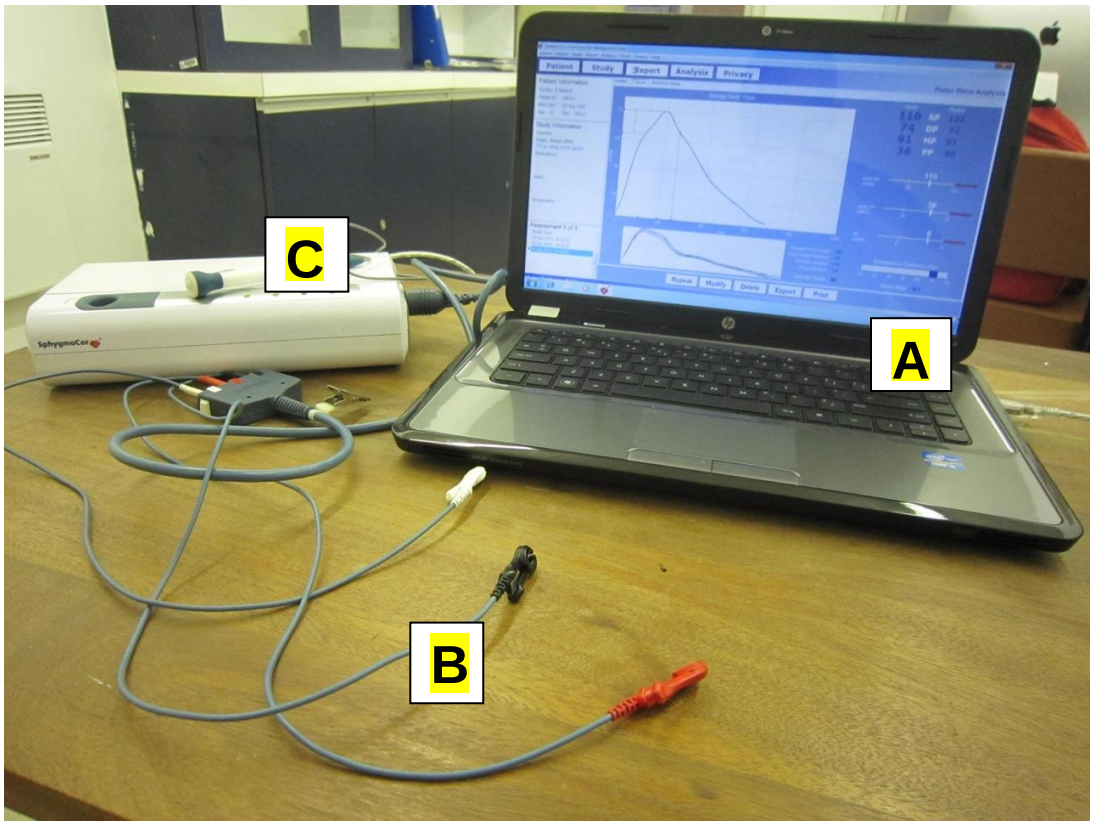


Figure 2.3 SphygmoCor device employed to determine aortic haemodynanmics. A is the SphygmoCor machine, B are electrocardiogram leads, and C is the pulse pen.

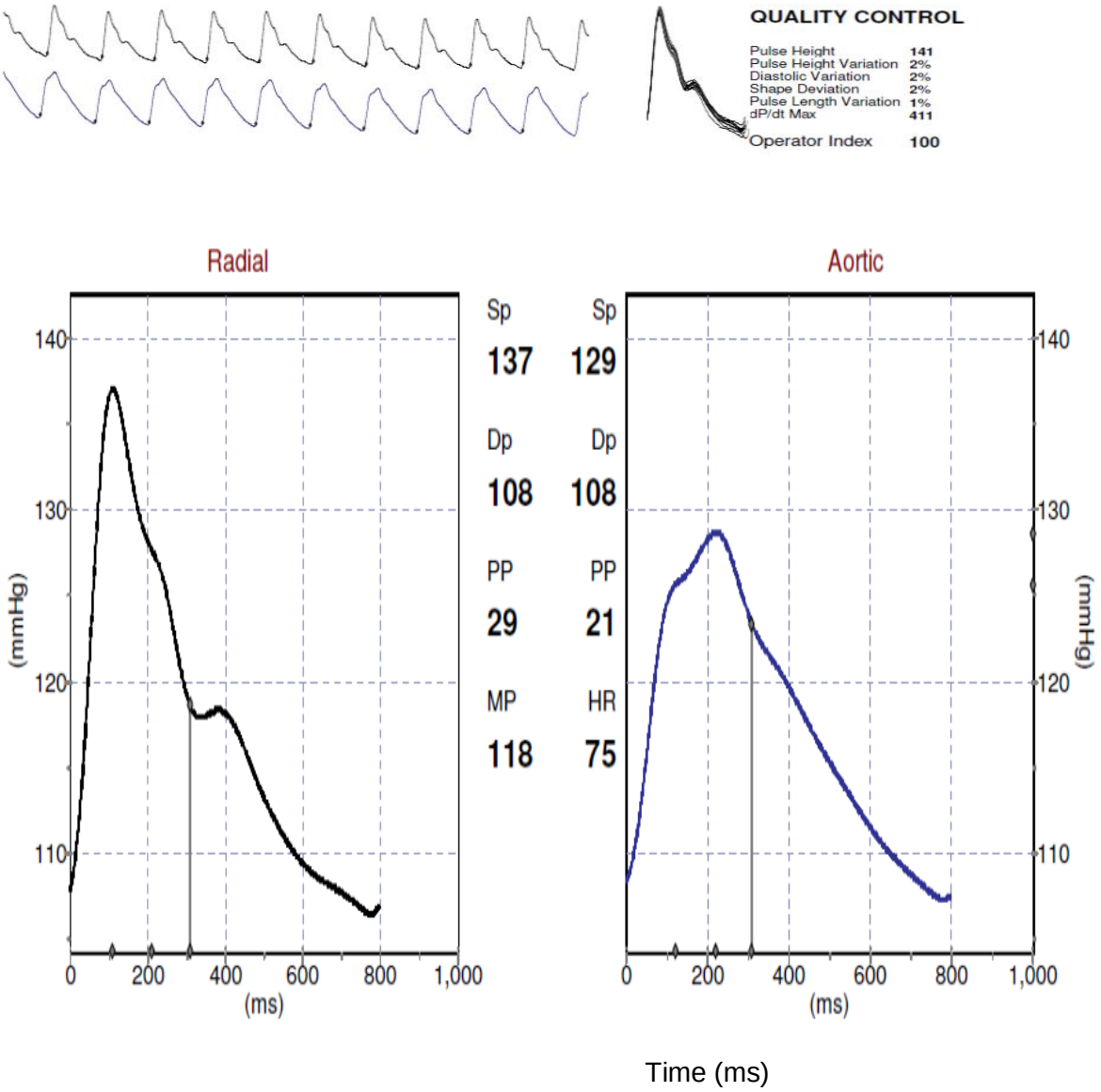
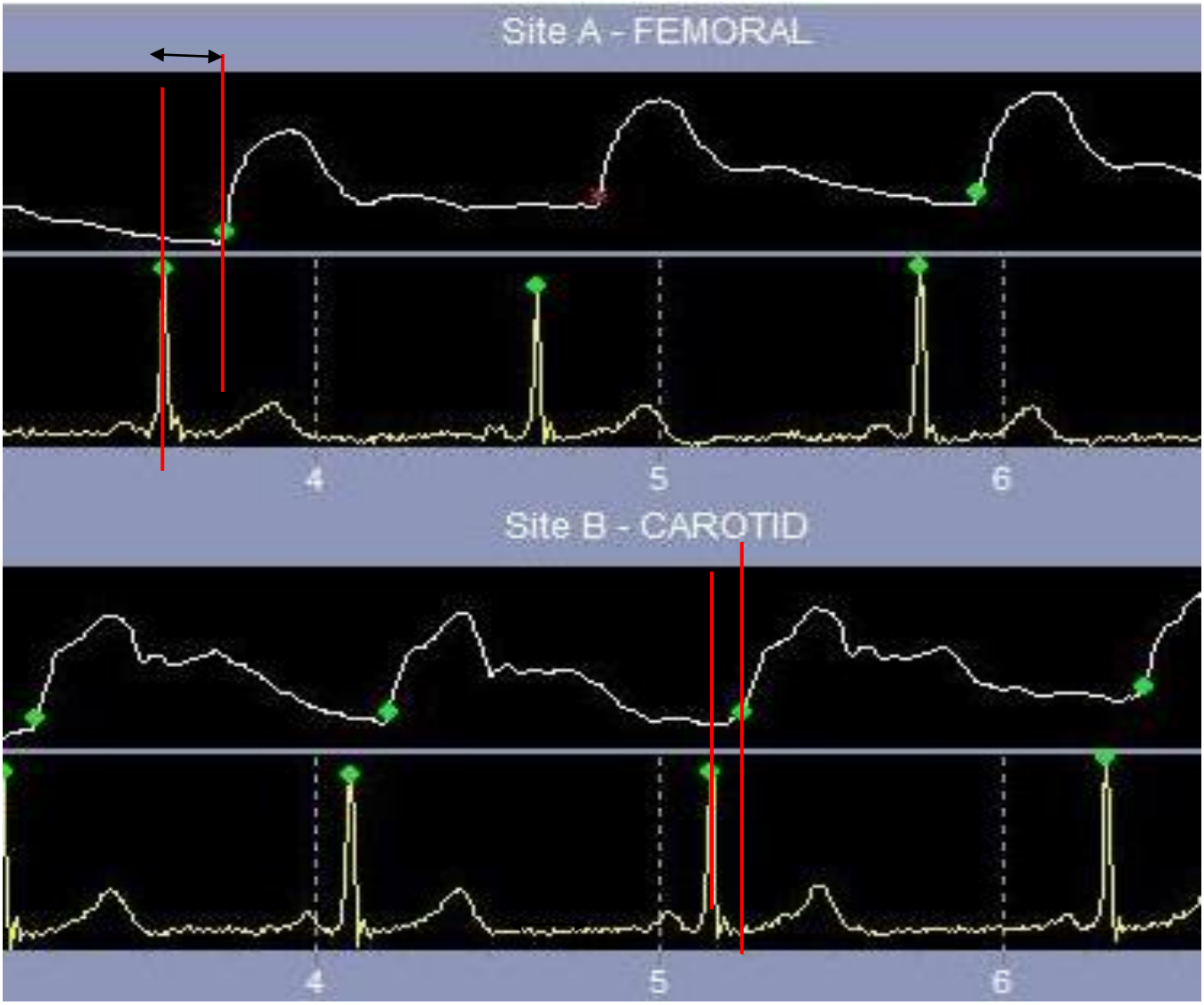


Figure 2.4 Examples of a pulse wave recording obtained to determine central haemodynamics. The figure shows the radial artery pulse wave obtained from applanation tonometry (lower left panel) and the aortic pulse wave derived from a population-based transfer function built into the software (lower right panel). See text for a further description. Quality control assessments are shown in the top panel. Sp, systolic blood pressure (BP); Dp, diastolic BP; MP, mean arterial pressure; PP, pulse pressure.

exceeded 5% or the amplitude of the pulse wave signal or were less than 80Mv were discarded. All measurements were made by a single experienced technician, unaware of the clinical history of the participants and with a low degree of intra-observer variability and a high degree of reproducibility.

Central aortic PP was determined as the difference between aortic systolic and the diastolic BP. Aortic backward wave pressure (Pb) and forward wave pressures (Pf) were determined using SphygmoCor software which separates the aortic waveform using a triangular flow wave (Tade *et al.*, 2017). In the present study I did not employ a "physiological aortic flow waveform" approach to wave separation analysis as in a pilot study conducted in 26 participants, the previously described physiological aortic flow waveform (Tade *et al.*, 2017), did not closely approximate aortic flow waveforms in the present community sample. Moreover, a wide variety of aortic flow waveforms were identified in the 26 participants studied, precluding the possibility of identifying a single "representative waveform" which could be used for wave separation analysis. Although the "triangular flow waveform" approach generates a hypothetical waveform, in 392 participants our group have previously validated this approach by separating the forward and backward waves using aortic flow waveforms derived from aortic velocity and diameter measurements obtained using echocardiography (five-chamber view) (Tade *et al.*, 2017). In this regard, our group have shown similar relations between Pf or Pb and LVMI when Pb or Pf were derived from wave separation analysis employing actual flow measurements versus Pb or Pf derived from the triangular waveform approach to wave separation analysis (Tade *et al.*, 2017).

Aortic PWV was measured from the sequential waveform measurements as described previously (Shiburi *et al.*, 2006). Pulse wave transit time i.e. the duration taken for the wave to travel from the carotid to the femoral site, was determined as the difference between the time taken to generate the femoral and the carotid pulse wave forms, as shown in **Figure 2.5.** To assess differences in time of the generation of the carotid and femoral pulse wave form, a 3-



Time of aortic wave travel=A-B

Figure 2.5 Assessment of aortic pulse wave velocity (PWV). Femoral and carotid artery pulse waves are obtained using applanation tonometry. Together with simultaneous electrocardiographic recordings aortic pulse wave velocity (PWV) is calculated. The red lines indicate the time between electrical events and the arterial pressure changes in the carotid and femoral arteries used to calculate PWV. See text for a further description.

lead ECG was performed simultaneously with pulse wave form sampling. The time delay in the pulse waves between the carotid and femoral locations was determined using the R wave of the ECG as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which pulse wave travels was determined as difference between distance from the femoral sampling site to the suprasternal notch (site B), and the distance from the carotid to the suprasternal notch is termed (site A). Pulse wave velocity was the calculated as a distance in meters divided by the transit time in seconds. Values of PWV >10 m/sec were considered as an elevated aortic PWV (Van Bortel *et al.*, 2012).

2.9 Data analysis

For database management and statistical analysis, SAS software, version 9.3 (SAS Institute Inc., Cary, NC) was employed. Multiple linear regression analysis was performed to determine the independent relations between aortic haemodynamic parameters and LVMI (continuous data). Multivariate adjusted logistic regression analysis was performed to determine the independent relations between aortic haemodynamic parameters and LVH (discrete data). Adjustments included in multivariate models were those correlated with central haemodynamic variables and LVMI in bivariate analysis. To determine probability values, further adjustments for non-independence of family members was performed using non-linear regression analysis (mixed procedure as defined in the SAS package). Regression coefficients were compared with Z statistics.

Chapter 3

Results

3.1 Characteristics of the participants.

Table 3.1 shows the clinical and demographic characteristics of all participants. In the sub-group that had echocardiography, 63.8% were females, while 36.2% were males. Aortic PWV and the proportion of participants receiving anti-hypertensive therapy were modestly greater in those in whom echocardiography was not available. Otherwise, no marked differences in the clinical and demographic characteristics of the participants included in the sub-study were noted as compared to those not included in the sub-study. The study sample was largely young-to-middle aged. 1.7% of the participants had a history of CVD. Importantly, a high proportion of participants had hypertension, and a significant proportion were not receiving anti-hypertension therapy. Moreover, 33.9% of all participants and 56.7% of participants receiving anti-hypertensives therapy had uncontrolled hypertension. 44.5% of participants had LVH.

3.2 Relationship between PWV and aortic haemodynamics.

Aortic PWV was directly correlated with aortic PPc, Pf, and Pb in both women and in men (Figure 3.1).

3.3 Relations between PWV or alternative aortic hemodynamic parameters and LVMI.

In multivariate adjusted models with either brachial SBP or PP (Figure 3.2) or mean arterial pressure (MAP) (Table 3.2) in the models, PPc and Pb, but not Pf were independently associated with LVMI in both women and men. However, independent of brachial BP, PWV was associated with LVMI in women, but not in men (Figure 3.2 and Table 3.2). Moreover, Rt

Table 3.1 Characteristics of the study sample.

	Echocardiographic Sub-study	No echocardiography
Sample size (% Female)	749 (63.8)	448 (67.4)
Age (years)	44.7±18.4	44.9±19.1
Body mass index (kg/m^2)	28.9±7.3	29.7±8.4
% Obese	41.7	43.6
Regular tobacco (% subjects)	16.2	15.7
Regular alcohol (% subjects)	19.9	22.3
% with DM or HbA_{1c}>6.1%	23.8	24.7
% Hypertensive	45.1	42.8
% treated for hypertension	25.9	19.8*
Pulse rate (beats/min)	66±11	66±12
Brachial SBP/DBP (mm Hg)	129±22/83±12	130±23/84±12
Brachial pulse pressure (PP)(mm Hg)	45±15	45±15
Central aortic SBP (mm Hg)	119±22	121±22
Central aortic PP (PPc) (mm Hg)	35±15	36±15
Aortic forward wave pressure (Pf) (mm Hg)	24±9	24±8
Aortic backward wave pressure (Pb) (mm Hg)	17±8	18±8
Aortic pulse wave velocity (PWV) (m/sec)	6.10±2.69	6.57±2.47**
Left ventricular mass index (g/m$^{1.7}$)	66.4±23.9	-

Data expressed as mean ± SD or proportions. DM, diabetes mellitus; HbA_{1c}, glycosylated haemoglobin; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP. *p<0.05, **p<0.005 vs echocardiographic sub-study group.

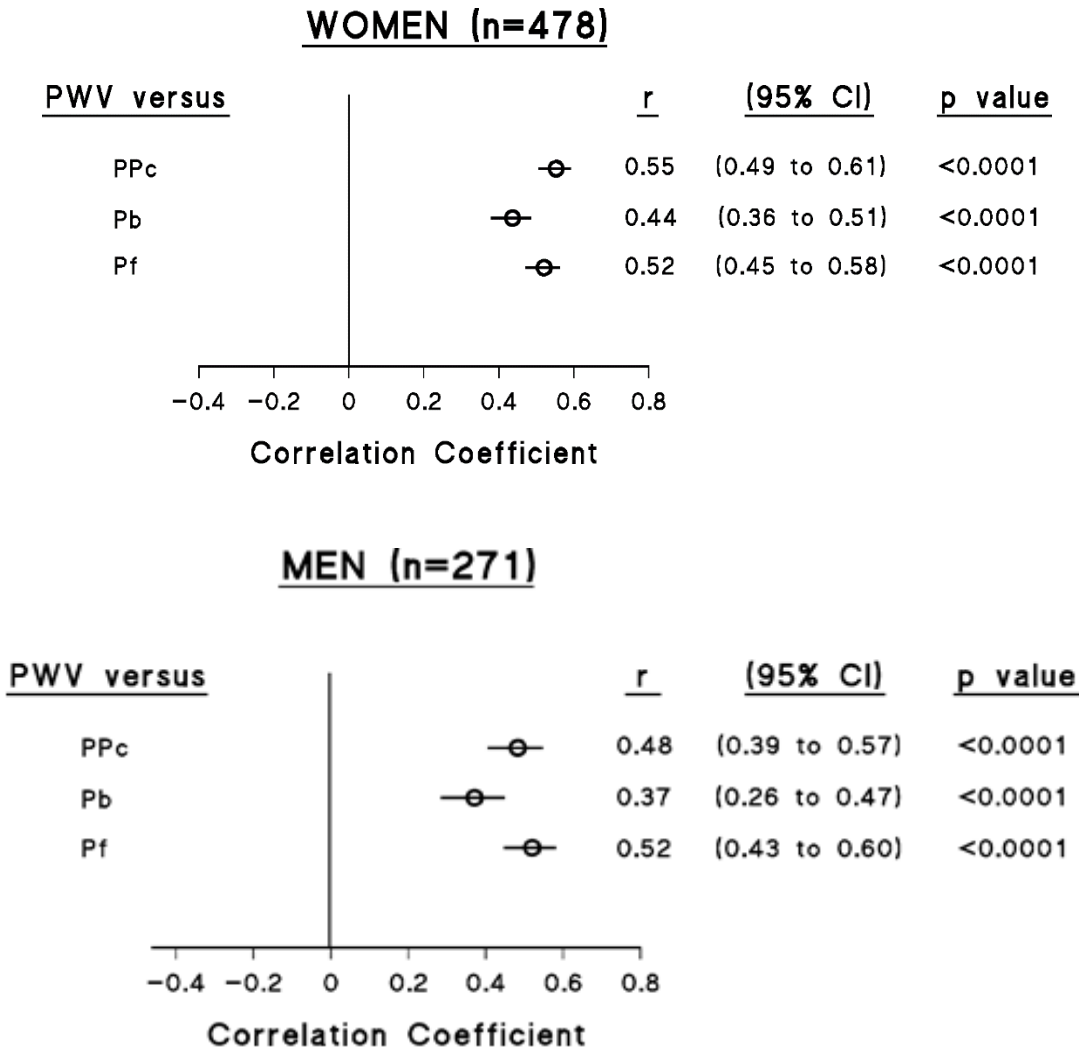


Figure 3.1 Bivariate relations between aortic (carotid-femoral) pulse wave velocity (PWV) and alternative haemodynamic factors in women and men of a community sample of African ancestry. See table 3.1 for additional abbreviations.

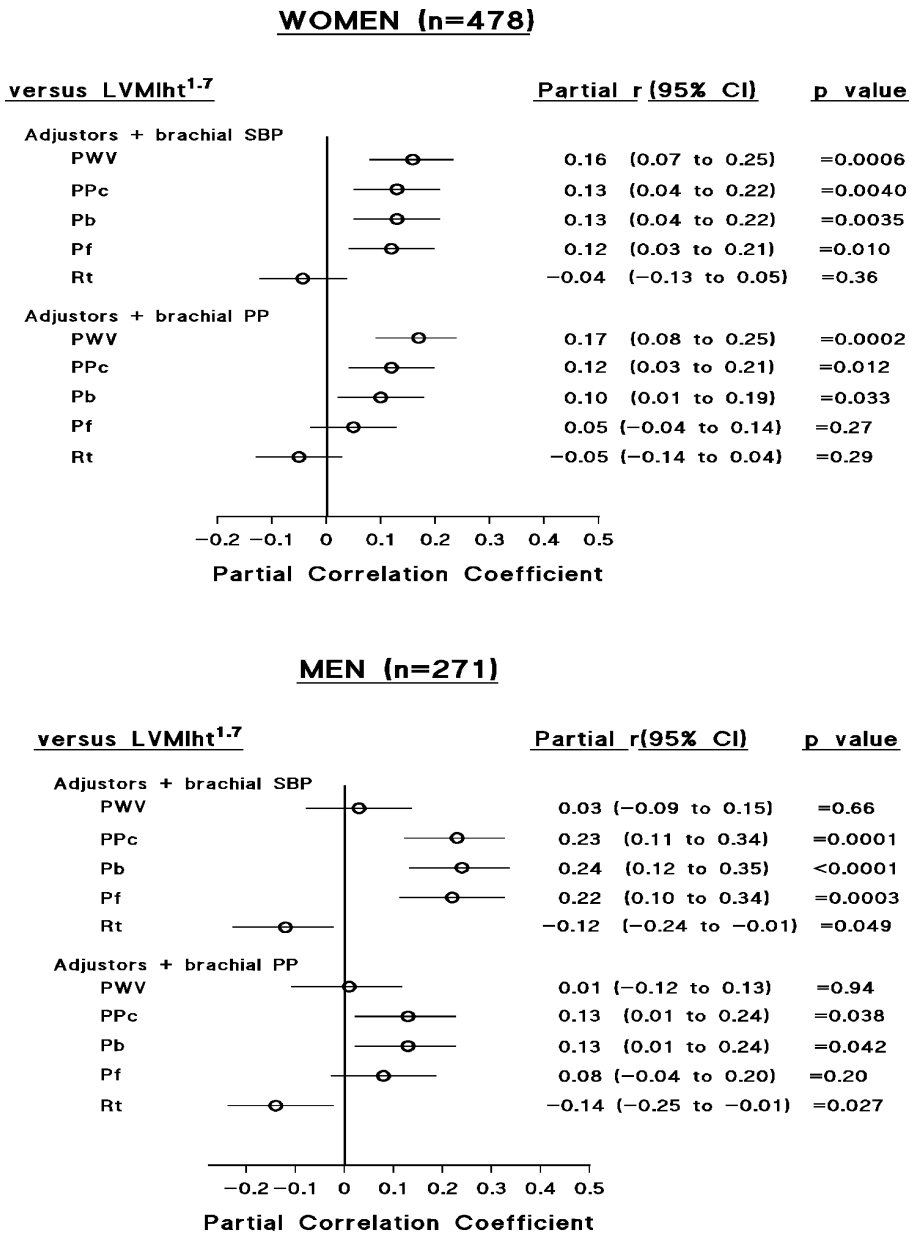


Figure 3.2. Brachial systolic blood pressure (SBP) or pulse pressure (PP) adjusted relations between aortic haemodynamic parameters and left ventricular mass indexed to height$^{1.7}$ (LVMI) in women and men of a community sample of African ancestry. See table 3.1 for additional abbreviations. *Adjustments are for haemodynamic parameter as stated as well as age, body weight, pulse rate, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and treatment for hypertension.

Table 3.2 Relationship between aortic haemodynamics parameters and left ventricular mass index before and after adjustments for mean arterial pressure (MAP).

		Women (n=478)		Men (n=271)	
	Adjustors	Partial r (95% CI)	p-value	Partial r (95% CI)	p-value
LVMI-ht$^{1.7}$ vs					
PWV	*	0.21 (0.12 to 0.29)	<0.0001	0.05 (0.07 to 0.17)	=0.38
	+MAP	0.16 (0.07 to 0.25)	=0.0005	0.04 (-0.08 to 0.16)	=0.50
Aortic PP	*	0.17 (0.08 to 0.25)	<0.0005	0.26 (0.14 to 0.37)	<0.0001
	+MAP	0.14 (0.05 to 0.22)	<0.005	0.27 (0.15 to 0.38)	<0.0001
Aortic Pf	*	0.11 (0.01 to 0.19)	<0.05	0.20 (0.08 to 0.31)	<0.005
	+MAP	0.05 (-0.04 to 0.14)	=0.31	0.19 (0.07 to 0.30)	<0.005
Aortic Pb	*	0.15 (0.06 to 0.24)	=0.001	0.26 (0.15 to 0.37)	<0.0001
	+MAP	0.13 (0.04 to 0.22)	<0.01	0.27 (0.15 to 0.32)	<0.0001

LVMI-ht$^{1.7}$, left ventricular mass indexed to height$^{1.7}$; LV stress, left ventricular systolic circumferential wall stress. See table 1 for additional abbreviations. *Adjustments are for MAP as indicated as well as age, body weight, pulse rate, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and treatment for hypertension.

was independently associated with LVMI in men, but not in women (Figure 3.2 and Table 3.2). With aortic Pb and Pf in the same regression models, Pb, but not Pf was independently associated with LVMI (Table 3.3) and the relationship between PPc and LVMI was attenuated with adjustments for Pb, but not Pf (data not shown).

3.4 Relations between PWV or alternative aortic haemodynamic parameters and LVH.

In multivariate adjusted models with either brachial SBP or PP (Figure 3.3) or mean arterial pressure (MAP) (Table 3.4) in the models, PPc and Pb, but not Pf were independently associated with LVH in both women and men. However, independent of brachial BP, PWV was associated with LVH in women, but not in men (Figure 3.3 and Table 3.4). Rt was not independently associated with LVH in either men or women (Figure 3.3 and Table 3.4). With aortic Pb and Pf in the same regression model, Pb, but not Pf was independently associated with LVH and the relationship between PPc and LVH was attenuated with adjustments for Pb, but not Pf (Table 3.5).

3.5 Relations between PWV and LVMI or LVH are not explained by aortic BP.

With adjustments for either PPc, Pf, or Pb in multivariate regression models, relations between aortic PWV and either LVMI or LVH in women were unchanged (Figure 3.4).

Table 3.3. Relations between aortic forward (Pf) or backward (Pb) wave pressures (in the same regression model) and left ventricular mass index (LVMI).

Models	Adjustors	Women (n=478)		Men (n=271)	
		Partial r	p-value	Partial r	p-value
LVMI-ht$^{1.7}$ vs		(95% CI)		(95% CI)	
1)	Pb * + brachial SBP	0.12 (0.03 to 0.21)	<0.05	0.19 (0.07 to 0.31)	<0.005
	Pf	0.05 (-0.05 to 0.14)	=0.33	0.07 (-0.05 to 0.19)	=0.27
2)	Pb * + brachial PP	0.10 (0.01 to 0.19)	<0.05	0.12 (0.002 to 0.24)	<0.05
	Pf	0.02 (-0.08 to 0.11)	=0.73	0.04 (-0.09 to 0.16)	=0.57

*Adjustments are for BP as indicated as well as age, body weight, pulse rate, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and treatment for hypertension.

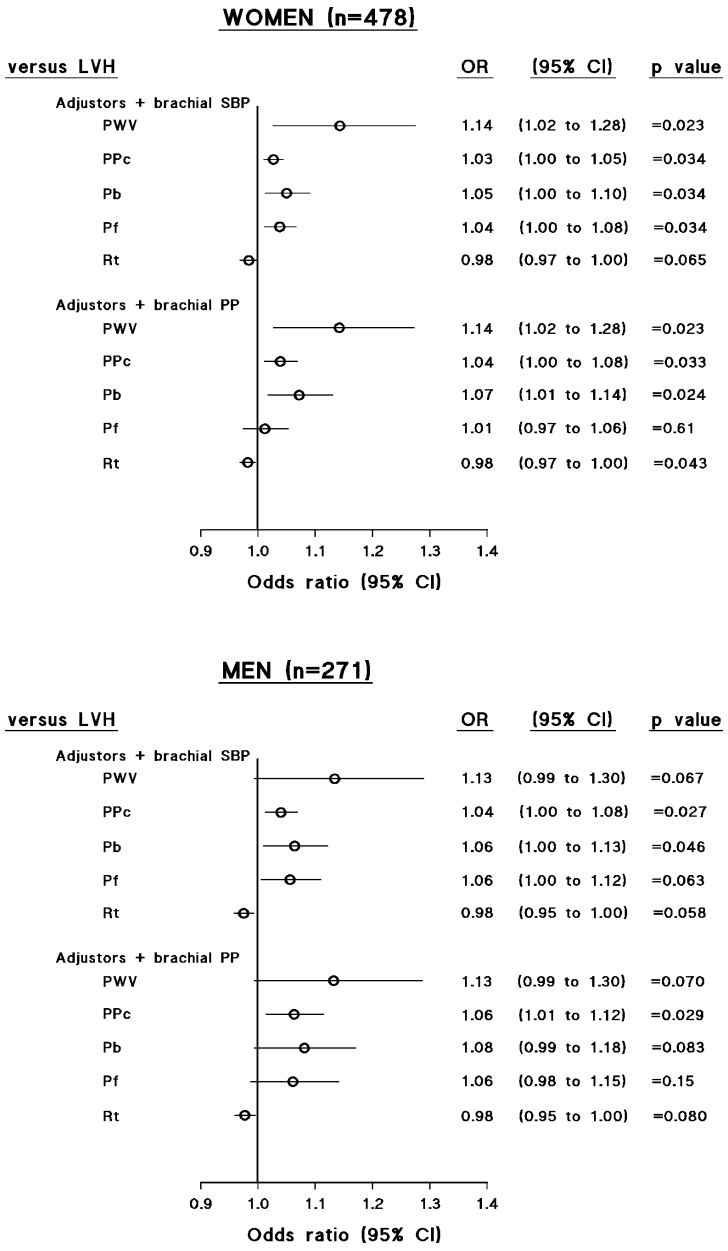


Figure 3.3. Brachial systolic blood pressure (SBP) or pulse pressure (PP) adjusted relations between aortic haemodynamic parameters and left ventricular hypertrophy (LVH) in women (n=273 of 478) and men (n=60 of 271) of a community sample of African ancestry. See table 1 for additional abbreviations. *Adjustments are for haemodynamic parameter as stated as well as age, body weight, pulse rate, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and treatment for hypertension.

Table 3.4 Relations between aortic haemodynamic parameters and left ventricular hypertrophy (LVH) before and after adjustments for mean arterial pressure (MAP) (steady-state pressure).

		Women (n=273 of 478)		Men (n=60 of 271)	
	Adjustors	OR (95% CI)	p-value	OR (95% CI)	p-value
LVH versus					
PWV	*	1.171 (1.051 to 1.305)	<0.005	1.139 (0.993 to 1.306)	=0.06
	+MAP	1.122 (1.002 to 1.256)	<0.05	1.135 (0.987 to 1.305)	=0.08
Aortic PP	*	1.029 (1.010 to 1.049)	<0.005	1.031 (1.006 to 1.057)	<0.05
	+MAP	1.026 (1.002 to 1.050)	<0.05	1.037 (1.007 to 1.068)	<0.05
Aortic Pf	*	1.041 (1.012 to 1.070)	<0.01	1.043 (1.002 to 1.085)	<0.05
	+MAP	1.027 (0.997 to 1.057)	=0.07	1.045 (1.000 to 1.093)	=0.05
Aortic Pb	*	1.055 (1.018 to 1.094)	<0.005	1.051 (1.006 to 1.098)	<0.05
	+MAP	1.064 (1.014 to 1.115)	=0.01	1.059 (1.004 to 1.117)	<0.05

OR, odds ratios. LV stress, left ventricular systolic wall stress. See table 1 for additional abbreviations. *Adjustors are for MAP as indicated as well as age, body weight, pulse rate, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and treatment for hypertension.

Table 3.5. Relations between aortic forward (Pf) or backward (Pb) wave pressures (in the same regression model) and left ventricular hypertrophy (LVH).

Models		Adjustors	Women (n=478)		Men (n=271)	
LVH vs			OR (95% CI)	p-value	OR (95% CI)	p-value
1)	Pb	* + brachial SBP	1.060 (1.005 to 1.118)	<0.05	1.068 (0.984 to 1.159)	=0.11
	Pf		0.990 (0.958 to 1.022)	=0.53	1.003 (0.933 to 1.079)	=0.93
2)	Pb	* + brachial PP	1.062 (1.001 to 1.127)	<0.05	1.077 (0.984 to 1.179)	=0.11
	Pf		0.989 (0.951 to 1.028)	=0.58	1.024 (0.957 to 1.097)	=0.49

OR, odds ratios. *Adjustors are for BP as indicated as well as age, body weight, pulse rate, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and treatment for hypertension.

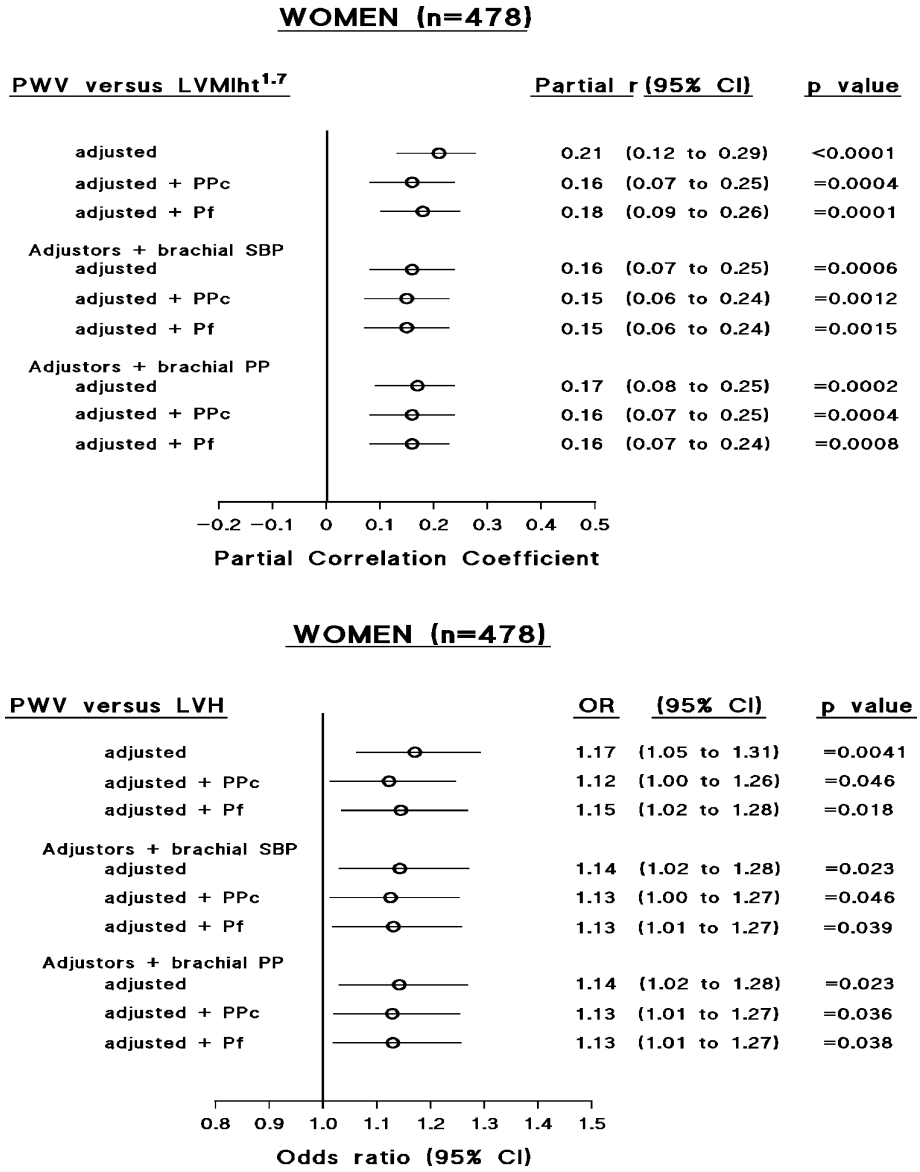


Figure 3.4. Relations between aortic pulse wave velocity (PWV) and left ventricular mass indexed to height$^{1.7}$ (LVMI) or LV hypertrophy (LVH) (n=273 of 478) before and after adjustments for alternative aortic haemodynamic parameters in women of a community sample of African ancestry. See table 1 for additional abbreviations. *Adjustments are for hemodynamic parameter a stated as well as age, body weight, pulse rate, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.1% and treatment for hypertension.

Chapter 4

Discussion

4.1 Summary of main findings.

The main findings of the present study are as follows: In a relatively large, randomly selected community sample of African ancestry, aortic (carotid-femoral) PWV was independently associated with LVMI and LVH in women, but not in men. However, this relationship in women persisted with adjustments for aortic PP and forward wave pressures. Moreover, this occurred despite the fact that aortic PP and backward wave pressures were also independently associated with LVMI and LVH in both women and in men and the relationships between either PWV or aortic BP and LVMI or LVH, although noted to occur together, were independent of each other.

4.2 Comparison with previous studies

As summarized in section 1.4.3 of chapter 1, many studies have shown that indices of arterial stiffness are associated with LVM (Baguet *et al.*, 2000; Bell *et al.*, 2015; Bouthier *et al.*, 1985; Boutouyrie *et al.*, 1995; Chen *et al.*, 1998; Deague *et al.*, 2001; Gates *et al.*, 2003; Iketani *et al.*, 2000; Kobayashi *et al.*, 1996; Leoncini *et al.*, 2006; Libhaber *et al.*, 2008; Ohyama *et al.*, 2016; Roman *et al.*, 2000; Roman *et al.*, 1996; Tatchum-Talom *et al.*, 1995). In this regard three human studies suggest that arterial stiffness effects on LVM are independent of brachial artery BP (Lekakis *et al.*, 2004; Leoncini *et al.*, 2006; Libhaber *et al.*, 2008). Of these studies, one was conducted in a very small study sample (Lekakis *et al.*, 2004) (n=48), another was a study relating the less well recognized ambulatory arterial stiffness index with LVM (Leoncini *et al.*, 2006) (the extent to which the information derived from the ambulatory arterial stiffness index is comparable with that of pulse wave analysis is uncertain as the correlation coefficient between the two is ~0.5) (Li *et al.*, 2006), but the third, performed by our group, showed strong independent relations between aortic PWV and LVM in women, but not in

men in a larger study sample (Libhaber *et al.*, 2008). These data were however described in a smaller study sample of men (n=123 as compared to the 271 men studied in the present analysis), and hence the lack of ability of aortic PWV to relate to LVMI independent of brachial BP in men could have been attributed to the fact that this study was underpowered to show such an effect (Libhaber *et al.*, 2008). However, the present study confirms these findings in a much larger study sample of both women (478 versus 204) and of men. Nevertheless, a number of studies have failed to demonstrate brachial BP or mean arterial pressure-independent relations between PWV and LVMI or on-treatment decreases in LVMI (Chen *et al.*, 1998; Coutinho *et al.*, 2016; Hashimoto *et al.*, 2008a; Hashimoto *et al.*, 2008b; Kaess *et al.*, 2016), even when sex-specific relations were evaluated (Coutinho *et al.*, 2016). Whether differences between the present and these prior studies can be attributed to several of these studies employing small samples of hypertensives (Baguet *et al.*, 2000; Bouthier *et al.*, 1985), where a narrow range of BP values only encompassing the upper tail of the distribution in the population at large might have reduced the power to detect an independent association between LVM index and PWV, is unclear. Differences between studies may also be attributed to differences in the demographic or clinical profiles of the different studies or to differences in measurement techniques several studies employed measurements other than PWV (Chen *et al.*, 1998; Roman *et al.*, 2000; Roman *et al.*, 1996). Importantly however, the present study showed robust probability values for such relations in women and hence is unlikely to reflect a false positive finding. Moreover, because none of the previous large studies could show a relation between measures of aortic stiffness and LVM independent of brachial BP, the present study is the only study where the potential mechanism of this relationship could be evaluated. What are the implications of the findings of the present study?

4.3 Possible implications of the present study

Although aortic PWV is an independent predictor of cardiovascular events (Ben-Shlomo *et al.*, 2014; Vlachopoulos *et al.*, 2010) the extent to which this relationship is explained by an impact of aortic stiffness on aortic PP, associated forward or reflected wave changes is uncertain. Although as indicated above, some studies have shown that the relations between aortic PWV and LVM are independent of brachial BP (Lekakis *et al.*, 2004; Libhaber *et al.*, 2008), none of these prior studies have explored whether these associations are explained by aortic BP effects. Indeed, as indicated in chapter 1, section 1.4.3, aortic PWV could produce increases in LVMI beyond brachial BP through an increased reflected wave amplitude or an wave time (by increasing the speed of wave reflection and by generating closer inflection points) and hence producing a greater aortic systolic PP augmentation (and thus aortic PP and systolic BP), which once again may not be adequately indexed by brachial PP. However, the results of the present study indicate that the brachial-BP independent relationship between aortic PWV and LVMI is not explained by increases in the aortic forward wave pressures, backward wave pressures, and hence increases in aortic PP, systolic BP.

The present results may explain some of the conflicting evidence on relations between aortic PWV and LVMI (Booysen *et al.*, 2015; Hashimoto and Ito, 2011; Sibiya *et al.*, 2015; Tade *et al.*, 2017; Westerhof *et al.*, 2006; Zamani *et al.*, 2015). The present study suggests that any relations which do exist, may not be through alterations in resting aortic forward or backward wave pressures and hence aortic PP. However, these results must be interpreted in context. These results do not suggest that an impedance mismatch mediated by proximal aortic stiffness (as opposed to stiffness along the whole length of the aorta) may not be causally related to increases in LVMI or LVH beyond central aortic pressures as previously demonstrated (Hashimoto and Ito, 2011; Zamani *et al.*, 2015). Moreover, these results do not exclude the possibility of total arterial compliance (reservoir function) significantly influencing

LVMI or LVH as also recently shown (Zamani *et al.*, 2015). In addition, aortic PWV may produce an earlier return of the reflected wave and subsequently enhance LV load during the systolic period of the cardiac cycle. This may be particularly important in women who have a shorter stature and hence have a shorter reflected wave travel distance, thus explaining the relations in women, but not men in the present study. As the assessment of the reflected wave time derived from the 'triangular flow wave' approach to wave separation analysis is inaccurate (Kips *et al.*, 2009) and simultaneous flow and pressure measurements are required for an accurate evaluation (Phan *et al.*, 2016), we could not accurately assess the speed (or timing) of wave reflection. Furthermore, the present results do not exclude the possibility that physical activity-related increases in forward wave and hence PP and systolic BP may be exacerbated by increases in aortic PWV and that these may contribute to LVMI or LVH. In this regard, further work is required to evaluate whether PWV enhances activity-related increases in aortic BP and that these effects contribute to increases in LVM.

Although the present study suggests that brachial BP–independent relations of aortic (carotid-femoral) PWV with LVMI may not be through an impact on resting aortic forward and backward wave pressures and hence aortic PP. Although the role of aortic PWV as a predictor of heart failure has not been adequately addressed (Ben-Shlomo *et al.*, 2014; Vlachopoulos *et al.*, 2010), aortic PWV in some (Coutinho *et al.*, 2016), but not other (Peterson *et al.*, 2016) studies may independently associate with a reduced LV diastolic function, a relationship which may translate into the development of heart failure with a preserved ejection fraction beyond brachial BP. Moreover, increases in aortic stiffness may produce adverse effects on the coronary circulation (Ikonomidis *et al.*, 2008; Watanabe *et al.*, 1993). Whether this explains the predictive power beyond brachial BP of aortic PWV for coronary events which normally involve plaque rupture (Ben-Shlomo *et al.*, 2014; Vlachopoulos *et al.*, 2010), is nevertheless unknown. Based on the present analysis it is possible that aortic PWV predicts coronary events

independent of brachial BP rather because it is an excellent index of long-term cardiovascular damage that is largely insensitive to the benefits of antihypertensive therapy.

There are further caveats to the interpretation of the results of the present study. A lack of brachial BP-independent relationship between aortic PWV and LVMI that cannot be explained by aortic pressure effects, does not imply that a portion of the aortic PWV-LVMI relationship cannot be explained by pulsatile haemodynamic effects which are readily detected at the brachial artery. Indeed, the strength of aortic PWV versus LVMI relations is reduced when adjusting for brachial PP. Because of the strong relationship that exists between brachial PP and either aortic PP, or forward wave pressures, one assumes that this means that aortic PWV-induced increases in forward wave pressure and aortic PP contribute to much of the brachial PP-LVMI relation.

4.4 Are there aortic pressure effects on LVMI beyond brachial BP?

The lack of ability of aortic PP to explain brachial BP-independent relationships between aortic PWV and LVMI or LVH does not exclude the possible importance of aortic BP as a determinant of LVMI beyond brachial BP. Importantly, consistent with several publications in the present study population (Booysen *et al.*, 2015; Norton *et al.*, 2012; Sibiya *et al.*, 2015), and with the findings of several other groups recently summarized in a review (Roman *et al.*, 2007) and a meta-analysis (Ben-Shlomo *et al.*, 2014), the present study shows consistent brachial BP-independent relations between aortic BP and LVMI or LVH. As with previous reports (Sibiya *et al.*, 2015), these findings are explained by the brachial BP- independent relationship between aortic backward, but not forward wave pressure and LVMI or LVH. Hence, the present study also suggests that aortic backward wave effects on end-organ measures should not be seen as representing an impact of aortic stiffness. Indeed, although aortic backward wave pressures contribute substantially to variations in aortic PP and LVMI (Booysen *et al.*, 2015), these effects

are independent of aortic PWV (present study). In this regard, it is important to note that the direct relations between backward wave pressures and aortic PWV that have been described (Sibiya *et al.*, 2015) are likely to be explained by reverse causality. That is, increases in backward wave magnitude cause damage to the aorta and this increases aortic stiffness, rather than increases in aortic stiffness cause an increase in aortic backward wave magnitude. Indeed, it has been argued that once aortic stiffness begins to increase dramatically with age it reduces the impedance mismatch between the aorta and large vessels which reduces wave reflection (Mitchell *et al.*, 2010).

4.5 Possible limitations of the present study

As the assessment of the reflected wave time derived from the 'triangular flow wave' approach to wave separation analysis may be inaccurate (Kaess *et al.*, 2016) and simultaneous flow and pressure measurements are required for an accurate evaluation (Peterson *et al.*, 2016), it could be argued that I cannot exclude a possible contribution of the timing of wave reflection to PWV-LVMI relations. However, the contribution of an earlier time of wave reflection to PWV-LVM relations would be explained by an enhanced aortic PP. As variations in aortic PP did not explain PWV-LVM relations, I assume that the time to wave reflection plays little role in mediating these relations. In addition, the use of the 'triangulation method' of aortic wave separation to derive aortic forward and backward wave pressures has also been questioned (Kaess *et al.*, 2016). However, as emphasized in the methods section, our group have previously shown similar relations between Pf or Pb and LVMI when Pb or Pf were derived from wave separation analysis employing actual flow measurements versus Pb or Pf derived from the triangular waveform approach to wave separation analysis (Bell *et al.*, 2015). Hence, at least in the present study, the use of the 'triangulation method' to derive backward and forward wave pressures is unlikely to have significantly affected the results.

There are several additional possible limitations to the present study that warrant consideration. First, in the present study, calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries (Watanabe *et al.*, 1993). Hence, aortic pressures are likely to have been underestimated using the current approach. However, both forward and backward wave pressures would have been affected by this calibration error and aortic backward, but not forward wave pressures were independently associated with LVMI and LVH. Second, the present study was conducted in one ethnic group and more women than men participated. The present findings may therefore be sex-specific and may not be translatable to other ethnic groups.

4.6 Conclusions

In conclusion, in the present study I show, in a relatively large randomly selected community sample that carotid-femoral PWV is associated with LVMI and LVH in women, but not in men and that these relations in women are independent of brachial PP and systolic BP. However, these relations between aortic PWV and LVMI or LVH in women were not attributed to increases in aortic forward or backward wave pressures and hence to increases in aortic PP or LV load as determined under resting conditions. Nevertheless, in addition to aortic PWV being independently associated with LVMI and LVH, both aortic PP and backward wave pressures were independently associated with LVMI and LVH beyond brachial BP. These data provide further insights into the mechanisms that account for relationship between aortic PWV and cardiovascular risk.

4.7 Potential clinical relevance

The results of this study provide further knowledge on the relationship between aortic

pulse wave velocity and other aortic hemodynamic parameters and LVMI. In this regard, the data provide some mechanistic insights into the ability of aortic pulse wave velocity to independently predict cardiovascular events. As the associations of aortic pulse wave velocity and Pb (and hence pulse pressure) with LVMI and LVH produce effects which were independent of each other, aortic pulse wave velocity and backward waves are likely to have a differential impact on LVMI. These data may potentially guide clinical management strategies in the future in that both aortic pulse wave velocity and aortic backward waves need to be targeted in order to prevent the development of LVH in response to increased stiffness and high pulse pressures.

References

Akpalu, A., Sarfo, F. S., Ovbiagele, B., Akinyemi, R., Gebregziabher, M., Obiako, R., Owolabi, L., Sagoe, K., Jenkins, C. & Arulogun, O. 2015. Phenotyping stroke in sub-Saharan Africa: stroke investigative research and education network (SIREN) phenomics protocol. *Neuroepidemiology,* 45**,** 73-82.

Alghatrif, M., Strait, J. B., Morrell, C. H., Canepa, M., Wright, J., Elango, P., Scuteri, A., Najjar, S. S., Ferrucci, L. & Lakatta, E. G. 2013. Longitudinal trajectories of arterial stiffness and the role of blood pressure. *Hypertension***,** HYPERTENSIONAHA. 113.01445.

Avolio, A. P., Van Bortel, L. M., Boutouyrie, P., Cockcroft, J. R., Mceniery, C. M., Protogerou, A. D., Roman, M. J., Safar, M. E., Segers, P. & Smulyan, H. 2009. Role of pulse pressure amplification in arterial hypertension. *Hypertension,* 54**,** 375-383.

Baguet, J.-P., Moreau-Gaudry, A., Siché, J.-P., Douchin, S., Tremel, F. & Mallion, J.-M. 2000. Carotid remodeling in essential hypertension: role of blood pressure, indexed parameters, and association with cardiac mass and aortic stiffness. *Clinical and Experimental Hypertension,* 22**,** 717-729.

Baumann, M., Wassertheurer, S., Suttmann, Y., Burkhardt, K. & Heemann, U. 2014. Aortic pulse wave velocity predicts mortality in chronic kidney disease stages 2–4. *Journal of Hypertension,* 32**,** 899-903.

Bell, V., Sigurdsson, S., Westenberg, J. J., Gotal, J. D., Torjesen, A. A., Aspelund, T., Launer, L. J., Harris, T. B., Gudnason, V. & De Roos, A. 2015. Relations Between Aortic Stiffness and Left Ventricular Structure and Function in Older Participants in the Age, Gene/Environment Susceptibility-Reykjavik StudyCLINICAL PERSPECTIVE. *Circulation: Cardiovascular Imaging.* doi:10.1161/CIRCIMAGING.114.003039.

Ben-Shlomo, Y., Spears, M., Boustred, C., May, M., Anderson, S. G., Benjamin, E. J., Boutouyrie, P., Cameron, J., Chen, C.-H. & Cruickshank, J. K. 2014. Aortic pulse wave

velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *Journal of the American College of Cardiology,* 63**,** 636-646.

Bennett, C., Guo, M. & Dharmage, S. 2007. HbA1c as a screening tool for detection of type 2 diabetes: a systematic review. *Diabetic Medicine,* 24**,** 333-343.

Birru, M. S., Matthews, K. A., Thurston, R. C., Brooks, M. M., Ibrahim, S., Barinas-Mitchell, E., Janssen, I. & Sutton-Tyrrell, K. 2011. African American ethnicity and cardiovascular risk factors are related to aortic pulse wave velocity progression. *American Journal of Hypertension,* 24**,** 809.

Booysen, H. L., Woodiwiss, A. J., Sibiya, M. J., Hodson, B., Raymond, A., Libhaber, E., Sareli, P. & Norton, G. R. 2015. Indexes of Aortic Pressure Augmentation Markedly Underestimate the Contribution of Reflected Waves Toward Variations in Aortic Pressure and Left Ventricular MassNovelty and Significance. *Hypertension,* 65**,** 540-546.

Boutayeb, A. 2006. The double burden of communicable and non-communicable diseases in developing countries. *Transactions of the Royal society of Tropical Medicine and Hygiene,* 100**,** 191-199.

Bouthier, J., De Luca, N., Safar, M. & Simon, A. C. 1985. Cardiac hypertrophy and arterial distensibility in essential hypertension. *American Heart journal,* 109**,** 1345-1352.

Boutouyrie, P., Bussy, C., Lacolley, P., Girerd, X., Laloux, B. & Laurent, S. 1999. Association between local pulse pressure, mean blood pressure, and large-artery remodeling. *Circulation,* 100**,** 1387-1393.

Boutouyrie, P., Laurent, S., Girerd, X., Benetos, A., Lacolley, P., Abergel, E. & Safar, M. 1995. Common carotid artery stiffness and patterns of left ventricular hypertrophy in hypertensive patients. *Hypertension,* 25**,** 651-659.

Campbell, N. R., Khalsa, T., Lackland, D. T., Niebylski, M. L., Nilsson, P. M., Redburn, K. A., Orias, M., Zhang, X. H., Burrell, L. & Horiuchi, M. 2016. High Blood Pressure 2016: Why

Prevention and Control Are Urgent and Important. The World Hypertension League, International Society of Hypertension, World Stroke Organization, International Diabetes Foundation, International Council of Cardiovascular Prevention and Rehabilitation, International Society of Nephrology. *The Journal of Clinical Hypertension,* 18**,** 714-717.

Cappuccio, F. P. & Miller, M. A. 2016. Cardiovascular disease and hypertension in sub-Saharan Africa: burden, risk and interventions. Internal and Emergency Medicine, 11, 299.

Casale, P. N., Devereux, R. B., Milner, M., Zullo, G., Harshfield, G. A., Pickering, T. G. & Laragh, J. H. 1986. Value of echocardiographic measurement of left ventricular mass in predicting cardiovascular morbid events in hypertensive men. *Annals of Internal Medicine,* 105**,** 173-178.

Cavalcante, J. L., Lima, J. A., Redheuil, A. & Al-Mallah, M. H. 2011. Aortic stiffness: current understanding and future directions. *Journal of the American College of Cardiology,* 57**,** 1511-1522.

Cecelja, M. & Chowienczyk, P. 2009. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension. *Hypertension,* 54**,** 1328-1336.

Chen, C.-H., Ting, C.-T., Lin, S.-J., Hsu, T.-L., Ho, S.-J., Chou, P., Chang, M.-S., O'connor, F., Spurgeon, H. & Lakatta, E. 1998. Which arterial and cardiac parameters best predict left ventricular mass? *Circulation,* 98**,** 422-428.

Chirinos, J. A., Kips, J. G., Jacobs, D. R., Brumback, L., Duprez, D. A., Kronmal, R., Bluemke, D. A., Townsend, R. R., Vermeersch, S. & Segers, P. 2012. Arterial wave reflections and incident cardiovascular events and heart failure: MESA (Multiethnic Study of Atherosclerosis). *Journal of the American College of Cardiology,* 60**,** 2170-2177.

Chirinos, J. A., Segers, P., De Buyzere, M. L., Kronmal, R. A., Raja, M. W., De Bacquer, D., Claessens, T., Gillebert, T. C., John-Sutton, M. S. & Rietzschel, E. R. 2010. Left ventricular mass. *Hypertension,* 56**,** 91-98.

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo Jr, J. L., Jones, D. W., Materson, B. J., Oparil, S. & Wright Jr, J. T. 2003. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *Jama,* 289**,** 2560-2571.

Clarke, R., Lewington, S., Youngman, L., Sherliker, P., Peto, R. & Collins, R. 2002. Underestimation of the importance of blood pressure and cholesterol for coronary heart disease mortality in old age. *European Heart Journal,* 23**,** 286-293.

Cohen, D. L. & Townsend, R. R. 2011. Central blood pressure and chronic kidney disease progression. *International Journal of Nephrology,* 2011. Doi:http//dx.doi.org.10.4046/2011/407801.

Connor, M. D., Modi, G. & Warlow, C. P. 2009. Differences in the Nature of Stroke in a Multiethnic Urban South African Population. *Stroke,* 40**,** 355-362.

Coutinho, T., Pellikka, P. A., Bailey, K. R., Turner, S. T. & Kullo, I. J. 2016. Sex differences in the associations of hemodynamic load with left ventricular hypertrophy and concentric remodeling. *American Journal of Hypertension,* 29**,** 73-80.

Dao, H. H., Essalihi, R., Bouvet, C. & Moreau, P. 2005. Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension. *Cardiovascular Research,* 66**,** 307-317.

Deague, J. A., Wilson, C. M., Grigg, L. E. & Stephen, B. 2001. Physiological relationships between central vascular haemodynamics and left ventricular structure. *Clinical Science,* 101**,** 79-85.

Devereux, R. B., Alonso, D. R., Lutas, E. M., Gottlieb, G. J., Campo, E., Sachs, I. & Reichek, N. 1986. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *The American Journal of Cardiology,* 57**,** 450-458.

Drazner, M. H., Rame, J. E., Marino, E. K., Gottdiener, J. S., Kitzman, D. W., Gardin, J. M., Manolio, T. A., Dries, D. L. & Siscovick, D. S. 2004. Increased left ventricular mass is a risk factor for the development of a depressed left ventricular ejection fraction within five years: the Cardiovascular Health Study. *Journal of the American College of Cardiology,* 43**,** 2207-2215.

Ebrahim, S. & Smith, G. D. 2001. Exporting failure? Coronary heart disease and stroke in developing countries. IEA. DOI: https://doi.org/10.1093/ije/30.2.201.

García-Espinosa, V., Curcio, S., Marotta, M., Castro, J. M., Arana, M., Peluso, G., Chiesa, P., Giachetto, G., Bia, D. & Zócalo, Y. 2016. Changes in Central Aortic Pressure Levels, Wave Components and Determinants Associated with High Peripheral Blood Pressure States in Childhood: Analysis of Hypertensive Phenotype. *Pediatric Cardiology,* 37**,** 1340-1350.

Gardin, J. M., Mcclelland, R., Kitzman, D., Lima, J. A., Bommer, W., Klopfenstein, H. S., Wong, N. D., Smith, V.-E. & Gottdiener, J. 2001. M-mode echocardiographic predictors of six-to seven-year incidence of coronary heart disease, stroke, congestive heart failure, and mortality in an elderly cohort (the Cardiovascular Health Study). *The American Journal of Cardiology,* 87**,** 1051-1057.

Gates, P. E., Tanaka, H., Graves, J. & Seals, D. R. 2003. Left ventricular structure and diastolic function with human ageing. *European Heart Journal,* 24**,** 2213-2220.

Ghali, J. K., Liao, Y. & Cooper, R. S. 1998. Influence of left ventricular geometric patterns on prognosis in patients with or without coronary artery disease. *Journal of the American College of Cardiology,* 31**,** 1635-1640.

Group, S. R. 2015. A randomized trial of intensive versus standard blood-pressure control. *N Engl J Med,* 2015**,** 2103-2116.

Guerin, A. P., Blacher, J., Pannier, B., Marchais, S. J., Safar, M. E. & London, G. M. 2001. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation,* 103**,** 987-992.

Hansen, T. W., Staessen, J. A., Torp-Pedersen, C., Rasmussen, S., Thijs, L., Ibsen, H. & Jeppesen, J. 2006. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation,* 113**,** 664-670.

Harald, K., Koskinen, S., Jousilahti, P., Torppa, J., Vartiainen, E. & Salomaa, V. 2008. Changes in traditional risk factors no longer explain time trends in cardiovascular mortality and its socioeconomic differences. *Journal of Epidemiology and Community Health,* 62**,** 251-257.

Hashimoto, J. & Ito, S. 2011. Central Pulse Pressure and Aortic Stiffness Determine Renal Hemodynamics. *Hypertension,* 58**,** 839-846.

Hashimoto, J., Nichols, W. W., O'rourke, M. F. & Imai, Y. 2008a. Association between wasted pressure effort and left ventricular hypertrophy in hypertension: influence of arterial wave reflection. *American Journal of Hypertension,* 21**,** 329-333.

Hashimoto, J., Westerhof, B. E., Westerhof, N., Imai, Y. & O'rourke, M. F. 2008b. Different role of wave reflection magnitude and timing on left ventricular mass reduction during antihypertensive treatment. *Journal of Hypertension,* 26**,** 1017-1024.

Hirai, T., Sasayama, S., Kawasaki, T. & Yagi, S. 1989. Stiffness of systemic arteries in patients with myocardial infarction. A noninvasive method to predict severity of coronary atherosclerosis. *Circulation,* 80**,** 78-86.

Hodson, B., Norton, G. R., Booysen, H. L., Sibiya, M. J., Raymond, A., Maseko, M. J., Majane, O. H., Libhaber, E., Sareli, P. & Woodiwiss, A. J. 2015. Brachial pressure control fails to account for most distending pressure-independent, age-related aortic hemodynamic

changes in adults. *American Journal of Hypertension***,** hpv140.DOI: https://doi.org/10.1093/ajh/hpv140.

Hope, S. A., Tay, D. B., Meredith, I. T. & Cameron, J. D. 2005. Waveform dispersion, not reflection, may be the major determinant of aortic pressure wave morphology. *American Journal of Physiology-Heart and Circulatory Physiology,* 289**,** H2497-H2502.

Ibrahim, E.-S. H., Johnson, K. R., Miller, A. B., Shaffer, J. M. & White, R. D. 2010. Measuring aortic pulse wave velocity using high-field cardiovascular magnetic resonance: comparison of techniques. *Journal of Cardiovascular Magnetic Resonance,* 12**,** 26.

Ibrahim, M. M. & Damasceno, A. 2012. Hypertension in developing countries. *The Lancet,* 380**,** 611-619.

Iketani, T., Iketani, Y., Takazawa, K. & Yamashina, A. 2000. The influence of the peripheral reflection wave on left ventricular hypertrophy in patients with essential hypertension. *Hypertension Research,* 23**,** 451-458.

Ikonomidis, I., Lekakis, J., Papadopoulos, C., Triantafyllidi, H., Paraskevaidis, I., Georgoula, G., Tzortzis, S., Revela, I. & Kremastinos, D. T. 2008. Incremental value of pulse wave velocity in the determination of coronary microcirculatory dysfunction in never-treated patients with essential hypertension. *American Journal of Hypertension,* 21**,** 806-813.

Irazola, V. E., Gutierrez, L., Bloomfield, G., Carrillo-Larco, R. M., Prabhakaran, D., Gaziano, T., Levitt, N. S., Miranda, J. J., Ortiz, A. B. & Steyn, K. 2016. Hypertension prevalence, awareness, treatment, and control in selected LMIC communities: results from the NHLBI/UHG network of Centers of Excellence for chronic diseases. *Global Heart,* 11**,** 47-59.

Izzo Jr, J. L. 2004. Arterial stiffness and the systolic hypertension syndrome. *Current Opinion in Cardiology,* 19**,** 341-352.

Jankowski, P., Kawecka-Jaszcz, K., Czarnecka, D., Brzozowska-Kiszka, M., Styczkiewicz, K., Loster, M., Kloch-Badełek, M., Wiliński, J., Curyło, A. M. & Dudek, D. 2008. Pulsatile but

not steady component of blood pressure predicts cardiovascular events in coronary patients. *Hypertension,* 51**,** 848-855.

Kaess, B. M., Rong, J., Larson, M. G., Hamburg, N. M., Vita, J. A., Cheng, S., Aragam, J., Levy, D., Benjamin, E. J. & Vasan, R. S. 2016. Relations of central hemodynamics and aortic stiffness with left ventricular structure and function: the Framingham Heart Study. *Journal of the American Heart Association*. doi:10.1161/JAHA.115.002693.

Kaess, B. M., Rong, J., Larson, M. G., Hamburg, N. M., Vita, J. A., Levy, D., Benjamin, E. J., Vasan, R. S. & Mitchell, G. F. 2012. Aortic stiffness, blood pressure progression, and incident hypertension. *Jama,* 308**,** 875-881.

Kahn, K., Tollman, S. M., Garenne, M. & Gear, J. S. 2000. Validation and application of verbal autopsies in a rural area of South Africa. *Tropical Medicine & International Health,* 5**,** 824-831.

Kips, J. G., Rietzschel, E. R., De Buyzere, M. L., Westerhof, B. E., Gillebert, T. C., Van Bortel, L. M. & Segers, P. 2009. Evaluation of noninvasive methods to assess wave reflection and pulse transit time from the pressure waveform alone. *Hypertension,* 53**,** 142-149.

Kobayashi, S., Yano, M., Kohno, M., Obayashi, M., Hisamatsu, Y., Ryoke, T., Ohkusa, T., Yamakawa, K. & Matsuzaki, M. 1996. Influence of aortic impedance on the development of pressure-overload left ventricular hypertrophy in rats. *Circulation,* 94**,** 3362-3368.

Kohro, T., Furui, Y., Mitsutake, N., Fujii, R., Morita, H., Oku, S., Ohe, K. & Nagai, R. 2008. The Japanese national health screening and intervention program aimed at preventing worsening of the metabolic syndrome. *International Heart Journal,* 49**,** 193-203.

Kovacic, J. C., Moreno, P., Hachinski, V., Nabel, E. G. & Fuster, V. 2011. Cellular senescence, vascular disease, and aging. *Circulation,* 123**,** 1650-1660.

Lam, C. S., Xanthakis, V., Sullivan, L. M., Lieb, W., Aragam, J., Redfield, M. M., Mitchell, G. F., Benjamin, E. J. & Vasan, R. S. 2010. Aortic root remodeling over the adult life course. *Circulation,* 122**,** 884-890.

Laurent, S. & Boutouyrie, P. 2013. Arterial Stiffness and Cardiovascular Events in Hypertensives. *Current Cardiovascular Risk Reports,* 7**,** 238-243.

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I. & Struijker-Boudier, H. 2006. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European Heart Journal,* 27**,** 2588-2605.

Lekakis, J. P., Zakopoulos, N. A., Protogerou, A. D., Kotsis, V. T., Papaioannou, T. G., Stamatelopoulos, K. S., Tsitsiricos, M. D., Pitiriga, V. C., Papamichael, C. M. & Toumanides, S. T. 2004. Cardiac hypertrophy in hypertension: relation to 24-h blood pressure profile and arterial stiffness. *International Journal of Cardiology,* 97**,** 29-33.

Leoncini, G., Ratto, E., Viazzi, F., Vaccaro, V., Parodi, A., Falqui, V., Conti, N., Tomolillo, C., Deferrari, G. & Pontremoli, R. 2006. Increased ambulatory arterial stiffness index is associated with target organ damage in primary hypertension. *Hypertension,* 48**,** 397-403.

Levy, B., Ambrosio, G., Pries, A. & Struijker-Boudier, H. 2001. Microcirculation in hypertension. *Circulation,* 104**,** 735-740.

Levy, D., Garrison, R. J., Savage, D. D., Kannel, W. B. & Castelli, W. P. 1990. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *New England Journal of Medicine,* 322**,** 1561-1566.

Levy, D., Salomon, M., D'agostino, R. B., Belanger, A. J. & Kannel, W. B. 1994. Prognostic implications of baseline electrocardiographic features and their serial changes in subjects with left ventricular hypertrophy. *Circulation,* 90**,** 1786-1793.

Li, Y., Wang, J.-G., Dolan, E., Gao, P.-J., Guo, H.-F., Nawrot, T., Stanton, A. V., Zhu, D.-L., O'brien, E. & Staessen, J. A. 2006. Ambulatory arterial stiffness index derived from 24-hour ambulatory blood pressure monitoring. *Hypertension,* 47**,** 359-364.

Liao, Y., Cooper, R. S., Mcgee, D. L., Mensah, G. A. & Ghali, J. K. 1995. The relative effects of left ventricular hypertrophy, coronary artery disease, and ventricular dysfunction on survival among black adults. *JAMA,* 273**,** 1592-1597.

Libhaber, E., Woodiwiss, A. J., Libhaber, C., Maseko, M., Majane, O. H., Makaula, S., Dessein, P., Essop, M. R., Sareli, P. & Norton, G. R. 2008. Gender-specific brachial artery blood pressure-independent relationship between pulse wave velocity and left ventricular mass index in a group of African ancestry. *Journal of Hypertension,* 26**,** 1619-1628.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R. & Ahn, S. Y. 2013. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet, 380, 2095-2128.

Mackenzie, I., Wilkinson, I. & Cockcroft, J. 2002. Assessment of arterial stiffness in clinical practice. *Qjm,* 95**,** 67-74.

Malhotra, R., Puoane, T., Hoyo, C., Hughes, G. & Østbye, T. 2008. Prevalence and awareness of hypertension in an urban township of South Africa: compelling need for action. *Ethn Dis,* 18**,** 401-402.

Mancia, G., Fagard, R., Narkiewicz, K., Redon, J., Zanchetti, A., Böhm, M., Christiaens, T., Cifkova, R., De Backer, G. & Dominiczak, A. 2013. 2013 ESH/ESC guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Blood Pressure,* 22**,** 193-278.

Maseko, M. J., Norton, G. R., Majane, O., Molebatsi, N. & Woodiwiss, A. J. 2011. Global cardiovascular risk profiles of untreated hypertensives in an urban, developing community in Africa: cardiovascular topics. *Cardiovascular Journal of Africa,* 22**,** 261-267.

Mceniery, C. M., Cockcroft, J. R., Roman, M. J., Franklin, S. S. & Wilkinson, I. B. 2014. Central blood pressure: current evidence and clinical importance. *European Heart Journal,* 35**,** 1719-1725.

Mceniery, C. M., Hall, I. R., Qasem, A., Wilkinson, I. B., Cockcroft, J. R. & Investigators, A. 2005. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *Journal of the American College of Cardiology,* 46**,** 1753-1760.

Mceniery, C. M., Mcdonnell, B. J., So, A., Aitken, S., Bolton, C. E., Munnery, M., Hickson, S. S., Maki-Petaja, K. M., Cockcroft, J. R. & Dixon, A. K. 2009. Aortic calcification is associated with aortic stiffness and isolated systolic hypertension in healthy individuals. *Hypertension,* 53**,** 524-531.

Mceniery, C. M., Spratt, M., Munnery, M., Yarnell, J., Lowe, G. D., Rumley, A., Gallacher, J., Ben-Shlomo, Y., Cockcroft, J. R. & Wilkinson, I. B. 2010. An analysis of prospective risk factors for aortic stiffness in men. *Hypertension,* 56**,** 36-43.

Menotti, A., Lanti, M., Angeletti, M., Botta, G., Cirillo, M., Laurenzi, M., Mancini, M., Panarelli, W., Scavizzi, P. & Terradura-Vagnarelli, O. 2009. Twenty-year cardiovascular and all-cause mortality trends and changes in cardiovascular risk factors in Gubbio, Italy: the role of blood pressure changes. *Journal of Hypertension,* 27**,** 266-274.

Mensah, G. A. 2008. Epidemiology of stroke and high blood pressure in Africa. *Heart,* 94**,** 697-705.

Mitchell, G. F. 2006. Triangulating the peaks of arterial pressure. *Hypertension,* 48**,** 543-545.

Mitchell, G. F. 2008. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *Journal of Applied Physiology,* 105**,** 1652-1660.

Mitchell, G. F. 2009. Clinical achievements of impedance analysis. *Medical & Biological Engineering & Computing,* 47**,** 153-163.

Mitchell, G. F. 2015. Arterial stiffness: insights from Framingham and Iceland. *Current Opinion in Nephrology and Hypertension,* 24**,** 1-7.

Mitchell, G. F., Hwang, S.-J., Vasan, R. S., Larson, M. G., Pencina, M. J., Hamburg, N. M., Vita, J. A., Levy, D. & Benjamin, E. J. 2010. Arterial stiffness and cardiovascular events. *Circulation,* 121**,** 505-511.

Mitchell, G. F., Parise, H., Benjamin, E. J., Larson, M. G., Keyes, M. J., Vita, J. A., Vasan, R. S. & Levy, D. 2004. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women. *Hypertension,* 43**,** 1239-1245.

Mitchell, G. F., Van Buchem, M. A., Sigurdsson, S., Gotal, J. D., Jonsdottir, M. K., Kjartansson, Ó., Garcia, M., Aspelund, T., Harris, T. B. & Gudnason, V. 2011. Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility–Reykjavik study. *Brain,* 134**,** 3398-3407.

Mulvany, M. & Aalkjaer, C. 1990. Structure and function of small arteries. *Physiological Reviews,* 70**,** 921-961.

Nichols, W., O'rourke, M. & Vlachopoulos, C. 2011. *McDonald's Blood flow in Arteries: Theoretical, Experimental and Clinical Principles*, CRC press.

Norton, G. R., Majane, O. H., Maseko, M. J., Libhaber, C., Redelinghuys, M., Kruger, D., Veller, M., Sareli, P. & Woodiwiss, A. J. 2012. Brachial Blood Pressure–Independent Relations Between Radial Late Systolic Shoulder-Derived Aortic Pressures and Target Organ Changes. *Hypertension,* 59**,** 885-892.

Norton, G. R., Maseko, M., Libhaber, E., Libhaber, C. D., Majane, O. H., Dessein, P., Sareli, P. & Woodiwiss, A. J. 2008. Is prehypertension an independent predictor of target organ changes in young-to-middle-aged persons of African descent? *Journal of Hypertension,* 26**,** 2279-2287.

O'brien, E., Asmar, R., Beilin, L., Imai, Y., Mallion, J.-M., Mancia, G., Mengden, T., Myers, M., Padfield, P. & Palatini, P. 2003. European Society of Hypertension recommendations for

conventional, ambulatory and home blood pressure measurement. *Journal of Hypertension,* 21**,** 821-848.

O'rourke, M. F., Safar, M. E. & Dzau, V. 2010. The Cardiovascular Continuum extended: aging effects on the aorta and microvasculature. *Vascular Medicine***.** DOI:10.1177/1358863X10382946.

O'rourke, M. F. & Hashimoto, J. 2007. Mechanical factors in arterial aging: a clinical perspective. *Journal of the American College of Cardiology,* 50**,** 1-13.

O'rourke, M. F., Staessen, J. A., Vlachopoulos, C. & Duprez, D. 2002. Clinical applications of arterial stiffness; definitions and reference values. *American Journal of Hypertension,* 15**,** 426-444.

Ohyama, Y., Ambale-Venkatesh, B., Noda, C., Chugh, A. R., Teixido-Tura, G., Kim, J.-Y., Donekal, S., Yoneyama, K., Gjesdal, O. & Redheuil, A. 2016. Association of Aortic Stiffness With Left Ventricular Remodeling and Reduced Left Ventricular Function Measured by Magnetic Resonance Imaging CLINICAL PERSPECTIVE. *Circulation: Cardiovascular Imaging*. doi: 10.1161/CIRCIMAGING.115.004426.

Oliver, J. J. & Webb, D. J. 2003. Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 23**,** 554-566.

Ott, C. E., Grünhagen, J., Jäger, M., Horbelt, D., Schwill, S., Kallenbach, K., Guo, G., Manke, T., Knaus, P. & Mundlos, S. 2011. MicroRNAs differentially expressed in postnatal aortic development downregulate elastin via 3′ UTR and coding-sequence binding sites. *PLoS One.* http://dxdoi.org/10.1371/Journal.pone.0016250.

Peterson, V. R., Woodiwiss, A. J., Libhaber, C. D., Raymond, A., Sareli, P. & Norton, G. R. 2016. Cardiac Diastolic Dysfunction is Associated With Aortic Wave Reflection, but Not Stiffness in a Predominantly Young-to-Middle–Aged Community Sample. *American journal of hypertension***.** doi: http//doi.org/10.1093/ajh/hpw052.

Phan, T. S., Li, J. K. J., Segers, P., Reddy-Koppula, M., Akers, S. R., Kuna, S. T., Gislason, T., Pack, A. I. & Chirinos, J. A. 2016. Aging is Associated With an Earlier Arrival of Reflected Waves Without a Distal Shift in Reflection Sites. *Journal of the American Heart Association.* DOI: 10.1161/JAHA.116.003733.

Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., Jones, D. W., Kurtz, T., Sheps, S. G. & Roccella, E. J. 2005. Recommendations for blood pressure measurement in humans and experimental animals. *Circulation,* 111**,** 697-716.

Reddy, K. S. 2002. Cardiovascular diseases in the developing countries: dimensions, determinants, dynamics and directions for public health action. *Public Health Nutrition,* 5**,** 231-237.

Redelinghuys, M., Norton, G. R., Scott, L., Maseko, M. J., Brooksbank, R., Majane, O. H., Sareli, P. & Woodiwiss, A. J. 2010. Relationship between urinary salt excretion and pulse pressure and central aortic hemodynamics independent of steady state pressure in the general population. *Hypertension,* 56**,** 584-590.

Roman, M. J., Devereux, R. B., Kizer, J. R., Lee, E. T., Galloway, J. M., Ali, T., Umans, J. G. & Howard, B. V. 2007. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure. *Hypertension,* 50**,** 197-203.

Roman, M. J., Ganau, A., Saba, P. S., Pini, R., Pickering, T. G. & Devereux, R. B. 2000. Impact of arterial stiffening on left ventricular structure. *Hypertension,* 36**,** 489-494.

Roman, M. J., Pickering, T. G., Schwartz, J. E., Pini, R. & Devereux, R. B. 1996. Relation of arterial structure and function to left ventricular geometric patterns in hypertensive adults. *Journal of the American College of Cardiology,* 28**,** 751-756.

Russo, C., Jin, Z., Palmieri, V., Homma, S., Rundek, T., Elkind, M. S., Sacco, R. L. & Di Tullio, M. R. 2012. Arterial Stiffness and Wave ReflectionNovelty and Significance. *Hypertension,* 60**,** 362-368.

Safar, M. E. 1989. Pulse pressure in essential hypertension: clinical and therapeutical implications. *Journal of Hypertension,* 7**,** 769-776.

Safar, M. E., Blacher, J., Pannier, B., Guerin, A. P., Marchais, S. J., Guyonvarc'h, P.-M. & London, G. M. 2002. Central pulse pressure and mortality in end-stage renal disease. *Hypertension,* 39**,** 735-738.

Safar, M. E., Levy, B. I. & Struijker-Boudier, H. 2003. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation,* 107**,** 2864-2869.

Safar, M. E., London, G. M. & Plante, G. E. 2004. Arterial stiffness and kidney function. *Hypertension,* 43**,** 163-168.

Sahn, D. J., Demaria, A., Kisslo, J. & Weyman, A. F. 1978. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation,* 58**,** 1072-1083.

Sehestedt, T., Jeppesen, J., Hansen, T. W., Rasmussen, S., Wachtell, K., Ibsen, H., Torp-Pedersen, C. & Olsen, M. H. 2012. Thresholds for pulse wave velocity, urine albumin creatinine ratio and left ventricular mass index using SCORE, Framingham and ESH/ESC risk charts. *Journal of Hypertension,* 30**,** 1928-1936.

Sehestedt, T., Jeppesen, J., Hansen, T. W., Wachtell, K., Ibsen, H., Torp-Petersen, C., Hildebrandt, P. & Olsen, M. H. 2010. Risk prediction is improved by adding markers of subclinical organ damage to SCORE. *European Heart Journal,* 31**,** 883-891.

Shiburi, C. P., Staessen, J. A., Maseko, M., Wojciechowska, W., Thijs, L., Van Bortel, L. M., Woodiwiss, A. J. & Norton, G. R. 2006. Reference values for SphygmoCor measurements in South Africans of African ancestry. *American Journal of Hypertension,* 19**,** 40-46.

Sibiya, M. J., Woodiwiss, A. J., Booysen, H. L., Raymond, A., Millen, A. M., Maseko, M. J., Majane, O. H., Sareli, P., Libhaber, E. & Norton, G. R. 2015. Reflected rather than

forward wave pressures account for brachial pressure-independent relations between aortic pressure and end-organ changes in an African community. *Journal of Hypertension,* 33**,** 2083-2090.

Smulyan, H., Asmar, R. G., Rudnicki, A., London, G. M. & Safar, M. E. 2001. Comparative effects of aging in men and women on the properties of the arterial tree. *Journal of the American College of Cardiology,* 37**,** 1374-1380.

Smulyan, H., Mookherjee, S. & Safar, M. E. 2016. The two faces of hypertension: role of aortic stiffness. *Journal of the American Society of Hypertension,* 10**,** 175-183.

Steyn, K., Bradshaw, D., Norman, R. & Laubscher, R. 2008. Determinants and treatment of hypertension in South Africans: the first Demographic and Health Survey. *SAMJ: South African Medical Journal,* 98**,** 376-380.

Sun, Z. 2015. Aging, arterial stiffness, and hypertension. *Hypertension,* 65**,** 252-256.

Szecsenyi, J., Rosemann, T., Joos, S., Peters-Klimm, F. & Miksch, A. 2008. German diabetes disease management programs are appropriate for restructuring care according to the chronic care model. *Diabetes care,* 31**,** 1150-1154.

Tade, G., Norton, G. R., Booysen, H. L., Sibiya, M. J., Ballim, I., Sareli, P., Libhaber, E., Majane, O. H. & Woodiwiss, A. J. 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**,** 300-309.

Tatchum-Talom, R., Niederhoffer, N., Amin, F., Makki, T., Tankosic, P. & Atkinson, J. 1995. Aortic stiffness and left ventricular mass in a rat model of isolated systolic hypertension. *Hypertension,* 26**,** 963-970.

Townsend, R. R., Wilkinson, I. B., Schiffrin, E. L., Avolio, A. P., Chirinos, J. A., Cockcroft, J. R., Heffernan, K. S., Lakatta, E. G., Mceniery, C. M. & Mitchell, G. F. 2015. Recommendations for improving and standardizing vascular research on arterial stiffness. *Hypertension,* 66**,** 698-722.

Urbina, E. M., Kimball, T. R., Mccoy, C. E., Khoury, P. R., Daniels, S. R. & Dolan, L. M. 2009. Youth with obesity and obesity-related type 2 diabetes mellitus demonstrate abnormalities in carotid structure and function. *Circulation,* 119**,** 2913-2919.

Urbina, E. M., Srinivasan, S. R., Tang, R., Bond, M. G., Kieltyka, L. & Berenson, G. S. 2002. Impact of multiple coronary risk factors on the intima-media thickness of different segments of carotid artery in healthy young adults (The Bogalusa Heart Study). *The American Journal of Cardiology,* 90**,** 953-958.

Van Bortel, L. M., Laurent, S., Boutouyrie, P., Chowienczyk, P., Cruickshank, J., De Backer, T., Filipovsky, J., Huybrechts, S., Mattace-Raso, F. U. & Protogerou, A. D. 2012. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *Journal of Hypertension,* 30**,** 445-448.

Vasan, R. S., Larson, M. G., Leip, E. P., Evans, J. C., O'donnell, C. J., Kannel, W. B. & Levy, D. 2001. Impact of high-normal blood pressure on the risk of cardiovascular disease. *New England Journal of Medicine,* 345**,** 1291-1297.

Verdecchia, P., Porcellati, C., Reboldi, G., Gattobigio, R., Borgioni, C., Pearson, T. A. & Ambrosio, G. 2001. Left ventricular hypertrophy as an independent predictor of acute cerebrovascular events in essential hypertension. *Circulation,* 104**,** 2039-2044.

Vlachopoulos, C., Aznaouridis, K. & Stefanadis, C. 2010. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *Journal of the American College of Cardiology,* 55**,** 1318-1327.

Vlachopoulos, C., Xaplanteris, P., Aboyans, V., Brodmann, M., Cífková, R., Cosentino, F., De Carlo, M., Gallino, A., Landmesser, U. & Laurent, S. 2015. The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: Endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society. *Atherosclerosis,* 241**,** 507-532.

Wagenseil, J. E. 2011. A constrained mixture model for developing mouse aorta. *Biomechanics and Modeling in Mechanobiology,* 10**,** 671-687.

Wagenseil, J. E. & Mecham, R. P. 2009. Vascular extracellular matrix and arterial mechanics. *Physiological Reviews,* 89**,** 957-989.

Wang, K.-L., Cheng, H.-M., Chuang, S.-Y., Spurgeon, H. A., Ting, C.-T., Lakatta, E. G., Yin, F. C., Chou, P. & Chen, C.-H. 2009. Central or peripheral systolic or pulse pressure: which best relates to target-organs and future mortality? *Journal of Hypertension,* 27**,** 461.

Wang, K.-L., Cheng, H.-M., Sung, S.-H., Chuang, S.-Y., Li, C.-H., Spurgeon, H. A., Ting, C.-T., Najjar, S. S., Lakatta, E. G. & Yin, F. C. 2010. Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities. *Hypertension,* 55**,** 799-805.

Watanabe, H., Ohtsuka, S., Kakihana, M. & Sugishita, Y. 1993. Coronary circulation in dogs with an experimental decrease in aortic compliance. *Journal of the American College of Cardiology,* 21**,** 1497-1506.

Webb, A. J., Simoni, M., Mazzucco, S., Kuker, W., Schulz, U. & Rothwell, P. M. 2012. Increased cerebral arterial pulsatility in patients with leukoaraiosis. *Stroke,* 43**,** 2631-2636.

Weber, T., Wassertheurer, S., Rammer, M., Haiden, A., Hametner, B. & Eber, B. 2012. Wave Reflections, Assessed With a Novel Method for Pulse Wave Separation, Are Associated With End-Organ Damage and Clinical OutcomesNovelty and Significance. *Hypertension,* 60**,** 534-541.

Weisbrod, R. M., Shiang, T., Al Sayah, L., Fry, J. L., Bajpai, S., Reinhart-King, C. A., Lob, H. E., Santhanam, L., Mitchell, G. & Cohen, R. A. 2013. Arterial Stiffening Precedes Systolic Hypertension in Diet-Induced ObesityNovelty and Significance. *Hypertension,* 62**,** 1105-1110.

Westerhof, B. E., Guelen, I., Westerhof, N., Karemaker, J. M. & Avolio, A. 2006. Quantification of wave reflection in the human aorta from pressure alone. *Hypertension,* 48**,** 595-601.

WHO fact sheets updated 2017 accessed at: www.who.int/mediacenter/factsheets/fs317/en.

Wilkinson, I. B. & Mceniery, C. M. 2012. Arteriosclerosis. *Hypertension,* 60**,** 3-5.

Wilkinson, I. B., Mceniery, C. M. & Cockcroft, J. R. 2009. Arteriosclerosis and atherosclerosis. *Hypertension,* 54**,** 1213-1215.

Williams, L. 2002. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation, 106, 3143-3143.

Woodard, T., Sigurdsson, S., Gotal, J. D., Torjesen, A. A., Inker, L. A., Aspelund, T., Eiriksdottir, G., Gudnason, V., Harris, T. B. & Launer, L. J. 2014. Mediation analysis of aortic stiffness and renal microvascular function. *Journal of the American Society of Nephrology***,** ASN. DOI:10.1681/ASN.2014050450.

Woodiwiss, A. J., Molebatsi, N., Maseko, M. J., Libhaber, E., Libhaber, C., Majane, O. H., Paiker, J., Dessein, P., Brooksbank, R. & Sareli, P. 2009. Nurse-recorded auscultatory blood pressure at a single visit predicts target organ changes as well as ambulatory blood pressure. *Journal of Hypertension,* 27**,** 287-297.

Zamani, P., Bluemke, D. A., Jacobs, D. R., Duprez, D. A., Kronmal, R., Lilly, S. M., Ferrari, V. A., Townsend, R. R., Lima, J. A. & Budoff, M. 2015. Resistive and Pulsatile Arterial Load as Predictors of Left Ventricular Mass and GeometryNovelty and Significance. *Hypertension,* 65**,** 85-92.

Zamani, P., Jacobs, D. R., Segers, P., Duprez, D. A., Brumback, L., Kronmal, R. A., Lilly, S. M., Townsend, R. R., Budoff, M. & Lima, J. A. 2014. Reflection Magnitude as a Predictor of Mortality. *Hypertension***,** HYPERTENSIONAHA. 114.03855.

Zieman, S. J., Melenovsky, V. & Kass, D. A. 2005. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 25**,** 932-943.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

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

CLEARANCE CERTIFICATE	**M1204108**
PROJECT	Gene Candidates as Determinants of Blood Pressure and Intermediary Phenotypes in Pathogenesis of Hypertension in Black South Africans (Previously M020472 and M070469)
INVESTIGATORS	Prof A/G Woodiwiss/Norton.
DEPARTMENT	School of Physiology
DATE CONSIDERED	Ad hoc
DECISION OF THE COMMITTEE*	Renewal Approved

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

DATE 2012/05/18 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof A Woodiwiss

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 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)

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)

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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

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DECLARATION OF INVESTIGATOR(S)

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

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