Aortic backward waves derived from wave separation analysis, and end-organ changes.

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Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Master of Science in Medicine

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Declaration

I, Imraan Ballim, declare that this dissertation is my own work. This dissertation is being submitted for the degree of Master of Science in Medicine in the Faculty of Health Science, at the University of the Witwatersrand, Johannesburg, South Africa. I declare that the dissertation submitted is entirely my own, original work and the copyright of this dissertation rests with the author or the University to which it was submitted. The work herein has not been submitted before any degree or examination at this, or any other University.

Imraan Ballim

Signed on the 18th day of November 2016

Supervisor 1…… Supervisor 2……

Conference presentations arising from this study

The following oral presentations were offered in support of this dissertation:

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Abstract

Aortic backward (reflected) waves are major determinants of cardiovascular events and their impact is independent of brachial blood pressure. Although aortic backward wave pressures (Pb) can be determined using a triangular flow wave form for wave separation analysis, which is a cheaper and time-efficient method, Pb derived from this approach correlates poorly with Pb derived from measured aortic flow waves. However, the comparative ability of these two Pb measurements to predict end-organ changes remains uncertain. Therefore, we aimed to compare Pb obtained using a triangular flow wave method (Pb^{tri}) and Pb obtained using echocardiographic derived aortic flow velocity waves (Pb^{echo}), and their relationships with left ventricular mass indexed to $height^{2.7}$ (LVMI). In 394 participants from a black African community sample (age>16years), aortic haemodynamics (applanation tonometry, SphygmoCor software), aortic flow velocity and LVMI (echocardiography) were determined. Bland-Altman analysis revealed that Pb^{tri} overestimated the backward wave pressure by an average of 3.65±3.17mmHg. However, the correlation between the two measurements was markedly high (r^2=0.82). Independent of confounders, including age, Pb^{tri} was associated with LVMI (partial r=0.14, p=0.02). Importantly, when comparing the association between Pb^{echo} and LVMI (partial r=0.14, p=0.01) no differences were noted (p=0.35, for comparison of partial r values, Z score). The triangular flow wave form employed for wave separation analysis produces Pb values that are as closely associated with LVMI as those derived from echocardiographic aortic flow wave measurements. Thus, risk prediction using simple approaches to aortic wave separation may be employed.

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LIST OF ABBREVIATIONS

AIx	Augmentation index
AP	Augmented pressure
APOGH	African Project on Genes and Hypertension
BH	Body height
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
BW	Body weight
CHF	Congestive heart failure
cPP	Central pulse pressure
CR	Cardiac revascularisation
CVD	Cardiovascular disease
CVE	Cardiovascular events
DBP	Diastolic blood pressure
DM	Diabetes mellitus
EF	Ejection Fraction
eGFR	Estimated glomerular filtration rate
HbA1c	Glycated haemoglobin
HF	Heart failure
HR	Heart rate
IMT	Intima-media thickness
IVS	Interventricular septum wall thickness
LV	Left ventricle
LVH	Left ventricular hypertrophy

LVID	Left ventricular internal diameter
LVM	Left ventricular mass
LVMI-$H^{1.7}$	Left ventricular mass indexed to height$^{1.7}$
LVMI-$H^{2.7}$	Left ventricular mass indexed to height$^{2.7}$
MAP	Mean arterial pressure
MI	Myocardial infarction
NHLS	National Health Laboratory Service
Pb	Backward wave pressure
Pb^{echo}	Backward waves obtained using echocardiographic aortic flow waves
Pb^{tri}	Backward waves obtained using the triangulation method
Pf	Forward wave pressure
Pf^{echo}	Forward waves obtained using echocardiographic aortic flow waves
Pf^{tri}	Forward waves obtained using the triangulation method
Pi	Incident wave
PP	Pulse pressure
PPamp	Pulse pressure amplification
PWT	Posterior wall thickness
PWV	Pulse wave velocity
RM	Reflected magnitude
RWT	Relative wall thickness
SBP	Systolic blood pressure
SD	Standard deviation
TChol	Total cholesterol
TIA	Transient ischaemic attack

Chapter 1

Introduction

Cardiovascular disease (CVD), which includes clinical conditions such as myocardial infarction, stroke, heart failure, peripheral vascular disease, and renal failure, account for a large number of deaths per year. CVD accounted for 17.5 million deaths worldwide in 2005 (Mendis et al 2007). By the year 2020, it is predicted that CVD will account for approximately 25 million deaths, mainly due to increased urbanisation and ageing population (Yusuf et al 2001, Perkovic et al 2007). Furthermore, it was previously noted that CVD was a disease mainly found in developed countries, however it is now understood that 80% of deaths caused by CVD occur in low-to-middle income countries (Mendis et al 2007).

Epidemiological studies regard hypertension as the main contributor to the global burden of disease (Poulter et al 2015). It is estimated that by the year 2025, the disease burden of hypertension will increase by 60% globally, with 1.56 billion hypertensive individuals in the world (Lackland and Weber 2015). The prevalence of people with hypertension is highest in developing countries (Ibrahim and Damasceno 2012). In South Africa, the risk of death from hypertension has increased by 25% in less than a decade (Ibrahim and Damasceno 2012). More importantly, in communities of black African ancestry in South Africa, hypertension is the most important risk factor for CVD, where hypertension accounts for up to a third of heart failure cases (Stewart et al 2008). Thus, controlling hypertension becomes an important factor in reducing the incidence of CVD.

Presently, hypertension is diagnosed through the measurement of brachial artery blood pressure. This measurement has been supported by the fact that high values of brachial artery blood pressure are a strong predictor of CVD (Trudeau 2014). Furthermore, data from over 50 years of randomised controlled trials demonstrate that lowering brachial blood pressure, in hypertensive individuals, reduces the risk cardiovascular events (Lewington et al 2002). Based on this

evidence, it became important to stratify, and classify, hypertension according to different levels of systolic blood pressure (SBP) and diastolic blood pressure (DBP). General consensus regards normal blood pressure (BP) as 120-129/80-84 mmHg, and hypertension as BP> 140/90 mmHg (Kjeldsen et al 2014). However, BP is a continuous trait. Indeed, studies have demonstrated that, independent of potential confounders, a significant proportion of CVD occurs within the normal BP range, and high-normal or pre-hypertensive (BP= 130-139/85-89 mmHg) BP range (Vasan et al 2001, Qureshi et al 2005). Based upon a threshold of 140/90 mmHg for the diagnosis of hypertension, a number of individuals would be regarded as not in need of hypertensive treatment. However, those individuals may well be at an increased risk of CVD. Therefore, there is a need for the development of new approaches in order to better understand blood pressure, and its impact on cardiovascular risk.

As will be discussed in subsequent sections, one of the continuously debated issues in understanding blood pressure is the measurement of central aortic blood pressure. Indeed, a significant proportion of pre-hypertensive patients have aortic blood pressures that are within the range of blood pressures noted in hypertensive patients (McEniery et al 2008, Kshirsagar et al 2006). Hence, measuring the aortic BP may be a useful tool to enhance risk prediction in pre-hypertensive patients. However, as will be highlighted, there is conflicting data with regards to aortic BP and risk prediction. This has led to several investigations assessing the value of further deconstruction of the aortic pressure wave into its component forward and backward waveforms. However, obtaining these waves relies on assumptions. In order to derive the forward and backward waves, an aortic flow waveform is required, which, to avoid having to acquire aortic flow waves, is assumed to be a triangle (triangulation method). This has led to dispute as to the value of this approach. Therefore, the present thesis investigated the validity of the triangular

flow waveform (triangulation method) in deriving the forward and the backward aortic waves. The waves derived from the triangulation method were compared to the waves obtained using echocardiographic-derived aortic flow waveforms. Furthermore, I investigated whether the backward and forward waves, obtained using the triangulation method and echocardiographic-derived aortic flow waves, differ in the strength of association with end organ measures. To provide the background to this aim, I have discussed the reasons for, and evidence in favour of, possible benefits of aortic BP measurement beyond brachial BP measurement. Thereafter, I have discussed the physiology of aortic BP, and its composition, whilst providing evidence as to why aortic BP may predict of cardiovascular events beyond brachial BP. I have then discussed the forward and backward waves, the physiology thereof, indices of wave reflection, and how they relate to end organ changes. Finally, I have discussed the methods of deriving aortic backward waves.

1.1 Brachial blood pressure and risk prediction

Initially it was thought that diastolic blood pressure (DBP) was the best measure that could predict the risk of developing CVD (Franklin et al 2001). However, Kannel et al (1971) demonstrated that there is an increase in the importance of systolic blood pressure (SBP) with age, and a decline in the relative importance of DBP (Kannel et al 1971). Thereafter, numerous studies have demonstrated that SBP increases with age until the ninth decade, whereas DBP only increased until middle age and thereafter either plateaus or decreases slightly (Staesson, Amery and Fagard 1990, Franklin et al 1997, Wang et al 2005). More importantly, most evidence points towards SBP being more important in cardiovascular risk prediction in the middle-aged and

elderly, while DBP tends to be more important in young adults (Chobanian et al 2003). Pulse pressure (PP) (PP= SBP-DBP), a proposed surrogate for large artery stiffness, was found to be a predictor of CVD (Franklin et al 2001, Madhaven et al 1994). Thus, there is a gradual shift in importance from DBP to SBP and then to PP, with an increasing age, when predicting coronary artery disease (Franklin et al 2001). The main question that arises is what causes the changes that occur in cardiovascular risk prediction with regards to BP components.

A possible explanation for why, in young adults, DBP may be more important than SBP, when risk predicting, could be because brachial SBP considerably overestimates aortic SBP (McEniery et al 2008). When comparing aortic SBP to brachial SBP, brachial SBP may be up to 40mmHg higher than aortic SBP, whereas aortic and brachial DBP remain constant (Ohte et al 2007). Since SBP, and not DBP, increases from the aorta to the brachial artery, the BP change that occurs is an amplification of PP (PP amplification). This phenomenon occurs due to an increase in arterial stiffness moving away from the heart (McEniery et al 2014). As the pressure wave travels from the highly elastic central arteries to the stiffer brachial artery, the time between the upstroke and the down stroke (at the peak) of the wave becomes narrower, and the systolic peak is more prominent, thus increasing systolic pressure (McEniery et al 2014). There is substantial evidence that, with ageing, aortic SBP may approximate brachial SBP due to the increase in stiffness of the aorta (McEniery et al 2008) and hence, brachial SBP, rather than DBP, better associates with cardiovascular damage. Since PP amplification is an indicator of arterial stiffness, it may be a useful tool in interpreting the health of the aorta, and risk prediction. Several studies (table 1.1) suggest that aortic blood pressure, or central blood pressure, is more strongly related to cardiovascular events than brachial BP (McEniery et al 2014). Hence, aortic

BP may be a better screening tool than brachial BP for patients at risk of developing cardiovascular events.

1.2 Aortic blood pressure

The ease of measuring brachial BP, and the wide variety of available devices, has been the driving force for its use clinically. Although brachial BP may be a poor surrogate of aortic BP, clinicians are unwilling to deviate from brachial BP measurements without robust evidence that cardiovascular risk stratification are better when based on central aortic measurements (McEniery et al 2014). Hence, what is the current understanding of central aortic BP?

Numerous studies have demonstrated that aortic BP is associated with, and predicts cardiovascular end-organ changes or cardiovascular outcomes better than, or independent of, brachial BP (Covic et al 2000, Wang et al 2009, Roman et al 2007, Roman et al 2009, Norton et al 2012, Safar et al 2002, Jankowski et al 2008) (table 1.1). The predictive value of central aortic PP or SBP, or PP amplification, beyond brachial BP, has been demonstrated in patients with end-stage renal disease (Safar et al 2002) and in patients undergoing coronary angiography (Jankowski et al 2008). Furthermore, evidence of aortic pressures better predicting cardiovascular events beyond brachial BP, were demonstrated in the elderly and general populations (Pini et al 2008, Roman et al 2007). However, a study conducted by Dart et al (2006) found that brachial BP, not aortic BP, predicted cardiovascular outcomes in elderly female hypertensive patients. However, the study by Dart et al (2006) was criticised for the method of calibration of aortic BP.

Table 1.1. Characteristics of studies comparing the impact of central aortic to brachial blood pressures on cardiovascular outcomes or mortality

Authors	Sample size	Study design	Outcomes evaluated	Result	Adjustors
Safar et al 2002	180	Prospective	All-cause mortality	PPamp is an I-P	Age, time on dialysis, previous CVE
Dart et al 2006	484	Prospective	MI, CR, HF, cerebral or coronary occlusion, stroke, TIA	cPP is an N-P	Age, Chol, Smoking
Williams et al 2006	2073	Prospective	CVE and CV procedures	cPP is an I-P	Age, baseline risk factors
Roman et al 2007	3520	Prospective	MI, stroke, CHF, CHD, CD	cPP is an I-P	Age, sex, BMI, smoking, Tchol:HDL, serum creatinine, fribrinogen, diabetes, HR
Pini et al 2008	854	Prospective	fatal and non-fatal CVE	cPP is an I-P	Age, sex
Jankowski et al 2008	1109	Prospective	CR, CD, HF, stroke, MI, heart transplant	cPP is an I-P	Age, sex, EF, CAS, HF, HR, CV history, GFR, Drug treatment, Risk factors
Wang et al 2009	1272	Prospective	All-cause mortality and CD	cPP is an I-P	Age, sex, HR, BMI, smoking, glucose, Chol, HDL, PWV, LVM, IMT, eGFR

Table 1.1 Continued

Roman et al 2009	2405	Prospective	MI, stroke, CHF, CHD	cPP is an I-P	Age, sex, BMI, smoking, Tchol, HDL, serum creatinine, fribrinogen, diabetes, HR
Mitchell et al 2010	2232	Prospective	MI, Angina, HF, stroke	cPP is N-P	Age, sex, SBPb, treatment, TChol
Vlachopoulos et al 2010	5648	Prospective	MI, stroke, CR, CD, All-cause mortality	cPP is an N-P	Meta-analysis
Regnault et al 2012	125121	Prospective	Mortality, CVE	PPamp is an I-P	Age, BMI, activity, sex, Charlson comorbidity index, previous CVD, treatment, MAP, HR
Chirinhos et al 2012	5960	Prospective	CVE, CHF	PPamp is N-P	Race, treatment, Tchol, HDL, HR, Smoking, SBP, DBP, sex, BH, BW,
Booysen et al 2013	1169	Prospective	PWV, eGFR, LVMI,	cPP is I-P	Age, sex, BMI, diabetes, smoking, alcohol, HR

cPP; Central aortic pulse pressure, CVE; Cardiovascular events, I-P; Independent predictor, N-P; Not an independent predictor, CV; Cardiovascular, PPamp; Pulse Pressure Amplification, SBPb; Brachial Systolic Blood Pressure, MI; Myocardial infarction, HF; Heart failure, CR; Cardiac revascularization, TChol; Total cholesterol, HDL; High-density lipoprotein, CD; Cardiovascular death, BMI; Body mass index, CVD; Cardiovascular disease, MAP; Mean arterial pressure, CHF; Congestive heart failire, eGFR; Estimated Glomerular Filtration Rate, BH; Body height, BW; Body weight, CA; Cardiac arrest, EF; Ejection fraction, CAS; Coronary artery stenosis, HR; Heart rate, GFR; Glomerular Filtration Rate, TIA; Transient ischemic event, Chol; Cholesterol, PWV; Pulse wave velocity, LVM; Left ventricular mass, IMT; Intima-media thickness

A meta-analysis was published in the year 2010 which compared the ability of aortic versus brachial BP to predict cardiovascular events (Vlachapoulos et al 2010). The meta-analysis, which deemed 11 studies eligible for the analysis yielded no significant differences between the two measurements, however a trend for better effect was noted ($p=0.057$). The study obviously excluded data from later studies which reported on relations between aortic versus brachial BP and cardiovascular events, as well as the Conduit Artery Function Evaluation (CAFE) based on the study design and the lack of baseline central haemodynamic data (Williams et al 2006).

A study conducted in Taiwan, which had not been published at the time of the meta-analysis, demonstrated a marked ability of aortic BP in cardiovascular risk prediction, compared to brachial BP (Wang et al 2009). However, the Framingham Heart Study published data, after the meta-analysis had been published, showed that neither aortic BP nor PP amplification demonstrated ability to risk predict beyond brachial BP (Mitchell et al 2010). In the study, little difference between aortic BP and PP amplification was noted across the adult lifespan, a finding which is difficult to understand given the considerably low average brachial BP values, which implies that PP amplification should have been high (Mitchell et al 2010). Hence, this could account for the lack of prognostic information that aortic BP or brachial BP provide beyond brachial BP, in the Framingham Heart Study.

With that being said, there are numerous studies which show that a decreased PP amplification provides strong prognostic information beyond brachial BP, however, in these studies, aortic BP failed to show an ability to risk predict beyond brachial BP (Benetos et al 2010, Regnault et al 2012, Benetos et al 2012). Moreover, Chirinos et al (2012) failed to show a relationship between PP amplification and cardiovascular outcomes. However, a recent meta-analysis demonstrated a

positive significant correlation between LVMI and central BP (r= 0.30; CI: 0.23 to 0.37) (Kollias et al 2016). This metal-analysis deemed 13 studies as appropriate for the analysis.

The evidence surrounding aortic BP and its ability to predict cardiovascular events, independent of brachial BP, points towards a possible use of this measurement. However, there remains some evidence in which aortic BP is not a better risk predictor than brachial BP. This implies that aortic BP, and its components, needs to be more thoroughly investigated in order to become clinically useful. The question that remains is what causes the difference in aortic and brachial BP?

1.3 Aortic and brachial blood pressure

As mentioned before, aortic BP is lower than brachial BP due to the increased arterial stiffness of the peripheral arteries compared to the aorta (Nichols et al 2011). In order to understand why aortic BP measurements yield different results to the conventional brachial BP measurements, we need to delve into the physical, and physiological, aspects of arterial distensibility and impedance. These principles affect blood flow and pressure waves throughout the arterial system.

Arteries serve a dual role in conducting blood to the peripheral tissues, and buffering the pressure pulsations created by intermittent ventricular ejection (Mitchell 2004). The pressure waveform, generated by left ventricular (LV) ejection, travels down the arterial tree causing changes in the pressure wave. Moving away from the aorta, the peripheral arteries become stiffer and have a smaller radius than the aorta, the upper portion of the wave becomes narrower and more prominent **(figure 1.1).** Thus amplifying systolic pressure while diastolic and mean arterial

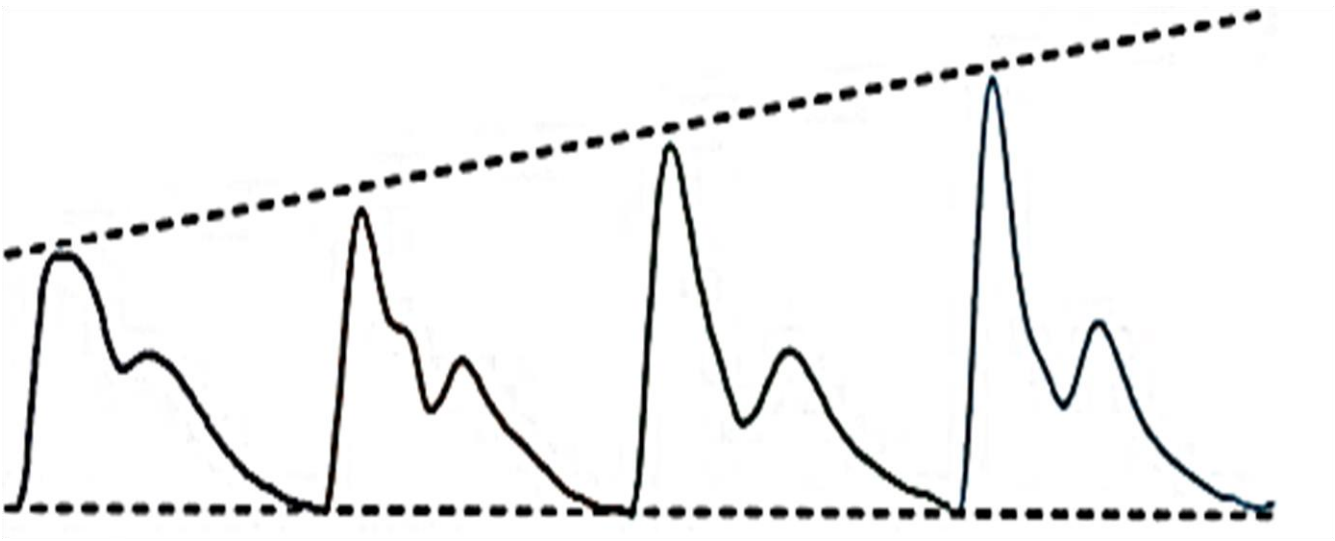

Figure 1.1. A representation of the amplification of the pressure waveform as it travels from the aorta to the periphery (McEniery et al 2014).

pressure remain constant (PP amplification) (McEniery et al 2014). Furthermore, the pressure waveform recorded at any site of the arterial tree is a summation of the forward wave (Pf), generated by LV ejection, and the backward wave (Pb) which is reflected at various sites of impedance such as bifurcations and travels back to the heart (Avolio et al 2009). The forward wave is dependent on the mechanical properties of central elastic arteries, whereas the backward wave is influenced by elastic and impedance factors of the arterial tree (Nichols and Singh 2002). As seen in **figure 1.2**, peak aortic pressure corresponds to peak Pb pressure, whereas peak Pf pressure corresponds to, what is termed as, the first systolic shoulder of the aortic pressure wave. Pb has also been shown to affect the shape of the arterial wave, and is therefore, a main determinant of PP, further justified by Booysen et al (2015) who demonstrated a strong relationship between Pb and central PP (Nichols and Singh 2002, Booysen et al 2015).

The reason that aortic pressure predicts cardiovascular events better than brachial BP is because it is aortic systolic pressure that the LV encounters during systole, and the aortic pressure during diastole is a determinant of coronary perfusion (Agabiti-Rosei et al 2007). However, in pathological circumstances, which are largely explained by ageing effects as well as diseases affecting the vascular system such as hypertension, the aorta and large elastic-type arteries stiffen, thus enhancing aortic pressures during aortic ejection when blood is pumped into a stiffer conduit. If we consider an instance where aortic distensibilty is reduced, the increased pressure is associated with a greater rise in systolic pressure and a slight rise, or no rise at all, in diastolic pressure (O'Rourke 1970). A reduced aortic distensibility is a complex process that is thought to involve the destruction of elastic tissue, increases in aortic collagen content and changes in the properties of collagen (Nichols et al 2011). However, the aortic impedance needs to be understood since it plays a role in the magnitude of the pressure waveform.

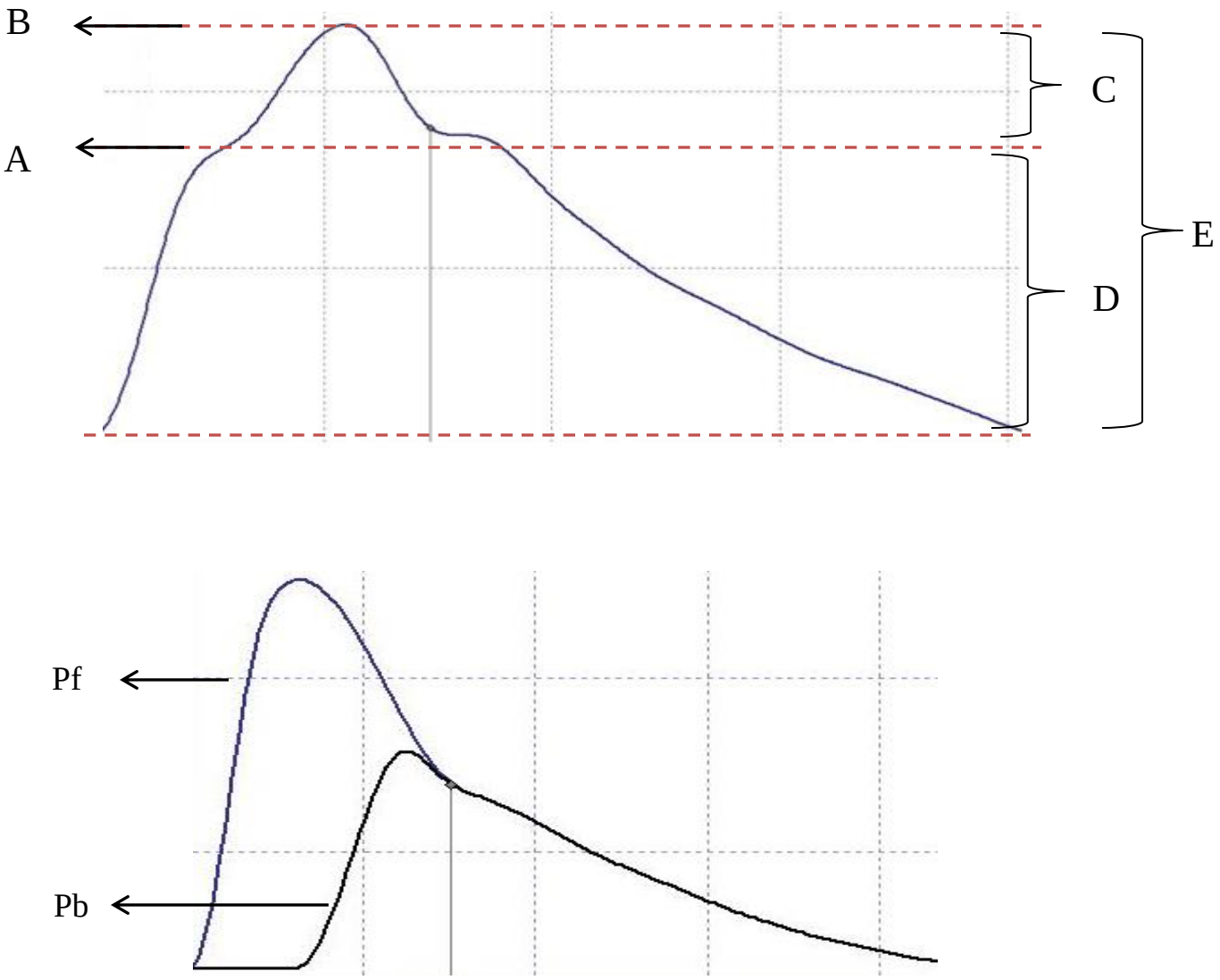

Figure 1.2. Aortic pressure wave as determined by the combined effect of the aortic forward (Pf) and aortic backward (Pb) pressure waves. Definitions of various measures of arterial pulse wave analysis are also shown. A: first systolic shoulder, B: peak systolic pressure, C: augmented pressure, D: forward wave pressure, E: central aortic pulse pressure.

Based on the fact that the heart pumps intermittently, and not continuously, resistance only describes one component of the load presented to the heart. Input impedance, a more complete description of this load, represents the relationship between the steady and oscillatory components of pressure and flow waves in the aorta (O'Rourke 1970). Impedance is determined by the inertial properties of blood, and by the elasticity, viscosity, and geometry of arteries (O'Rourke 1970). Thus an increase in the impedance, due to an increase in stiffness, could lead to enhanced pressures. As the aorta and large arteries stiffen, mainly a natural ageing process, the smaller peripheral arteries stiffen to a lesser degree across the adult lifespan (Nichols et al 2011). Therefore, in older individuals, the aortic BP approximates brachial BP (Nichols et al 2011).

When comparing the aortic pressure wave morphology to the brachial pressure wave morphology, there is a stark difference observed between the two waveforms **(figure 1.3)**. Peak brachial pressure, termed as the percussion wave, corresponds to the first systolic shoulder of the aortic wave, and as mentioned above, is generated by the peak Pf pressure. The figure also reveals that peak aortic pressure, which is generated by peak Pb pressure, corresponds to the tidal wave of the brachial pressure wave. More importantly, **figure 1.4** demonstrates the change in wave morphology as a person ages. In an old individual, we see that the percussion wave and the tidal wave have similar pressures, thus peak aortic pressure might equal peak brachial pressure, which is far from the case in a young individual. These characteristic differences in brachial and aortic pressure waves, and the determinants thereof, may explain why aortic pressures better predict cardiovascular outcomes compared to brachial BP measurements.

As previously mentioned in section 1.1, PP amplification is a useful tool for cardiovascular risk prediction. If the aortic BP approximates brachial BP, mainly an aging effect, then PP amplification has decreased (figure 1.4). A study conducted by Hodson et al (2016), whereby

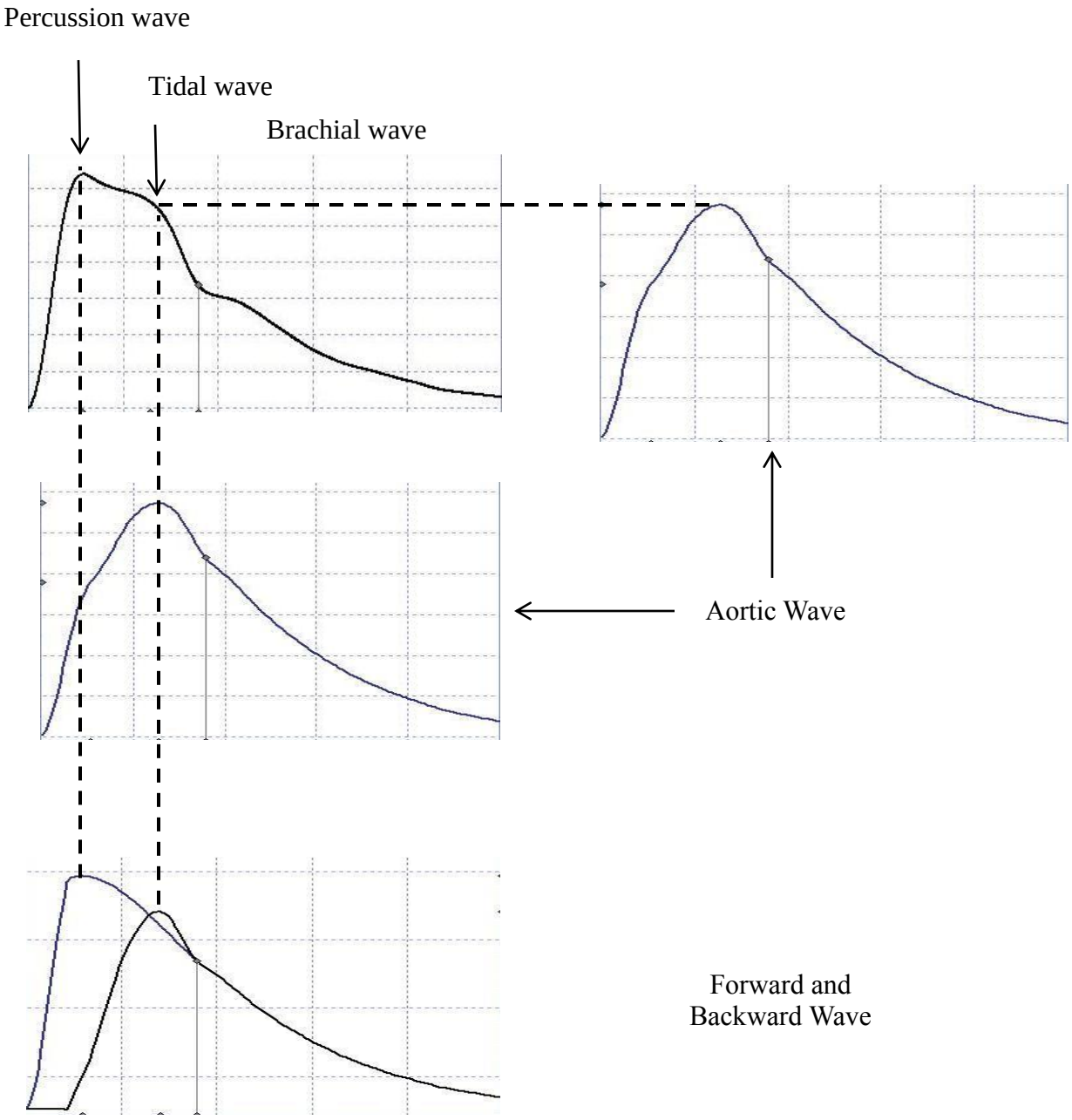

Figure 1.3. The contribution of aortic forward and aortic backward waves to aortic and radial (approximate of brachial) pressure waves. The dashed lines show temporal alignment and alignment of the magnitude (left versus right panels) of pressure waves.

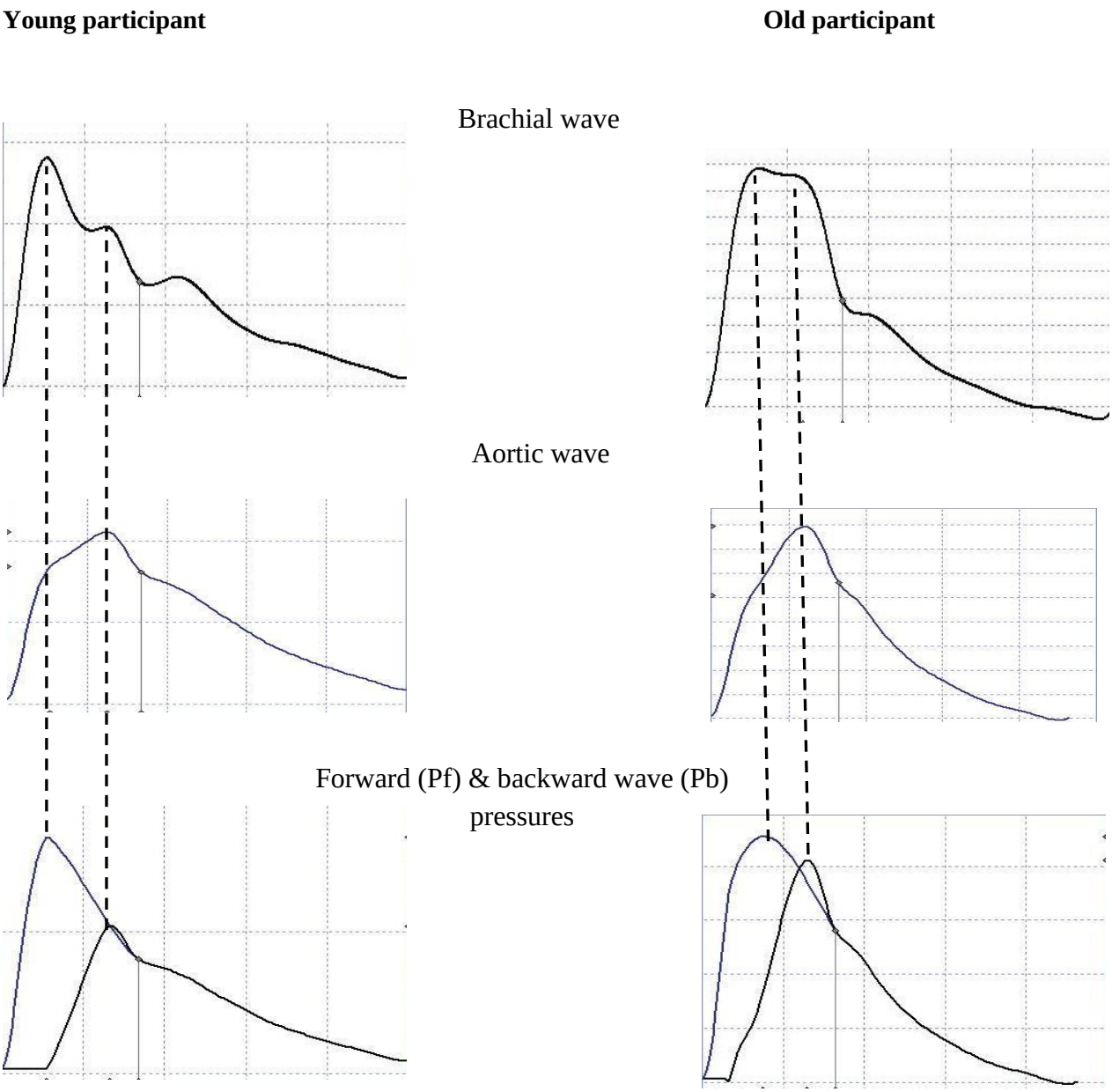

Figure 1.4. Age effects on aortic and radial artery pressure waves (which approximate brachial pressure waves). The figure shows changes in the combined effect of the aortic forward and aortic backward waves on pressure waveforms with age. The dashed line show how the forward and backward pressure waves contribute to radial and aortic pressure waves.

they assessed the age associated changes in aortic function, demonstrated a decrease in PP amplification from the age of 30 years. Along with the decrease in PP amplification, they found an increase in Pb and Pf magnitude occurring at the same age. However, according to Sibiya et al (2015), the backward wave is closely correlated to PP amplification than Pf. The age associated changes in aortic function are almost inevitable, but since Pb is closely correlated with PP amplification, Pb could be the main driver the variations in PP amplification independent of age.

Upon further investigation, **figure 1.4** reveals that peak Pb occurs quite close to Pf in an older individual. Since the timing of the return of Pb is determined by arterial stiffness, and aortic stiffness, an early return of Pb could be indicative of cardiovascular pathophysiology. Hence, the early return of Pb causes a change in aortic wave morphology, or an augmented pressure, which will be described in a subsequent section.

An established index of arterial stiffness, known as pulse wave velocity (PWV), measures the time it takes for the pulse wave to travel to the carotid and femoral arteries (Laurent, Marais and Boutouyrie 2016). The velocity of the pulse wave is dependent of the elasticity of the arteries. A stiff artery would conduct the pulse wave much quicker than that of a pulse wave travelling via an elastic artery (Mitchell et al 2009). Hence, PWV is an indication of arterial stiffening. Therefore, if we apply this principle to that of wave reflection, a stiff artery, and quick PWV, would inevitably affect the timing of the backward wave travel. Thus, it is important to understand how PWV affects the backward wave.

Wang et al (2010) looked at wave reflection, and indices of wave reflection and arterial stiffness, in predicting cardiovascular mortality. Wang et al (2010) demonstrated that Pb was independent of PWV in predicting cardiovascular mortality. With age related arterial stiffness, PWV

increases which propagates Pf, and increases the timing of the return of Pb. Furthermore, Sibiya et al (2015) observed an association between PWV and Pb; however, it is suggested that the association may be that Pb has an effect on aortic stiffness, rather than aortic stiffness on Pb (Sibiya et al 2015). Hence, the timing of the return of Pb depends on arterial stiffness, and has a minimal effect on the magnitude of Pb.

With that being said, numerous factors affect the magnitude of Pb. The intensity of Pf has an effect on the magnitude of Pb such that an increase in Pf would cause a relative increase in the magnitude of Pb. However, this does not explain an increase in Pb beyond an increase in Pf. A possible explanation is an increase in total peripheral resistance and muscular artery tone. At muscular arteries, vasoconstriction would cause an increase in the impedance mismatch and thus increasing the magnitude of Pb.

Since aortic pressures better predict cardiovascular outcomes than brachial pressures, is it possible that the main drivers of those differences could be associated with Pb and Pf? Furthermore, we have established that PWV does not inform us of the magnitude of Pb. Hence, what are the methods of obtaining Pb and Pf?

1.4 Forward and backward waves

The shape of the aortic wave is influenced by Pb and Pf (Mitchell at el 2003). As mentioned previously, an early return of Pb, caused by an increase in arterial stiffness, causes an increase in peak aortic pressure, or an augmented pressure (AP).

Numerous studies have demonstrated the impact of reflected waves, determined from indices of augmented pressures, in predicting cardiovascular outcomes, and cardiovascular damage, beyond brachial blood pressure (Hashimoto, Imai and O'Rourke 2007, Weber et al 2006, Westerbacka et al 2005, Sibiya et al 2014, Chirinos et al 2005, London et al 2001, Weber et al 2005). These studies illustrated the importance of backward waves in cardiovascular risk prediction using indices of augmented pressure, a point further emphasised by a meta-analysis conducted on the aforementioned studies (Vlachopoulos et al 2010). The indices of backward waves, which will be explained, are augmented pressure (AP) and augmentation index (AIx).

Augmented pressure is defined as the difference between peak systolic pressure and the pressure at the first systolic shoulder of the aortic pressure wave (figure 1.2). Aortic augmentation index is calculated as AP/ aortic PP x 100, or to avoid negative values in young individuals as aortic SBP/ pressure at the first systolic shoulder x100. However, despite the positive results for AP and AIx, the Framingham Heart Study failed to show that the indices of augmented pressures predicted cardiovascular outcomes, beyond brachial BP (Mitchell et al 2010). The obvious inaccuracy that occurs in these indices is that the point at which Pf ends, and Pb begins is obscure. However, those are not the only limiting factors with regards to indices of augmented pressure.

It has been demonstrated that AIx is influenced by body height and sex (Hughes et al 2013). Furthermore, AIx was found to be lower in females (Hughes et al 2013). Gatzka et al (2001) demonstrated that older females have stiffer arteries compared to males of the same age; hence, the gender effects on AIx. Furthermore, a study conducted by Mitchell et al (2010) demonstrated that the known accelerated increase in systolic pressure and PP with advancing age is attributable to the Pf. Moreover, with advancing age, if the Pf amplitude increases and Pb remains the same,

the use of AIx as an index of wave reflection becomes ambiguous since the variations in AIx are caused by the increase in Pf (Mitchell et al 2010). Increases in arterial reservoir function and Pf, and not Pb, were found to contribute to variations in AP and AIx (Davies et al 2010). Subsequently, several studies suggest that an overlap between Pf and Pb may confound AP and AIx (Cheng et al 2012, Fok et al 2014, Torjesen et al 2014, Schultz et al 2013, Booysen et al 2015).

It is quite clear that AIx and AP are influenced by numerous factors, and are therefore inaccurate in determining cardiovascular damage. That being said, reports suggest that wave reflection, based on AIx and AP, is inaccurate in predicting cardiovascular outcomes. However, as mentioned above, AIx and AP is an interaction between Pf and Pb, and therefore cannot be indicative of wave reflection only. Thus, AIx and AP are indices of wave reflection, but only inform us of the timing of Pb, and not the magnitude of Pb.

One way of revealing the magnitude of reflection is by wave separation analysis which separates aortic pressure into its Pf and Pb components (Westerhof et al 2006). The separation of these waves requires an aortic pressure and flow waveform. The following formula is used to calculate Pb and Pf:

$$Pf(t) = [P(t) + Zc \bullet F(t)] /2$$

$$Pb(t)= [P(t) – Zc \bullet F(t)] /2$$

P(t) is the measured pressure wave, F(t) is the flow wave, and Zc is the characteristic impedance of the proximal aorta (Westerhof et al 2006). Zc is derived from the averaged value of the 4th to

the 7th harmonic (Westerhof et al 2006). Furthermore, from the above equations it can be seen that the product Zc • F(t) appears in the calculation for both Pf and Pb. Thus Zc= Pf/Ff = -Pb/ Fb. Therefore, the amplitude of flow is eliminated, and Zc • F(t) is independent of the flow calibration (Westerhof et al 2006).

Wave separation analysis provided a way of identifying Pb, and determining its full impact on cardiovascular outcomes. Numerous studies have demonstrated the contribution, and association, of wave reflection to end-organ changes (table 1.2). The Framingham Heart Study found that Pf, rather than Pb, provides a major contribution to variations in aortic pressure across the adult age range (Mitchell et al 2010). However, as mentioned previously, Sibiya et al (2015) showed that Pb plays a more important role than Pf in increases in PP amplification. A study by Weber et al (2012) found that peak Pb is associated with hypertensive end-organ damage, and is an independent predictor of cardiovascular events in high risk patients (Weber et al 2012). Since the Framingham study conducted analysis in a sample with a normal average BP, it explains the negative results found in the study, as explained in section 1.2. Moreover, Pb was found to be a strong risk factor for congestive heart failure, as well as a strong predictor for long-term cardiovascular mortality in both men and women, independent of arterial stiffness (Chirinhos et al 2012, Wang et al 2010). It is noticeable that Pb and wave reflection holds much promise in being able to predict cardiovascular mortality, meaning that the method of obtaining Pb, that is wave separation analysis, still needs to be scrutinised.

If we inspect the equations for wave separation analysis mentioned above, we notice that an aortic flow wave is required. In order to obtain the aortic flow waveform, either a catheter equipped with an electromagnetic flow velocity sensor or Doppler ultrasound was used. This method can be costly as well as time consuming. With this in mind, a study conducted by

Table 1.2. Characteristics of studies assessing the impact of aortic backward waves on cardiovascular damage, and outcomes

Authors	Sample size	Study design	Outcomes evaluated	Result	Adjustors
Wang et al 2010	1272	Community based survey	All-cause mortality and CVE	I-P	None
Weber et al 2012	725	Prospective	Mortality, Mi, Stroke, CR, Peripheral and cerebrovascular revascularisation	I-P	Sex, age, systolic function, Statin use, MAP, LA diameter, GFR, CAD, Aortic PWV, angioscore, diabetes, smoking, NT-proBNP, Hypertension, E/E, HR, previous MI, treatment, LV end-diastolic pressure
Chirinos et al 2012	5960	Prospective	Heart Failure	I-P	Race, treatment, Tchol, HDL, eGFR, smoking
Sibiya et al 2015	1174	Cross-sectional study	LVMI, PWV, IMT, E/A, eGFR	Pb is an I-P	Sex, BMI, smoking, alcohol, diabetes, HR,

Table 1.2 continued

Zamani et al 2014	5984	Prospective	All-cause mortality and CD	I-P	HR, age, sex, SBP, Urinary Albumin/Creatinine ratio, Tchol, LDL, Chol, HDL, treatment, smoking, BMI, previous MI, diabetes, C-reactive protein, education, family income, alcohol, calorie intake, calories from fat, physical activity, ankle-brachial index, IMT, NT-proBNP, eGFR, Aortic Agaston calcium score, Agaston calcium score
Booysen et al 2015	1174	Cross-sectional study	LVMI, Age effects on PPc	Pb is an I-P	Sex, MAP, BH, BW, HR, diabetes, smoking, alcohol

LVMI; Left Ventricular Mass Index, IMT; Intima Media Thickness, eGFR; Estimated Glomerular Filtration Rate, PWV; Pulse Wave Velocity, CVE; Cardiovascular Events, I-P; Independent predictor; GFR; Glomerular Filtration Rate, LA; Left Atrium, LV; Left Ventricle, MI; Myocardial Infarction, CR; Coronary Revascularization, Hr; Heart Rate, CAD; Coronary Artery Disease, TChol; Total Cholesterol, HDL; High-density Lipoprotein, CD; Cardiovascular Death, SBP; Systolic Blood Pressure, NT-proBNP; NT-probrain Natriuretic peptide, Chol; Cholesterol, LDL; Low-density lipoprotein, BMI; Body Mass Index, MAP; Mean Arterial Pressure, E/E, Moderate-to-Severe Diastolic Dysfunction, MI; Myocardial Infarction, E/A; Mild Diastolic Dysfunction, Pb; Backward Wave, BH; Body Height, BW; Body Weight, PPc; Central Aortic Pulse Pressure.

Westerhof et al (2006) demonstrated that the aortic flow waveform required for wave separation analysis could be assumed to be a triangular shape which approximates the flow during cardiac ejection (Westerhof et al 2006). The beginning, peak flow, and the end of aortic flow could be determined from the aortic pressure waveform. As seen in **figure 1.5**, the time of end-diastolic aortic pressure is the time of the valve opening and hence the beginning of ejection. The incisura corresponds to the end of systole, and thus the end of ejection and the closure of the valve. The peak aortic flow corresponds to the first inflection point of the measured pressure wave during systole (Westerhof et al 2006).

With the fundamentals of the theory laid out, Westerhof (2006) set out to compare the wave reflection results obtained from the triangulation method and catheter-derived aortic flow waves. The study used reflection magnitude (RM) as the basis for comparison of the backward wave, which is the ratio of the amplitudes of Pb and Pf. In 19 participants, a regression value of 0.79 was obtained for the relationship between the triangulation method and measured flow. Interpretation of the results demonstrated that the use of triangular flow tends to overestimate the amplitude of Pf and underestimate the amplitude of Pb, however, the authors claim that the differences are small and not significant (Westerhof et al 2006). The results of this study allowed for an economically feasible method of obtaining Pf and Pb amplitudes. Indeed, the use of catheters, or Doppler ultrasound, is not easily available, and costly in certain economic environments.

This led to a study conducted by Kips et al (2009), whereby the triangulation method was compared to a non-invasive method of obtaining aortic flow waves, in this case using Doppler ultrasound. In a substantially larger sample size, Kips et al (2009) yielded an R^2 value of 0.55, using RM as the basis for comparison as well. This value is much lower than that found by

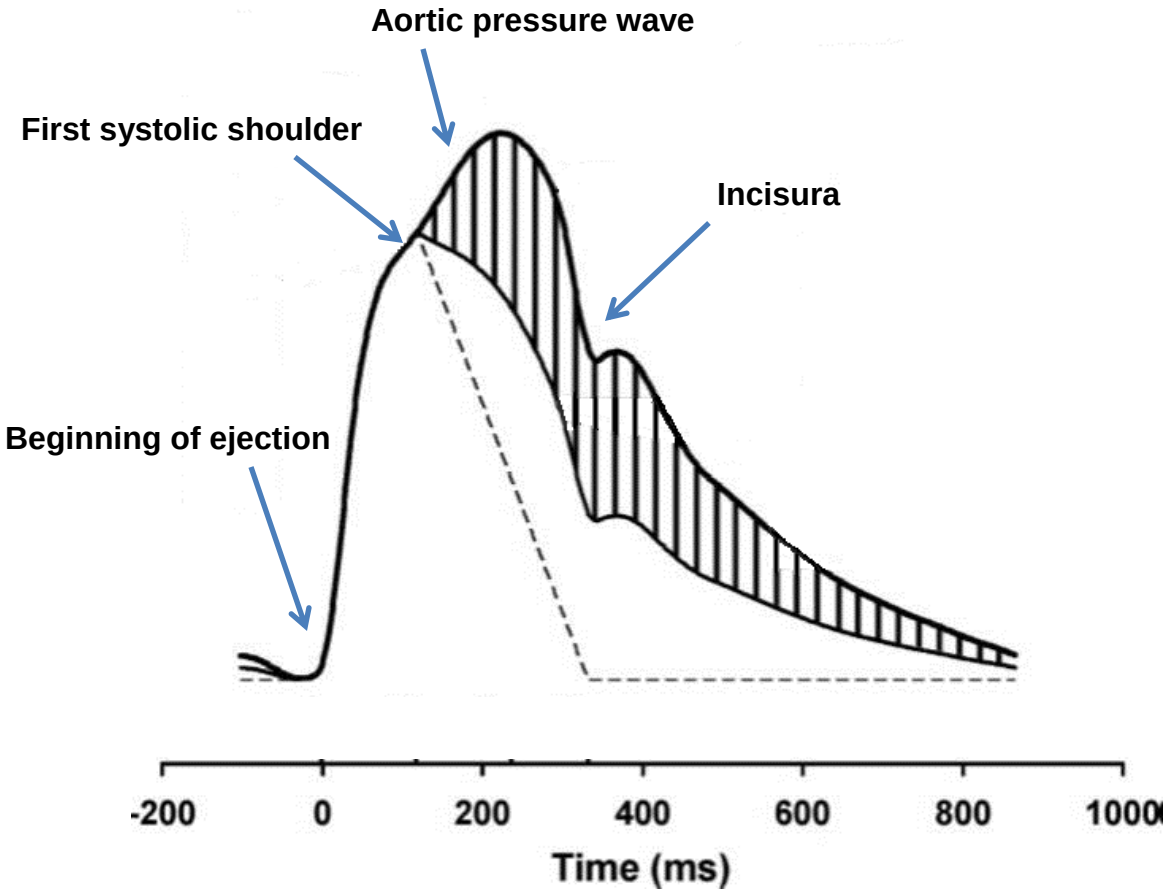

Figure 1.5 Figure representing the triangulation method of obtaining backward waves (Mitchell 2006). Dotted line represents triangular flow wave. The beginning of the triangular flow wave is lined up with the beginning of ejection. Peak flow is lined up with the first systolic shoulder. The end of the flow wave is lined up with the incisura, the end of systole.

Westerhof (2006), basically questioning the validity of the triangulation method. Kips et al (2009) suggested an alternative method called the physiological waveform. The physiological waveform is an average echocardiographic waveform obtained from many individuals which was shown to perform better than the triangulation method.

The use of RM as a basis for comparison is justified in the sense that it eliminates the need for calibration since it is a ratio. In a normal circumstance, an increase in cardiac contractility would increase Pf, and a likely increase in Pb, thus RM would remain the same. However, in a situation of cardiac failure, cardiac contractility would decrease, thus reducing Pf, but since Pb is not only influenced by Pf, Pb would not necessarily decrease as well. Thus RM would increase, but this increase in RM is attributable to changes in Pf. Therefore, we are unable to distinguish the independent changes of Pb by using RM.

Subsequently, a study compared the validity of the aforementioned methods of deriving Pb in patients with reduced ejection fraction (Parragh et al 2015). The authors suggested a new approach to deriving Pb called the Windkessel model, which they compared to the triangulation method, and the physiological waveform described by Kips et al (2009) (Parragh et al 2015). The study showed that the magnitude of Pb derived using all three methods were well correlated with Pb derived using echo-derived aortic flow waves. Furthermore, the Windkessel model and the physiological waveform performed better than the triangulation method in the normal group with normal ejection fraction. However, the triangulation method performed better than the physiological waveform in the group with reduced ejection fraction (Parragh et al 2015). The implications of these results mean that the triangle may be a poor approximation of the actual aortic flow waveform. Therefore, based on these results, the triangulation method overestimates Pb and Pf.

The studies by Westerhof et al (2006), Kips et al (2009), and Parragh et al (2015) demonstrate discrepancies in results, which could be associated with different methodological approaches. Where Westerhof used catheters to determine the aortic flow waveform, which is an invasive procedure, Kips determined the aortic flow waveform using Doppler ultrasound. One could associate the different results between the two studies to the differences in obtaining the aortic flow waveform. Furthermore, aortic flow waves of 74 individuals were used to derive the physiological waveform which is not a true reflection of the population. Moreover, Parragh et al (2015) demonstrated a better performance of the triangulation method in patients with reduced ejection fraction, thus there remains validity to the triangulation method which could be applied to clinical settings.

As demonstrated, the backward wave provides a useful tool for the prediction of end-organ changes. Furthermore, the triangulation method offers an efficient and cost-effective method of determining the magnitude of Pb. However, it remains unclear whether the triangulation method yields values which are dramatically different to a gold-standard (echocardiographic derived aortic flow velocity waveforms). The more important consideration is whether these two methods of obtaining Pb differ in assessing end-organ changes. If the triangulation method produces Pb values which differ to the gold standard method, but these two methods yield results which are not different when assessing end-organ changes, then the differences in Pb values are somewhat irrelevant. Therefore, the question arises as to how the triangulation method and Doppler ultrasound method of obtaining Pb differ in assessing end-organ changes?

1.5 Study objectives

Therefore, the aim of my dissertation is to assess the validity of the triangulation method in deriving Pb, by comparing it to Pb derived using echocardiographic aortic flow waves. Thereafter, I will analyse their associations with various indices of LVM, and compare the associations for the two methods of obtaining Pb.

Chapter 2

Methods

2.1 Study group

The present study was conducted according to the principles outlined in the Helsinki Declaration. The University of the Witwatersrand Committee for Research on Human Subjects approved the protocol (M02-04-72 renewed as M07-04-69 and M12-04-108). All participants gave informed, written consent. The present study is a sub-study, which is part of a large cross-sectional study known as the African Project on Genes and Hypertension (APOGH). The study design of APOGH has been previously described (Maseko et al 2006, Shiburi et al 2006). Participants were randomly recruited from nuclear families of Black African descent, mainly the Nguni (Zulu, Xhosa, Ndebele, Swati), Sotho (South, North Sotho and Tswana) and Venda chiefdoms, with siblings older than 16 years of age, living in households from formal dwellings, from the South West Township (SOWETO) of Johannesburg, South Africa. We required assent from participants between the age of 16-18 as well as consent from their legal guardian. 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. No subjects of mixed, Asian, Khoi-San, or Europeans ancestry were recruited. From the year 2001 to the present year, 1319 participants have been recruited for APOGH. In the last four years, echocardiographic aortic flow waveforms were acquired on 394 participants needed for the present study.

2.1.1 Clinical, demographic, and anthropometric measurements

Demographic and clinical data were obtained using a standardised questionnaire as previously described (Norton et al 2008, Woodiwiss et al 2009). The questionnaire was not translated into an African language, but research assistants familiar with all languages spoken in SOWETO and who either previously lived in SOWETO or currently reside in SOWETO assisted with the completion of each questionnaire, in order to avoid translational errors. Research assistants first visited the homes of the subjects who agreed to participate in the study. At this visit, participants were familiarised with the questionnaire. The questionnaire was only completed at a subsequent clinic visit. Ambiguities in answers were detected by an independent observer, and were rectified by performing a follow-up home visit. If family members were absent at the follow-up home visit, data was checked with them personally via telephonic conversation whenever possible.

The questionnaire requested specific answers to date of birth, gender, previous medical history including the presence of hypertension, diabetes mellitus, and kidney disease, previous cardiovascular events including stroke, myocardial infarction or heart failure, the presence of angina pectoris, prior and current drug therapy, and family history of hypertension. The questionnaire also asked about smoking habits including average number of cigarettes per day, alcohol consumption (daily consumption in any form), and caffeine consumption (number of cups of tea or coffee per day). For females, menstrual history, history of pregnancies and or oral contraceptive use was recorded.

Height and weight were measured using a stadiometer and calibrated scale. Hip and waist circumference were measured with a tape measure. Participants were classified as overweight if their body mass index (BMI) was ≥25 kg/m^2, and obese if their BMI was ≥30 kg/m^2.

2.1.2 Blood collection and measurements

In order to identify medical conditions and syndromes, fasting blood samples were collected by a nursing sister on the participant's first visit to the clinic. The blood was sent to the National Health Laboratory Service (NHLS) of South Africa to ensure result reliability and reproducibility. A full blood count with a differential count was requested as well as analyses for blood glucose, renal function (urea, creatinine, and electrolyte concentrations), and percentage glycated haemoglobin (HbA1c) (Roche Diagnostics, Mannheim, Germany). Diabetes mellitus, or abnormal blood glucose control was defined as the use of insulin or oral hypoglycaemic agents, or an HbA1c (Roche Diagnostics, Mannheim, Germany) value >6.1% (Bennet et al 2007).

2.1.3 Blood pressure

High quality nurse-derived conventional BP measurements were obtained by a trained nurse-technician according to the European Society of Hypertension, and the American Heart Association recommendations using a standard mercury sphygmomanometer (O'Brien et al 2003, Pickering et al 2005). A standard adult cuff (22cm x 12cm) was employed except in cases where the participant's arm circumference exceeded 31cm in which case a large cuff (31cm x 15cm), or an extra-large adult cuff (38cm x 50cm) was used. Blood pressures were recorded to the nearest 2 mmHg. Korotkov phases I and V were employed to identify systolic and diastolic BP respectively. Care was taken to avoid auscultatory gaps. BP was measured 5 times

consecutively, at least 1 minute apart, after the participant had rested for 5-10 minutes in the sitting position. The average of the 5 BP measurements was taken as the conventional BP. BP measurements were performed between 09:00 and 12:00 hours. Only 3.8% of visits had fewer than planned BP recordings. The frequency of identical consecutive recordings was 0.12% for systolic BP, and 0.51% for diastolic BP. The occurrence of BP values recorded as an odd number was 0.4%. Of the systolic and diastolic BP readings, 30.7% ended on a zero.

2.2 Echocardiography

2.2.1 Left ventricular mass

Echocardiographic measurements were performed using a Sonosite M-Turbo ultrasound device (SonoSite Inc., Bothell, Washington, USA) with the participant in the partial left decubitus position. All measurements were recorded and analysed by two experienced investigators (CL and AJW) whom were unaware of the clinical data of the participants. All participants were assessed for mitral valve abnormalities as determined using 2-dimensional and colour Doppler imaging. Left ventricular dimensions were determined using 2-dimensional M-mode echocardiography in the parasternal long axis view of the heart, and these recordings were analysed according to the American Society of Echocardiography convention (Sahn et al 1978). From the images recorded, only images of acceptable quality whereby both the right and left septal walls were clearly visible were analysed. The left ventricular internal diameter, posterior wall end-diastolic thickness, and intraventricular septal wall thickness were measured, as shown in figure 2.1. Using these measurements, LV mass (LVM) was calculated according to an anatomically validated formula (Devereux et al 1986):

$$LVM = 1.04\ [(IVSd + LVIDd + PWTd)^3 - LVID^3]$$

where IVSd is the interventricular septum wall thickness during diastole, LVIDd is the left ventricular internal diameter during diastole, and PWTd is the posterior wall thickness during diastole. LVM was indexed height $^{2.7}$ (LVMI-$H^{2.7}$) (De Simone et al 2013), and height$^{1.7}$ (LVMI-$H^{1.7}$) (Chirinos et al 2010), as there is no consensus as to which index should be used (Woodiwiss and Norton 2015). Left ventricular hypertrophy (LVH) was defined as an LVMI-$H^{2.7}$ $\geq$50 g/$m^{2.7}$ for males and $\geq$46g/$m^{2.7}$ for females (de Simone et al 2013), and LVMI-$H^{1.7}$ $\geq$80 g/$m^{1.7}$ for males and $\geq$60 g/$m^{1.7}$ for females (Chirinos et al 2010). Left ventricular relative wall thickness (RWT) was calculated as (LV diastolic posterior wall thickness x 2)/ LV end diastolic diameter.

2.2.2 Aortic flow velocity

The aortic flow wave velocities were obtained from an apical 5-chamber view of the heart. The pulse-wave Doppler cursor was placed approximately 5mm proximal to the aortic valve, in order to measure the flow velocity through the left ventricular outflow tract (figure 2.2a). The flow velocity wave seen in figure 2.2a represents the velocity-time integral which is the integral of all flow velocities during the time of flow across the aortic valve (Quinones et al 2002). According to standard procedures, the flow velocity wave was traced along the outer edge or the most dense, or brightest, portion of the spectral tracing (Quinones et al 2002) (figure 2.2b). The shape of the aortic flow wave was used to derive Pf and Pb, which will be explained in section 2.3.2.

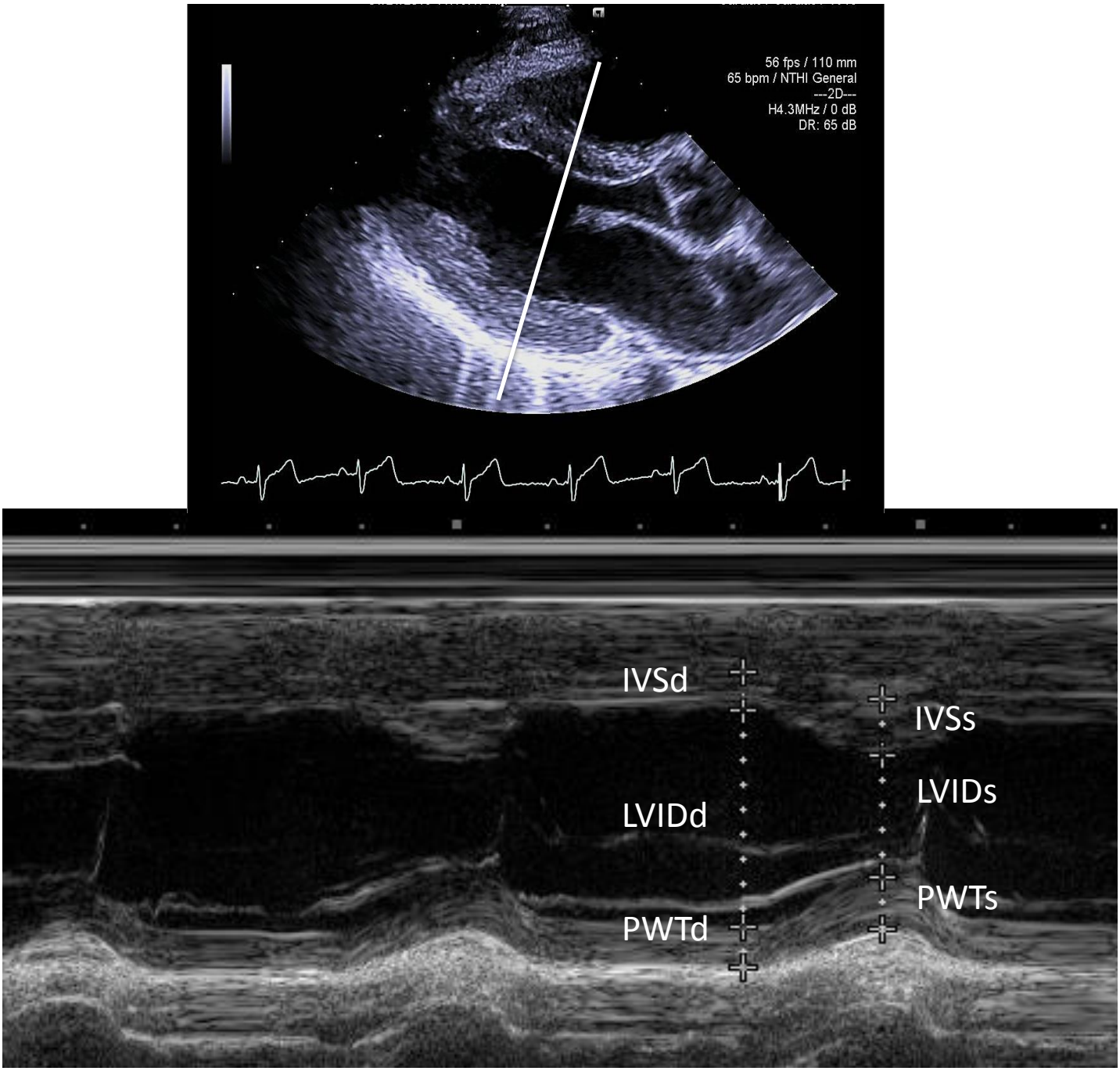

Figure 2.1 An example of a two-dimensional directed M-mode echocardiogram in the parasternal long-axis view of the left ventricle, used to obtain left ventricular dimensions for the calculation of left ventricular mass. IVSd= Intraventricular septum wall thickness at end diastole. IVSs= Intraventricular septum wall thickness at end systole. PWTd= posterior wall thickness at end diastole. PWTs= posterior wall thickness at end systole. LVIDd= left ventricular internal diameter at end diastole. LVESD= left ventricular internal diameter at end systole.

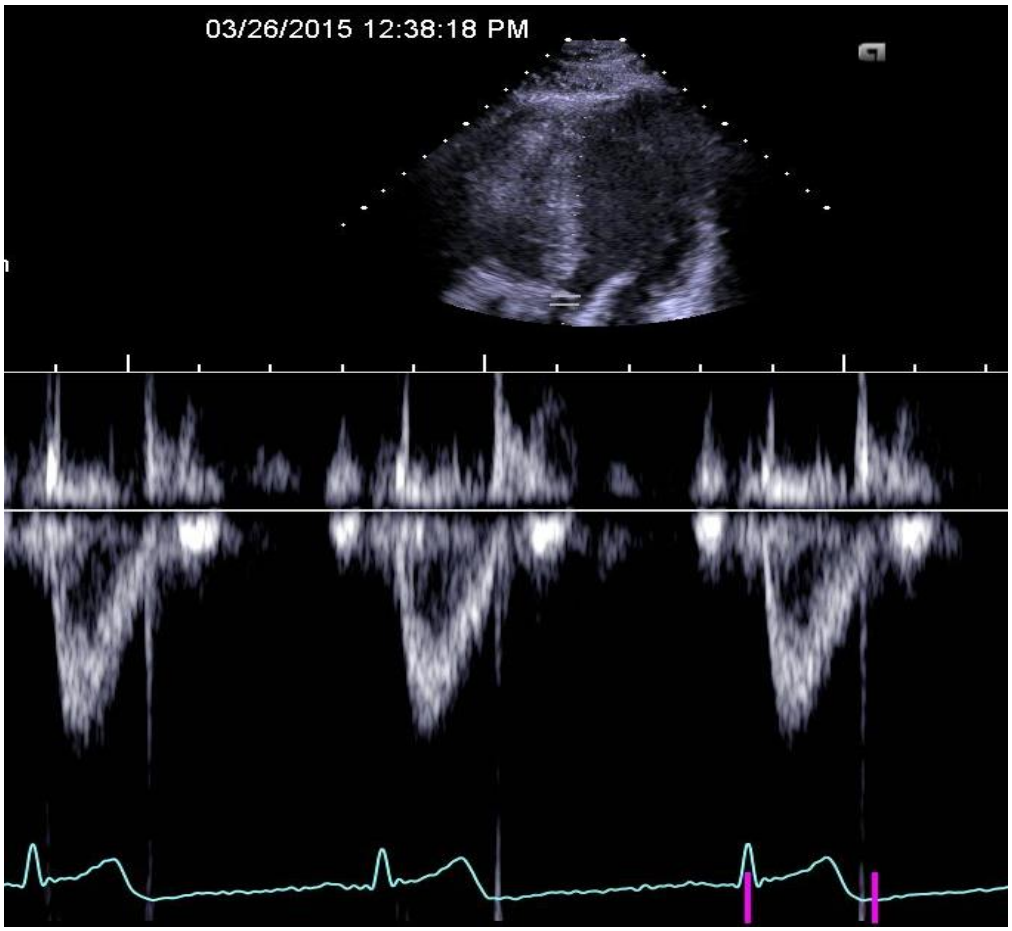

Figure 2.2a An example of three aortic flow velocity waves obtained using echocardiography, in the apical 5-chamber view of the heart. Electrocardiograph traces below the aortic flow velocity waves signifying the beginning and end of systole.

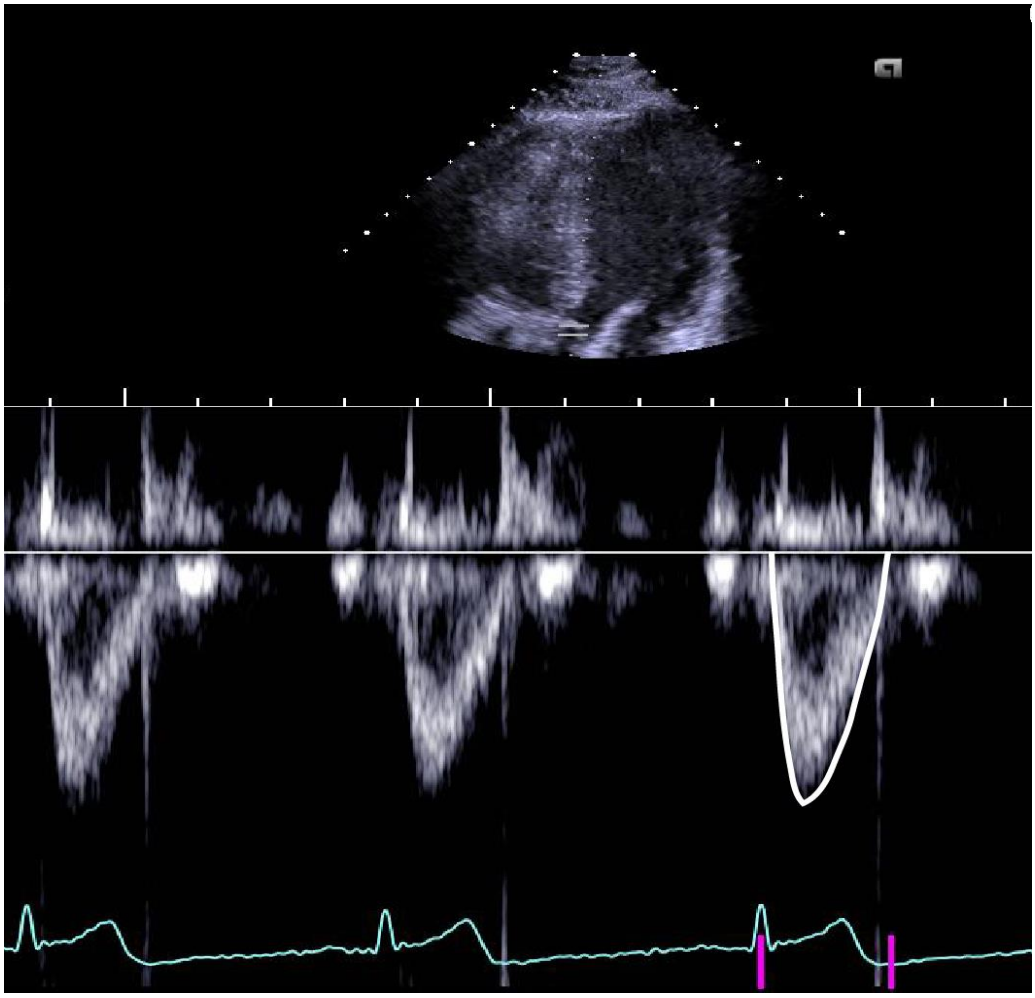

Figure 2.2b An example of an echocardiographic-derived aortic flow velocity wave, with a drawn outline of the flow waveform (in white). Electrocardiograph traces below the aortic flow velocity waves signifying the beginning and end of systole. The outline of this wave was used to determine the backward wave by superimposing the outline of the flow waveform on the aortic pressure wave.

2.3 Aortic haemodynamics

2.3.1 Pulse wave analysis

Central aortic BP was measured by radial applanation tonometry during an 8 second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc, Houston, TX) which was interfaced with a computer using SphygmoCor, version 9 software (AtCor Medical Pty Ltd, West Ryde, New South Wales, Australia) (figure 2.3). After the participant had rested for 15 minutes in the supine position, arterial pressure waveforms at the radial pulse were recorded. Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5%, or the amplitude of the pulse wave signal was less than 80mV, were discarded. The radial pressure wave was calibrated as provided for in the SphygmoCor software by the manual measurement of brachial systolic and diastolic BP immediately before the recordings. From the radial pressure waveform, a validated generalised transfer function built into the SphygmoCor software was used, to derive the aortic pressure waveform, and hence the central aortic systolic, diastolic, and mean arterial BP (figure 2.4). The magnitude of the incident wave (Pi) was determined as the difference between the inflection point at the end of the first systolic shoulder and central diastolic BP (i.e. the height of the first systolic shoulder). Augmented pressure (AP) was determined as the difference between central systolic BP and the inflection point at the end of the first systolic shoulder. Central aortic PP (cPP) was calculated as the difference between the central systolic BP and central diastolic BP. Central mean arterial pressure was calculated as [central diastolic BP + 1/3(cPP)]. Augmentation index (AIx) was determined as AP/cPP, expressed as a percentage. Both these measurements were obtained using the SphygmoCor software.

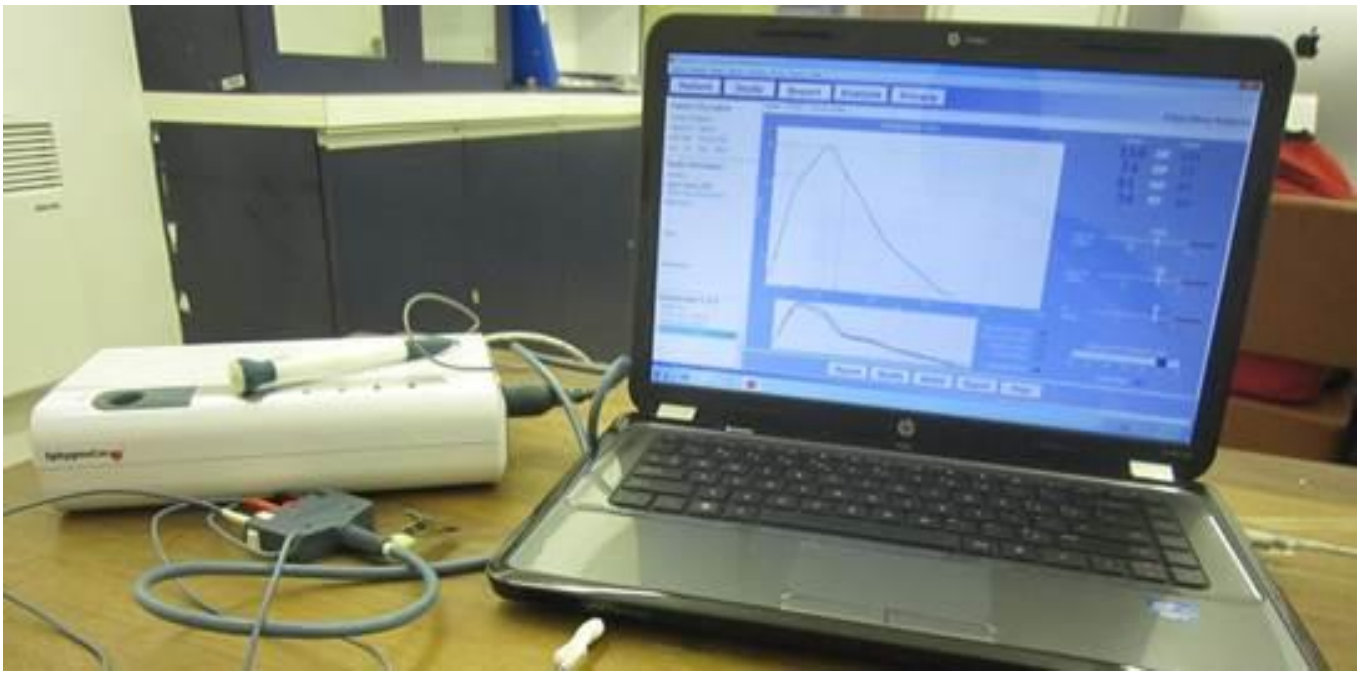

Figure 2.3 Applanation tonometer coupled to SphygmoCor device, which was attached to a computer, and used to determine aortic haemodynamics.

Radial Pressure Waveform **Aortic Pressure Waveform**

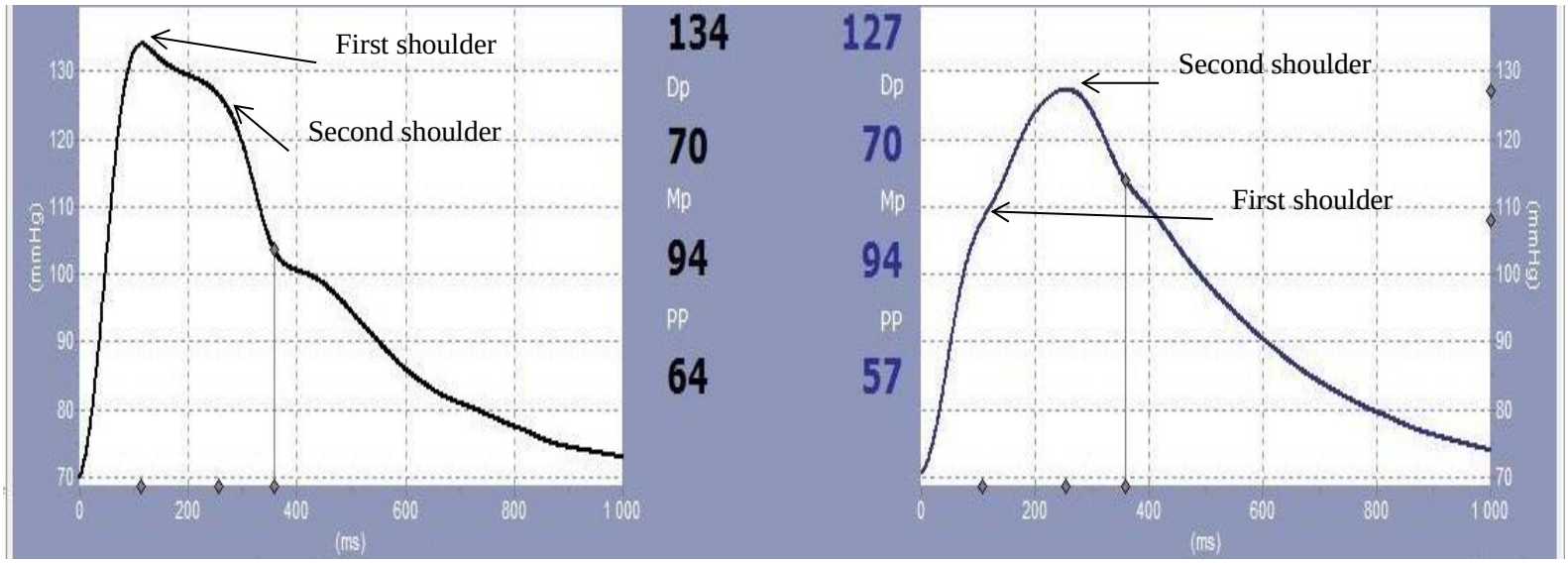

Figure 2.4 Example of pressure waveform recordings obtained using radial applanation tonometry and SphygmoCor software. The figure shows the radial pressure waveform (left) obtained using the tonometer. The aortic pressure waveform (right) was derived from the radial pressure waveform using a generalised transfer function built into the SphygmoCor software.

2.3.2 Wave separation analysis

The backward wave (Pb^{tri}) and forward wave (Pf^{tri}) pressure obtained using the triangulation method were derived using SphygmoCor wave separation software. This method assumes a triangular shape of the aortic flow velocity wave. Assuming that there is no flow during diastole, the beginning and end of the triangle are lined up with the beginning (foot of the upstroke of the aortic pressure wave) and end of systole (dicrotic notch) on the aortic pressure waveform respectively, and the peak of the triangle with the first systolic shoulder of the aortic pressure waveform, as shown by the dashed line in figure 2.5. Pf is the arithmetic mean between the measured aortic pressure waveform and the triangle (flow waveform), and Pb is the area between Pf and the aortic pressure waveform (shaded area in figure 2.5). Thus, peak Pb would be the maximum distance between Pf and the aortic pressure waveform.

In order to derive Pb and Pf using the echocardiographic derived flow velocity waveform (Pb^{echo}, Pf^{echo}), an image of the aortic flow velocity wave was needed, as seen in figure 2.2a. The outline of the echocardiographic derived aortic flow velocity wave was drawn (figure 2.2b). The outline of the aortic flow velocity wave was superimposed on the aortic pressure waveform by lining up the beginning and end of the flow velocity wave with the beginning (foot of the upstroke of the pressure wave) and end (dicrotic notch) of systole respectively, and lining up the peak aortic flow velocity wave with the first systolic shoulder of the aortic pressure waveform. Pf is the arithmetic mean between the measured aortic pressure waveform and the aortic flow velocity waveform, and Pb is the area (shaded area in figure 2.6) between Pf and the aortic pressure waveform (figure 2.5).

Using the following formulae for Pb and Pf:

$$Pf(t) = [P(t) + Zc \bullet F(t)] /2$$

$$Pb(t)= [P(t) – Zc \bullet F(t)] /2$$

Where P(t) is the measured pressure wave, F(t) is the flow wave, and Zc is the characteristic impedance of the proximal aorta (Westerhof et al 2006), it can be seen that the product Zc • F(t) appears in the calculation for both Pf and Pb. Thus Zc= Pf/Pf = -Pb/ Fb. Therefore, the amplitude of flow is eliminated, and Zc • F(t) is independent of the flow calibration, and is unit-less (Westerhof et al 2006). Therefore, the use of an echocardiographic derived aortic flow velocity wave allows us to derive Pb without calibration.

Intra-observer variability analysis on aortic flow velocity waveforms conducted on 21 individuals yielded an R^2 value of 0.990 for Pf^{echo} and an R^2 value of 0.986 for Pb^{echo}.

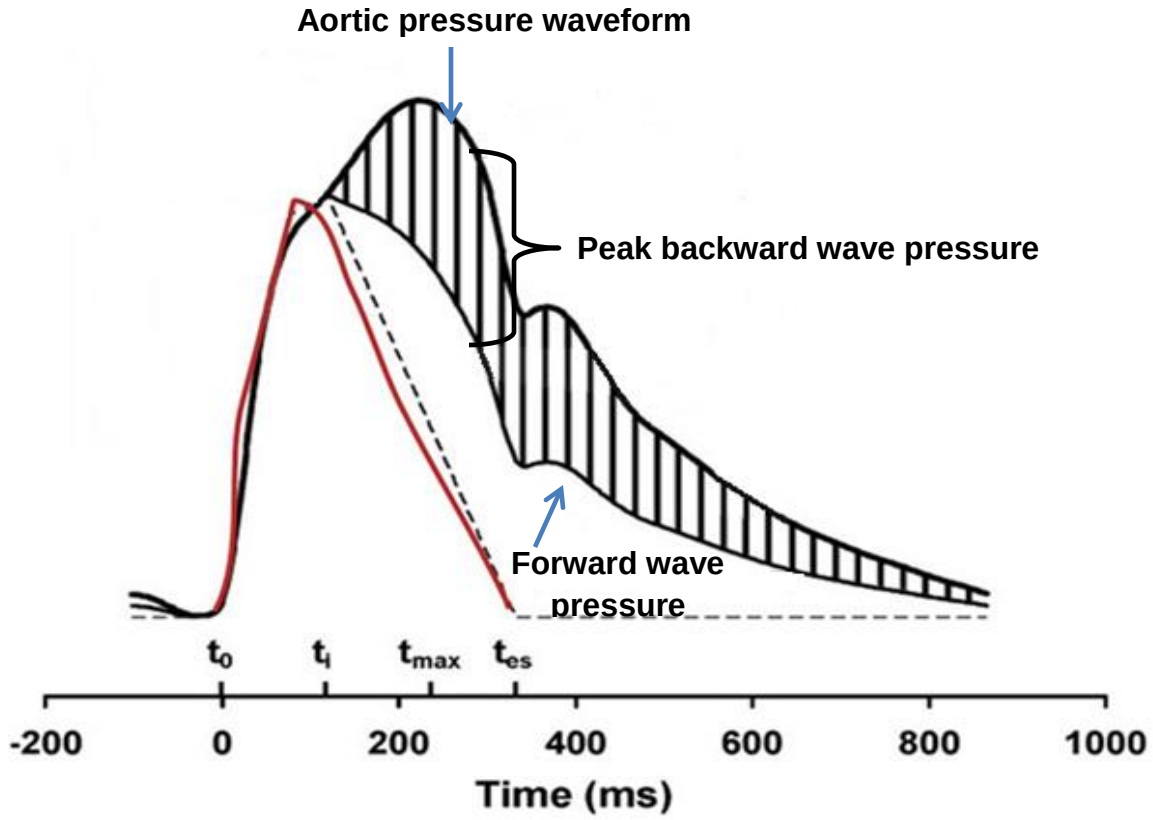

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Figure 2.5 An adaptation from Mitchell (2006), illustrating the derivation of Pb and Pf. The red line indicates the outline of the echo derived aortic flow wave, as seen in figure 2.2b. t_0= beginning of systole; t_1= time to first systolic shoulder; t_{max}= time to peak aortic pressure; t_{es}= time to end systole. The dotted line represents the triangular flow waveform, used for the triangulation method. The shaded area represents Pb, where the maximum distance between Pf and the aortic pressure waveform represents peak Pb. The red line represents the outline of the aortic flow velocity waveform.

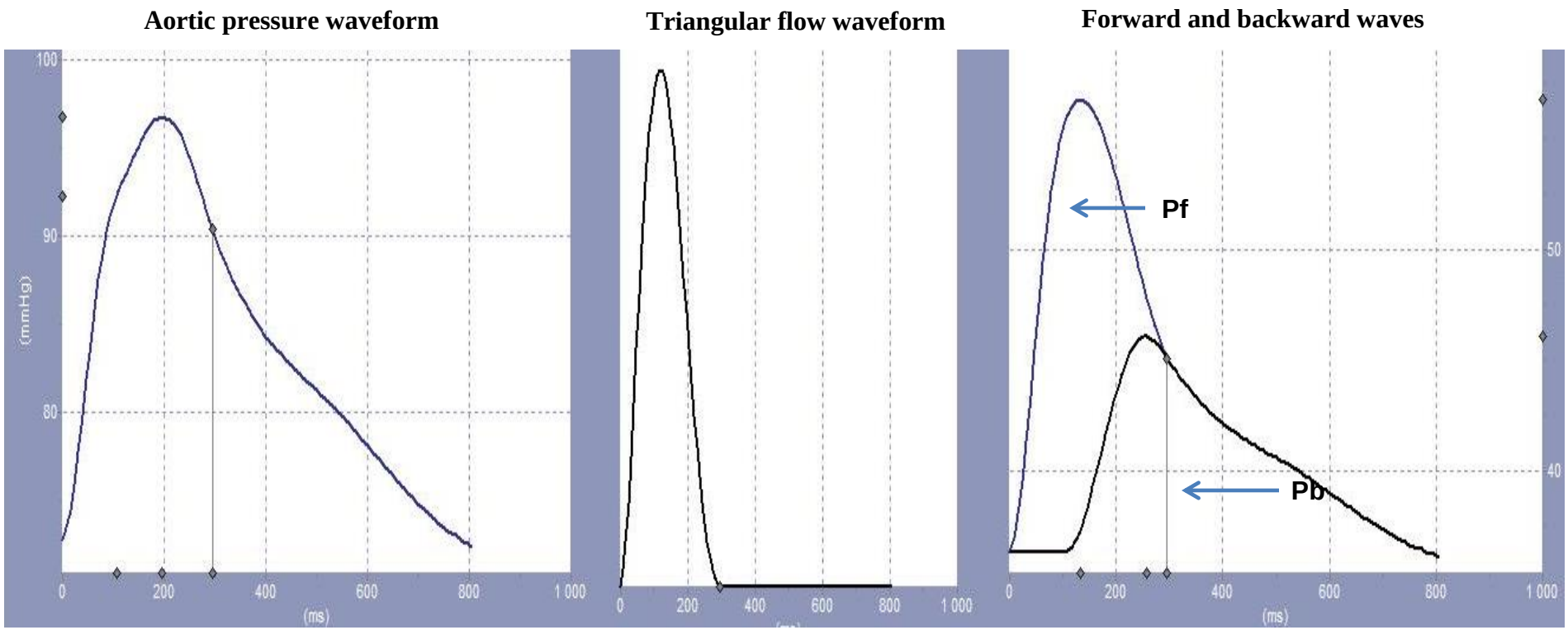

Figure 2.6 An example of wave separation obtained from SphygmoCor software. The software uses the triangulation method. See text for full description.

2.4 Statistical analysis

Database management and statistical analyses were performed with SAS software, version 9.4 (SAS Institute Inc., Cary, NC). Demographic data and characteristics of participants are given as means ± SD or as frequencies (%). To determine the relationship between Pb^{tri} and Pb^{echo}, or Pf^{tri} and Pf^{echo}, Pearson's correlation was used. However, the correlation only determines the relationship between the two methods, and does not inform us of the levels of agreement (Bland and Altman 1986). Hence, agreement between Pb^{tri} and Pb^{echo}, and Pf^{tri} and Pf^{echo}, measurements were assessed using Bland-Altman analyses. This analysis describes the agreement between two measurements by using the difference of the two methods against the mean value (Bland and Altman 1986). The mean is used in order to avoid any statistical artefact.

In order to assess the ability of Pb and Pf measurements to associate with end-organ changes, partial correlation analysis was used. Correlation coefficients were compared using z statistics. Adjustors included: smoking, alcohol use, diabetes, body height and weight, age, sex, and heart rate. These are variables known to contribute to cardiovascular damage. It is well documented that advancing age contributes to cardiovascular damage (Booysen et al 2015). Various differences noted in the cardiovascular system and the way those pathologies manifest could be explained by sex differences. Furthermore, heart rate plays an influential role in Pf and Pb. In addition, smoking and alcohol use are acknowledged as risk factors for cardiovascular disease, and hence may impact on Pf, Pb, and/or LVM. Therefore, we needed to eliminate the potential influences these variables may have when doing regression analysis. Moreover, by adjusting for these variables, we were able to determine the independent relationships between Pf and Pb and LVM.

Chapter 3

Results

3.1 Participant characteristics

The clinical and demographic characteristics of the participants are shown in table 3.1. Of the total community sample, 394 participants had echocardiographic aortic flow velocity waveforms. Of these participants, 44.7% were characterised as obese, and 21.6% were classified as overweight. Of the participants, 16.5% were regular tobacco users, and 21.4% had diabetes. Approximately 40% of the participants were hypertensive. The current sample is a good representation of the entire community sample since none of the characteristics differed between those with, and without, echocardiographic aortic flow wave data (table 3.1).

The aortic haemodynamic and blood pressure measurements of the participants are shown in table 3.2. The brachial BP of the 394 participants was similar to those without echocardiography. Furthermore, the Pf values obtained using the triangulation method were the same as those obtained using the echocardiographic derived aortic flow velocity waveform. Pb^{tri} yielded an average value of 17 mmHg, and Pb^{echo} yielded an average value of 13 mmHg. The echocardiographic data of the participants are shown in table 3.3. The percentage of LVH according to LVMI-$H^{2.7}$ of the 394 participants was not significantly different from the larger cohort without aortic flow waves.

Table 3.1 Characteristics of study participants

	With aortic flow wave	Without aortic flow wave
Sample size (n)	394	925
Age (years)	47.8 ± 18.1	43.7 ± 18.4
Sex (% female)	64.1	65.5
BMI (kg/m^2)	29.2 ± 7.7	29.6 ± 8.1
% Overweight	21.6	22.5
% Obese	44.7	42.7
% with DM or HbA1c >6.1%	21.4	25.4
% Hypertensive	40.6	43.8
% treated for hypertension	24.4	22.5
Regular tobacco intake (%)	16.5	14.3
Regular alcohol intake (%)	18.8	21.3

Data expressed as mean ± SD or proportions. BMI, body mass index; DM, diabetes mellitus; HbA1c, glycated haemoglobin. P> 0.05 for all variables between the 2 groups

Table 3.2 Aortic haemodynamics of study participants

	With aortic flow wave	Without aortic flow wave
Sample size (n)	394	925
Brachial SBP (mmHg)	127 ± 21	129 ± 23
Brachial DBP (mmHg)	82 ± 12	84 ± 13
Central SBP (mmHg)	118 ± 20	121 ± 23
Central DBP (mmHg)	82 ± 12	84 ± 13
Central PP (mmHg)	34 ± 14	36 ± 15
Augmented pressure (AP) (mmHg)	10.4 ± 7.1	11.0 ± 8.1
Augmentation index (AIx)	27.7 ± 12	27.3 ± 12.9
Heart rate (beats per minute)	66 ± 11	66 ± 11
Pf^{tri} (mmHg)	24 ± 8	–
Pf^{echo} (mmHg)	24 ± 8	–
Pb^{tri} (mmHg)	17 ± 7	–
Pb^{echo} (mmHg)	13± 5	–

Data expressed as mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressre; PP, pulse pressure; Pf^{tri}, aortic forward wave obtained using the triangulation method; Pf^{echo}, aortic forward wave obtained using echo-derived aortic flow waveform; Pb^{tri}, aortic backward wave obtained using the triangulation method; Pb^{echo}, aortic backward wave obtained using echo-derived aortic flow waveform. P> 0.05 for all variables between the 2 groups

Table 3.3 Echocardiographic data of the study participants

	With aortic flow wave	Without aortic flow wave
Sample size (n)	372	527
LVID (cm)	4.61± 0.62	4.78 ± 0.56
PWT (cm)	0.85± 0.15	0.92 ± 0.16
IVS (cm)	0.84 ± 0.17	0.95 ± 0.19
LVM indexed to height$^{2.7}$ (g/m$^{2.7}$)	37.9 ± 14.7	44.0 ± 14.6
LVM indexed to height$^{1.7}$ (g/m$^{1.7}$)	60.8 ± 24.9	70.8 ± 22.8
% LVH g/m$^{2.7}$	22.6	26.8
% LVH g/m$^{1.7}$	30.1	43.2
RWT	0.37 ± 0.08	0.31 ± 0.08

Data expressed as mean ± SD or proportions. LVID, left ventricular internal diameter during diastole; PWT, posterior wall thickness during diastole; IVS, intraventricular septal wall thickness during diastole; LVM, left ventricular mass; LVH, left ventricular hypertrophy; RWT, relative wall thickness. P> 0.05 for all variables between the 2 groups

3.2 Association between Pb^{tri} and Pb^{echo}, and Pf^{tri} and Pf^{echo}

Bivariate regression analysis of Pb^{tri} and Pb^{echo} demonstrated a strong positive relationship between the two measurements (R^2= 0.82) (Figure 3.1). The levels of association between the two measurements of Pb are presented in the Bland-Altman plot (Figure 3.2) whereby Pb^{tri} was higher than Pb^{echo} by an average of 3.65 ± 3.35 mmHg. If we observe the spread of the data in the Bland-Altman plot of Pb^{tri} and Pb^{echo} (figure 3.2), we notice a consistent error. This consistent (systematic) error allows us to correct Pb^{tri} by multiplying it by the slope of the regression line (figure 3.1). Thus, after correcting Pb^{tri} for this error, the R^2 value of the linear regression was not changed, however, the slope of the line was now 1 (figure 3.3). Furthermore, the Bland-Altman plot revealed an average difference of -2.2 ± 2.23 mmHg (figure 3.4).

Bivariate regression analysis of Pf^{tri} and Pf^{echo} revealed a strong positive relationship (R^2= 0.86) (figure 3.5). Furthermore, the Bland-Altman plot revealed an average difference of -0.19 ± 4.28 mmHg between Pf^{tri} and Pf^{echo} (figure 3.6). Furthermore, the Bland-Altman plot revealed no consistent error between Pf^{tri} and Pf^{echo}, therefore we could not correct.

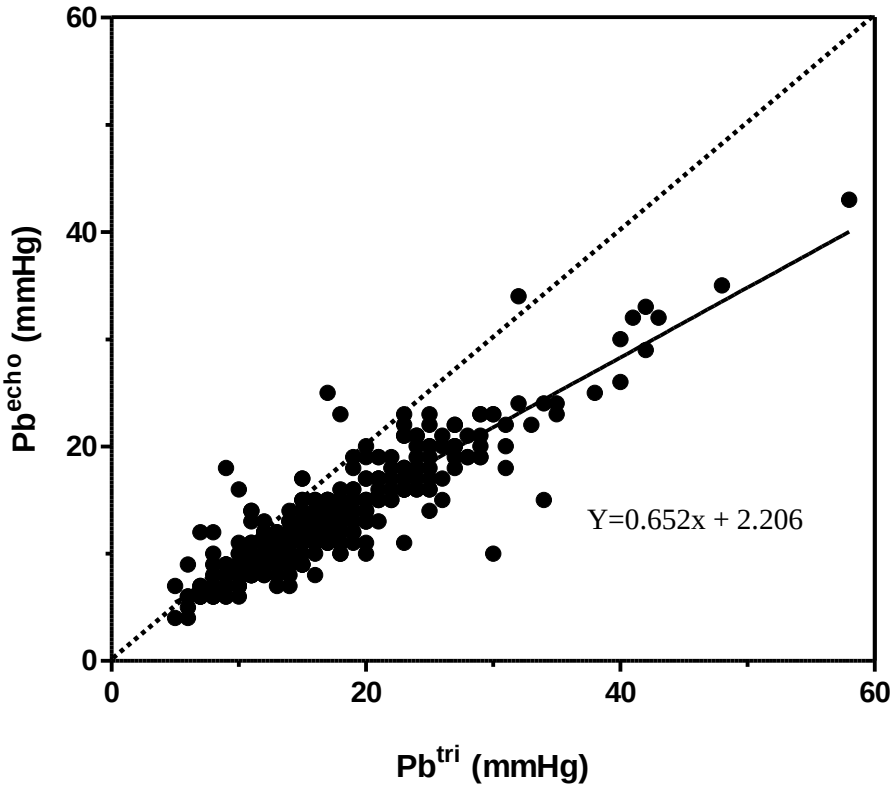

Figure 3.1 Linear regression between Pb obtained using triangulation method (Pb^{tri}) and Pb obtained using echo derived aortic flow waves (Pb^{echo}). Dashed line represents line where y= x.

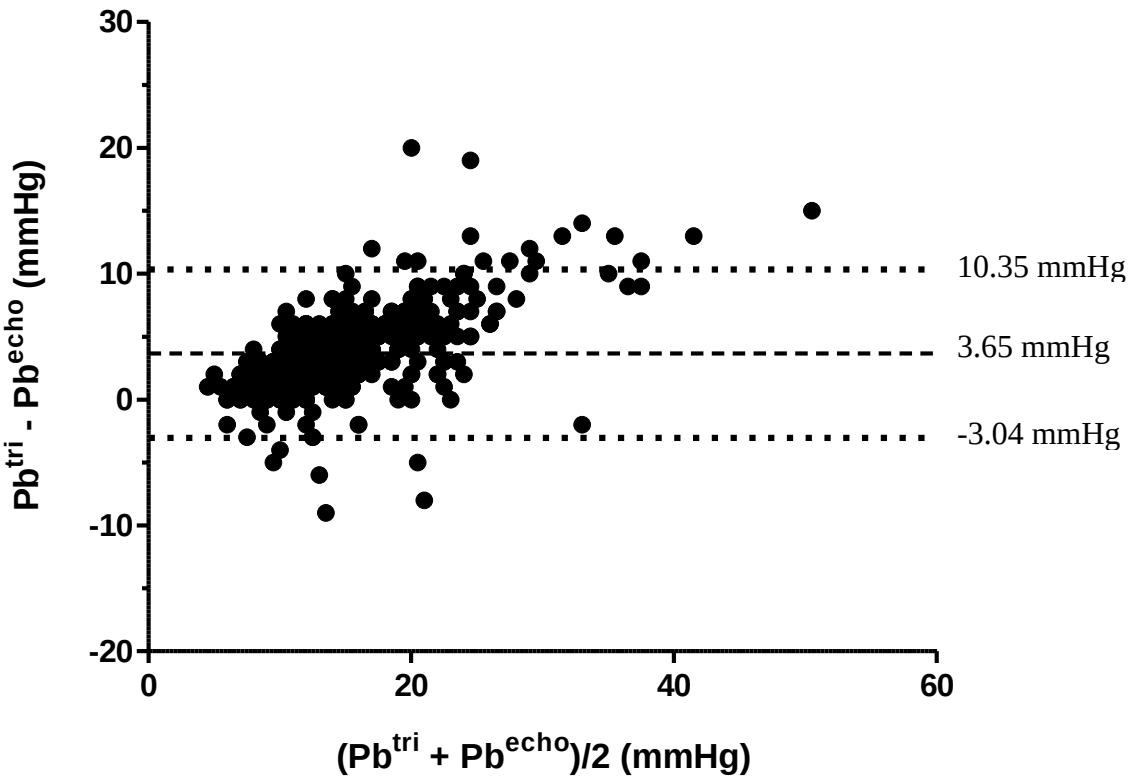

Figure 3.2 Bland-Altman plot of Pb obtained using the triangulation method (Pb^{tri}) and Pb obtained using echocardiographic derived aortic flow waves (Pb^{echo}). The average difference is displayed as the dashed line, and the dotted lines are ± 2 SD.

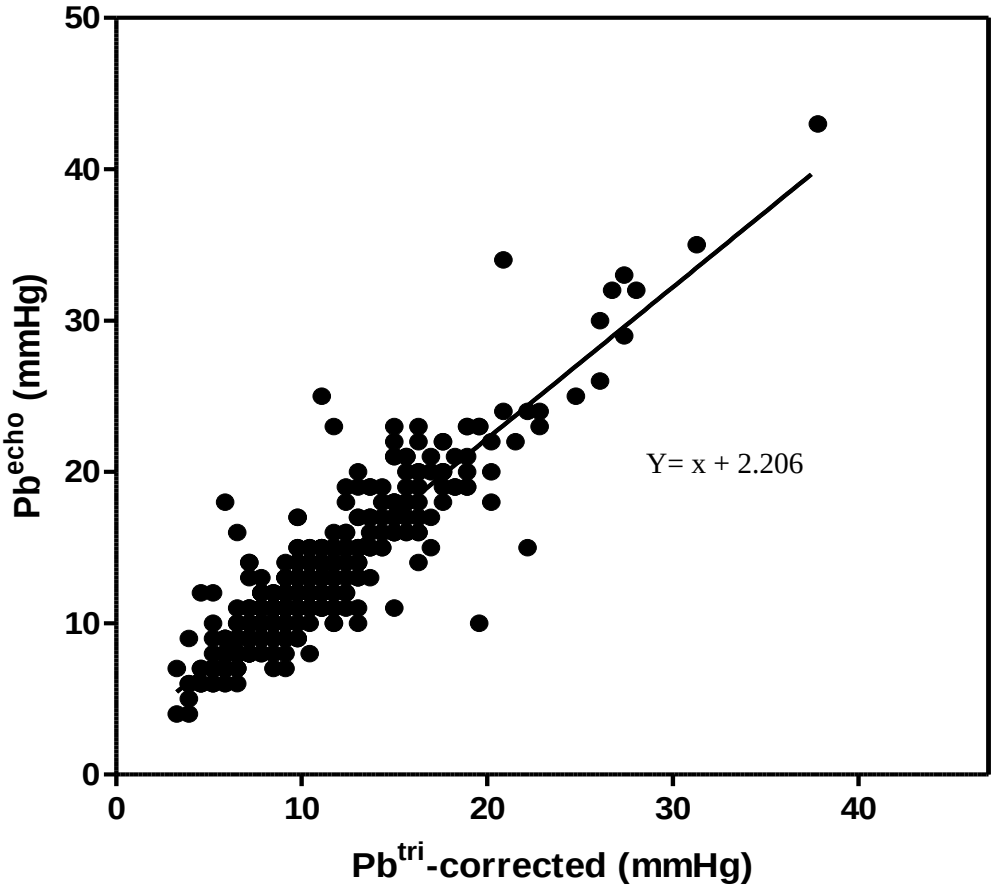

Figure 3.3 Linear regression between Pb obtained using echocardiographic derived aortic flow velocity waves (Pb^{echo}) and the corrected Pb obtained using the triangulation method.

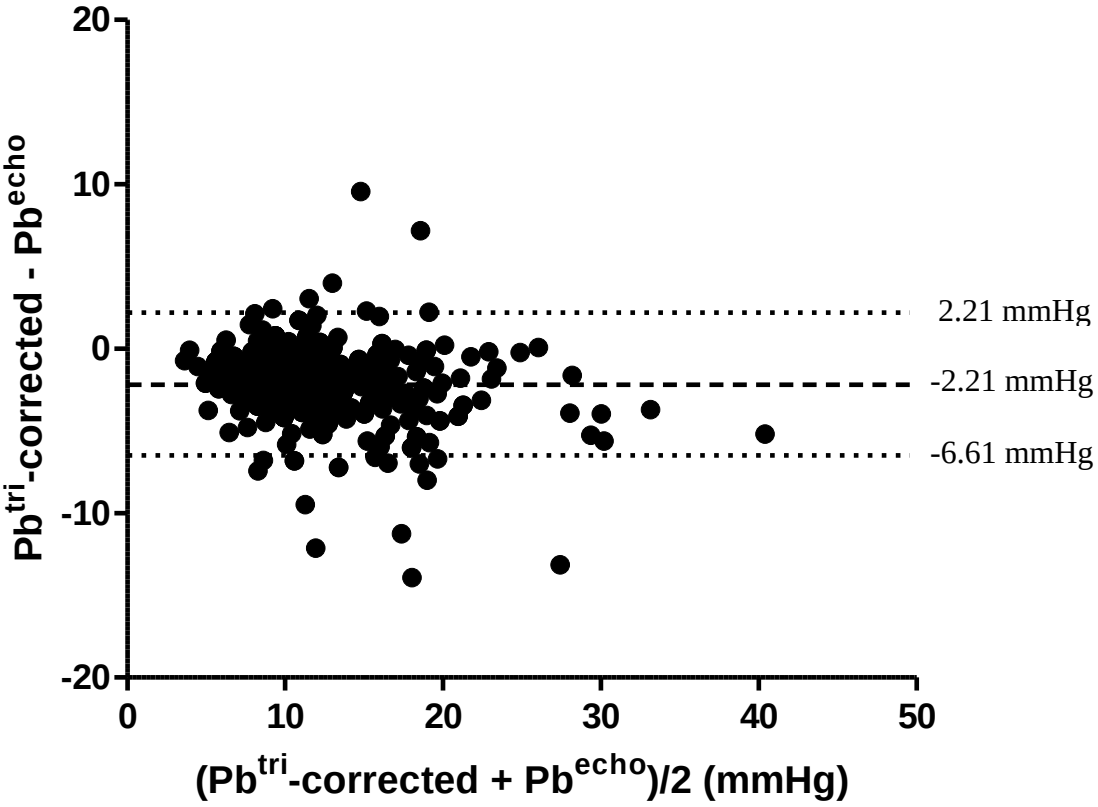

Figure 3.4 Bland-Altman plot of the corrected Pb obtained using the triangulation method (Pb^{tri}corrected) and Pb obtained using echo derived aortic flow waves (Pb^{echo}). The average difference is displayed as the dashed line, and the dotted lines displayed are ±2 SD.

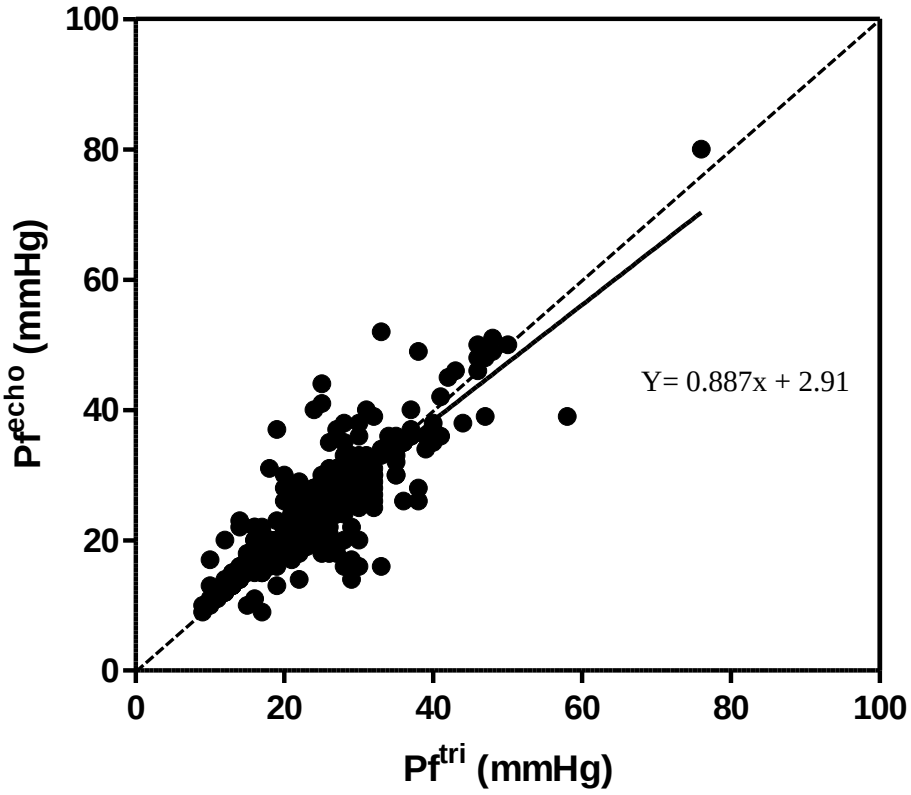

Figure 3.5 Linear regression between Pf obtained using triangulation method (Pf^{tri}) and Pf obtained using echocardiographic derived aortic flow velocity waves (Pf^{echo}). Dashed line represents line where y= x.

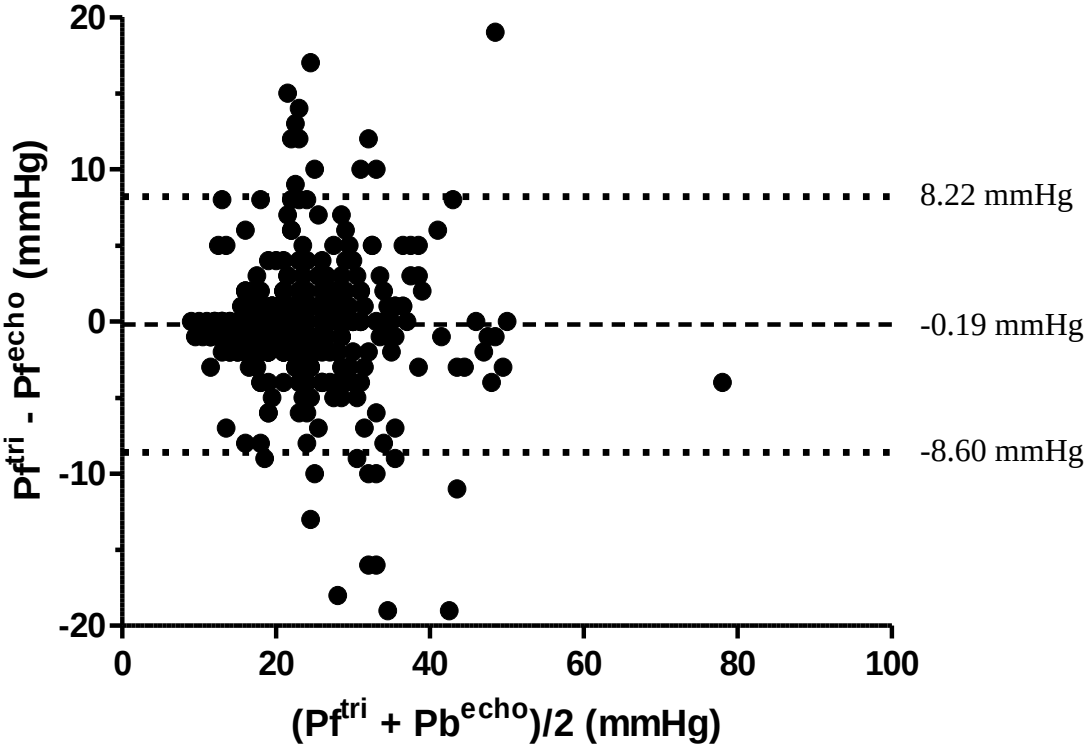

Figure 3.6 Bland-Altman plot of Pf obtained using the triangulation method (Pf^{tri}) and Pf obtained using echocardiographic derived aortic flow velocity waves (Pf^{echo}). The average difference is displayed as the dashed line, and the dotted lines are ±2 SD.

3.3 Relationship between backward, and forward, waves and LVM indexed to height$^{2.7}$

With regards to LVMI-$H^{2.7}$, Pb^{tri}-corrected and Pb^{echo} were positively associated, before adjusting for potential confounders (figure 3.7). After adjusting for potential confounders excluding age, similar results were obtained and positive associations were yielded between LVMI-$H^{2.7}$ and Pb^{tri}-corrected and Pb^{echo} (figure 3.7). Furthermore, after adjusting for potential confounders, including age, both methods of obtaining Pb were significantly associated with LVMI-$H^{2.7}$ (figure 3.7). When comparing the r values, obtained with and without adjusting for potential confounders, no significant differences were noted ($p>0.05$).

Before and after adjusting for potential confounders, excluding age, Pf^{tri} and Pf^{echo} were positively associated with LVMI-$H^{2.7}$ (figure 3.8). However, after adjusting for potential confounders, including age, both Pf^{tri} and Pf^{echo} were not significantly associated with LVMI-$H^{2.7}$.

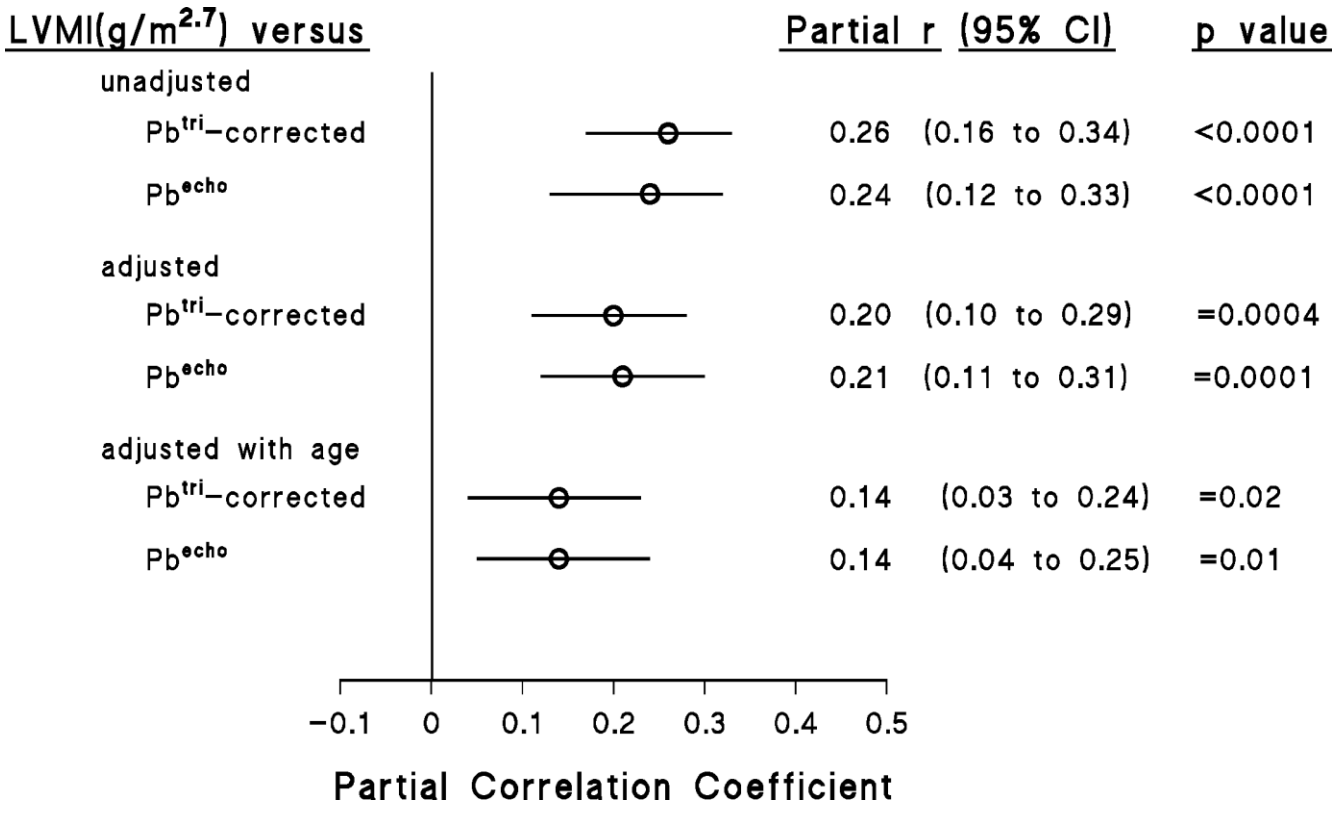

Figure 3.7 Unadjusted and adjusted correlations between the two methods of obtaining the backward wave (Pb^{tri}-corrected and Pb^{echo}), and left ventricular mass index to $height^{2.7}$. Potential confounders included in the model are age, sex, body weight, diabetes mellitus or an HbA1c> 6.1%, regular alcohol and tobacco usage.

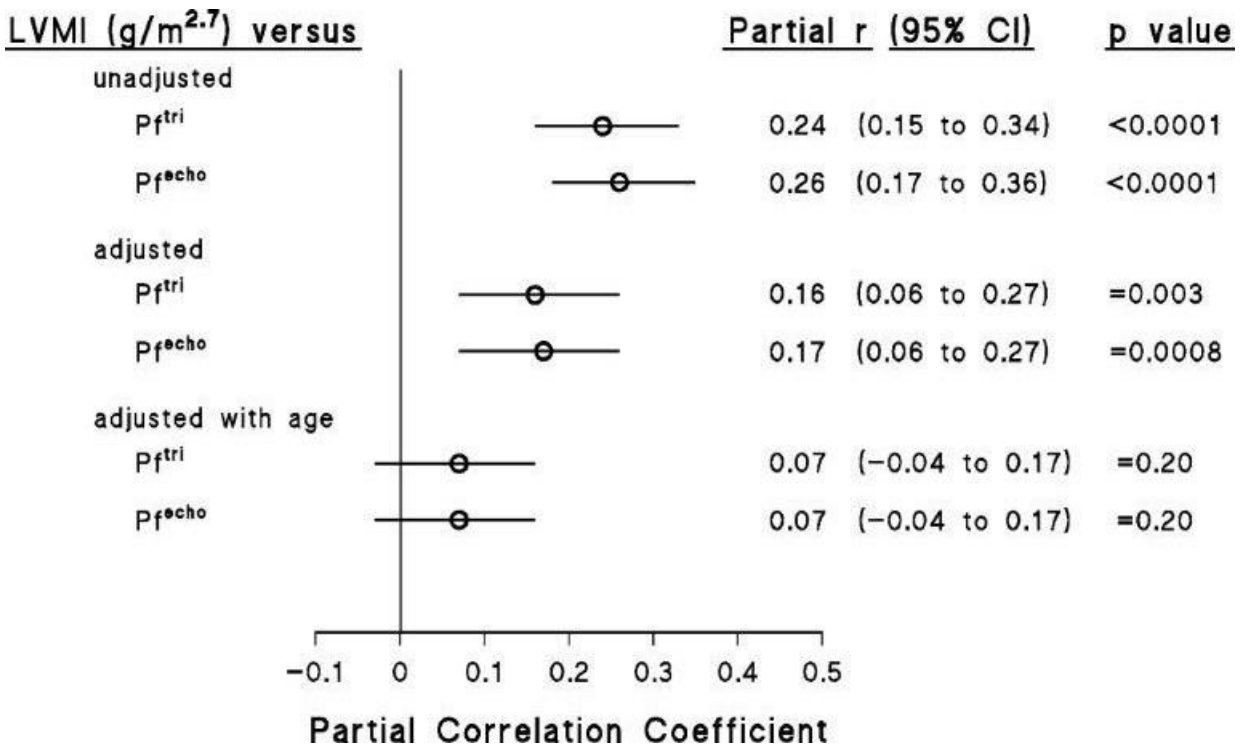

Figure 3.8. Unadjusted and adjusted correlations between the two methods of obtaining the forward wave (Pf^{tri} and Pf^{echo}), and left ventricular mass index to $height^{2.7}$. Potential confounders included in the model are age, sex, body weight, diabetes mellitus or an HbA1c> 6.1%, regular alcohol and tobacco usage.

3.4 Relationship between backward and forward waves and LVM indexed to height$^{1.7}$

Before adjusting for potential confounders, Pb^{tri}-corrected and Pb^{echo} were positively associated with LVMI-$H^{1.7}$ (figure 3.9). After adjusting for potential confounders, excluding age, Pb^{tri}-corrected and Pb^{echo} were positively associated with LVMI-$H^{1.7}$ (figure 3.9). Similarly, after adjusting for potential confounders, including age, both methods of obtaining Pb were significantly associated with LVMI-$H^{1.7}$ (figure 3.9). Furthermore, when comparing the r values, obtained with and without adjusting for potential confounders, no significant differences were noted ($p> 0.05$).

With regards to Pf and LVMI-$H^{1.7}$, Pf^{tri} and Pf^{echo} had a significant positive association with LVMI-$H^{1.7}$, before adjusting for potential confounders (figure 3.10). After adjusting for potential confounders, excluding age, Pf^{tri} and Pf^{echo} were significantly associated with LVMI-$H^{1.7}$ (figure 3.10). However, neither Pf^{tri} nor Pf^{echo} were significantly associated with LVMI-$H^{1.7}$ when age was included as a potential confounder (figure 3.10).

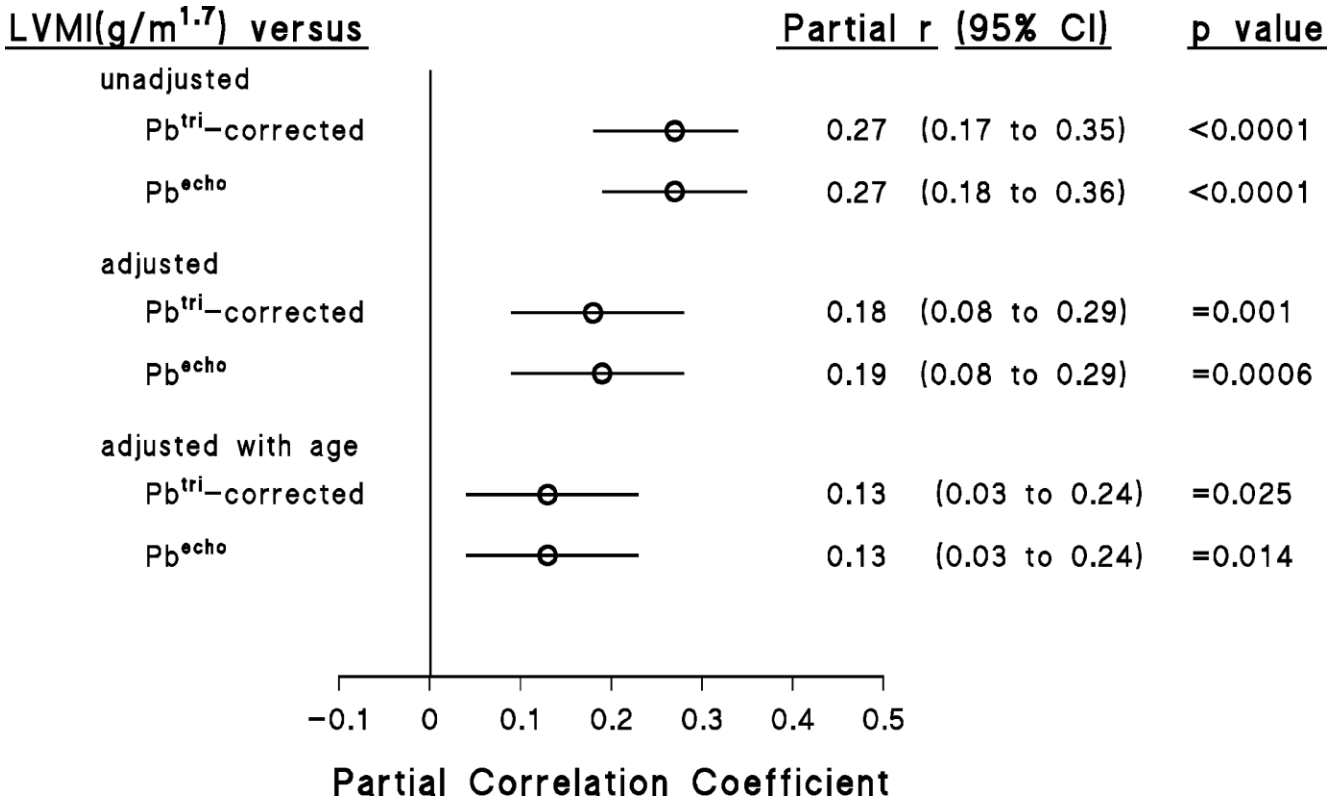

Figure 3.9 Unadjusted and adjusted correlations between the two methods of obtaining the backward wave (Pb^{tri}-corrected and Pb^{echo}), and left ventricular mass index to $height^{1.7}$. Potential confounders included in the model are age, sex, body weight, diabetes mellitus or an HbA1c> 6.1%, regular alcohol and tobacco usage.

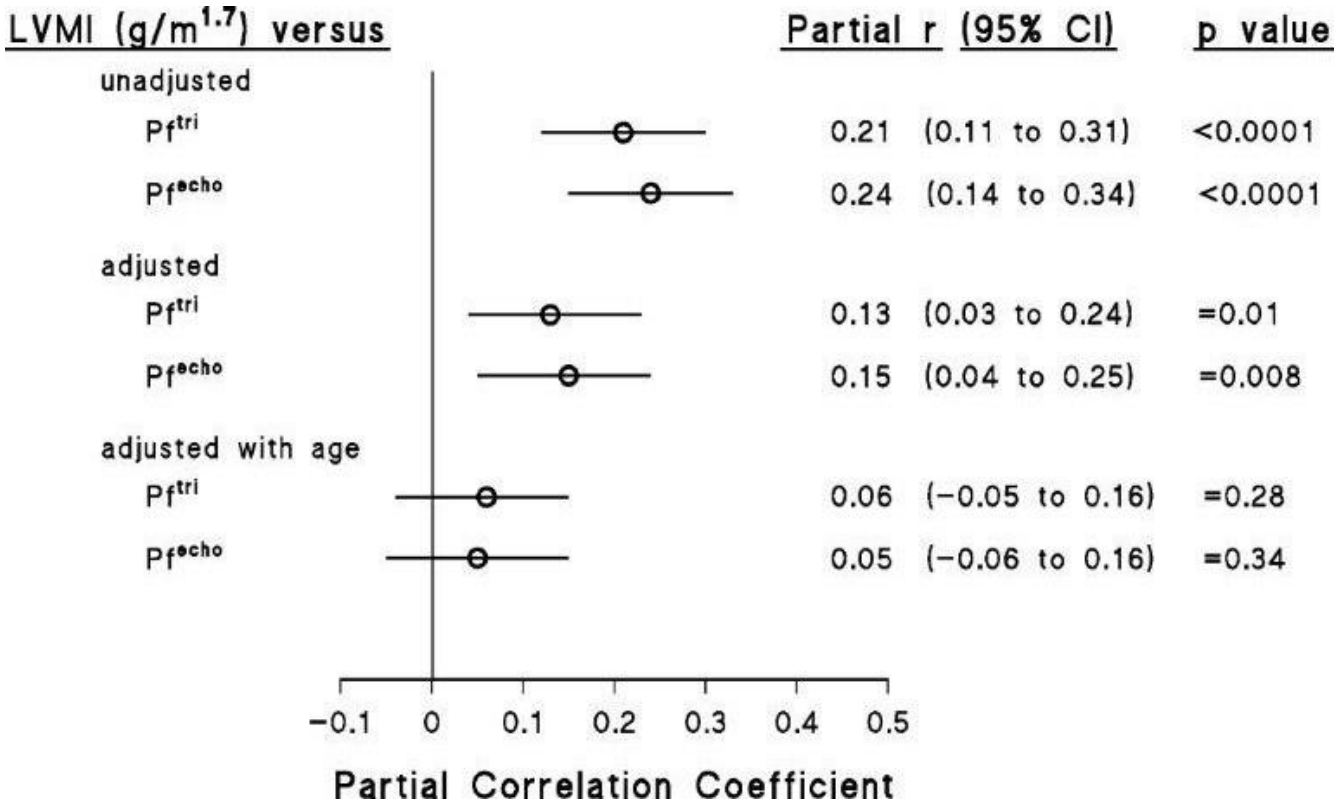

Figure 3.10 Unadjusted and adjusted correlations between the two methods of obtaining the forward wave (Pf^{tri} and Pf^{echo}), and left ventricular mass index to height$^{1.7}$. Potential confounders included in the model are age, sex, body weight, diabetes mellitus or an HbA1c> 6.1%, regular alcohol and tobacco usage.

3.5 Relationship between backward, and forward waves, and relative wall thickness

Before adjusting for potential confounders, both Pb^{tri}-corrected and Pb^{echo} were significantly associated with relative wall thickness (RWT) (figure 3.11). However, after adjusting for confounders, excluding age, the associations showed borderline significance (figure 3.11). Indeed, after including age as a potential confounder, the associations remained insignificant (figure 3.11). Furthermore, comparison of the r values, with or without adjusting for potential confounders, revealed no significant differences ($p> 0.05$).

Pf^{tri} and Pf^{echo} had a significant positive association with RWT, before adjusting for potential confounders, as seen in figure 3.12. However, after adjusting for confounders excluding age, these associations were no longer evident for both methods of obtaining Pf (figure 3.12). The same effects were noted after adjusting for age (figure 3.12).

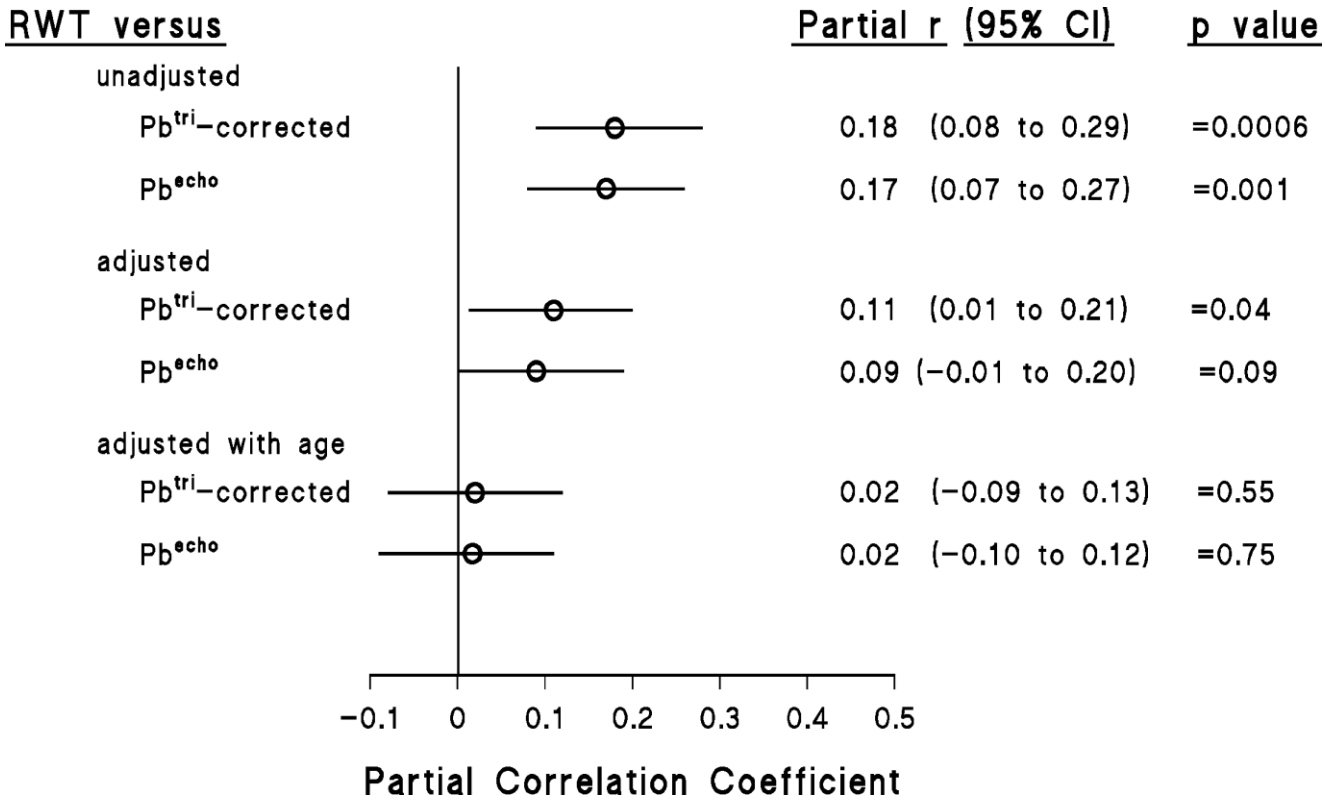

Figure 3.11 Unadjusted and adjusted correlations between the two methods of obtaining the backward wave (Pb^{tri}-corrected and Pb^{echo}), and relative wall thickness (RWT). Potential confounders included in the model are age, sex, body weight, body height, diabetes mellitus or an HbA1c> 6.1%, regular alcohol and tobacco usage.

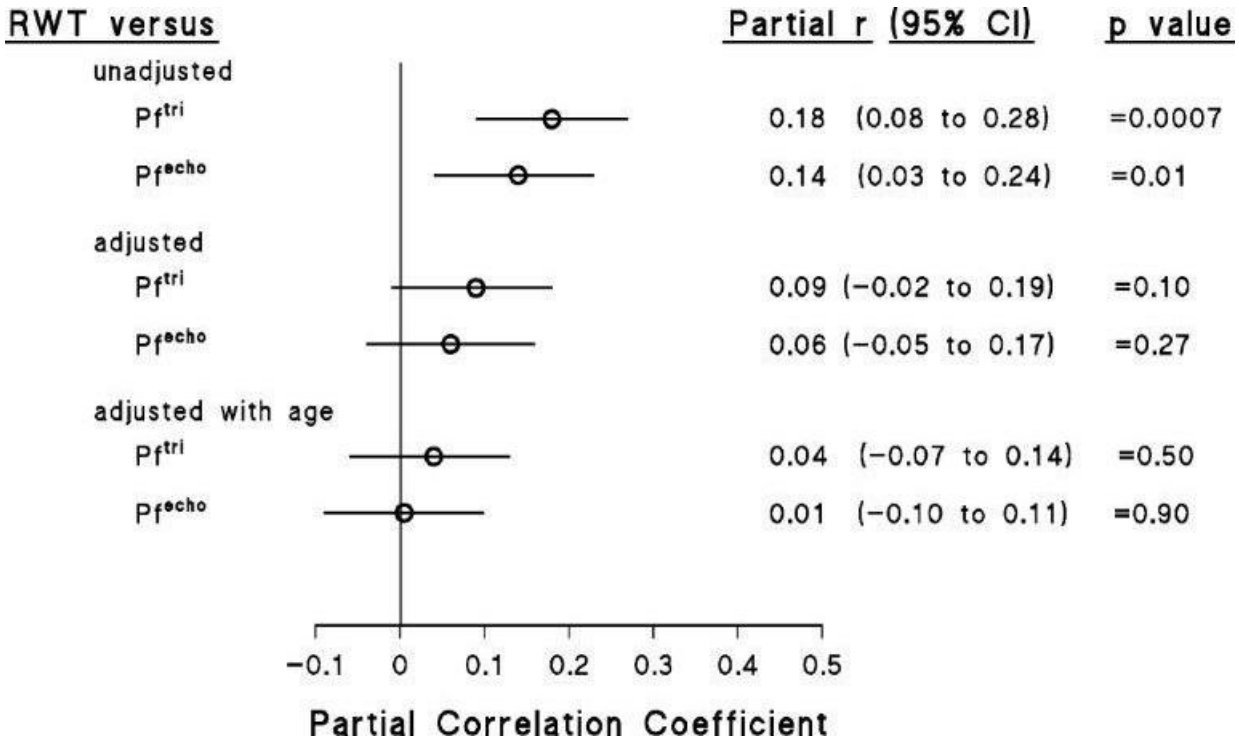

Figure 3.12 Unadjusted and adjusted correlations between the two methods of obtaining the forward wave (Pf^{tri} and Pf^{echo}), and relative wall thickness (RWT). Potential confounders included in the model are age, sex, body weight, body height, diabetes mellitus or an HbA1c> 6.1%, regular alcohol and tobacco usage.

Chapter 4

Discussion

The main findings of the present study are as follows. In a randomly selected community sample of black African descent, the backward wave pressure (Pb) obtained using the triangulation method (Pb^{tri}) and echocardiographic derived aortic flow waveforms (Pb^{echo}) had a strong positive correlation. The limits of agreement between the two methods of obtaining Pb differed by an average of 3.65 mmHg. After correcting for the systematic error observed in the Bland-Altman plot, the average difference for the two Pb values was -2.2 mmHg. However, we have demonstrated significant, and independent, positive associations between Pb^{tri} and Pb^{echo} with LVM, and these associations were not significantly different from each other. The present study demonstrates that the contribution of Pb to LVM is similar when using the triangulation method of wave separation analysis, which is a simpler method of obtaining Pb, versus that obtained using echocardiographic derived aortic flow waves.

4.1. Comparing the triangulation method with other models of obtaining the backward wave.

In the original study by Westerhof et al (2006), where the triangulation method of wave separation analysis was first described, 19 subjects were studied, 17 of whom were male, and who were patients and underwent catheterisation. Kips et al (2009) who proposed the physiological waveform as an alternative to the triangulation method, conducted measurements on 2325 subjects, using echocardiographic aortic flow velocity waveforms, with a fairly equal split between males and females. Westerhof et al (2006) obtained an R^2 value of 0.79 using RM, compared to Kips' et al (2009) R^2 value of 0.55. These discrepancies could be explained by the differences in sample size or subject characteristics. In comparison with these studies, although we derived and analysed Pb, we obtained an R^2 value of 0.82. These results point toward the same direction that Westerhof et al (2006) had indicated. However, the R^2 values do not tell us

about the level of agreement between the two methods of obtaining Pb. However, studies conducted by Kips et al (2009) and Westerhof et al (2006) assessed the potential differences in the triangulation method and a gold-standard by comparing the RM, a ratio of Pb to Pf, and not explicitly assessing Pb on its own. By only looking at RM, it is unclear whether the significant results are explained by Pf. We do know that Pb has a larger contribution to LVM than Pf, as demonstrated by Sibiya et al (2015), therefore it was important to understand the differences in the methods of obtaining Pb by only analysing Pb.

A previous study conducted by Wang et al (2010) assessed the agreement between the backward waves obtained using the triangulation method and echo derived aortic flow waves. In 30 participants, Bland-Altman analysis revealed an average Pb difference of 1 mmHg, and a correlation coefficient value of 0.91, compared to the present study where the corrected average Pb difference was -2.21 mmHg and the correlation coefficient was 0.82. The average Pf difference obtained by Wang et al (2010) was -1.9 mmHg and the correlation coefficient was 0.90, compared to the present study where the average Pf difference was -0.19 mmHg and correlation coefficient of 0.89. The major difference between the study by Wang et al (2010) and the present study is the larger study sample employed by us. We have demonstrated that our study cohort is representative of a larger population sample, whereas there remains some uncertainty as to whether the cohort used by Wang et al (2010) was representative of a population.

The average difference observed between the two methods of obtaining Pb was -2.2 mmHg, using Bland-Altman analysis, after correcting for systematic error. This implies that the triangulation method produces, on average, a lower Pb value by -2.2 mmHg. Westerhof et al (2006) obtained an average difference of 1.7 mmHg between the two methods of obtaining Pb.

This is lower than the average difference obtained in our study; but that could be explained by the sample size. However, before we corrected for the systematic error, we obtained an average difference of 3.65 mmHg. This is over 20% of the average value obtained for Pb^{echo}, which, in context, is a fairly high difference. However, there are issues surrounding the models used to derive Pb.

Kips et al (2009) introduced the hypothesis of using a physiological flow waveform (average waveform) to derive Pb and Pf, as opposed to a triangular flow waveform. The method of obtaining a physiological flow waveform requires the normalisation and averaging of measured flow waveforms (Kips et al 2009). The first claim made by Kips et al (2009) is that the triangulation method produces Pf and Pb values which are different to those derived using echocardiographic aortic flow velocity waveforms. However, the average difference of 3.65 mmHg we obtained points in the same direction as Kips et al (2009). Nevertheless, Kips et al (2009) demonstrated a stronger correlation for the physiological waveform (R^2= 0.74) than the triangular flow waveform. Furthermore, the claims of the study state that the physiological waveform is a more accurate method of deriving Pb and Pf in a healthy population. However, in this study, the physiological waveform was generated from 74 of the 2325 participants. Moreover, the population consisted of healthy participants of a narrow age range. The issue is whether the 74 individuals truly reflect the population, or whether the total sample reflects a general population, considering the healthy nature and narrow age range of the sample.

A study by Hametner et al (2013) proposed a new method of obtaining Pb called the Windkessel model. The Windkessel model used a continuous parameter for arterial resistance, arterial resistance, and arterial compliance, as described by Hametner et al (2013). The study by Hametner et al (2013) found that the triangulation method produces a lower correlation with

echocardiographic aortic flow velocity waveforms than the Windkessel model and the physiological waveform. Thereafter, a study by Parragh et al (2014) evaluated different models of deriving aortic flow waves, the triangular flow waveform, the physiological flow waveform, and the Windkessel model, for wave separation analysis. Parragh et al (2014) compared these three methods of obtaining Pb in people with normal and reduced ejection fractions (EF). They discovered that in the group with reduced EF, the triangulation method performed better than the physiological waveform (Parragh et al 2014). This is due to the fact that patients with systolic heart failure have modified ejection patterns (Parragh et al 2014). Patients with systolic heart failure, or reduced EF, produce concave aortic flow waveforms and a late maximum (Nichols et al 2011). Furthermore, they discovered that, in the control group, the triangular flow waveform performs just as well as the physiological flow waveform, which contradicts the findings of the study by Kips et al (2009). The most likely explanation is that the physiological waveform was constructed using normotensive healthy patients, and thus cannot represent a population other than that. Therefore, the triangular flow waveform better represents the aortic flow wave in patients with reduced EF, potentially due to the late maximum (Parragh et al 2014). The study by Parragh et al (2014) demonstrates the validity of the triangulation method, beyond the proposed physiological waveform.

In a pilot study conducted in our laboratory, echocardiography was used to obtain aortic flow waveforms in 26 participants of black African descent. The averaged flow waveform for these participants did not equate well with the prescribed physiological waveform. From the 26 participants, a wide variation of aortic flow shapes was obtained. Thus, it is unlikely that the physiological waveform, prescribed by Kips et al (2009) fully represents the population we are

dealing with. However, the positive correlation in favour of the triangulation method obtained in our study nullifies the need for a physiological waveform in the present community sample.

Previous studies have examined the validity of the triangulation method. Indeed, these studies have compared the triangulation method to other methods of deriving Pb. Furthermore, the triangulation method, according to the above mentioned studies, is inconclusive. In a population of black African descent, we have shown that the triangulation method produces an average difference that is too high, when interpreted in context. The correlation coefficient we obtained was high, but not 1. Kips et al (2009) proposed a new method for obtaining Pb when the triangulation did not work. However, we need to understand the implications of the differences in Pb obtained using the triangulation method and echocardiographic aortic flow velocity waveforms in assessing LV mass before we should consider a new approach.

4.2. Backward waves and its associations with left ventricular mass

The backward wave has been shown to have good associations with LVM, with many studies demonstrating a stronger positive correlation between Pb and LVM than Pf, or other indices of aortic function (Wang et al 2010, Booysen et al 2015, Sibiya et al 2015). With regards to LVM indexed to height$^{2.7}$ and height$^{1.7}$, both methods of obtaining Pb yielded positive results, specifically after adjusting for age. Relative wall thickness (RWT) yielded similar positive associations, with no differences between the two methods of obtaining Pb. However, after adjusting for age, we found that relationship between Pb and RWT disappeared entirely. It has been previously demonstrated that age is one of the main influences in producing end organ changes (Booysen et al 2015). Thus, once we adjusted for age as a confounder, Pb did not have a significant relation with RWT.

The use of different indices of LVM are employed to normalise LVM to body size, where LVM indexed to body surface area (BSA) eliminates the impact of body size (including obesity), whereas LVM indexed to height$^{2.7}$ and height$^{1.7}$ eliminate the impact of growth but not obesity (Woodiwiss and Norton 2015). LVM indexed to BSA dramatically underdiagnoses LVH in overweight and obese patients, compared to LVM indexed to height$^{2.7}$ and height$^{1.7}$ (Woodiwiss and Norton 2015). The European Society for Hypertension (ESH) and the European Society of Cardiology (ESC) recommend the use of LVM indexed to height$^{2.7}$ and height$^{1.7}$ in preference of BSA in order to avoid possible underdiagnosis of obesity-related pathological LVH (Woodiwiss and Norton 2015). This was the justification for using LVM indexed to height$^{2.7}$ and height$^{1.7}$. However, we did evaluate the associations of both methods of obtaining Pb with LVM indexed to BSA, not included in results, and both methods were not significantly related to LVM indexed to BSA after adjusting for age.

The positive relationships between the two methods of obtaining Pb and LVM indexed to height$^{2.7}$ and height$^{1.7}$ demonstrates the influence of Pb on the progression of LVM. This relationship is independent of age associated effects. More importantly, Pb obtained using the triangulation method and Pb obtained using echo derived aortic flow waves did not differ in their relationship with these two indices of LVM. Furthermore, a total of 44.7% of our sample was regarded as obese, and the average BMI value was in the overweight category. This is representative of the population (as seen in table 3.1); hence we are dealing with a population where the prevalence of obesity is high. Moreover, when analysing the influence of Pb on RWT, after adjusting for BMI, the significance disappeared. This was before adjusting for age.

In this study, we evaluated the associations between Pb and Pf with LVM. However, previous studies used Reflection magnitude (RM) to evaluate the associations with end-organ changes.

Reflection magnitude is an effective way of determining the contribution of Pb and Pf with end-organ changes. The measurement is a ratio and therefore does not require calibration. A study by Zamani et al (2014) found that RM is a strong predictor of heart failure and cardiovascular mortality. They demonstrated that, from the RM results, Pb and Pf produce opposite hazard ratios, and when subjects who developed heart failure were censored, Pb remained predictive of all-cause mortality (Zamani et al 2014). The results of the study by Zamani et al (2014) justified by a study whereby Sibiya et al (2015) demonstrated the same odds ratio with regards to Pb and Pf. In both studies, Pb remains the constant variable which has a significant independent relationship with end-organ changes. Sibiya et al (2015) demonstrated the independent relations between RM and Pb with end-organ changes. Therefore, we could deduce, from the results of these two studies, that the independent relationship between RM and LVM could largely be contributed by the independent relationships between Pb and LVM.

This effect is noted in our study when we analysed the relationship between Pf and LVM. After adjustments, especially after adjusting for age, the relationship between Pf and LVM disappeared. This is documented in numerous studies which emphasise the relationship between Pb and LVM. This is not to say that Pf does not contribute to end organ changes, however, the associations between Pb and LVM had a much larger magnitude. Despite these differences noted between Pb and Pf, what is more important is that Pf obtained from the triangulation method and the echo-derived aortic flow wave performed similarly when predicting LVM.

Our study demonstrates a significant relationship between Pb, whether obtained using triangular flow waveform or echo derived aortic flow waveform, and end organ changes. Furthermore, this relationship remains significant after adjusting for age. The results of our study agree with the findings of Sibiya et al (2015). The study used LVM indexed to height$^{2.7}$ and showed similar

independent relationships (partial r= 0.09, CI: 0.02 to 016) (Sibiya et al 2014). However, we demonstrated that LVM indexed to height$^{1.7}$ has a significant relationship with Pb as well, independent of age.

4.3. Limitations and future studies

The present study was a cross-sectional design. Therefore, we cannot make causal claims about the Pb and LVM, nor can we comment on the progression of Pb and LVM. Furthermore, a large proportion of the study population was female. Moreover, in the present study, calibration of the radial waveform required brachial BP measurements. This ignores the amplification of BP from brachial to radial arteries, and could potentially lead to underestimation of aortic pressures (Davies et al 2010, Verbeke et al 2005). However, a recent study conducted by Mitchell et al (2016) evaluated the aortic pressures which have been calibrated using radial, brachial, and carotid tonometry. Mitchell et al (2016) demonstrated no significant difference in the ability of each calibrating technique to predict cardiovascular disease (Mitchell et al 2016).

When deriving Pb and Pf, using echo derived aortic flow waves, there is a degree of subjectivity which could lead to an overestimation or underestimation. However, deriving Pb and Pf using echo derived aortic flow waves were restricted to one observer only, which eliminated inter-observer variability. Similarly, intra-observer variability analysis revealed a positive correlation extremely close to 1.

Although the present study demonstrates the validity of the triangulation method in the present population, Hametner et al (2012) introduced a new method of deriving Pf and Pb, known as the Windkessel model. Hence, the next step would be to determine the applicability of this model in

our current population. Furthermore, it would be interesting to note how a reduced ejection fraction affects the validity of the triangulation method in a black African population.

4.4 Conclusion

Our study demonstrates the validity of the triangulation method in deriving backward waves, when compared to backward waves derived using echocardiographic aortic flow velocity waves, in a black African population. Furthermore, the triangulation method produces Pb values that are as closely associated with LVM as those derived from echocardiographic aortic flow wave measurements. Thus, risk prediction using a simple approach to aortic wave separation may be employed.

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Appendices

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)

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

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

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

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

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

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

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

DECISION OF THE COMMITTEE *

Approved unconditionally

This clearance is valid and within the with 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.

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES