CENTRAL ARTERIAL FUNCTION IN REFRACTORY HYPERTENSIVES TREATED WITH VERSUS WITHOUT β-ADRENORECEPTOR BLOCKERS

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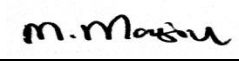
Declaration

I Mohlabani Masiu declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Masters in Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

7th .day of July 2020 in Johannesburg.

Dedication

I dedicate this work to my grandparents, Thotholoane V Matsie and Maselloane MJ Matsie, who from my youth until today, have stood by me through my years of study. To my parents, Selloane Claudia Masiu and Steve Molefi Masiu and my closest companion Boteng Maluke thank you for the support.

Presentations arising from this study

Conference poster presentation

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Abstract

Although atenolol is not recommended for first line therapy in the treatment of hypertension, it is commonly used to control blood pressure particularly in resource limited settings and where blood pressure control is resistant or refractory to therapy. However, for a given decrease in brachial blood pressure, atenolol does not reduce central arterial blood pressure as effectively as alternative antihypertensive agents. The mechanisms of this effect are nevertheless, poorly understood. Hence, using advanced approaches to the assessment of aortic function (central arterial pressure and simultaneous aortic velocity and diameter assessments in the left ventricular outflow tract [echocardiography]) in 61 resistant or refractory hypertensive participants I therefore compared the peripheral and central hemodynamic correlates of BP between 28 participants receiving atenolol and 33 not receiving atenolol, but who were receiving similar alternative agents. Atenolol therapy was associated with similar peripheral blood pressure values, but a lower heart rate ($p<0.01$), and hence cardiac output ($p<0.05$), prolonged ejection duration ($p<0.05$), and an increase in total peripheral resistance ($p<0.01$). Atenolol-treated hypertensives showed an increased slope of the relationship between maximal compression (forward travelling) wave pressures (determined from the product of peak aortic flow and aortic characteristic impedance) and aortic pulse pressure ($p<0.01$). Thus, at higher compression wave pressures, atenolol-treated participants had increased pulse pressure values. The higher pulse pressure at increased compression wave pressures was attributed to an enhanced reflected wave magnitude and wave reflection and re-reflection pressures at lower heart rates ($p<0.01$), but not to an increased TPR or to an increased overlap of the compression with the reflected wave mediated by a prolonged ejection duration. In conclusion, the adverse effects of heart rate reducing agents such as atenolol on central arterial pulsatile load is a class effect likely to be mediated by harmonic effects on oscillating waves at lower frequencies. Indeed, these relations between atenolol use and central aortic haemodynamics were not through atenolol's limited vasoactive properties or through an increased overlap of backward with forward waves (following extended ejection duration). The adverse effects are therefore, likely to be common to all heart rate reducing agents, but are only observed when the amplitude of forward travelling compression waves are high.

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β	Beta
BB	β-adrenergic receptor blocker, atenolol
CVD	Cardiovascular disease
BP	Blood pressure
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
HF	Heart failure
MI	Myocardial infarction
CAD	Coronary artery disease
CHD	Coronary heart disease
DM	Diabetes Mellitus
HR	Heart rate
LVEF	Left ventricular ejection fraction
CCB	Calcium channel blockers
PP	Pulse pressure
Zc	Characteristic impedance
Pf	Forward wave pressure
Pb	Backward wave pressure
TPR	Total peripheral resistance
MAP	Mean arterial pressure
RM	Reflection magnitude/ Reflected wave magnitude
ED	Ejection duration
PPc	Central/ aortic pulse pressure
Pa	Aortic augmented pressure
AIx	Augmentation index
Pi	Incident wave pressure
CNS	Central nervous system
CO	Cardiac output
SV	Stroke Volume
SVR	Systemic vascular resistance
BNP	Brain natriuretic peptide

Q	Aortic flow
CKD	Chronic kidney disease
BMI	Body mass index
SNS	Sympathetic nervous system
RAAS	Renin- angiotensin- aldosterone system
GFR	Glomerular filtration rate
HFpEF	Heart failure with a preserved ejection fraction
HFrEF	Heart failure with a reduced ejection fraction
SHR	Spontaneously hypertensive rats

CHAPTER 1: INTRODUCTION

β-adrenergic receptor blockers and central aortic haemodynamics

1.1 Hypertension as a cardiovascular risk factor

Of all the accepted risk factors for cardiovascular disease (CVD) and because of its high prevalence in most communities worldwide, hypertension is the risk factor that carries the most population attributable risk. In this regard, hypertension is estimated to affect over 1 billion people globally (Chobanian *et al*., 2003; Hilas and Ezzo, 2009; DiNicolantonio *et al*., 2015). South Africa is no exception to the high prevalence rates of hypertension worldwide with approximately 39.6 % of rural formal and 37.1 % of urban formal South Africans affected (Berry *et al*., 2017). Based on data from the Global Burden of Disease Study, hypertension is indeed the major cause of death and disability worldwide (Lim *et al*., 2012; Poulter *et al*., 2015) possibly accounting for 9.4 million deaths per year (Haldar, 2013). Importantly, mortality and morbidity related to this risk factor have increased by 25% in the past ten years (Ibrahim and Damasceno, 2012).

In most countries hypertension is defined as a blood pressure (BP) over 140 mm Hg systolic (SBP) or 90 mm Hg diastolic (DBP) BP (Kjeldsen *et al*., 2014). However, BP values from as low as 115/75 (SBP/DBP) mm Hg are well recognised as contributing toward a variety of cardiovascular events (Lewington *et al*., 2002), and these include heart failure (HF), stroke, renal failure and acute myocardial infarction (MI) (Chobanian *et al*., 2003). In Africa, hypertension is frequently identified as a major comorbidity in acute HF or MI with 79% of patients presenting with acute HF or coronary artery disease (CAD) having hypertension (Ogah *et al*., 2015; Irazola *et al*., 2016). Furthermore, in all economically developing countries the leading cause of stroke (Mensah, 2008; Connor *et al.*, 2009; Akpalu *et al.*, 2015) is hypertension. Thus, the management of hypertension is the most effective approach to the prevention of cardiovascular events. In this regard, the pharmacological treatment of hypertension is the most effective way of reducing hard clinical end points.

Antihypertensive agents exert their therapeutic effects on BP through several mechanisms but the mechanisms of these effects largely go beyond the scope of the present dissertation. Importantly however, all are designed to decrease both systolic and diastolic BP and every 10 mm Hg decrease in SBP translates into a 17% reduction in coronary heart disease (CHD), a 27% and 28% decrease in stroke and HF respectively, a 13% decline in all-cause mortality and thus an overall 20% decrease in the risk of major cardiovascular events (Ettehad *et al*., 2016). Thus,

presently all guidelines recommend that patients with BP values above the threshold of 140/90 mm Hg should receive therapy to reduce BP although lower thresholds may be considered in high risk patients. There is nevertheless controversy as to which BP threshold should be employed and since high risk patients are more susceptible to cardiovascular damage (Vasan *et al*., 2001; Blake *et al*., 2003; Liszka *et al*., 2005; Zhang *et al*., 2006; Hsia *et al*., 2007; Dorjgochoo *et al*., 2009; Gu *et al*., 2009; Butler *et al*., 2011) it has been posited that there is an urgent need of therapy to treat even those whose BP may be considered normal or high/normal (BP=120-139/80-89 mm Hg). Indeed, in some controlled, randomised trials and meta-analyses of high risk patients, antihypertensive therapy has beneficial effects (Staessen and Jiguang, 2001; Schrier *et al*., 2002; Patel, 2007; Nissen *et al*., 2004; Law *et al*., 2009; Remme *et al*., 2009) especially if patients have hypertension-induced CVD or diabetes mellitus (DM) (Mancia *et al*., 2007). Nevertheless, other intervention studies also suggest that in patients with hypertension-induced CVD or at a high risk whose BP lies in the normal or high/normal range, further lowering of BP is ineffective at preventing events (Staessen and Jiguang, 2001; Schrier *et al*., 2002; Nissen *et al*., 2004; Patel, 2007; Yusuf *et al*., 2008; Law *et al*., 2009; Remme *et al*., 2009; Califf *et al*., 2010; Cooper-DeHoff *et al*., 2010; Evans *et al*., 2010). However, in high risk cases with a normal/high-normal BP, it may take up to 10 years for 80% of these patients to develop cardiovascular risk factor associated damage (Moreira *et al*., 2008; Fukuhara *et al*., 2012) and hence the benefits of lowering BP may not be readily observable over the short periods usually employed for study in large scale clinical trials.

1.2 Beta adrenergic receptor blocker-based therapy in hypertension

Antihypertensive agents exert their therapeutic effects in hypertension through several different mechanisms. In this regard, activation of the sympathetic nervous system is well established as contributing to the pathophysiology of primary hypertension (Colle *et al*., 2007). As such, agents that block the sympathetic nervous system have evolved as central to managing hypertension. In this regard, β-adrenergic receptor blockers (β-blockers) are among the most frequently used antihypertensive medications (Psaty *et al*., 1997; Clarke *et al*., 2002; Wiysonge *et al*., 2012; Mancia *et al*., 2013; Larochelle *et al*., 2014). β-blockers are well established as agents that lower BP (Agabiti- Rosei *et al*., 2007; Bangalore *et al*., 2008; Ettehad *et al*., 2016) and hence decrease morbidity and mortality in hypertensive patients (Hollenberg, 2005; DiNicolantonio *et al*., 2015).

However, the general use of these agents in the management of hypertension is more controversial.

As a consequence of major clinical trials, national and international guidelines (Williams *et al*., 2018) do not recommend β-blockers as first-line antihypertensive agents for general use, but only in those with HF, MI, angina pectoris and atrial fibrillation or alternative abnormalities affecting the heart's cardiac conduction system (e.g. long QT syndrome) (Caterina and Leone, 2010, Hackam *et al*., 2013). A recommendation for their use in several cardiac conditions is made for a number of reasons. β-blockers are well established as preventing MI in hypertension not only through reductions in BP, but also heart rate (HR) and myocardial oxygen demand, effects which are cardioprotective (Freemantle *et al*., 1999; Caterina and Leone, 2010; Trudeau, 2014). The beneficial actions of β-blockers are particularly notable in patients below the age of 60 years (Trudeau, 2014). Through several effects on the myocardium including reverse remodelling, β-blockers improve left ventricular ejection fraction (LVEF) and hence reduce hospitalizations and improve outcomes in patients with HF with a reduced LVEF (Sobotka *et al*., 2011; Kotecha *et al*., 2014). Although β-blocker based therapy does not prevent cardiovascular events in patients with angina pectoris, (Psaty *et al*., 1997; Lindholm *et al*., 2005) β-blockers along with other antihypertensive drugs like calcium channel blockers (CCB), are the only antianginal antihypertensive agents (Bradley *et al*., 2006). In atrial fibrillation, through rate rather than rhythm control, β-blocker therapy is well recognised as improving outcomes (Helfand *et al*., 2009) and in disorders such as long QT syndrome, β-blocker therapy is essential for preventing sudden cardiac death. Thus, there are several cardiovascular conditions, where in the treatment of hypertension, the judicious use of β-blockers will have major clinical benefits. However, in the absence of HF, MI, angina pectoris, atrial fibrillation, or disorders which herald the onset of sudden cardiac death, the benefit of β-blockers for the treatment of hypertension is less clear. In this regard, in the absence of compelling conditions, β-blockers are recommended as fourth or fifth line antihypertensive agents (Williams *et al*., 2018) and presently are discouraged from use. What is the evidence that suggests that β-blockers should not be the preferred agents in the treatment of uncomplicated hypertension?

Several large clinical trials including the Swedish Trial in Old Patients with hypertension (STOP- trial) (Dahlöf *et al*., 1991); the Heart Outcomes Prevention Evaluation study (HOPE trial) (Yusuf *et* al., 2000); the Losartan Intervention For Endpoint reduction (LIFE) trial (Dahlöf *et al*., 2002); and the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) (Dahlöf *et al*., 2005) have demonstrated that as compared to alternative antihypertensive agents that therapy

based on the use of the β-blocker, atenolol, is inferior at risk reduction in the treatment of uncomplicated hypertension. Importantly, the inferiority of atenolol-based therapy at reducing events in the aforementioned clinical trials is despite similar reductions in brachial BP as compared to comparator groups. The comparatively inferior impact on events produced by atenolol is mirrored by inferior effects on end organ changes. Indeed, several studies have provided the evidence that β-blocker-based therapy is not as effective as alternative agents at producing beneficial effects on several cardiac and vascular end organ measures including carotid intimal thickness, resistance artery structure, endothelial function and left ventricular hypertrophy (Dahlöf *et al*., 1991; Schiffrin *et al*., 2002; Buus *et al*., 2004; Devereaux *et al*., 2004; Paliotti *et al*., 2005) or alterations in the myocardium well-recognised as contributing to the transition from compensated hypertrophy to dysfunction such as myocardial collagen content (Ciulla *et al*., 2004). Although the relatively adverse effect of atenolol on end organ measures suggests a diverse impact on several organ systems, a Cochrane meta-analyses of several studies indicates that a primary limitation of the use of β-blockers in preventing events is in the prevention of stroke (Wiysonge *et al*., 2007; Caterina and Leone, 2010). Data that suggests mainly an adverse vascular effect of atenolol. Indeed, the HR reducing effects of atenolol are thought to prevent cardiac events. However, alternative studies indicate that amlodipine-based antihypertensive treatment is much better at lowering the risk of stroke as well as coronary events as compared to the atenolol-based therapy despite a slight increase in heart rate (Dahlöf *et al*., 2005, Caterina and Leone, 2010). Thus the adverse effects of atenolol-based therapy may not be specific to vascular events (stroke). The adverse events of atenolol-based therapy may also include alterations that increase the risk of cardiac events despite atenolol's benefits to the myocardium as a consequence of HR reduction.

As a consequence of the greater adverse effects and inferiority of β-blocker-based therapy (atenolol therapy) when compared to alternative agents at reducing events in the treatment of hypertension (despite being equally as effective as other agents at reducing brachial BP), there is a general consensus that β-blockers should not be used as first line agents in primary hypertension (Bangalore *et al*., 2008; Larochelle *et al*., 2014). In this regard, the recommendation is that β-blockers should rather be used as add-on therapy to other antihypertensive agents (Mancia *et al*., 2013). Based on this, a better understanding of when and how to use β-blocker agents is required and to this end, understanding the possible reasons that may explain why they have suboptimal therapeutic benefits is necessary. Regardless of whether β-blocker agents are better therapeutic agents when used as first or second line therapy, these

agents are still better tolerated (Larochelle *et al*., 2014) antihypertensive agents than many alternative agents and hence play a major role in the treatment of hypertension. To this extent, β-blockers remain the most commonly prescribed class of antihypertensive agents when compared to other antihypertensive agents (Larochelle *et al*., 2014).

1.3 Possible mechanisms that may explain the inferiority of β adrenergic receptor blockers in uncomplicated hypertension

There are several possible reasons why atenolol-based therapy may be inferior to other therapeutic approaches in the treatment of uncomplicated hypertension. First, over time patients receiving atenolol develop insulin resistance and hence glucose intolerance (Caterina and Leone, 2010; DiNicolantonio *et a*l., 2015). In this regard, β-blockers cause weight gain, a reduced blood flow in the skeletal muscle microcirculation, dyslipidaemia (Bakris *et al*., 2004; Manrique *et al*., 2009**)**, and impair the secretion of insulin from pancreatic β cells all of which are responsible for insulin resistance and reduced glucose uptake (Lithell, 1991). The evidence for a diabetogenic effect of β-blockers comes from several different studies. In this regard, in the Atherosclerosis Risk In Communities (ARIC) cohort study, those participants receiving atenolol for the treatment of hypertension had a 28% increased risk of developing type 2 DM (Dahlof *et al*., 2002; DiNicolantonio *et al*., 2015). In a meta-analysis of 150,000 patients without DM, patients that received the β-blocker agent, atenolol, along with a diuretic for the treatment of hypertension when compared to those that received different classes of antihypertensive agents demonstrated an increased risk of developing DM (Elliot and Meyer, 2007). Based on several large clinical trials such as the HOPE trial and the Captopril Prevention Project (CAPPP) randomised trial, it is now well accepted that the new-onset of DM is dependent on the use of atenolol-based therapy (Hansson *et al*., 1999; Yusuf *et al*., 2000; Dahlöf *et al*., 2002). The adverse effects of atenolol on metabolic changes may or may not be class specific as both atenolol and metoprolol (non-vasodilator β_1 selective blockers) have been found to increase the risk of type 2 DM through an association with weight gain and dyslipidaemia (DiNicolantonio *et al*., 2015). The extent of the diabetogenic effects of β-blockers is evidenced by the United Kingdom Prospective Diabetes Study (UKPDS) where β-blocker-based therapy was discontinued because the patients receiving this treatment developed marked weight gain together with an increased requirement for glucose lowering agents (Holman *et al*., 1998).

Besides increasing insulin resistance and in many instances causing frank DM, atenolol-based therapy does not lower all cause and cardiovascular mortality in elderly hypertensives (DiNicolantonio *et a*l., 2015). Therefore, in older individuals β-blockers may have a minimal effect at reducing the risk of cardiovascular outcomes (Chen *et al*., 2010). Although this may in part be attributed to the susceptibility of older individual to weight gain and glucose intolerance, one has to consider alternative age-related effects. In this regard, aging is well recognised as being associated with increases in mainly SBP, whilst DBP increases until 60 years of age and then declines (Staessen *et al*., 1990; Franklin *et al*., 1997; Wang *et al*., 2005). Hence, with aging increases in pulse pressure (PP) and hence SBP are the main BP changes associated with hypertension (Chobanian *et al*., 2003). In this regard, as will be discussed in subsequent sections, SBP and PP differ in the brachial as compared to central (aortic) arteries, with central pressures being much lower than peripheral (brachial) pressures. Importantly, there is now substantial evidence that although atenolol-based therapy may reduce peripheral SBP and PP to a similar extent as alternative antihypertensive agents, for the same decrease in brachial SBP and PP, atenolol-based therapy has far less of an effect on central arterial SBP and PP when compared to alternative antihypertensive agents (Williams *et al*., 2006; Williams *et al*., 2009, Boutouyrie *et al*., 2010; Pucci *et al*., 2015; Sluyter *et al*., 2016). Indeed, in the Conduit Artery Function Evaluation (CAFE) a sub-study (Williams and O'Rourke, 2001) of the ASCOT (Sever *et al*., 2001; Dahlöf *et al*., 2005), the inferiority of β-blockers in preventing cardiovascular events was associated with an inability to decrease central aortic pressures as effectively as non-β-blocker-based therapeutic approaches to treating hypertension despite similar effects on brachial BP (Williams *et al*., 2006). Thus, atenolol is thought to produce a pseudo-antihypertensive effect where brachial BP decreases to a similar extent as alternative agents, but atenolol fails to reduce those BP values (central arterial) that may in fact cause cardiovascular damage. However, as will be discussed in later sections, whether this effect of atenolol on central arterial pressures is only mediated through the non-vasodilator β_1 selective blockers atenolol and metoprolol, or is a class effect associated with reductions in HR, is nevertheless unclear (Agabiti-Rosei *et al*., 2007). Thus, whether the newer vasodilator β-blockers, nebivolol and carvedilol show similar effects on BP and hence possibly on cardiovascular mortality and morbidity, is unclear (Caterina and Leone, 2010). Based on atenolol effects on outcomes, unfortunately β blockers as a class have been ruled out as the choice of therapy for primary hypertension and their use has further been discouraged in future hypertension trials (Lindholm *et al*., 2005). This approach may exclude the possibility that the newer β blockers have unrealised benefits if they do not produce the same

effects on central BP as atenolol. As will be discussed however, the adverse impact of atenolol on aortic BP may or may not be a class effect mediated by HR changes.

1.4 Central aortic blood pressure and its determinants

As indicated in the preceding section, the differential impact of β-blocker-based versus alternative therapies on outcomes in hypertension despite a similar impact on brachial BP may in-part be attributed to a differential effect on aortic versus brachial BP. To understand how these differential effects occur, it is first important to understand the differences that exist in central versus brachial BP and the explanations for these differences. Central aortic systolic BP may be 10-20 mm Hg lower than brachial artery systolic BP whilst central and peripheral diastolic BP are similar (Ohte *et al*., 2007). There are several differences in the determinants of central aortic and brachial PP that must be considered. What are the determinants of aortic and brachial PP?

Pulse pressure is generated by summation of forward and backward travelling pressure waves (Li *et al*., 2017) (Figure 1.1). The forward travelling pressure wave is generated by the flow of blood in the proximal aorta produced by contraction of the left ventricle (Avolio *et al*., 2009; Schultz *et al*., 2013) where blood is ejected into an elastic conduit (the aorta). As with any conduit conducting pulsatile flow, the aorta will produce resistance to flow during the ejection of blood (impedance), which in the absence of wave reflection is called characteristic impedance (Zc). The pressure generated by left ventricular contraction into the aorta is thus the product of impedance to flow (Zc) and flow itself (Nichols *et al*., 2011). However, the healthy aorta is not a resistance vessel and normally has a very low Zc and hence most of the pressure generated during ventricular contraction is produced by flow (Nichols *et al*., 2011). The pressure wave produced by ventricular ejection is rapidly conducted in a forward direction down the arterial tree so that within a very short period (literally milliseconds), the pressure wave will have reached peripheral (including the brachial) arteries. As with any pulsatile pressure wave travelling through a conduit there will be some dampening, but the pressure wave generated in the proximal aorta is the same wave seen in all arterial beds downstream. The pulsatile pressure wave transmitted to all arterial beds is called a forward travelling pressure wave and the peak pressure of this wave is peak forward wave pressure (Pf) (Figure 1.1). This peak pressure (Pf) is a reflection of not only the magnitude of ventricular contraction, but also the extent to which the aorta buffers load on the cardiovascular system during contraction. Furthermore, as the aorta

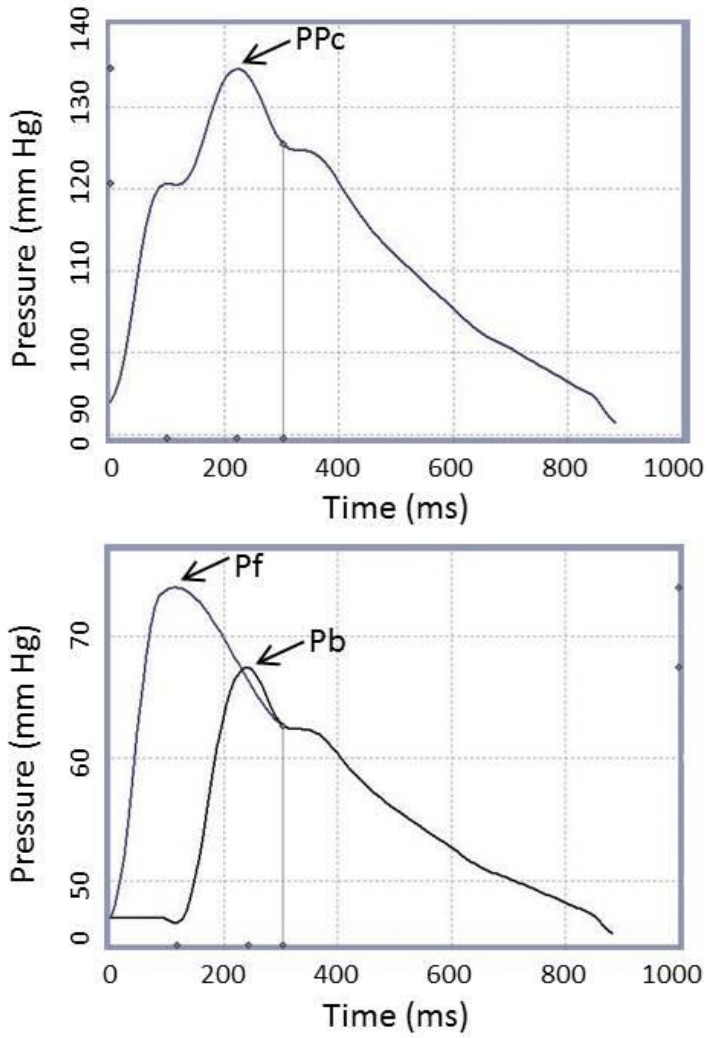

Figure 1.1 Aortic pressure wave (upper panel) and forward and backward travelling pressure waves (lower panel) showing (arrows) peak aortic pulse pressure (PPc), and the component pressure waves, peak forward (Pf) and peak backward (Pb) pressures.

stores energy during systole, which is then employed to drive blood flow when it recoils, Pf is also an index of the extent of aortic elastic recoil which contributes to diastolic flow in vessels (Nichols *et al*., 2011). Thus, Pf is an index of ventricular function and also of central elastic artery mechanical properties (Nichols and Edwards, 2001).Whilst aortic flow (Q) is determined by many variables including any factor which influences cardiac contraction, Zc is determined mainly by two factors, an increased stiffness and a decreased diameter of the aorta (Nichols *et al*., 2011). In this regard, it is thought that the main determinant of Zc is an increased aortic stiffness which increases with age, whilst the contribution of diameter is controversial. In this regard, aortic diameter also increases (which will reduce Zc) with age. However, this increase in diameter may not be as extensive in those with an increase in PP (Mitchell *et al*., 2003). Moreover, an increase in aortic diameter may alter the impact of stiffness by increasing the degree of strain placed on collagen fibres in the aorta (Nichols *et al*., 2011). Thus, indirectly, increases in aortic diameter may enhance rather than reduce aortic Zc. The critical determinant of aortic stiffness is arteriosclerosis, which is determined by fragmentation of elastin fibres, an increase in collagen, an enhanced cross-linking of collagen and calcification of the aorta, all changes that are produced by uncontrolled conventional risk factors including hypertension, diabetes mellitus, smoking and dyslipidaemia (Lam *et al*., 2010; Kovacic *et al*., 2011). In short, risk factor-related arteriosclerotic changes are thought to be the main determinants of aortic stiffness and hence Zc and thus Pf.

As with any conduit which transmits flow in a pulsatile manner, where tapering occurs (such as at points of bifurcation or narrowing of vessels in the arterial system), forward travelling pressure waves generated by intermittent flow in the conduit will reflect and generate multiple backward travelling pressure waves which return to the point of origin (O'Rourke and Yaginuma, 1984). As with the forward travelling pulsatile pressure waves, backward travelling pulsatile pressure waves travel at a very high speed in a normal aorta (around 3-6 m/sec). The high speed of wave travel of reflected waves will then result in the reflected waves returning to points in large arteries where the forward travelling pressure waves are at their antinodes and summation with the forward wave occurs (Figure 1.2). At points close to reflection sites summation is maximal (the two waves literally superimpose) (Figure 1.2). However, the further from reflection sites (such as the point of origin of the forward travelling pressure wave in the proximal aorta), the more chance that the reflected wave will arrive at the forward wave's pressure node and hence summation of the two waves will be less than in the periphery (Figure 1.2). Where summation of forward and backward travelling waves occurs, the pulse will appear

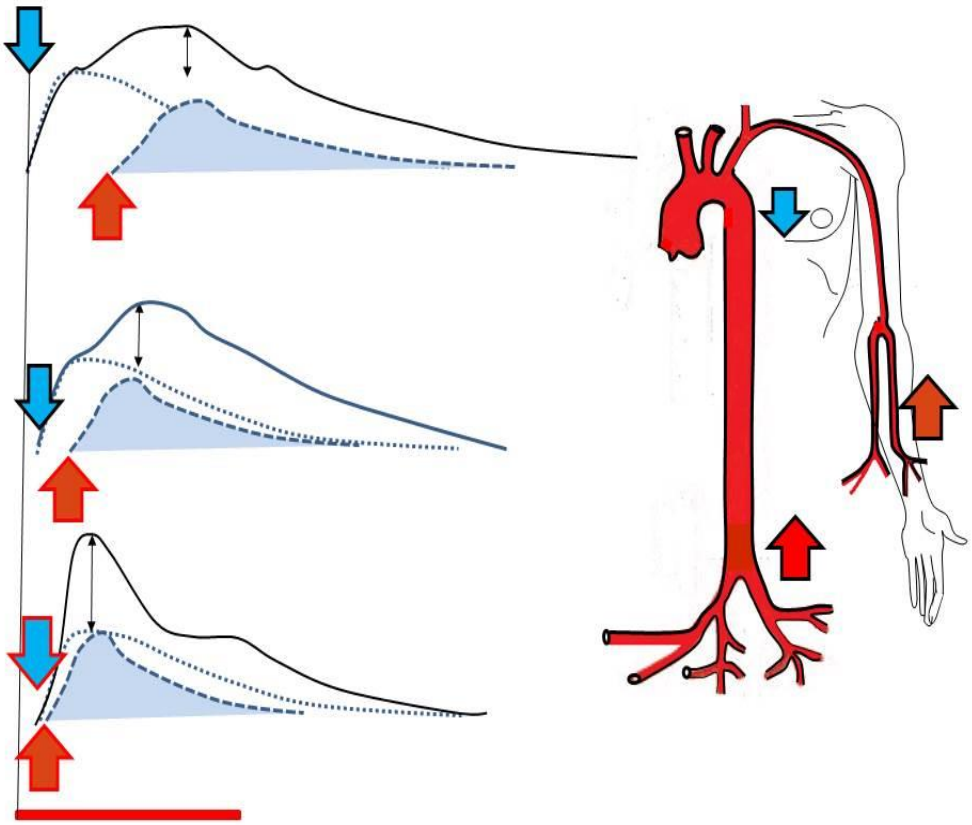

Figure 1.2 Impact of the backward travelling pressure wave (interrupted line with light blue shading) generated by wave reflection from points of vascular tapering (red arrows) on the summed aortic pressure wave (continuous line). The summed pressure wave is generated by the pressure produced by the forward travelling wave (interrupted line with no shading) (blue arrows) adding to that of the pressures generated by the backward wave. At distal sites there is a greater chance of summation because antinodes of oscillating waves overlap, whilst closer to the aorta there is a better chance that nodes and antinodes overlap and hence summation is reduced.

as a single pressure wave (Avolio *et al*., 2009; Phan *et al*., 2016). The further from the aorta (that is, the closer to reflection sites) where maximal overlap between forward and backward travelling waves occurs, summation will generate what appears to be a single pulsatile wave (Figure 1.2). In contrast, the closer to the aorta (i.e. the further from reflection sites), where the least overlap between forward and backward waves occurs, the pulse will appear more as two discernible waves that have coincided with a first and second systolic shoulder (Figure 1.2). In this regard, the first systolic shoulder is the point where the forward wave begins to generate less of a pressure change over time as peak velocity ends and the backward wave then increases the pressure change over time yet again as it overlaps with the forward wave (inflection point).

If the speed of wave reflection is sufficiently slow then backward travelling pressure wave will arrive well after forward waves in the aorta and add to or augment diastolic more than systolic BP. This occurs in childhood and explains central arterial waveforms in some mammals with very elastic aorta or where a longer distance for wave travel exists (Nichols *et al*., 2011). The advantage of this system is that it will promote coronary blood flow which largely occurs in the diastolic period of the cardiac cycle and which strongly depends on the pressure gradient across the coronary bed. However, in most human adults from as early as 16 years of age the speed of wave travel is sufficiently fast that backward wave pressures will arrive in systole and thus summate with the forward travelling pressure wave. In this circumstance, backward waves thus augment aortic PP and are thus considered as essential determinants of peak aortic PP (Agabiti-Rosei *et al*., 2007; Avolio *et al*., 2009). What are the determinants of backward wave pressures?

Through Newton's 1st (inertial forces) and 3rd (for every action there is an equal and opposite reaction) Laws of Motion the major determinant of maximal backward wave pressure (Pb in figure 1.1) is the maximal pressure generated by the forward travelling pressure wave (Pf in figure 1.1). Thus, as Pf increases, so does Pb. This relationship has rendered the opinion that the primary target to reduce PP should always be Pf as reductions in Pb will automatically follow. However, there is now significant evidence that strong determinants of Pb are arteriolar tone (indexed by total peripheral resistance [TPR] and mean arterial pressure [MAP]) and vascular tone in more proximal arteries (Cecelja *et al*., 2009; Liao *et al*., 2011). In this regard, to identify the role of vascular tone as a determinant of wave reflection obviously requires an approach that excludes the effects of aortic forward wave pressures on wave reflection. To do this, reflection magnitude (RM) is calculated as the percentage Pb/Pf. Importantly, with aging RM increases

from young adulthood to old age and this change is independent of MAP and hence generally considered to be independent of arteriolar tone (Hodson *et al*., 2017).

1.5 Explanation for differences between aortic and brachial BP

The differences between peripheral and aortic systolic BP are driven by amplification of pulsatile pressures (PP amplification) from the aorta to the brachial artery (McEniery *et al*., 2008; Vlachopoulos *et al*., 2010). Indeed, over the same age range (20 to 80 years) peripheral BP increases by 25 mm Hg whilst central BP increases by 40 mm Hg (Vlachopoulos and O'Rourke, 2000). There are several explanations for aortic to peripheral artery PP amplification. In this regard, the wider radius and greater degree of elasticity (decreased stiffness) of the aorta as compared to more peripheral muscular arteries generates an impedance mismatch. The higher impedance of peripheral arteries thus may amplify the pulse as it travels down the arterial system (Nichols *et al*., 2011; McEniery *et al*., 2014). Through aging and risk factors, the aorta loses its elasticity (increases its stiffness), an effect attributed to arteriosclerotic changes. However, stiffness in more distal arteries changes little with age (Mitchell *et al*., 2004). Consequently, with aging and risk factors, whilst the impedance in the aorta increases, distal arterial impedance changes little, thus decreasing the impedance mismatch between the aorta and more distal vessels (Smulyan *et al*., 2016). Thus with aging, aortic to brachial PP amplification decreases and brachial PP more closely resembles central arterial PP.

The second explanation for aortic to peripheral artery PP amplification is that illustrated in Figure 1.2. In this regard, because reflected waves in distal arteries return more in synchrony with Pf antinodes in these distal sites, summation of the waves is maximal and the impact of wave reflection on the peripheral pulse is striking (Nichols *et al*., 2011). In contrast, because reflected waves arrive in the central arteries more at a time when Pf nodes occur, the extent of summation of the two pressure waves in central arteries is thus far less than in the periphery (Nichols *et al*., 2011). Consequently, central arterial PP is augmented less than peripheral arterial PP and peripheral pressures are markedly amplified (Nichols *et al*., 2011). With aging, three changes occur which enhance the extent to which reflected waves augment central arterial PP. First, Pb increases linearly across the adult lifespan (Hodson *et al*., 2017) and this correlates well with the decrease in PP amplification that occurs with age (Sibiya *et al*., 2015). Second, with age-induced increases in ventricular ejection duration (ED), the time to the peak of the Pf

increases and this enhances the chance that the reflected wave returns in synchrony with the Pf antinode (Tade *et al*., 2017). Age-induced increases in the time to the peak of the Pf therefore markedly influence aortic, but not peripheral PP, and decrease PP amplification. Third, age-induced increases in the speed of wave reflection, presumably due to increases in aortic stiffness or a movement of reflection points closer to the heart, also enhances the chance that the reflected wave returns in synchrony with the Pf antinode (Nichols *et al*., 2011). In short, several age-induced changes in factors that influence wave reflection or the impact thereof are responsible for PP amplification and age-related decreases in PP amplification.

1.6 Indices of aortic reflected wave effects

As reflected pressure waves summate with forward wave pressures and augment central/ aortic PP or PPc, for several decades the impact of wave reflection has been determined by the extent of aortic pressure augmentation (pressure at the second systolic shoulder of the aortic pulse– pressure at the first systolic shoulder) [Figure 1.3] and is referred to as aortic pressure augmentation (Pa). The degree of wave reflection has thus been estimated as augmented pressure (Pa)/aortic PP or aortic augmentation index (AIx) (Burns *et al*., 2018). However, the determinants of the quantitative contribution of Pa to PPc or AIx (Nichols *et al*., 2011) are complex. Augmentation index is indeed strongly influenced by wave reflection, but in addition by several other factors including left ventricular contraction (Hughes *et al*., 2013; Schultz *et al*., 2013; Torjesen *et al*., 2014), the time to the peak of the forward wave in which an extended time increases the chances that Pb will maximally augment aortic PP (Tade *et al*., 2017), and by the speed of wave reflection, where the greater the speed of wave reflection the greater the chances that reflected waves will augment aortic PP. It is nevertheless, argued that it is not only reflected wave magnitude effects that are important, but also the time to the peak of the aortic forward wave and the speed of wave reflection that one should consider, as all are important determinants of aortic PP. However, more recently it has been recognised that a substantial portion of the Pf is driven by re-reflection of backward wave pressures (Phan *et al*., 2016). Thus, Pa indexes only half of the overall contribution of reflected waves to aortic PP (Figure 1.3). Indeed, our group has demonstrated that when Pf and Pb are included in the same regression model, that despite Pf being quantitatively greater than Pb, Pb, but not Pf makes a major contribution to variations in aortic PP in either those younger or older than 50 years of age (Booysen *et al*., 2015). In contrast,

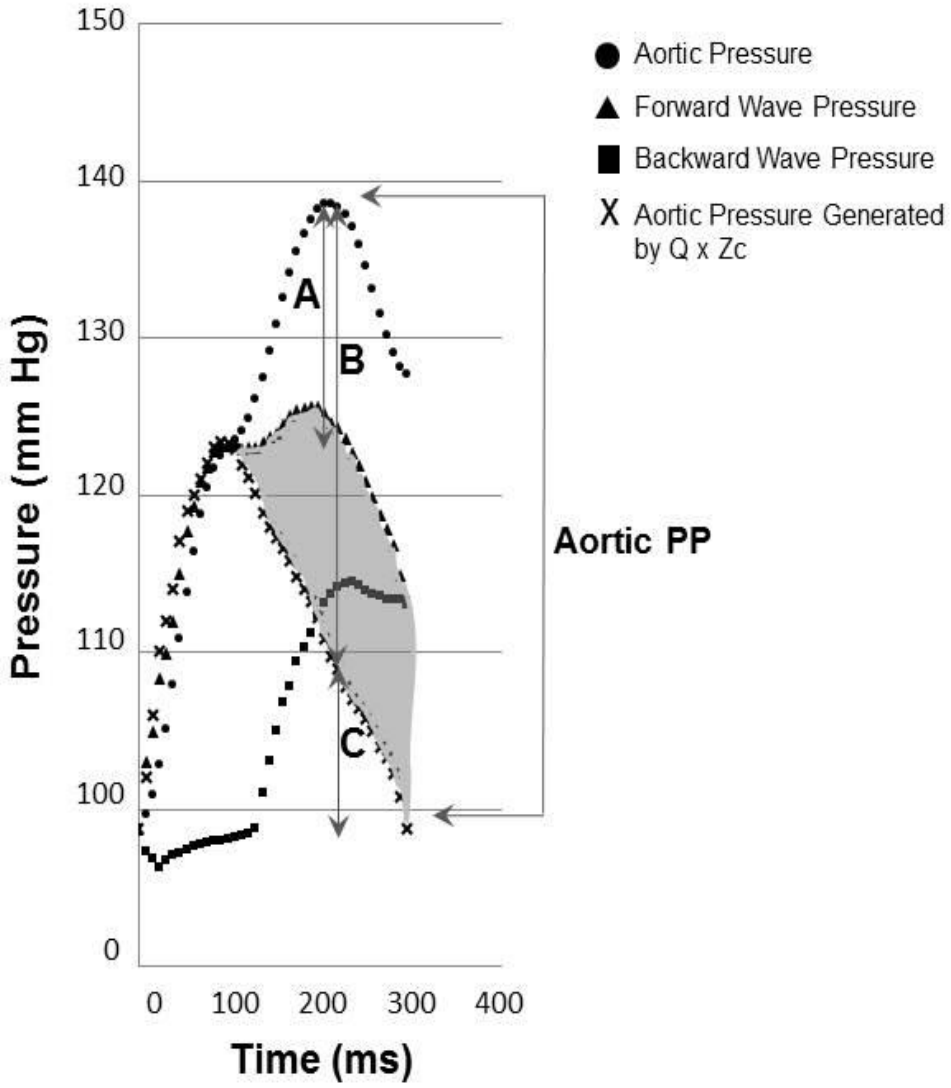

Figure 1.3 Components of the aortic pressure wave. A, aortic augmented pressure (Pa); B, reflected + re-reflected wave pressures; C, pressure generated by the product of aortic flow (Q) and characteristic impedance (Zc) at peak PP; shaded area, re-reflected wave.

the contribution of reflected waves as indexed by Pa to variations in aortic PP is substantially less (Booysen *et al*., 2015). In other words, the impact of reflected waves is markedly underestimated when employing Pa rather than Pb as an index of wave reflection (Booysen *et al*., 2015). Thus, to determine the effects of wave reflection, aortic Pb should be assessed from wave separation analysis using either measured (Hametner *et al*., 2013), or assumed physiological (Kips *et al*., 2009) or triangular (Westerhof *et al*., 2006) aortic flow waves, and to assess changes in wave reflection independent of Pf, RM must be determined.

1.7 Relative importance of forward and reflected pressure waves

As will be discussed in subsequent sections, as β-blockers may have differential effects on aortic and brachial PP mainly through an impact on reflected wave effects, it is essential to understand the evidence that exists to suggest whether Pf or Pb are more important in mediating effects on aortic PP and consequently cardiovascular damage. In this regard, the relative contribution of forward and reflected waves to variations in PP has been debated for many years (Cecelja *et al*., 2009; Namasivayam *et al*., 2009; Mitchell *et al*., 2010; Namasivayam *et al*., 2016; Hodson *et al*., 2017). Most earlier studies (Cecelja *et al*., 2009, Namasivayam *et al*., 2009) demonstrated that Pa increases linearly across the adult lifespan, whilst the forward travelling pressure wave up until the inflection point (called incident wave or Pi) only begins to increase at 50-60 years of age. Thus, whilst Pa contributes to age-related increases in aortic PP across the adult lifespan; Pi (the incident or Pf up until the first systolic shoulder) only begins to contribute after the age of 50 years (Namasivayam *et al*., 2009). However, as indicated in the above discussion Pa and Pi are poor indices of forward and backward wave pressure effects. Soon thereafter, using wave separation analysis data from the Framingham Heart Study showed a major contribution of Pf, but a relatively minor contribution of Pb to age-related increases in aortic PP (Mitchell *et al*., 2010). However, the Framingham Heart Study included a high proportion of hypertensives with well controlled BP values (Mitchell *et al*., 2010). What is now clear from recent data from our group in a large community-based study where half the hypertensives were not receiving antihypertensive therapy is that in untreated participants Pb begins to increase from early adulthood and this effect is independent of MAP (and thus arteriolar function) whilst Pf only begins to increase from around 50 years of age and that both Pf and Pb are important in driving age-related increases in aortic PP (Hodson *et al*., 2017). Thus, while Pb is the main determinant of aortic PP in early adult life, Pf and Pb may either contribute equally in later adult life

(Namasivayam *et al*., 2016; Hodson *et al*., 2017) or in populations with well controlled BP values, Pf may be more important than Pb in mediating age-related increases in aortic PP (Mitchell *et al*., 2010). There is nevertheless an additional consideration which has not been accounted for in any of these studies.

As indicated in previous discussion, more recently it has been recognised that Pf is not only determined by the product of Zc and aortic flow (Q), but also by the re-reflection of Pb (Phan *et al*., 2016). This may explain why when the relative contribution of Pf and Pb considered in the same regression model to variations in aortic PP, Pb rather than Pf is noted to be the main determinant of aortic PP at any age (Booysen *et al*., 2015; Sibiya *et al*., 2015). However, further work is required to assess the contribution of reflected wave effects together with that portion of Pf that indexes re-reflection of Pb to variations in aortic PP and the end organ effects thereof. Although a meta-analysis of several studies assessing the ability of aortic AIx to predict events demonstrated robust effects (Vlachopoulos *et al*., 2010), subsequent studies have failed to demonstrate similar effects beyond steady-state pressures (Mitchell *et al*., 2010). However, as indicated in the aforementioned, in the context of wave re-reflection, aortic AIx is likely to markedly underestimate the impact of wave reflection on aortic PP and hence end organ effects (Booysen *et al*., 2015). What of data on the role of Pb? Few studies have assessed the relative impact of Pf and Pb on cardiovascular end organ damage or the ability to predict events. In this regard, aortic Pb has been shown to be more closely associated with end organ changes (mainly left ventricular mass index) than forward wave pressures (Zamani *et al*., 2014; Booysen *et al*., 2015; Sibiya *et al*., 2015), whilst some studies have demonstrated a similar impact (Wang *et al*., 2010) and others have failed to show an effect (Kaess *et al*., 2015). Moreover, whilst the Multi-Ethnic Study in Atherosclerosis (MESA) demonstrated an ability of Pb to predict events (mainly HF) (Chirinos *et al*., 2012; Zamani *et al*., 2014), the Framingham Heart Study showed that Pf was more important in predicting events (Cooper *et al*., 2015).

Importantly, in all of the aforementioned studies adjustments were made for the impact of MAP, which excludes the effects of arteriolar tone on wave reflection. To what extent arteriolar effects on wave reflection influence end organ measures or predict events is therefore unknown. Furthermore, in none of the aforementioned studies did the authors report on reflected wave effects accounting for that component of forward wave pressures that is actually a re-reflected and hence indirectly a reflected wave effect. Consequently, it is very possible that through the direct effect of reflected waves and through wave re-reflection, the total impact of reflected

waves (Figure 1.2) accounts for far more of the adverse effects of PP than that component of Pf that is driven by the product of flow and Zc than previously thought. In the context of the current debate as to the role of forward and reflected waves to central arterial PP, what is the contemporary understanding of the mechanisms that explain the limited benefits of β blockers on central as compared to brachial artery PP?

1.8 Mechanisms of the limited benefit of atenolol on aortic BP

Before discussing the possible differences in the impact of β-blockers on central as compared to peripheral arterial PP, it is important to first discuss the mechanisms that may account for the BP-lowering effects of β-blockers in general. In this regard, β-blockers may reduce BP through several mechanisms. Inhibition of β-adrenergic receptors decreases sympathetic output from the central nervous system (CNS), attenuates renin release (renal β-adrenergic receptors) and reduces the rate and strength of cardiac contraction by blocking cardiac β-adrenergic receptors (Trudeau, 2014). Thus, theoretically β-blockers reduce both cardiac output (CO) through decreases in heart rate (HR) and stroke volume (SV) and total peripheral resistance (TPR) or systemic vascular resistance (SVR). However, reductions in CO will limit the ability to perform physical activity. Thus, compensatory changes occur. In this regard, as long as MAP is within autoregulatory ranges, vasodilation may contribute to maintaining a normal SV and hence limit reductions in CO. In addition, through force-frequency relationships (a decrease in HR may result in an increased SV by enhancing the force of cardiac contraction), SV will increase in proportion to the decrease in HR and thus possibly maintain a normal CO even though HR is at the lower end of the normal range. Consequently, through autoregulatory changes and possibly through effects on renin release (through β-adrenergic receptors, sympathetic activation has a strong effect on renin release), the impact of β-blockers on BP may occur not only through reductions in HR and hence CO, but also in-part through a decrease in TPR or SVR. However, unlike most newer classes of antihypertensive agents (carvedilol or nebivolol), atenolol and alternative agents such as metoprolol are unable to influence vascular resistance through direct vasoactive properties and these agents are therefore called non-vasodilating β-blockers (Heffernan *et al*., 2011). In this regard, the impact of these agents on TPR (SVR) may therefore be minimal with the main effect on BP being mediated by a reduction in HR and hence CO and MAP (assuming right atrial pressures are 0 mm Hg, MAP=CO x TPR). Importantly, as reviewed in the aforementioned sections (see section 1.1 and 1.2), for the same effect on brachial BP,

atenolol-based therapy does not decrease central aortic BP as effectively as alternative antihypertensive agents. What could be the possible mechanisms of this effect?

All β-blockers including atenolol reduce HR (Fares and Ventura, 2012). This would normally produce marked decreases in CO (Marshall and Parratt, 1976) if compensatory changes did not occur. In this regard, as indicated above it is well recognised that a reduction in the frequency of cardiac contraction may result in an increased force of contraction (force-frequency relationship), an effect that could in-part be explained by enhanced filling volumes produced by a slower HR and hence through the Frank-Starling relationship, an increase in the force of cardiac contraction. The increased force of contraction will enhance SV and aortic flow (Q). The enhanced Q, although limiting the decreases in CO produced by a reduced HR, will nevertheless elevate Pf, PPc and hence central arterial SBP (Williams *et al*., 2009). This is the traditional view of why PP increases on β-blocker therapy. If this were the case however, because this effect is through increases in Pf, which contributes toward the generation of the brachial pulse, atenolol's effect on Pf should be well indexed by changes in brachial PP and this is not the case (see above discussion). Indeed, although SV may increase with a reduced HR, this may be achieved by extending the ED, thus allowing for a longer period to eject more blood per beat. Consequently, although SV may increase with a reduced HR, this may not be achieved by enhancing peak aortic flow and hence Pf, but by increasing the ejection time. For this reason, one has to consider alternative possibilities. How then could atenolol influence central aortic as opposed to brachial PP?

There are several mechanisms related to factors that influence central aortic (as opposed to brachial) PP that may explain the impact of atenolol on aortic as opposed to brachial PP. In this regard, several studies (Williams *et al*., 2006), with the largest being the CAFE study conducted in a subset of the ASCOT study (Dahlöf *et al*., 2005) have demonstrated that for the same degree of brachial BP reduction, atenolol-based antihypertensive therapy leads to increases in aortic augmentation index (AIx) as compared to the impact of alternative antihypertensive agents. This effect was initially interpreted as indicating that β-blockers without direct vasodilator properties are unable to produce the direct vascular effects (vasodilation) that result in a decrease aortic wave reflection that alternative vasodilator antihypertensives (CCB or angiotensin-converting enzyme inhibitors) are able to produce (Williams *et al*., 2006). A proposed mechanism for the inability of non-vasodilator β-blockers to decrease AIx was that these agents possibly do not modify circulating brain natriuretic peptide (BNP) concentrations as well as other agents

(Williams *et al*., 2006). However, there is little evidence to show that BNP has specific effects on wave reflection beyond alternative substances with vascular (vasodilator or vasoconstrictor) properties. The proposal that atenolol is unable to influence AIx because of a lack of effect on vascular changes that mediate wave reflection, of course would suggest that as long as one employs a β-blocker with direct vasodilator properties (Agabiti-Rosei *et al*., 2007) β-blockers would be safe to use as first-line therapy (Caterina and Leone, 2010). Indeed, there is some evidence to support this contention as β-blockers such as nebivolol and carvedilol lower aortic PP to a greater extent than atenolol or metoprolol for the same brachial PP and indeed increase the difference between central aortic and brachial PP (increase PP amplification) (Protogerou *et al*., 2009). However, at the time of the initial reports on β-blocker effects on aortic haemodynamics, the mechanism of atenolol's effect on AIx was not apparent. What could be the alternative possible mechanisms that may explain the impact of β-blockers on AIx?

The effect of atenolol on AIx is well correlated with reductions in HR (Williams and Lacy, 2009) and hence the authors proposed that this effect was produced by extending the ejection duration (ED) and thus allowing Pb more time to augment Pf (that is, increase the chances of more of the compression pressure wave summating with the reflected pressure waves) (Williams *et al*., 2006; Williams and Lacy, 2009). Thus, it was postulated that atenolol therapy reduces HR and as a result increases AIx and PP by increasing ED and thus the ability of the reflected wave to augment PP by extending the time to the peak of the forward wave (Williams *et al*., 2006). Nevertheless, the impact of atenolol therapy on neither Pf nor Pb, nor the extent to which extended ED enhances the ability of the reflected wave to maximally augment aortic PP as derived from wave separation analysis has been assessed. Hence this proposal is speculative. The notion that the adverse effects of atenolol-based therapy on central aortic PP is through HR associated effects is supported by the fact that adjustments for HR abolished the variations in the impact of β-blockers on central aortic AIx and PP (Williams and Lacy, 2009; Pucci *et al*., 2015). However, this is an association and hence does not provide definitive evidence for cause and effect. Importantly, in contrast to the hypothesis that atenolol's adverse effect on AIx and hence aortic PP is because it lacks vasodilator properties and hence is not necessarily a class effect, the hypothesis that atenolol is unable to reduce AIx or aortic PP by extending the ED suggests that the adverse effect is a class effect and that all β-blockers should be avoided as first line therapy in primary prevention (Bangalore *et al*., 2008; Larochelle *et al*., 2014). More recently, the mechanisms of HR effects on wave reflection have been modelled and in this regard, decreases in HR are predicted to show marked increases in wave reflection through a major impact on the

frequency of oscillating waves (harmonic effects) and to some degree through alterations in the visco-elastic properties of the aorta (Xiao *et al*., 2018). Indeed, an inverse relationship between HR and RM has been well described (Van Den Bogaard *et al*., 2011; Tan *et al*., 2016). Thus, the adverse effects of atenolol on AIx and central aortic PP could be explained by the direct effects of HR on wave reflection mediated by mechanical changes, rather than through vascular effects *per se*. This suggests that the adverse effects of atenolol are indeed through an impact on wave reflection, but are a class effect that once again cannot be prevented with the use of vasodilator agents or rather using β-blockers with direct vasodilator properties. This conundrum has major clinical implications for the use of vasodilator β-blockers. Subsequent work has demonstrated that an increased time to the peak of Pf does indeed strongly determine whether Pb is independently associated with end-organ measures (Tade *et al*., 2017). These data (Tade *et al*., 2017) therefore provide the evidence to support the argument that atenolol's limited effect on outcomes could be explained by an extended time to the peak of the Pf. However, to the best of my knowledge I can find no evidence to show, using wave separation analysis, that atenolol either fails to decrease Pb as with other antihypertensive agents or by extending ED, enhances the ability of the reflected wave to maximally augment aortic pressure, and if so which factor contributes the most to variations in PP augmentation. Furthermore, there is no evidence that an extended ED produced by β-blocker therapy will necessarily translate into a greater contribution of the pressure generated by the product of flow and characteristic impedance (Zc) (compression wave pressures) to aortic augmented and pulse pressures. Thus, the role of β-blocker-based therapy in the treatment of hypertension remains uncertain and there is an urgent need to identify, using wave separation analysis the impact of atenolol, when used as second or third or fourth line antihypertensive therapy, on central aortic haemodynamics. In other words, is atenolol associated with increases in AIx and aortic PP without similar changes in brachial PP because of an inability to decrease wave reflection through a lack of vasodilator properties and thus represents an adverse effect specific to some β-blockers; is atenolol associated with increases in AIx because of an extended time to the peak of the Pf and thus represents a class effect of all β-blockers not amenable to a solution; or is atenolol associated with increases in AIx because of an increased wave reflection produced by the impact of HR on mechanical properties of the aorta (harmonic or visco-elastic effects on wave reflection) and thus represents a class effect of all β-blockers, also not amenable to a solution? Importantly, if the effects of atenolol on central aortic PP are through either an extended ED producing an increased overlap of the Pf with the returning reflected pressure wave, or because of vascular or mechanical effects on wave reflection, then the chances of atenolol producing an adverse effect on PP would be greater at

higher compression wave pressures. In contrast, if the adverse effects are through an enhanced peak aortic flow at a slower HR, then the impact on central aortic PP would be driven by the compression wave, rather than being sensitive to the magnitude of the compression wave. This distinction is critical as whilst the former suggests that this effect would only occur in those with an enhanced compression wave pressures and is readily addressed by ensuring that sufficient antihypertensive therapy is employed to maintain a low compression wave, the latter, which is driven by an enhanced aortic flow (Q) has no ready solution. To better understand the impact of the former, consider the following. Increases in compression wave pressures are driven by either an enhanced Q, or an increased aortic stiffness and hence Zc. In hypertension, increases in compression wave are determined by aortic stiffness and Zc. Aortic stiffness and Zc can be readily modified by reducing distending pressures in the aorta which are determined by MAP. This reduces aortic stiffness through passive processes (moves the point down the aortic pressure-volume relationship). By decreasing compression wave pressures, through Newton's Laws of Motion, the reflected wave is diminished and hence although the extent to which wave reflection occurs may still be enhanced beyond compression waves, the absolute reflected wave magnitude is reduced. Consequently, even if wave reflection cannot be detected at the peripheral pulse, a simple solution is to decrease MAP to values lower than normal (intense BP reduction) and this should address the impact of a high reflected wave. However, the extent to which atenolol's effect on wave reflection is sensitive to compression wave pressure changes is unknown. If this is indeed the case, the solution to the adverse effects of atenolol is not to employ vasodilator β-blockers but any agent or combinations thereof that are able to achieve a much lower MAP than that normally achieved.

To address the questions posed above, in the present dissertation to provide proof of principle, I compared the differences in aortic wave characteristics in patients whom often receive atenolol as part of their antihypertensive therapy to the wave characteristics of those patients who did not receive atenolol as part of their therapy. In this regard, atenolol is frequently employed as either third or fourth-line therapy in patients with resistant or refractory hypertension. I therefore evaluated aortic wave characteristics in patients with resistant or refractory hypertension approximately half of whom were receiving atenolol as one of the agents to control BP. As resistant and refractory hypertensives were evaluated in the present dissertation, in the next section I will outline key points on the topic of resistant and refractory hypertension.

1.9 Resistant or Refractory hypertension and its management

Resistant or refractory hypertension is a phenotype of hypertension in which BP is uncontrolled despite the use of 3 to 4 (resistant) or 5 or more (refractory) antihypertensive agents of different classes including both long acting thiazide or thiazide-like-diuretic agents and mineralocorticoid receptor antagonist or even just a diuretic if tolerated (Chobanian *et al*., 2003; Calhoun *et al*., 2008; Dudenbostel *et al*., 2016). In other words, resistant or refractory hypertension is identified when BP control is only achieved when 3 to 4 or 5 or more antihypertensive agents are employed. Resistant or refractory hypertension occurs in 10-15% of hypertensives (Sim *et al*., 2013; Muntner *et al*., 2014) with the greatest prevalence observed in those of African descent, obese or overweight individuals, patients diagnosed with chronic kidney disease (CKD) and in elderly people (Dudenbostel *et al*., 2015). In this regard, a higher body mass index (BMI), old age and African descent are identified as independent predictors of resistant or refractory hypertension (Thomas *et al*., 2016). Resistant or refractory hypertension carries a considerable risk of events with a higher ambulatory BP reading for a given automated office BP reading, substantially more target organ damage and markedly adverse overall cardiovascular risk profiles (Calhoun *et al*., 2014; Siddiqui *et al*., 2016; Armario *et al*., 2017). The higher ambulatory BP is attributed to increases in nocturnal BP and to less BP variation (Salles *et al*., 2008; Oliveras *et al*., 2011; De La Sierra, 2013; Armario *et al*., 2017).

The pathophysiologic processes leading to the onset of resistant or refractory hypertension are multifactorial and include fluid retention, activation of the sympathetic nervous system (SNS) and stimulation of the renin-angiotensin-aldosterone system (RAAS) (Edwards and DiPette, 2016). The multifactorial process thus requires targeting several systems to achieve BP control. Importantly, in resistant or refractory hypertension, appropriate treatment regimens should include combinations of different classes if diuretic agents involving both spironolactone (a mineralocorticoid receptor antagonist) when contraindications are not present (including a reduced glomerular filtration rate [GFR]), and chlorthalidone (a thiazide type diuretic) when available (these are not available in South Africa in appropriate preparations and thus thiazide diuretics are required) (Nishizaka *et al*., 2003; Khosla *et al*., 2005; Williams *et al*., 2015). Indeed, in order to achieve goal BP levels in resistant or refractory hypertension, it is acknowledged that lowering sodium intake and employing approaches that achieve advanced diuresis or natriuresis are required (Graves *et al*., 1989). Importantly, in the Prevention and

Treatment of Hypertension With Algorithm based Therapy (PATHWAY-2) study spironolactone was noted to be the most effective antihypertensive agent to improve BP control over a short period of time (Williams *et a*l., 2015). Unfortunately, although spironolactone is one of the most effective agents at controlling BP in resistant or refractory hypertension, it frequently causes hyperkalaemia and often hyponatraemia and hence can only be used in settings where regular potassium measurements can be performed (not in primary care settings in developing countries). Moreover, men often develop breast pain and breast enlargement as a well-recognised side effect of spironolactone. Although an aldosterone receptor blocker without this side effect in men is available in South Africa, the public and private sector hospitals limit the use because of the cost. Hence, to achieve BP control, resistant or refractory hypertension often requires the judicious use of alternative agents including atenolol. Although several newer treatment approaches such as device based (including renal sympathetic denervation) and pharmacologic therapies have been suggested to better manage resistant or refractory hypertension (Edwards and DiPette, 2016), conflicting results have emerged regarding their efficacy, and the use would be cost-prohibitive in the public and private sector. Hence, the stepwise approach in the primary care setting (use of multi antihypertensive drugs) is currently the best route to treat resistant or refractory hypertension (Edwards and DiPette, 2016). The frequent use of atenolol as 3rd of 4th line therapy in resistant or refractory hypertension raises the question of the impact on central arterial pulsatile load in the presence of the simultaneous use of several alternative agents, many of which have vascular effects.

1.10 Problem statement

Although the use of β-blocker therapy is a well-recognised approach to treating hypertension, several clinical trials provide strong evidence that atenolol shows inferiority to alternative antihypertensive agents at event reduction. This adverse effect of atenolol is thought to be in-part through an increased central aortic pressure augmentation and hence pulsatile load. However, the exact mechanisms of this effect are uncertain. In this regard, this effect could be attributed to a class effect produced by a reduction in HR and an increased Q. This change is not amenable to targeting with alternative therapeutic approaches. Alternatively, this effect could be attributed to a class effect produced by a reduction in HR and an extended ED, thus enhancing overlap between the forward and backward travelling pressure waves. This change could be amenable to intense BP lowering with subsequent reductions in compression wave pressures. The adverse

effects of atenolol could also be attributed to a class effect produced by a reduction in heart rate and mechanical changes in the amplitude of the reflected wave through harmonic effects or alterations in the visco-elastic properties of the aorta. This change could also be amenable to intense BP lowering with subsequent reductions in compression wave pressures. Alternatively, the adverse effects of atenolol on central pulsatile load may represent an effect specific to non-vasodilator β-blockers rather than a class effect, where the lack of impact of atenolol on vascular tone fails to reduce reflected wave pressures. This adverse effect would be shared by some but not other β-blockers. Vasodilator β-blockers may therefore be as efficacious in the impact on events in general as alternative antihypertensive agents but with an additional cardioprotective action associated with HR reduction. However, there are no studies that have identified the exact mechanisms that explain the adverse effects of atenolol on aortic PP.

1.11 Aims

As a consequence of the uncertainty as to the mechanisms that explain the adverse effects of atenolol on central aortic PP, I compared the characteristics of aortic pressure waves in resistant or refractory hypertensives either receiving or not receiving atenolol as part of their therapy. Using formal wave separation analysis and aortic flow and diameter measurements in the outflow tract, I aimed to identify the mechanisms that may explain either an increased central aortic PP or the impact of compression waves on central aortic PP between treatment groups. In this regard, I considered whether increases in:

1. Q,
2. a prolonged ED and hence
3. enhanced overlap of the Pf with the Pb at higher compression wave pressures,
4. an increased wave reflection at higher compression wave pressures as determined by the impact of HR on harmonics, or
5. an increased wave reflection at higher compression wave pressures as determined by the impact of vascular effects beyond arteriolar function,

explain differences in aortic PP in resistant or refractory hypertensives either receiving or not receiving atenolol therapy as part of their treatment.

CHAPTER 2: METHODS

2.1 Study participants

The present study was conducted according to the guidelines outlined in the Helsinki Declaration (version 2013). The Committee for Research on Human Subjects of the University of the Witwatersrand approved and reviewed all procedures (clearance number: M17-02-71). All participants gave informed written consent in order to participate. Briefly, 61 resistant or refractory hypertensives, (men and women), defined as receiving 3 to 4 or 5 or more antihypertensive agents with or without brachial BP controlled to target (<140/90 mm Hg) were evaluated. Participants of different ethnic groups were recruited from the Hypertension Clinic at Charlotte Maxeke Johannesburg Academic Hospital. A clinical diagnosis of hypertension was established by the treating clinician prior to inclusion in the study. Data collected in 28 individuals who were receiving atenolol was compared to data collected in 33 participants who were not receiving atenolol.

2.2 Demographic and clinical data

A standardized questionnaire was administered to obtain demographic data. The questionnaire was made available in English, but trained study assistants (a nursing sister and trained technician) familiar with the home (first) languages assisted with the completion of each questionnaire. Assistance was only provided when requested. Nonetheless, the majority of participants were reasonably proficient in the English language (primary and secondary education is largely conducted in the English language). The questionnaire sought specific answers to date of birth and gender (designated as either male of female). Most of these questions required a simple response of either “YES” or “NO” as answers. Clinical data were extracted from the clinical file. These data included associated clinical conditions and classes of antihypertensive agents and the doses employed. Each participant’s anthropometric measurements (height and weight) were determined using standard approaches. For anthropometry, height (in millimetres) and weight (in kilograms) were measured using a stadiometer (Seca 217) and a bathroom scale (Healthometer 160LBS) (Manufacturer: Medline Industries, Inc. Chicago, United States of America). Body mass index (BMI) was then derived from weight and height as weight in kg, divided by height squared (kg/m^2).

Brachial BP was obtained using both an oscillometric BP monitor (Omron HEM-7113, Manufacturer: Gandhi Bag, Nagpur, India) and a sphygmomanometer (Mercurial Alpk2, Manufacturer: Advanced Technocracy Inc. Grain Market, Ambala, India). Recordings obtained with the oscillometric device were used to calibrate SphygmoCor recordings with participants in the supine position. Auscultatory BP recordings were obtained in the seated position to record the actual BP on that day. Auscultatory BP was measured by a trained nurse-technician, according to guidelines of the American Heart Association and European Society of Hypertension recommendation using a standard mercury sphygmomanometer (O'Brien *et al.*, 2003; Pickering *et al.*, 2005). Blood pressure was recorded to the nearest 2 mm Hg. Korotkoff phases I and V were employed to identify systolic and diastolic BP respectively, and care was taken to avoid auscultatory gaps. Five consecutive BP readings were obtained, at least 30 seconds apart in a sitting position after 10 minutes of rest using an appropriately sized cuff for each of the participants, and the average of the five readings was subsequently employed for statistical analysis. For both auscultatory and oscillometric BP measurements, standard cuffs were used with an inflatable bladder with a length of 22 cm and a width of 12 cm, except when arm circumference exceeded 31 cm larger cuffs with a 31 x 15 cm bladder were employed.

2.3 Aortic pressure

An aortic pressure waveform was obtained using a SphygmoCor system (Figure 2.1). After participants had rested for 15 minutes in the supine position, the waveform at the radial (dominant arm) pulse was recorded by applanation tonometry during an 8-seconds period using high-fidelity SPC-301 micromanometer (Miller Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, Version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) (Figure 2.1). The pulse wave was calibrated by measurement of brachial BP, taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software (Figures 2.2). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal or were less than 80 Mv were discarded. Central aortic PP (PPc) was determined as the difference between aortic systolic BP (SBP) and diastolic BP (DBP) and aortic augmented pressure (Pa) as

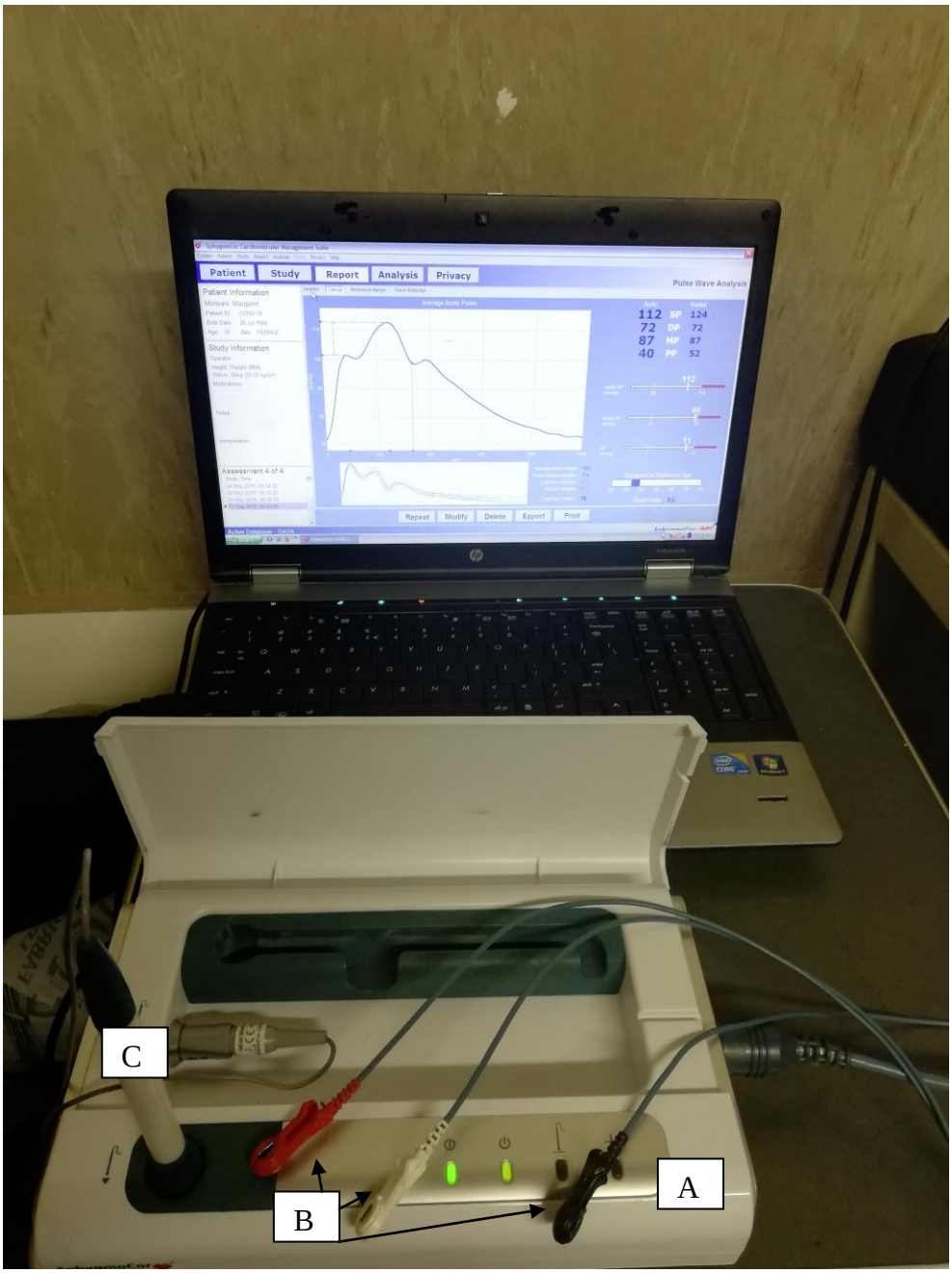

Figure 2.1 SphygmoCor system used to determine aortic haemodynamics. A is the SphygmoCor device, B, are electrocardiogram leads, and C is the tonometer.

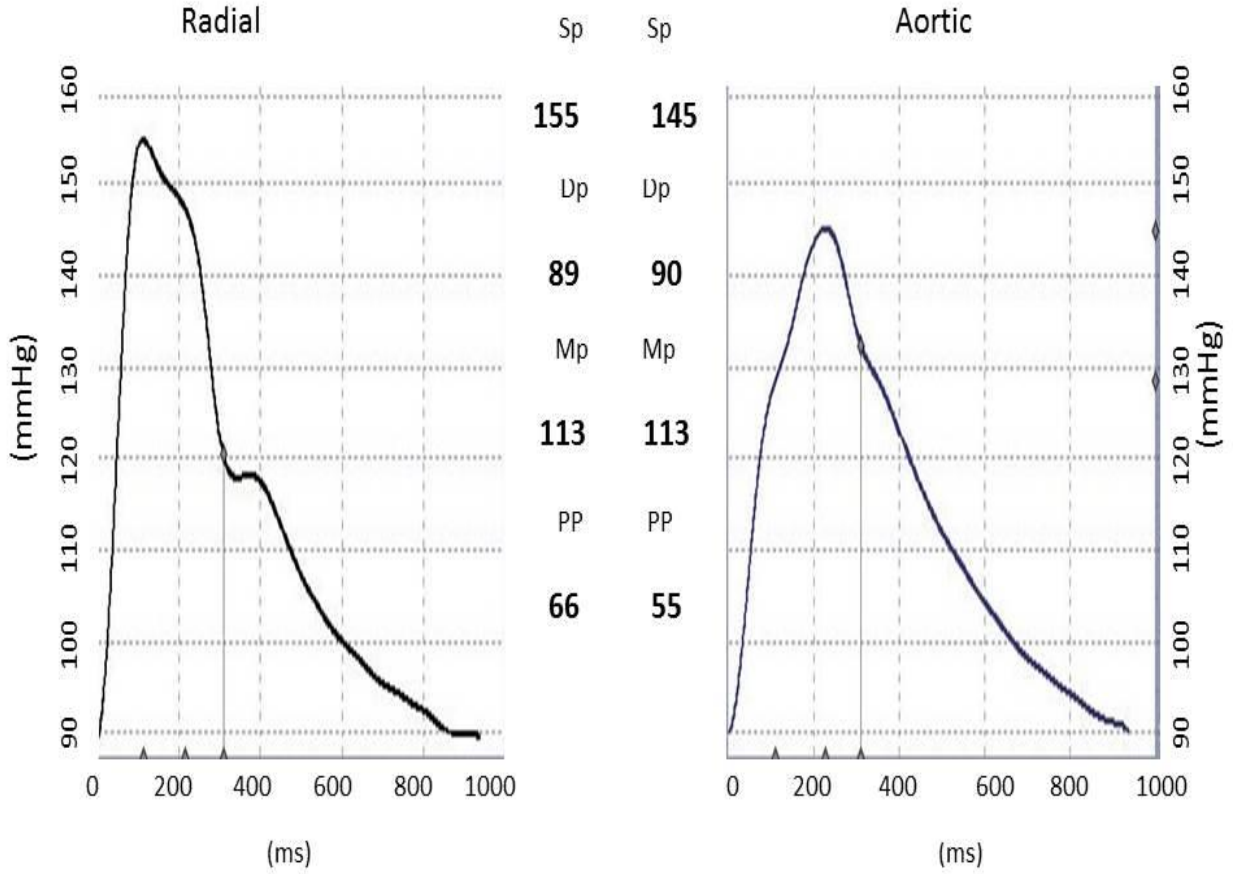

Figure 2.2 Peripheral (radial) pressure trace (left panel) and central (aortic) pressure trace (right panel) derived from a generalised transfer function incorporated in SphygmoCor software. Sp, systolic blood pressure; Dp, diastolic blood pressure; MP, mean arterial pressure; PP, pulse pressure.

the difference between PPc – (height of the first systolic shoulder-diastolic BP). Augmentation index (AIx) was determined as Pa/PPc expressed as a percentage.

2.4 Aortic flow and systemic haemodynamics

Whilst central arterial pressures were recorded using radial applanation tonometry as described above, echocardiographic measurements were obtained by an experienced observer (Professor Angela Woodiwiss) with the participants in the left lateral decubitus position using an Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.) equipped with a standard linear array transducer (1-4 MHz) and electrocardiogram (Figure 2.3). Aortic flow waveforms were generated from aortic diameter and velocity measurements. Aortic diameter measurements were obtained just proximal to the aortic leaflets in the long axis parasternal view (Figure 2.4). The largest diameter recorded in early systole was employed to construct the flow waveform (Mitchell *et al*., 2010). Aortic velocity waveforms were obtained in the apical five-chamber view (Figure 2.5). To generate an aortic flow waveform, taking care to avoid any overshoot of the image, the leading (outer) edge or the most dense, or brightest, portion of the spectral image of the velocity waveform was outlined using ultrasound graphics software (Sante DICOM viewer free(64-bit).Ink) and using aortic diameter measurements employed to construct a flow waveform. In this regard, to determine aortic flow at each time point along the velocity wave, the velocity x aortic root area (3.142 x (aortic root diameter/2)^2 formula was used. Peak aortic flow (peak Q) was determined at peak velocity. Aortic velocity and diameter were also employed to determine the time to the peak of aortic flow, ejection duration (ED) and stroke volume (SV) using standard approaches. In this regard, time to the peak of flow was determined from the foot to the peak of the velocity wave, ED from the duration of the velocity wave from foot to foot and SV from the velocity-time integral x aortic root area (3.142 x (aortic root diameter/2)^2 formula. Cardiac output (CO) was determined from the product of SV and heart rate (HR). Systemic vascular resistance (SVR) was estimated from mean arterial pressure (MAP) derived from SphygmoCor software and CO using the formula SVR = (MAP-right atrial pressure)/CO), where right atrial pressure was assumed to be 0 mm Hg.

Figure 2.3 Echocardiographic device (Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.)) used to obtain aortic velocity waveforms in the 5-chamber view and aortic root diameter from the parasternal long axis view. A= electrocardiography cables, B= 1-4 MHz probe.

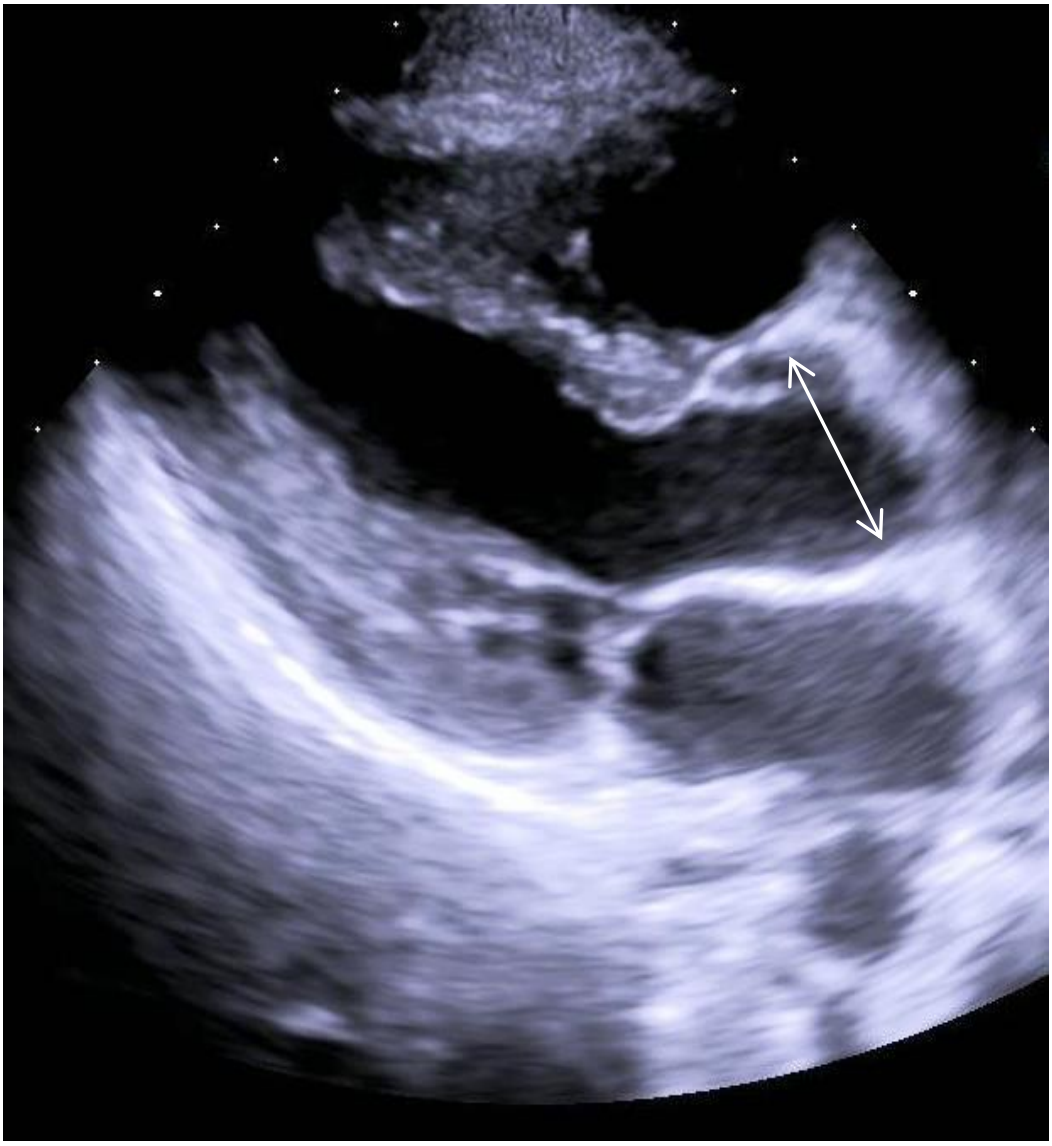

Figure 2.4 Aortic root diameter obtained at the tips of aortic valves during systole in parasternal long axis view.

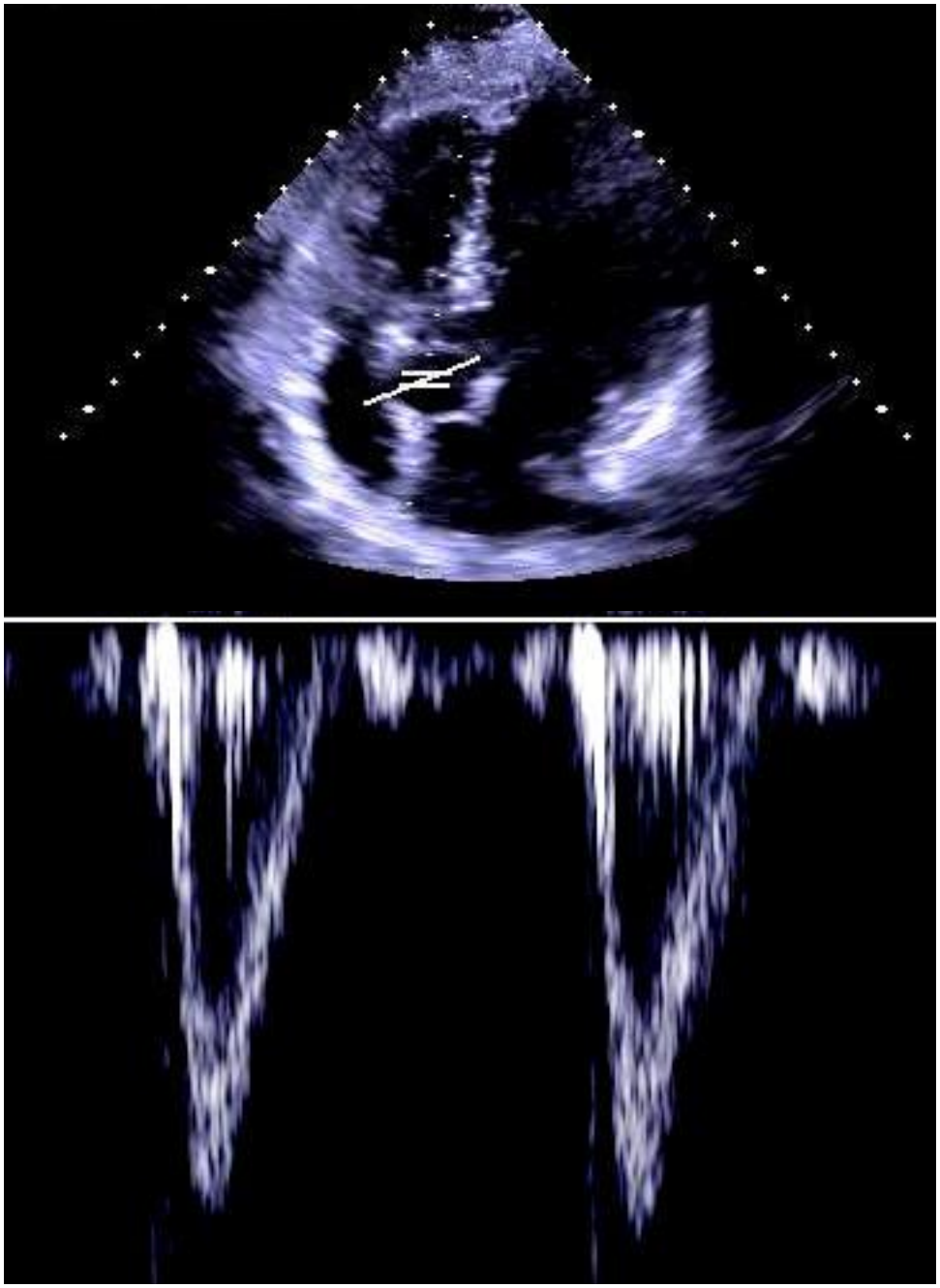

Figure 2.5 Aortic velocity waveform obtained in the apical 5 chamber view. Upper panel shows apical 5 chamber view and the lower panel shows the aortic root velocity waveforms.

2.5 Determinants of aortic pulse pressure

Aortic characteristic impedance (Zc) was calculated in the time domain (Westerhof *et al*., 1972; Dujardin and Stone, 1981; Mitchell *et al*., 2010) as change in pressure/change in flow from the foot of the pulse wave up until 95% of peak aortic flow (Q) (Mitchell *et al*, 2010). Compression waves were generated from the product of Q at each time point on the aortic flow wave and Zc (P_{QxZc}) (Phan *et al*., 2016). The maximal pressure generated by P_{QxZc} (max P_{QxZc}) was taken as the actual compression wave pressure (Figure 2.6). To identify the forward and the backward wave pressures (Figure 2.6), wave separation analysis was performed based on the following formulae: Forward wave pressure= (Pc + QxZc)/2) and Backward wave pressure= (Pc – QxZc)/2) (Murgo *et al*., 1981; Westerhof *et al*., 2006). Wave separation analysis was performed in the time domain based upon the consensus that this analysis can be conducted in either the time or the frequency domain (Segers *et al*, 2017). To determine the contribution of wave re-reflection to aortic PP (Phan *et al*., 2016) the difference between forward wave pressures and P_{QxZc} was identified at different time points on the downslope of the compression wave. The pressure generated by P_{QxZc} at the peak of aortic PP (downslope of the compression wave) was taken as the degree to which extended ED enhances aortic PP by increasing the extent (summation) of that component of the compression wave which adds to the reflected wave (Figure 2.6). Time from the foot of the aortic pulse until the foot of the reflected wave was taken as the speed of wave reflection. Changes in wave reflection were not only determined from the peak of the Pb, but also from the sum of the reflected+ re-reflected wave pressures at peak aortic PP (Figure 2.6). Forward pressure wave (Pf) effects were identified as the peak of aortic Pf (Figure 2.6). To identify changes in wave reflection beyond forward wave pressures, data were expressed as reflected wave magnitude (RM= Pb/Pf x 100). However, because forward wave pressures are determined in-part by wave re-reflection, to identify that component of wave reflection that is beyond compression wave pressures we also expressed Pb as a factor of max P_{QxZc}.

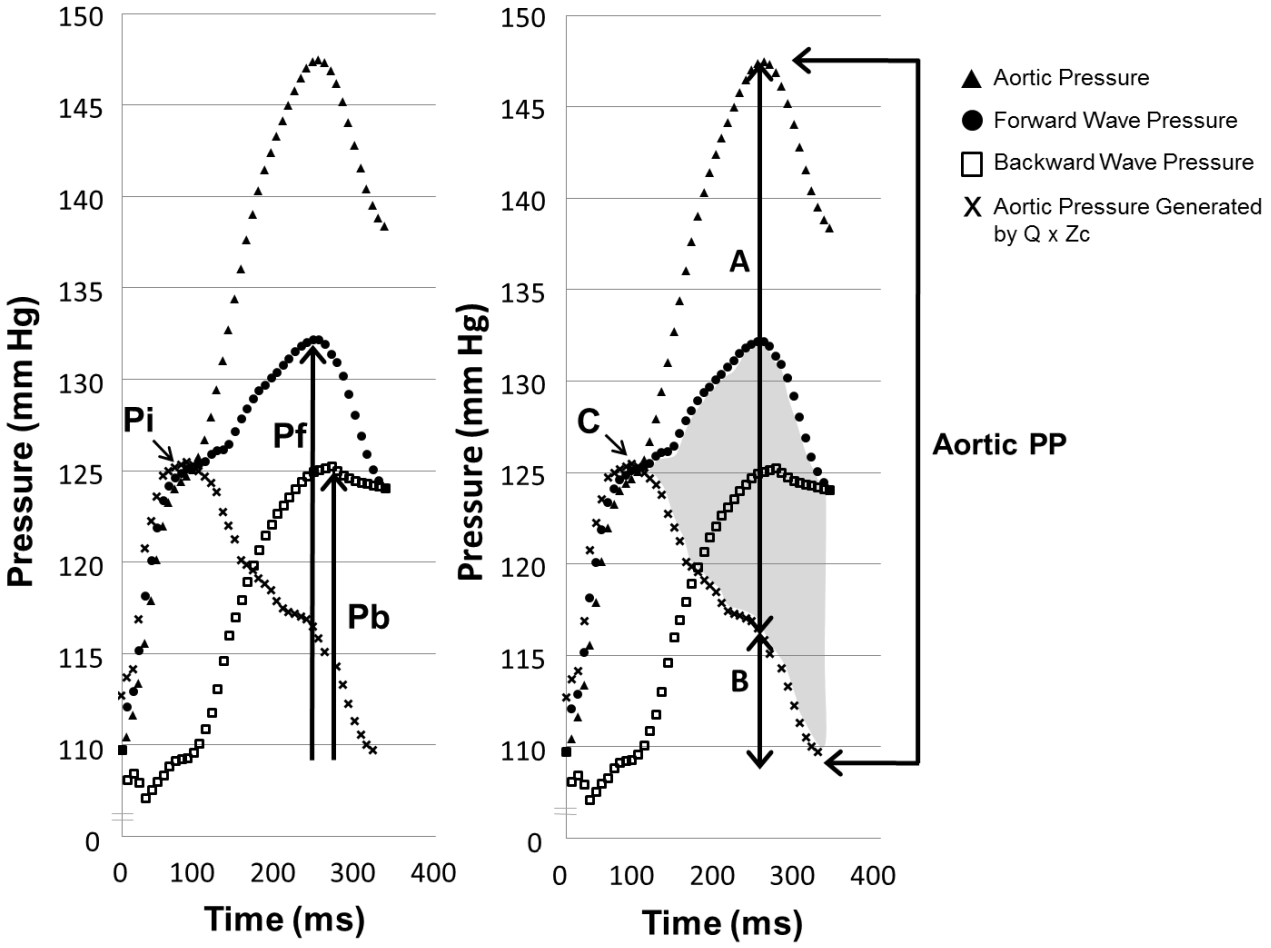

Figure 2.6 Wave components of aortic pulse pressure (PP) superimposed on the aortic pressure wave. Pb, backward wave pressure; Pf, forward wave pressure; Pi, incident wave pressure; A, reflected + re-reflected wave pressures; B, pressure generated by the product of aortic flow (Q) and characteristic impedance (Zc) at peak PP; C, Maximum pressure generated by the product of Q and Zc (Max P_{QxZc}); shaded area, re-reflected wave.

2.6 Data analysis

For statistical analysis, statistical analysis system (SAS) software version 9.4 (SAS Institute Inc. Cary, NC, USA) was employed. As the impact of atenolol on aortic PP may be sensitive to the magnitude of compression wave pressures, comparisons were made between both mean data within each group and the slope of the relations between compression wave versus alternative aortic functional parameters between groups. Where slopes of relations are shown, data are also shown grouped across tertiles of compression wave pressures. An unpaired t test was performed to compare the unadjusted means for various haemodynamic parameters and wave components between groups and multivariate adjusted regression analysis to compare adjusted means between groups. Bivariate analysis and multivariate adjusted regression analysis was performed to compare relationships between groups with adjustments for age, sex, BMI and MAP where appropriate.

CHAPTER 3: RESULTS

3.1 Characteristics

Table 3.1 shows the general characteristics and the different alternative antihypertrensive classes employed in participants receiving atenolol versus those not. Importantly, no differences in the general characteristics or in the use of alternative classes of agents were noted between the groups. Table 3.2 compares the general hemodynamics between the participants receiving atenolol versus those not. Importantly, those receiving the β-blocker, showed a markedly lower HR. The reduction in HR was associated with a prolonged ED. Although, with a prolonged ED, SV was maintained, the decreased HR was associated with a reduced CO. Although MAP was similar between groups, the reduced CO was associated with an increased TPR in patients receiving atenolol. No differences in brachial artery systolic or diastolic BP or PP were noted.

3.2 Aortic versus brachial pulse pressure

Table 3.3 shows that despite marked differences in HR between participants receiving atenolol versus those not, that multivariate adjusted aortic PP was not different between the groups. However, as demonstrated in figure 3.1, through Newton's Laws of Motion, aortic PP was markedly sensitive to an increased magnitude of compression wave pressures (max P_{QxZc}), and that participants receiving atenolol therapy showed a strikingly steeper relationship so that at higher max P_{QxZc} values, aortic PP was greater in participants receiving atenolol versus those not. Importantly, with adjustments for HR, these differences in the relations were abolished (Figure 3.1). In contrast to the increased slope of the relationship between max P_{QxZc} and aortic PP in participants receiving atenolol versus those not, no differences in relations between max P_{QxZc} and brachial PP were noted (Figure 3.2).

Table 3.1 Characteristics of the study sample.

Variable	β blocker (n=28)	Non-β blocker (n=33)
Sample size [% men]	10 [35.7]	11 [33.3]
Age (years)	59.3±13.1	59.5±14.4
Body mass index (kg/m^2)	31.9±7.6	33.1±9.0
Antihypertensive medication (%)		
Diuretics	17 (61)	20 (61)
Calcium channel blockers	21 (75)	28 (85)
ACE Inhibitors	14 (50)	22 (67)
Alpha blockers	3 (11)	6 (18)
Hydralazine	7 (25)	7 (21)
Methyldopa	3 (11)	2 (6)
Spironolactone	4 (14)	7 (21)
Losartan	2 (7)	2 (6)

Data expressed as mean±SD and n (%). ACE-inhibitors indicates angiotensin converting enzyme inhibitors.

Table 3.2 General haemodynamic parameters.

	β blocker (n=28)	Non β blocker (n=33)	p-value
Peripheral SBP (mmHg)	141±14	146±21	=0.32
Peripheral DBP (mmHg)	80±10	81±10	=0.51
Peripheral PP (mmHg)	62±12	65±16	=0.44
Heart rate (beats/min)	61±10	70±11	<0.01
SV (ml/beat)	120±43	133±32	=0.21
CO (l/min)	7±3	9±2	=0.01
TPR (mmHg/l/min)	16±6	12±4	<0.01
MAP (mmHg)	100±10	103±13	=0.36
Ejection duration (msec)	333±27	317±27	=0.04

Abbreviation: SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; MAP, mean arterial pressure. Data presented are not adjusted for sex, age, body mass index (BMI) and MAP. Data expressed as mean±SD.

Table 3.3 Multivariate-adjusted central aortic haemodynamic parameters.

	β blocker (n=28) Mean±SD	Non β blocker (n=33) Mean±SD	p-value
Aortic SBP (mmHg)	135±7	133±7	=0.19
Aortic PP (mmHg)	54±9	50±10	=0.16
Pf (mmHg)	34.5±6.8	36.1±6.7	=0.36
Pb (mmHg)	27.1±6.8	24.1±6.9	=0.10
Aortic AIx (%)	151±24	147±24	=0.47
Aortic Pa (mmHg)	19±5	17±5	=0.15
Aortic flow (Q) (ml/s)	398±92	437±104	=0.20
Zc (dynes.s/cm^5)	91.7±30.9	80.7±30.9	=0.18
Max P_{QxZc} (mmHg)	37.2±7.4	37.1±7.6	=0.99
P_{QxZc} at peak PPc(mmHg)	17.7±7.3	18.9±7.5	=0.56

Abbreviation: Zc, characteristic Impedance; Q, flow; Pa, augmentation pressure; PPc, aortic pulse pressure; Pf, forward wave pressure; Pb, backward wave pressure; SBP, systolic blood pressure. *Adjustments are for age, sex, body mass index (BMI) and mean arterial pressure (MAP).

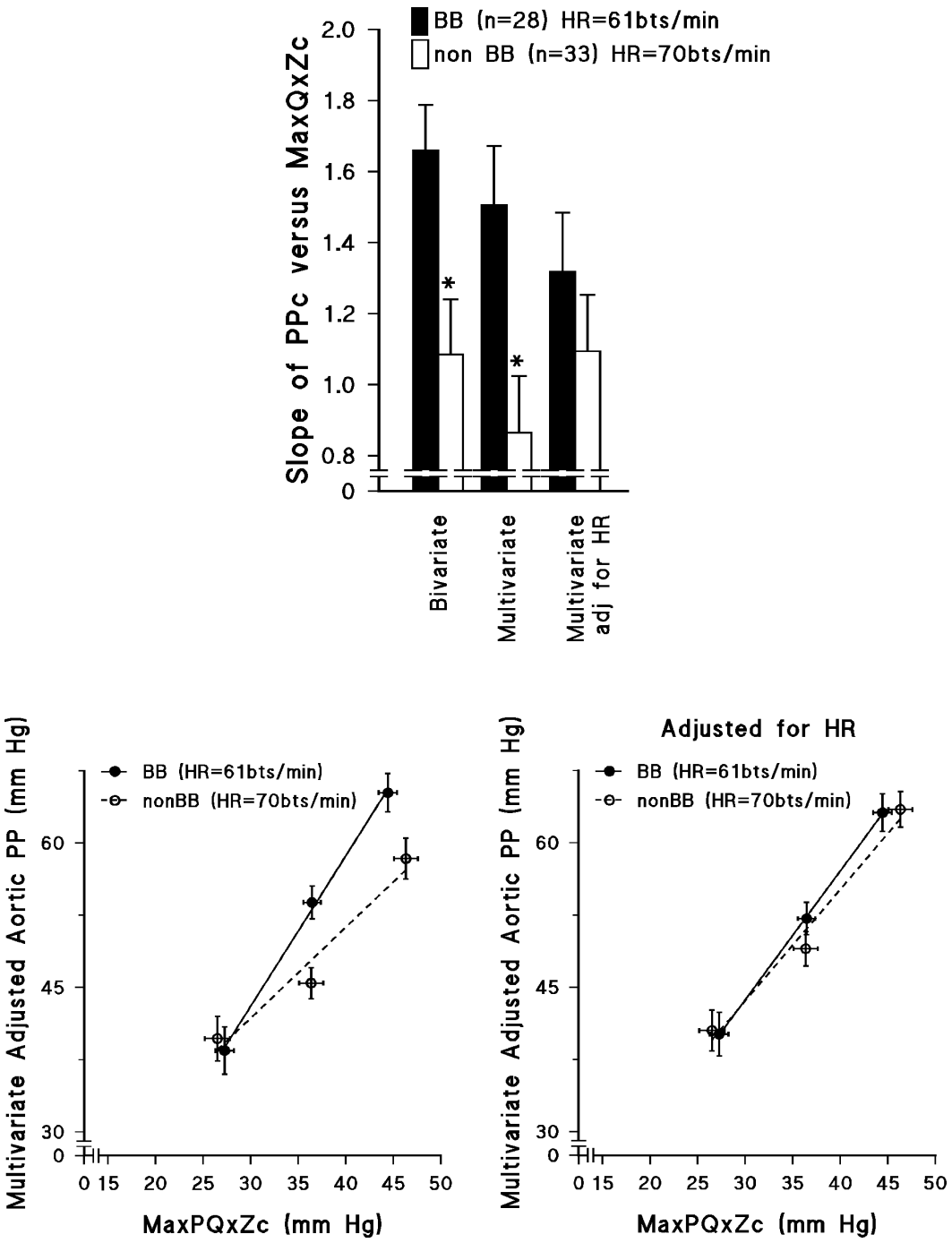

Figure 3.1 Comparison of relationship between compression wave pressures (Max P_{QxZc}) and aortic pulse pressure (PP) in participants receiving atenolol (BB, β-adrenergic receptor blocker) versus those not (Non BB). Adjustments are for age, sex, BMI, MAP and HR as indicated.

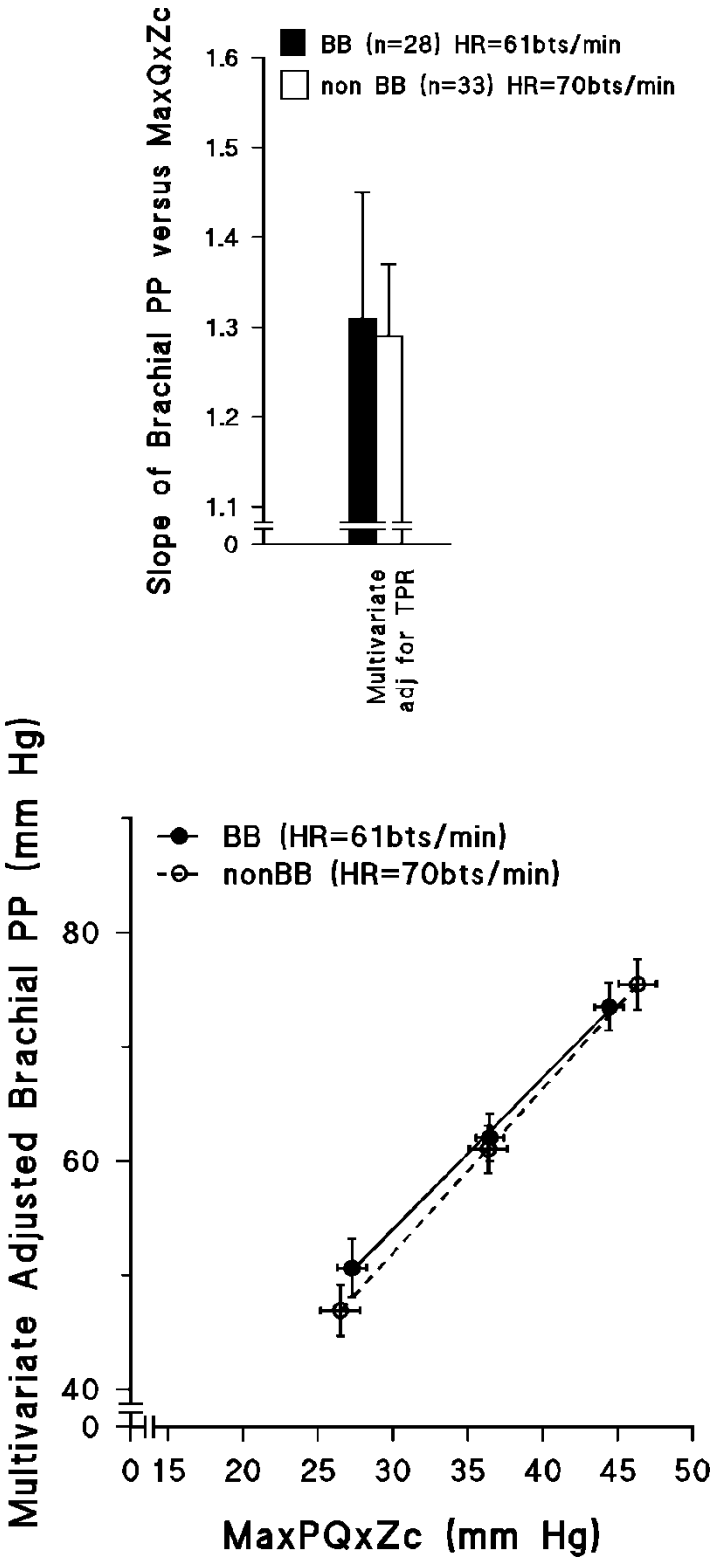

Figure 3.2 Comparison of relationship between compression wave pressures (Max P_{QxZc}) and brachial pulse pressure (PP) in participants receiving atenolol (BB, β-adrenergic receptor blocker) versus those not (Non BB). Adjustments are for age, sex, BMI and MAP.

3.3 Augmentation index

Table 3.3 also shows that despite marked differences in HR between participants receiving atenolol versus those not, that similar to aortic PP, multivariate adjusted aortic AIx was no different between the groups. However, also similar to that for aortic PP (Figure 3.1), aortic AIx was markedly sensitive to an increased magnitude of compression wave pressures (max P_{QxZc}), and that participants receiving atenolol therapy showed a strikingly steeper relationship so that at higher max P_{QxZc} values, aortic AIx was greater in participants receiving atenolol versus those not (Figure 3.3). Importantly, with adjustments for HR, these differences in the relations were abolished (Figure 3.3).

3.4 Contribution of compression wave characteristics to the impact of atenolol on aortic pulse pressure.

No differences in peak aortic flow (Q) and hence compression wave pressures (max P_{QxZc}) were noted between participants receiving atenolol versus those not (Table 3.3). Furthermore, no differences in peak Q at incremental compression wave pressures (Figure 3.4) could explain the differences in relations between compression wave pressures and aortic PP (Figure 3.1) or AIx (Figure 3.3) between participants receiving atenolol versus those not (Figure 3.4). Although ED was increased in participants receiving atenolol versus those not (Table 3.2), this failed to translate into an enhanced contribution of the downslope of the compression wave to peak PP. Indeed, no differences in the pressure generated by the product of Q and Zc at peak aortic PP (P_{QxZc} at peak PP) were noted (Table 3.3). Moreover, differences in P_{QxZc} at peak PP at incremental compression wave pressures (Figure 3.4) could not explain the differences in relations between compression wave pressures and aortic PP (Figure 3.1) or aortic AIx (Figure 3.3) between participants receiving atenolol versus those not.

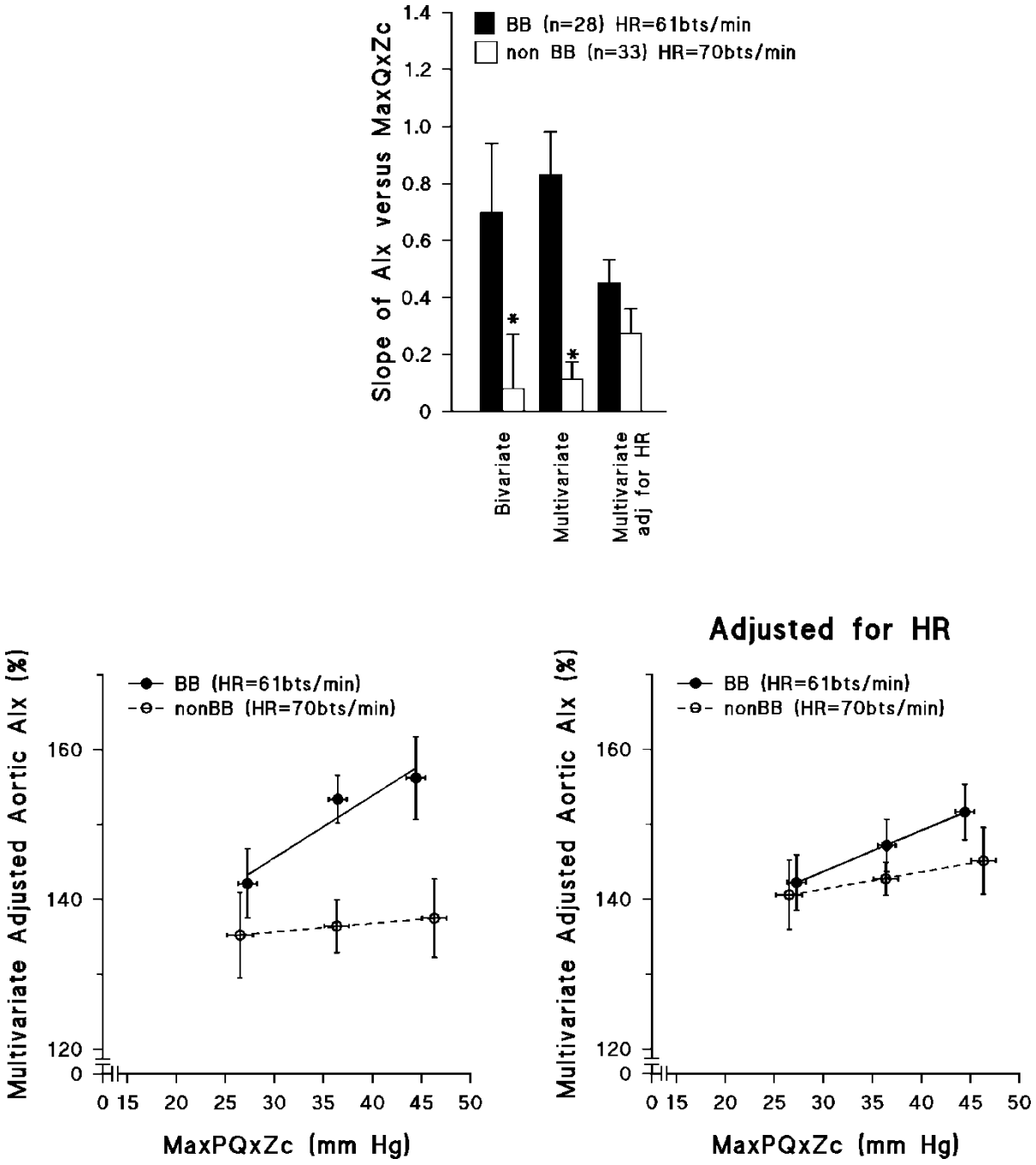

Figure 3.3 Comparison of relationship between compression wave pressures (Max P_{QxZc}) and aortic augmentation index (AIx) in participants receiving atenolol (BB, β-adrenergic receptor blocker) versus those not (Non BB). Adjustments are for age, sex, BMI, MAP and HR as indicated.

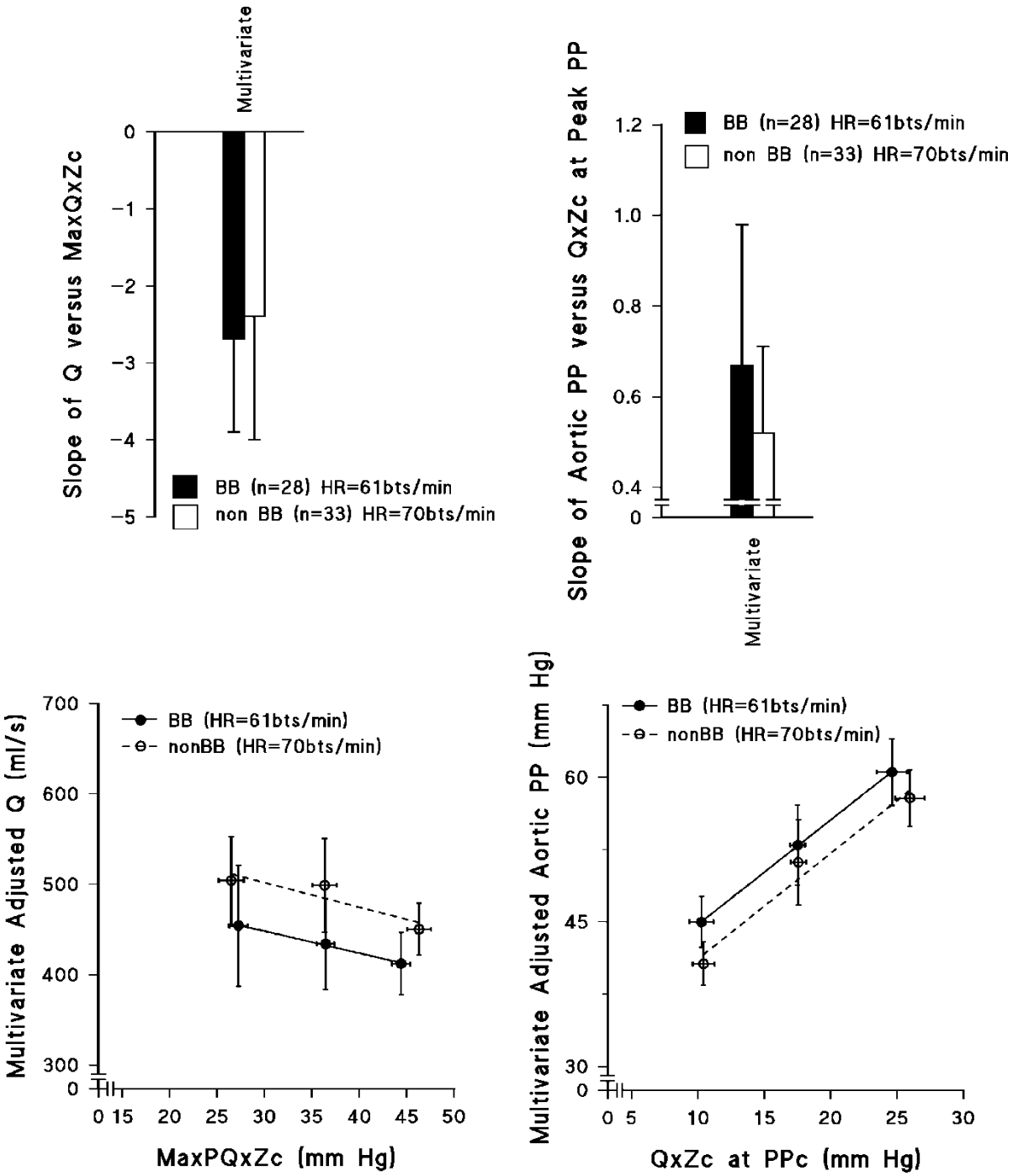

Figure 3.4 Comparison of relationship between compression wave pressures (Max P_{QxZc}) and peak aortic flow (Q) (left panel) or the contribution of P_{QxZc} at peak aortic pulse pressure (PP)(downslope of the compression wave indexing overlap of the Pf with the Pb) to aortic PP (right panel) in participants receiving atenolol (BB, β-adrenergic receptor blocker) versus those not (Non BB). Adjustments are for age, sex, BMI, MAP and heart rate as indicated.

3.5 Contribution of wave reflection to the impact of atenolol on aortic pulse pressure.

Although no differences in backward wave pressures (Pb) were noted between participants receiving atenolol versus those not (Table 3.3), marked differences in several indices of wave reflection were noted between treatment groups (Figure 3.5). These differences were eliminated by adjustments for HR (Figure 3.5). Importantly, the combined impact of wave reflection and re-reflection and that of the impact of reflection magnitude was greater in participants receiving atenolol versus those not, effects that were abolished by adjustments for HR (Table 3.3). These differences in wave reflection also translated into differences in wave reflection at incremental compression wave pressures (Figure 3.6) changes that explained the differences in relations between compression wave pressures and aortic PP (Figure 3.1) and aortic AIx (Figure 3.3) between participants receiving atenolol versus those not. The marked differences in the slopes of the relations between compression waves and indices of wave reflection were also abolished with further adjustments for HR (Figure 3.6).

3.6 Atenolol effects on wave reflection and hence aortic PP are not through a lack of vasodilator properties

Across a range of compression wave pressures, TPR remained higher in the atenolol treated refractory hypertensives (Figure 3.7). However, adjustments for TPR failed to influence the increased slope of the relations between compression wave pressures and either aortic PP, AIx or RM in the atenolol-treated resistant or refractory hypertensives (Figure 3.8). Thus, the impact of atenolol on wave reflection and hence AIx and aortic PP is not attributed to the lack of arteriolar vasodilator properties of atenolol. In all participants considered as a single group (irrespective of whether receiving atenolol or not) a strong independent relationship was noted between HR and indices of wave reflection (Figure 3.9). Importantly, these relations were unaffected by adjustments for atenolol use (Figure 3.9). Thus, once again, atenolol's effects on wave reflection (and hence AIx and aortic PP) are attributed to HR effects and not to the inability of atenolol to produce arteriolar vasodilation.

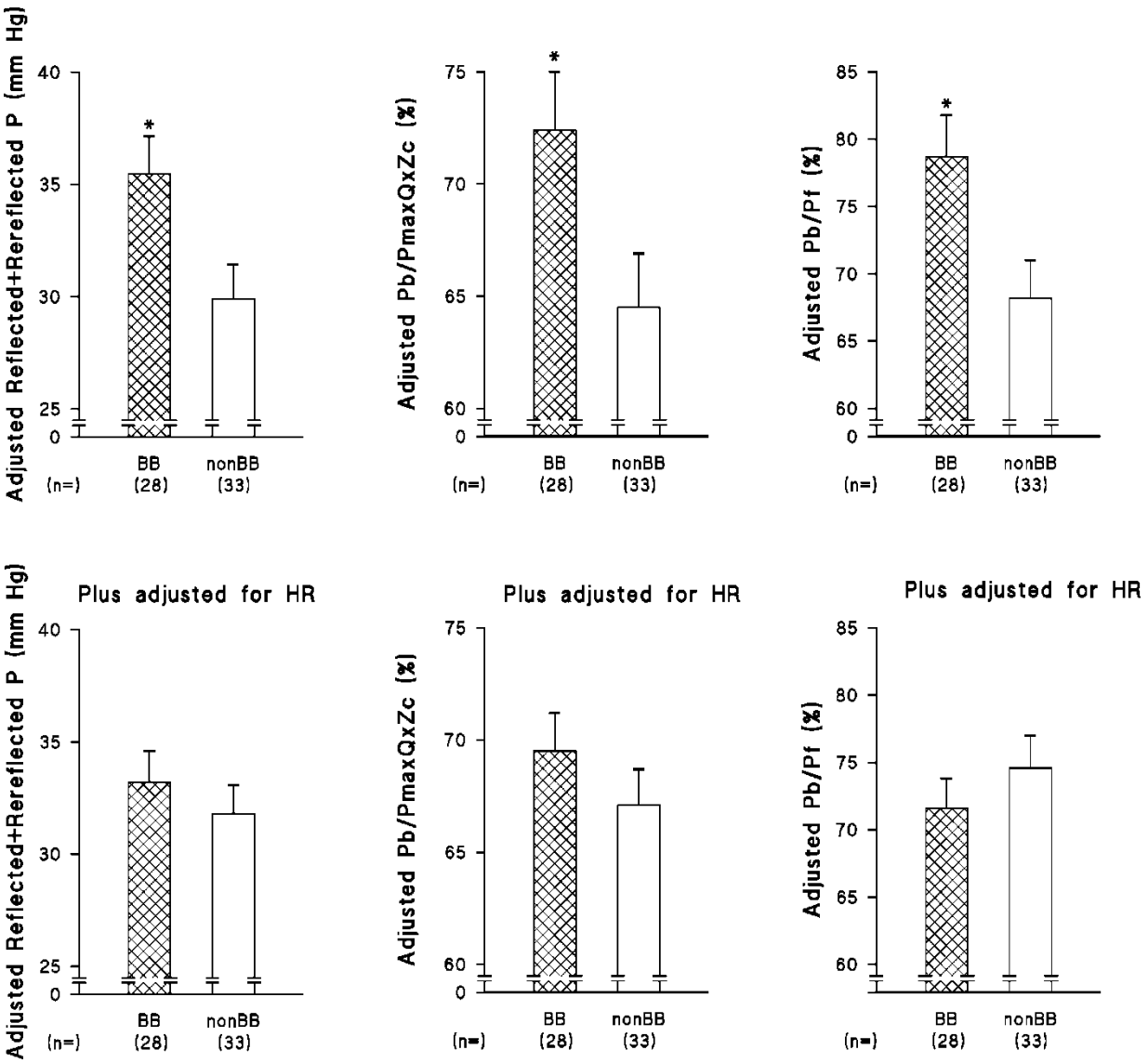

Figure 3.5 Impact of β blocker (BB) therapy on wave reflection and re-reflection. See figure 2.6 for abbreviations. Values in upper panels are adjusted for age, sex, BMI and MAP and values in lower panels are further adjusted for HR. Pb is expressed as a proportion of either Pf or compression wave pressures (Max P_{QxZc}) to exclude the impact of compression waves on wave reflection *p<0.005.

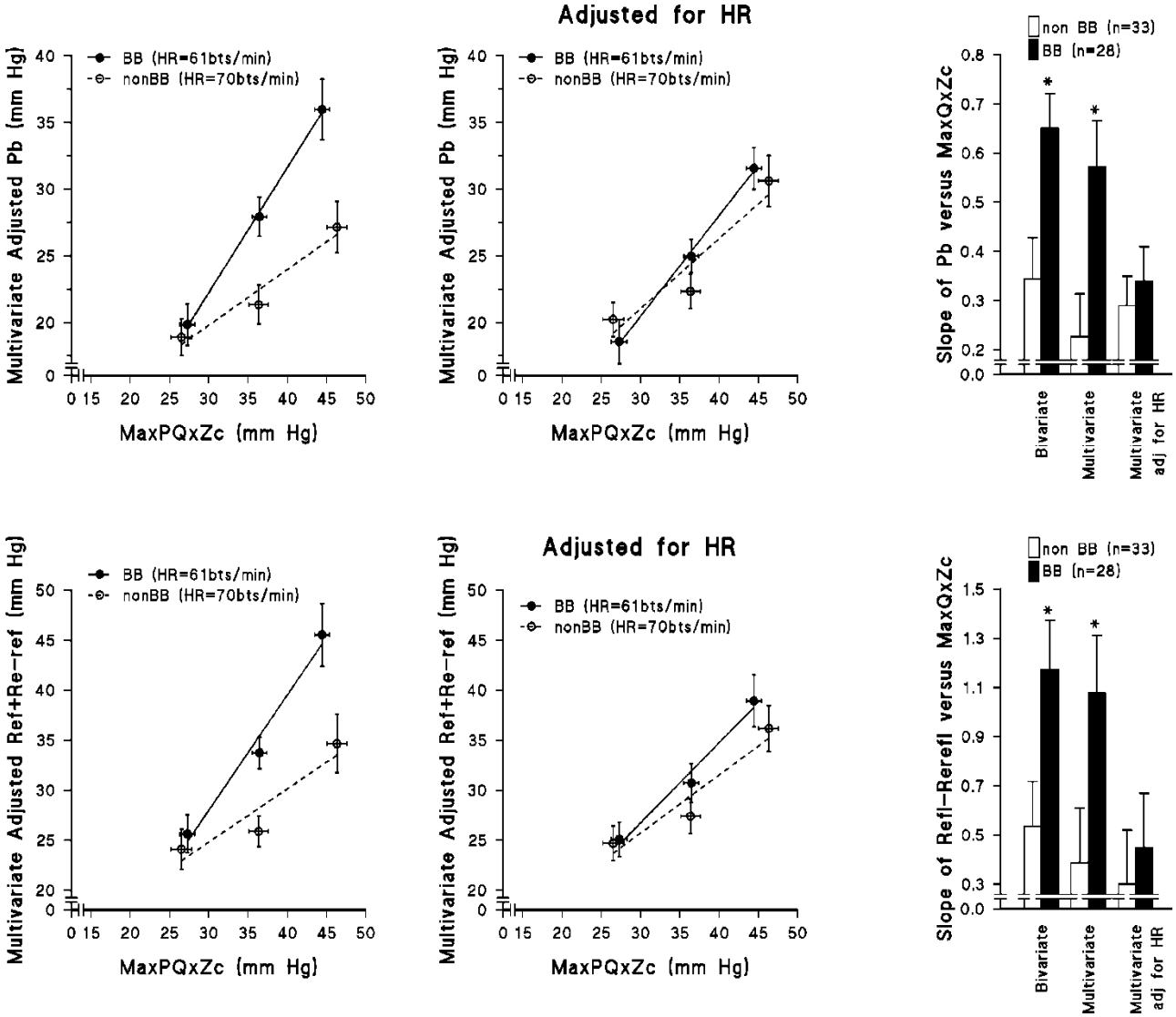

Figure 3.6 Comparison of relationship between compression wave pressures (Max P_{QxZc}) and either backward wave pressures (upper panels) or reflected and re-reflected wave pressures combined in participants receiving atenolol (BB, β-adrenergic receptor blocker) versus those not (Non BB). Adjustments are for age, sex, BMI, MAP and HR as indicated.

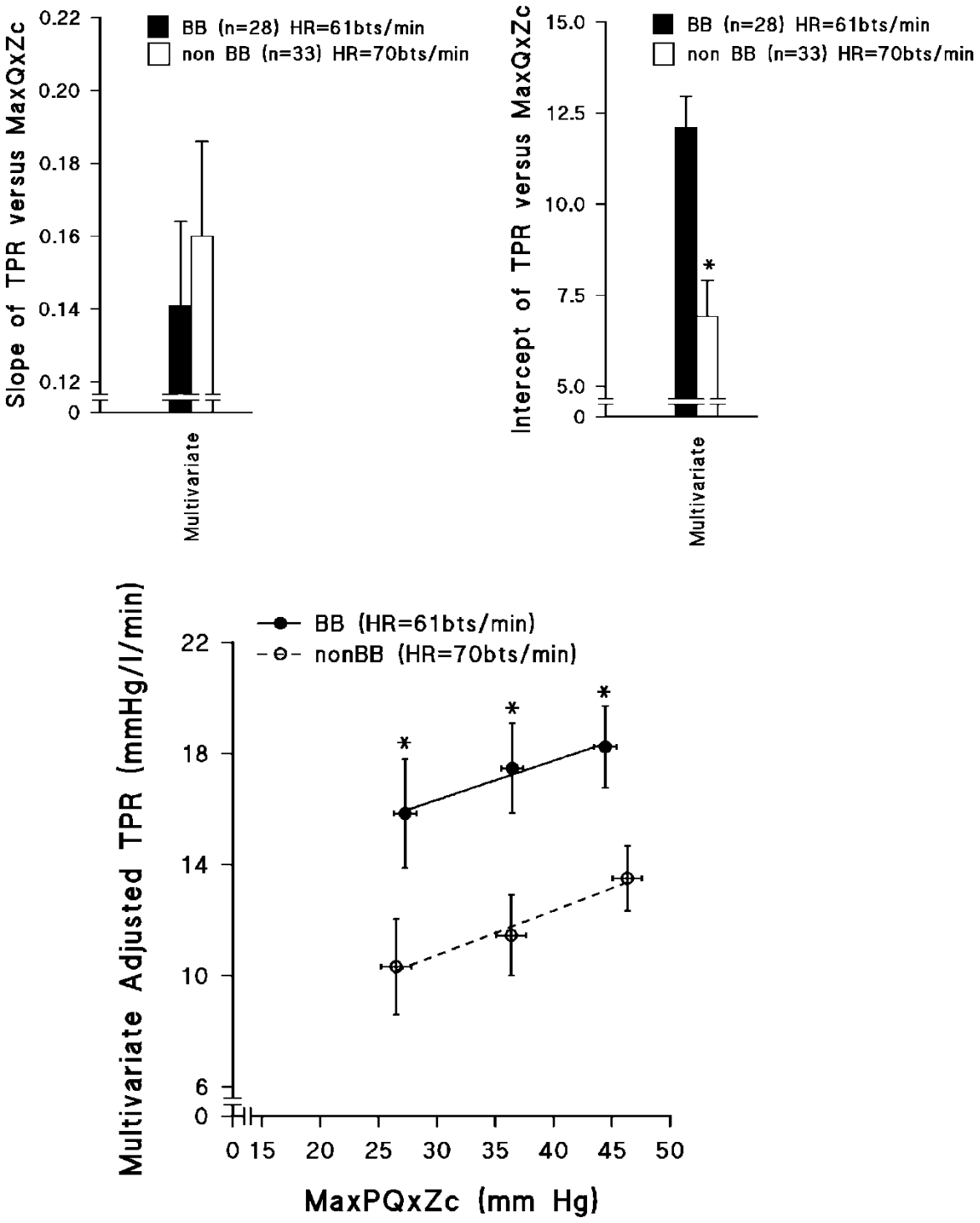

Figure 3.7 Comparison of relationship between compression wave pressures (Max P_{QxZc}) and total peripheral resistance (TPR) in participants receiving atenolol (BB, β-adrenergic receptor blocker) versus those not (Non BB). Adjustments are for age, sex, BMI, and MAP.

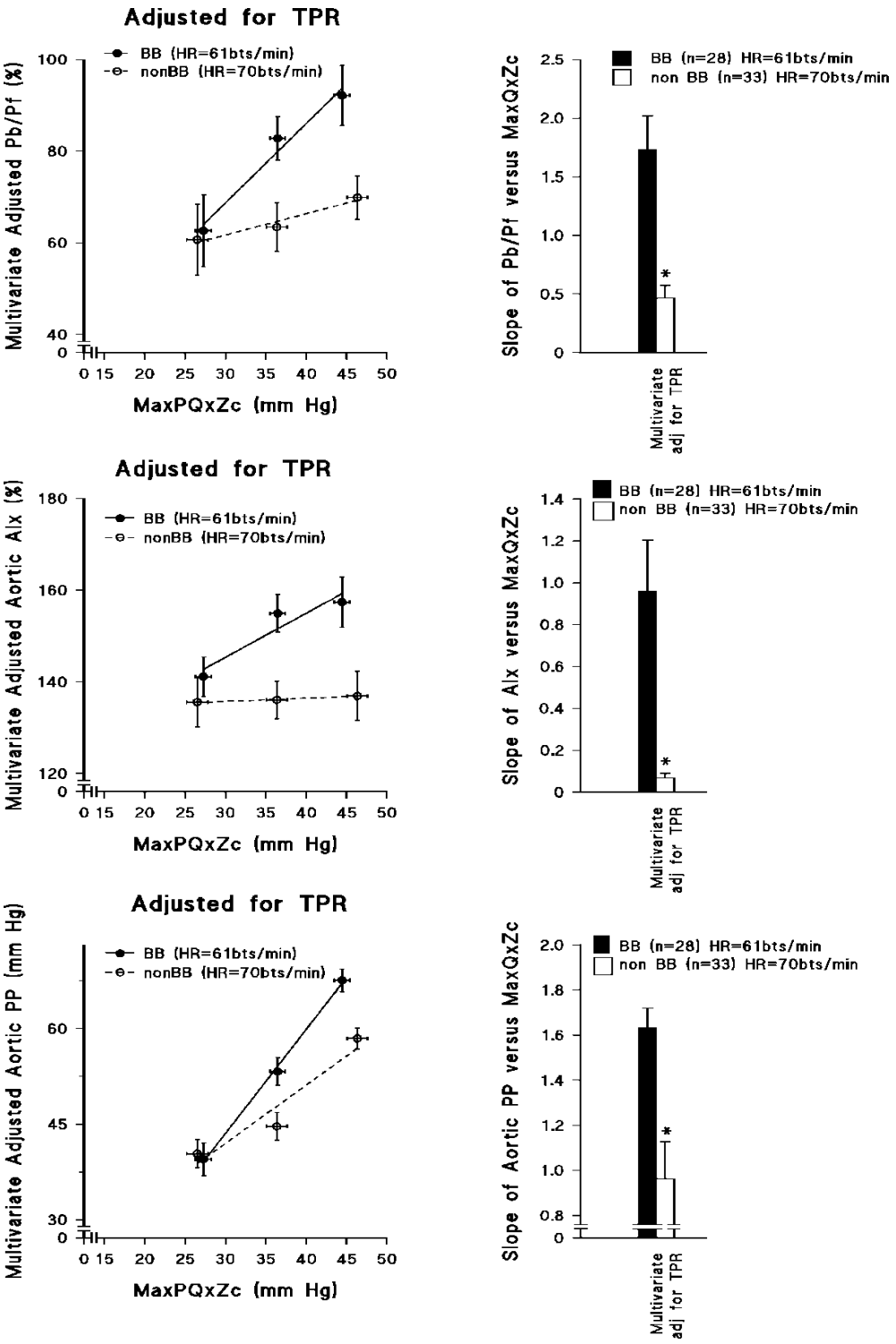

Figure 3.8 Impact of adjustments for total peripheral resistance (TPR) on the relationship between compression wave pressures (Max P_{QxZc}) and aortic reflected wave magnitude (RM), augmentation index (AIx) and aortic pulse pressure (PP) in participants receiving atenolol (BB, β-adrenergic receptor blocker) versus those not (Non BB). Adjustments are for age, sex, BMI, MAP and TPR as indicated.

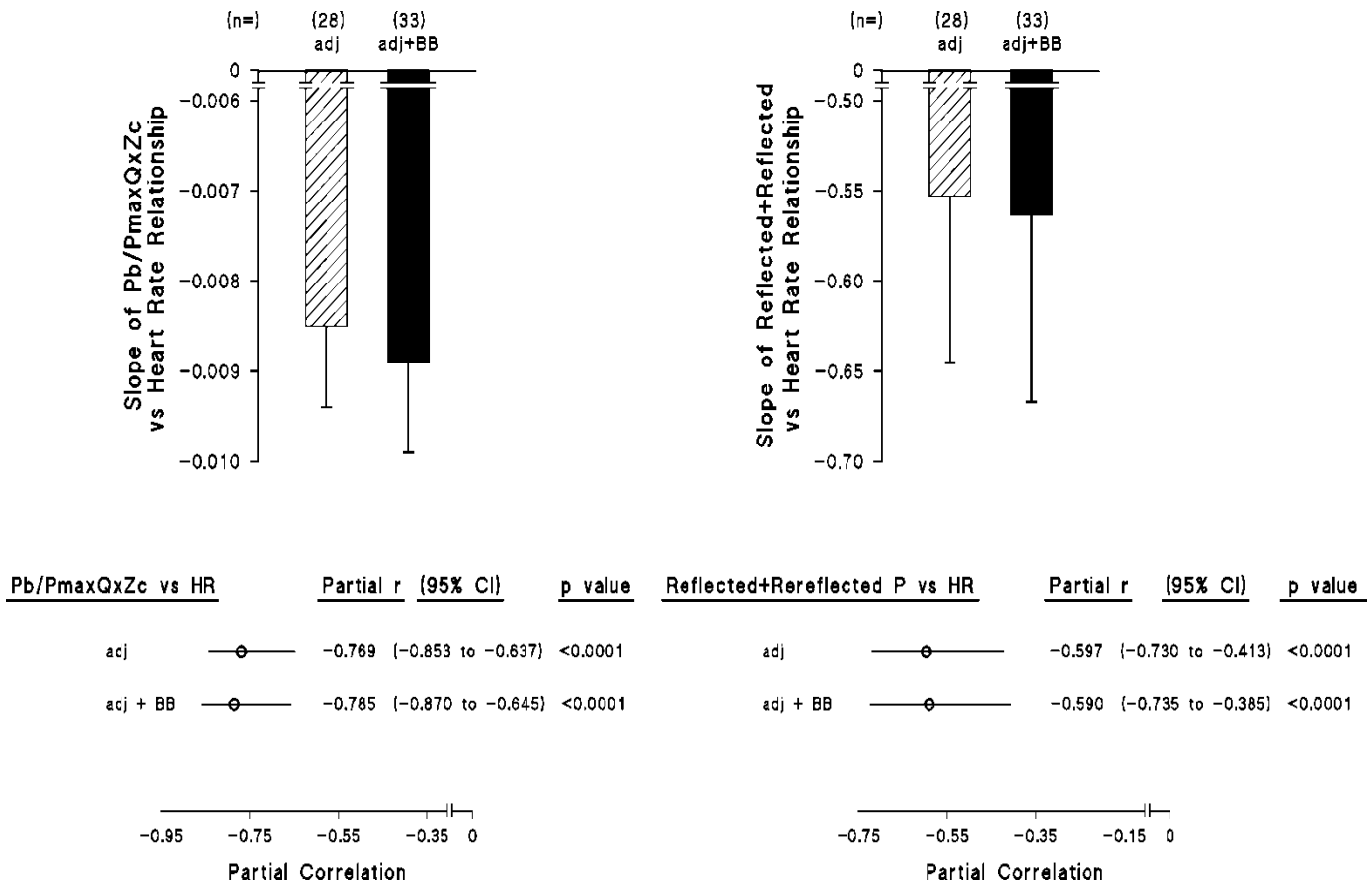

Figure 3.9 Impact of adjustments for the use of atenolol therapy on the independent relations between HR and indices of wave reflection. Upper panels show the impact (slopes) of the relations and lower panels the strength (partial r values) of the relations. BB, β-adrenergic receptor blocker, atenolol. Adjustments are for age, sex, BMI, MAP and atenolol as indicated.

CHAPTER 4: DISCUSSION

4.1 Summary of main findings

The main findings of the present study are as follows: As compared to resistant or refractory hypertensives not receiving the β-adrenergic receptor blocker (atenolol), those receiving atenolol or alternative agents with direct negative chronotropic actions, showed a lower HR, prolonged ED, and increased TPR (systemic vascular resistance) but no difference in peak aortic flow, aortic PP and aortic AIx values. However, at higher maximal compression wave pressures, an increased multivariate adjusted aortic but not brachial PP (increased slope of the relationship between maximal compression wave pressures and aortic PP) was noted in the atenolol-treated group, changes associated with a parallel increase in the slope of the relationship between compression waves and aortic AIx. Although related to a longer ED, atenolol therapy was not associated with an increased contribution of the downslope of compression wave pressures (overlap with the reflected wave) to peak aortic PP. However, reflection magnitude and reflected and re-reflected waves were increased in atenolol-treated resistant or refractory hypertensives, effects that were similarly greatest at higher compression wave pressures (increased slope of the relationship). Moreover, TPR was higher in the atenolol-treated resistant or refractory hypertensives at comparable compression wave pressures. Although the greater slope of the relationship between compression and reflected waves in the atenolol-treated group was eliminated with adjustments for HR, the striking independent relationships between HR and wave reflection in all participants was not affected by adjustments for atenolol use. Moreover, adjustments for differences in TPR failed to modify the increased slope of the relationship between compression waves and aortic PP, AIx or RM in the atenolol-treated group. Thus, the impact of atenolol on wave reflection is attributed entirely to HR effects, rather than to a lack of vasodilator effect on wave reflection.

4.2 Mechanisms of the impact of atenolol on aortic pulse pressure

A number of studies have demonstrated that atenolol-based therapy may reduce peripheral SBP and PP to a similar extent as alternative antihypertensive agents. However, for the same decrease in brachial SBP and PP, atenolol-based therapy has far less of an effect on central arterial SBP and PP when compared to alternative antihypertensive agents (Williams *et al*., 2006; Williams *et al*., 2009; Boutouyrie *et al*., 2010; Pucci *et al*., 2015; Sluyter *et al*., 2016). As discussed in the introduction, these effects are in-part thought to account for the inferiority of atenolol-based

antihypertensive therapy as compared to therapy based on alternative agents on event reduction in several large clinical trials (Dahlöf *et al*., 1991; Yusuf *et* al., 2000; Dahlöf *et al*., 2002; Dahlöf *et al*., 2005). Indeed, in the CAFÉ study a sub-study (Williams and O'Rourke, 2001) of the ASCOT (Sever *et al*., 2001; Dahlöf *et al*., 2005), the inferiority of the β-blocker, atenolol in preventing cardiovascular events was associated with an inability to decrease central aortic pressures as effectively as non-β-blocker-based therapeutic approaches to treating hypertension, despite similar effects on brachial BP (Williams *et al*., 2006). However, whether this effect of atenolol on central arterial pressures is only mediated through the non-vasodilator β_1 selective blockers atenolol and metoprolol, or is a class effect associated with reductions in HR, is nevertheless unclear (Agabiti-Rosei *et al*., 2007). Thus, whether the newer vasodilator β-blockers, nebivolol and carvedilol have unrealised benefits, is unknown. As highlighted in the introduction to the present thesis, to address this question, a better understanding of the mechanisms of action of atenolol on central arterial PP is required and the present study is the first to address this question employing a comprehensive approach to aortic haemodynamic function. How have the findings of the present study advanced our knowledge of the adverse effects of atenolol on central arterial PP?

4.2.1 Contribution of compression wave characteristics

As reviewed in the introductory chapter, atenolol's effect on central arterial PP has been suggested to be the consequence of several factors (Williams and Lacy, 2009). In this regard, one suggestion is that this effect may occur through reductions in HR and the impact of the force-frequency relationship (a decreased HR is compensated for by an increased force of cardiac contraction). Through an enhanced cardiac contraction the peak of Q and hence the peak of the compression wave pressure is increased and aortic PP is enhanced. However, in the present study although HR was markedly lower in those receiving atenolol, this failed to translate into an increased Q or compression wave pressures. Thus, it is unlikely that β-blocker-associated reductions in HR cause increases in aortic PP through an impact of the force-frequency relationship.

Reductions in HR are not only compensated for by an increased peak Q, but also by an extended ED, a change that allows for a greater time for ejection. The increased ejection time in-part maintains SV without necessarily increasing peak flow and compression wave pressures.

However, as long as the reflected wave returns at the same speed, the extended ED may increase the chances of the reflected wave augmenting aortic PP. In this circumstance, the extent to which the compression wave pressures on the downslope contribute to pressures generated by the reflected wave at peak PP (P_{QxZc} at peak PP) will increase. The potential consequence is that increased peak aortic PP will occur even when compression wave pressures are unchanged. This is generally thought to be the main mechanism that accounts for the impact of reductions in HR produced by atenolol and alternative β-blockers on central arterial AIx and PP (Williams and Lacy, 2009). However, in the present study I have shown that the increases in aortic PP that occur at incremental compression wave pressures in those receiving atenolol as compared to those not receiving atenolol are not associated with an enhanced P_{QxZc} at peak PP. Consequently, although extended ED occurs in those receiving atenolol, this does not account for an increased aortic AIx or PP.

4.2.2 Contribution of wave reflection

Atenolol-induced increases in central aortic PP are well documented as being associated with increases in central aortic augmented pressures and augmentation index (chapter 1). These data have been interpreted to suggest that atenolol increases wave reflection. However, as also reviewed in chapter 1, and in-part indicated above, AIx is influenced by several other factors including left ventricular contraction (Hughes *et al*., 2013; Schultz *et al*., 2013; Torjesen *et al*., 2014) and hence peak Q, and an extended ED which increases the chances that the reflected wave will maximally augment aortic PP (Tade *et al*., 2017). However, as indicated in the aforementioned sections of the discussion, in the present study, neither peak Q, nor the contribution of pressures generated by the product of Q and Zc at peak PP were increased at any compression wave pressure in patients receiving atenolol as compared to those that were not. Thus, neither of these mechanisms can explain the impact of atenolol treatment on aortic PP at higher compression wave pressures.

In contrast to alternative antihypertensive agents which reduce arteriolar tone and decrease TPR (systemic vascular resistance), non-vasodilator β-blockers, such as atenolol do not produce the same effect. Consequently, decreases in BP are largely attributed to reductions in HR and hence CO (MAP=CO x TPR). Although there are several possible changes that may contribute to vasodilation in the presence of atenolol therapy (autoregulation of blood flow, decreased

sympathetic output from the CNS, decreased renin release from the kidney), in the present study patients receiving atenolol had a higher TPR. Thus, in the present study we considered whether the impact of atenolol on wave reflection and hence central arterial AIx and PP could be accounted for by increases in TPR at incremental compression wave pressures. If this is the case, then this effect on central arterial pressures is specific to non-vasodilator β-blockers and would readily be addressed by employing vasodilator β-blockers. However, in the present study we show that adjustments for differences in TPR had no effect on either wave reflection, AIx or central arterial PP. Thus, the impact of atenolol on central arterial pressures cannot be attributed to the lack of vasodilator properties of specific β-blockers.

Decreases in HR are predicted to show marked increases in wave reflection through a major impact on the frequency of oscillating waves (harmonic effects) and to some degree through alterations in the visco-elastic properties of the aorta (Xiao *et al*., 2018). Indeed, an inverse relationship between HR and appropriate indices of wave reflection (not just AIx) has been described (Van Den Bogaard *et al*., 2011; Tan *et al*., 2016). In keeping with this notion, in the present study I demonstrated a marked increase in backward wave pressures, RM and combined reflected and re-reflected wave pressures at peak aortic PP over a range of increasing compression wave pressures in atenolol-treated patients. These effects were eliminated by adjustments for HR, just as the greater impact of compression waves on aortic PP and AIx in atenolol-treated patients were eliminated by adjustments for HR. Thus, a direct effect of atenolol on wave reflection, produced by mechanical changes, is likely to explain the impact of atenolol on aortic AIx and PP. Importantly, a striking relationship between HR and wave reflection was noted in all participants and adjustments for atenolol failed to modify this relationship. Thus, the impact of atenolol on wave reflection is unlikely to be attributed to the lack of impact of atenolol on either arteriolar function or more proximal vascular beds that may modify wave reflection.

4.3 Clinical implications

There are several implications of the present study that require consideration. The present study suggests that the adverse effects of atenolol on central aortic PP are a class effect caused by the impact of heart rate-related mechanical alterations in the aorta (harmonics and visco-elastic properties) on wave reflection. These adverse effects are thus not specific to non-vasodilator β_1-adrenoreceptor blockers and hence the use of vasodilator β-adrenoreceptor blockers is not a

solution. Importantly, this class effect of β-blockers (HR-induced alterations in wave reflection mediated by mechanical changes) cannot be specifically targeted. However, an important finding of the present study is that the impact is remarkably sensitive to compression wave pressures. That is, a lower heart rate only translates into a marked increase in wave reflection and hence aortic PP at higher compression wave pressures. In this regard, the recommendations by guidelines that in the treatment of uncomplicated hypertension, β-adrenoreceptor blockers should be reserved for use as second or third line agents only (Chen *et al*., 2010), is in-part appropriate. In this regard, through an impact of reductions in MAP produced by combination therapy, a passive reduction in aortic stiffness and hence Zc will occur and hence compression wave pressures will be reduced to levels where lower HR values will not translate into increases in wave reflection and hence aortic PP. The present data suggest that these values will be achieved only at compression wave pressures of <30 mm Hg. Whether this requires central pressure measurements to identify target BP values, is uncertain. Indeed in patients with increases in aortic stiffness (which often accompanies coronary heart disease, hypertensive HF or atrial fibrillation), increases in brachial SBP may not faithfully parallel changes in compression wave pressures (through a reduced impedance mismatch between central and peripheral arteries compression wave pressures may increase more than brachial PP). Alternatively, in the absence of central aortic BP monitoring, to ensure appropriate central BP targets in those on β-adrenoreceptor blockers, intense SBP lowering may be required (to ensure as low as possible compression wave pressure).

Importantly, the current findings are in-part against recommendations by guidelines, which indicate that when compelled to use these agents (heart failure with a reduced ejection fraction [HFrEF], coronary artery disease and atrial fibrillation or alternative electrical disturbances of the heart), that they can be employed as first-line therapy. The present data rather suggest that if their use is necessary, as in those with cardiac conditions, they should always be employed together with vasodilators or diuretic agents and that intense brachial BP lowering is required or central arterial BP measurements should be employed to guide therapy. In this regard, the vasodilator β_1-adrenoreceptor blockers may have some advantage in that they may serve the dual purpose of reducing HR and simultaneously vasodilating, thus increasing the chances of producing a more striking effect on MAP and hence on compression wave pressures. This may in-part explain how for the same brachial BP reduction, nebivolol and carvedilol lower aortic PP to a greater extent than either atenolol or metoprolol (Dhakam *et al*., 2008; Mahmud and Feely, 2008; Protogerou *et al*., 2009). However, as indicated by the present study, the approach of

simply employing simultaneous vasodilators or vasodilator β-adrenoreceptor blockers does not ensure that reflected wave function and hence aortic PP is maintained at normal levels. Indeed, the adverse impact of atenolol is only observed centrally, whilst no adverse effects on brachial PP are noted. Thus, simply employing agents with direct acting vasodilator properties and targeting brachial BP provides no guidance as to whether appropriate aortic PP values are achieved. In this regard, either central aortic BP-guided therapy is required or intense BP lowering should be achieved with alternative agents.

Of importance, the sensitivity of the adverse effects of HR on wave reflection to compression wave pressures also suggests that patients with HF and systolic dysfunction (heart failure with a reduced ejection fraction [HFrEF]) and a reduced Q are unlikely to develop increases in aortic PP when receiving β-blockers. However, they may require more vasodilation and intense BP lowering as systolic dysfunction improves and Q along with compression wave pressures increases with therapy. Wave reflection is well recognised as being influenced by vascular tone, with arteriolar vasoconstriction increasing RM. However, in the present study despite the increments in TPR noted in those receiving atenolol, adjustments for TPR had no impact on the differences in reflected wave magnitude (RM) noted between the groups. These data highlight the limited impact that arteriolar tone may have on wave reflection beyond compression wave pressure effects (increases in compression waves enhance wave reflection through Newton's Laws of Motion). Importantly, this notion is in-keeping with the impact of vasodilator antihypertensives on wave reflection. Indeed, prior studies that have assessed the impact of vasodilator antihypertensive agents on reflected wave function as indexed by AIx, have provided equivocal results, with a meta-analysis (Manisty and Hughes, 2013) and a large more recent study (Agnoletti *et al*., 2013) demonstrating little effect. Thus, to decrease wave reflection, it is unlikely that any current antihypertensive agent will achieve much of a benefit through direct actions on the vasculature reducing wave reflection, except if large reductions in BP are achieved. Most of the benefits of current antihypertensive agents on wave reflection are likely to be produced by decreasing MAP (aortic distending pressures) and consequently compression wave pressures.

Of importance, the present study was conducted in resistant or resistant hypertensives that are particularly at risk for events. Despite the number of agents employed to manage these patients, although the average diastolic BP noted in these patients reflects a reasonable proportion at target values (in either the group receiving atenolol or not), the average brachial SBP recorded

suggests that only approximately a half of all patients were at target SBP (<140 mm Hg SBP). Of further concern is that well over a half had central arterial SBP at values above target (<130 mm Hg). The present data therefore suggest that resistant or refractory hypertensives are at a particular risk for pulsatile damage even when diastolic BP targets are achieved and hence that compression wave pressures (which drive brachial PP) are higher than what should be achieved. The added central arterial burden of the impact of reductions in HR on wave reflection and peak aortic PP in the atenolol-treated group may create an even greater risk for pulsatile damage. In these resistant or refractory hypertensives the original decision to treat with atenolol was largely historical (initiated prior to when the potential adverse effects of atenolol were identified) rather than based on the presence of compelling indications. The decision to continue treatment is based on the fact that most are reluctant to change medication because of their initial experience with repeated additions or changes in medication often causing side effects. Thus, replacing atenolol is not an option in these patients. Importantly, in resistant or refractory hypertensive patients in general, reductions in HR with atenolol therapy is often useful when diuretic or vasodilator agents produce side effects or are particularly harmful. Moreover, atenolol therapy is often an effective addition to other agents as it reduces BP through decreases in CO rather than TPR, thus acting in synergy with alternative agents. The present study nevertheless, highlights the need to attempt to achieve as low as possible SBP when atenolol therapy is necessary in resistant or refractory hypertensives. In this regard, the level of brachial PP that must be achieved to lower compression waves centrally to a level where HR effects on wave reflection are abolished requires identification. Further large studies are presently underway to identify the brachial PP necessary to achieve this effect.

4.4 Augmentation index and wave reflection

In the present study while reflection magnitude (RM) and reflected + re-reflected wave pressures were increased in atenolol-treated patients as a group, AIx was unchanged except at higher compression wave pressures. In this regard, AIx may underestimate the extent of the change in wave reflection and hence the impact of wave reflection on aortic PP (Booysen *et al*., 2015) for several possible reasons. Augmentation index may be determined not only by wave reflection, but also by left ventricular contraction (Hughes *et al*., 2013; Schultz *et al*., 2013; Torjesen *et al*., 2014), the time to the peak of the Pf in which an extended time increases the chances that reflected waves will maximally augment aortic PP (Tade *et al*., 2017), and by the speed of wave

reflection, where a greater speed will also increase the chances that reflected waves will augment aortic PP. Importantly however, a substantial portion of the Pf is driven by re-reflection of backward wave pressures (Phan *et al*., 2016). Thus, augmented pressure indexes only half of the overall contribution of reflected waves to aortic PP (Figure 1.3). This may explain why when forward and backward wave pressures are included in the same regression model, that despite Pf being quantitatively greater than Pb, Pb but not Pf makes a major contribution to variations in aortic PP in either those younger or older than 50 years of age (Booysen *et al*., 2015). In contrast, the contribution of reflected waves as indexed by Pa to variations in aortic PP is substantially less (Booysen *et al*., 2015). In other words, the impact of reflected waves is markedly underestimated when employing augmented pressures rather than backward wave pressures as an index of wave reflection (Booysen *et al*., 2015). The importance of the use of wave separation analysis rather than the use of AIx as an index of wave reflection is highlighted by the relationships often noted between RM or Pb but not AIx and end organ changes (Booysen *et al*., 2015) or the ability of RM of backward wave pressures to predict events when AIx fails to do so (Chirinos *et al*., 2012; Zamani *et al*., 2014).

4.5 Possible limitations of the present study

Importantly, β-blockers are not recommended as first or even second line therapy unless there are compelling indications for their use (Chen *et al*., 2010). It is therefore, difficult to perform intervention studies to evaluate the impact on aortic function of atenolol when employed as either monotherapy or as add-on therapy in the absence of underlying conditions which may influence aortic function (such as HF, CAD or atrial fibrillation all of which influence ventricular function). Consequently, the present study was conducted as a case-control analysis with important limitations inherent in such analyses. In this regard, unidentified or unmeasured factors (residual confounding) other than the use of atenolol may have explained differences in aortic function between groups. Moreover, aortic function was assessed across incremental compression wave pressures in different rather than the same individuals. Importantly however, in an intervention study performed on spontaneously hypertensive rats (SHR) where simultaneous invasive central arterial pressures, outflow tract velocity and diameter measurements were performed to assess the same parameters as those described in the present dissertation, we have also recently demonstrated that when atenolol is employed as monotherapy, we obtain the same findings as the present study (unpublished data). In this regard,

we evaluated central arterial function over a range of compression wave pressures, produced by injecting the vasoconstrictor, phenylephrine. Thus central arterial function was assessed over a range of compression waves pressures in each rat as opposed to in different individuals as in the present study. In that study conducted in SHR we showed that central arterial PP and AIx were increased at higher compression wave pressures (increased slope of the relations) and that although associated with a reduced HR, longer ED and increased TPR, the longer ED and higher TPR failed to explain the differences noted. As with the present study, the differences noted between atenolol and non-atenolol-treated SHR in aortic function were entirely driven by HR effects on wave reflection and these effects were reproduced by the HR-reducing agent ivabradine. Although data in SHR were collected under anaesthesia, which may influence wave reflection, the consistency of the data across the present study in unanesthetised humans and in anaesthetized rats was striking. Thus, together, these studies complement each other by accounting for limitations present in the other study and at the same time providing data to support the findings of each other.

A second possible limitation of the present study is that calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries (Picone *et al*., 2015). Hence, aortic pressures may have been underestimated using the current approach. However, central arterial PP and all waveform components (forward, backward, re-reflected, etc) would have been similarly affected by this calibration error. A third possible limitation of the present study is that the majority of the participants were of black African ancestry and there were more women than men who participated. Therefore, the present findings may be specific to the dominant ethnic group and gender. However, the similar findings noted in male SHR not reported in the present study suggests that these effects are noted even across species and are therefore unlikely to be ethnic or sex-specific.

4.6 Conclusion

In conclusion, in the present study I provide evidence to show that the adverse effect of the β-blocker, atenolol on central arterial pulsatile load is a class effect mediated by the impact of mechanical changes (harmonic effects on oscillating waves) produced by a decreased HR on wave reflection, rather than through the lack of vasodilator properties of non-vasodilator β-blockers. In this regard, in resistant or refractory hypertensives receiving atenolol I have shown

that HR is reduced and that at higher compression wave pressures, central arterial AIx and PP, but not brachial PP were increased in association with an enhanced wave reflection. Importantly, although these changes were related to an increased ED and TPR, neither of these factors accounted for increases in AIx, wave reflection and hence central arterial PP. However, through mechanical effects of a lower frequency of oscillating waves, and possibly through changes in visco-elastic properties of central arteries, reductions in HR accounted for the impact of atenolol on wave reflection and hence AIx and central arterial PP. As the impact of a lower HR on wave reflection was noted mainly at higher compression wave pressures, these data suggest that when employing any β-blocker (with or without vasodilator properties) intense BP reduction may be required to maintain optimal compression wave pressures and hence prevent the adverse effect of a low end of normal HR on central arterial pulsatile load.

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Appendix

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Mr M Masiu et al
School of Physiology
Medical School
University

E-mail: 789702@students.wits.ac.za

CC: Supervisor: Professors AJ Woodiwiss and G Norton
<Angela.Woodiwiss@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 01/10/2018

REF: *R14/49*

PROTOCOL NO: **M180898** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Impact of heart rate on wave refection in treated refractory hypertensives*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG

R14/49 Mr M Masiu et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180898

NAME: (Principal Investigator)	Mr M Masiu et al
DEPARTMENT:	School of Physiology Medical School University
PROJECT TITLE:	Impact of heart rate on wave refection in treated refractory hypertensives
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M170271
SUPERVISOR:	Professors AJ Woodiwiss and G Norton
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/10/2018

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date of the meeting when the study was initially reviewed. In this case, the study was initially reviewed in **August** and will therefore reports and re-certification will be due early in the month of **August** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES