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Impact of Aortic Pulse Pressure Determinants on the Heart Rate-Aortic Pulse Pressure Relationship: An Intervention Study in Rats

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Impact of Aortic Pulse Pressure Determinants on the Heart Rate-Aortic Pulse Pressure Relationship: An Intervention Study in Rats

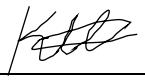
Katleho Nelson Khanye

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

Johannesburg, 2024

Declaration

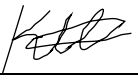
I Katleho Nelson Khanye declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

31st day of March 2025 in Johannesburg.

I certify that the studies contained in this dissertation have been approved by the Animal Ethics Committee of the University of the Witwatersrand, Johannesburg, under the ethics approval number (2021/03/06C).



(Signature of student)

31st day of March 2025

Angela J. Woodiwiss (Supervisor) Vernice R. Peterson (Supervisor)





(Signature of supervisor) (Signature of supervisor)

Presentation Arising from This Study

Conference Oral presentation

Katleho N Khanye, Angela J Woodiwiss, Nonhlanhla Mthembu, Hamza Bello, Glenda Norman, Suraj M Yusuf, Vernice R Peterson. Impact of determinants of aortic pulse pressure on the relationship between heart rate and aortic pulse pressure: an intervention study in rats. Southern African Hypertension Society Biennial Congress 16 - 18 August 2024. Recipient of the Lionel Opie Prize for Third Place in the oral presentation category.

Candidate's role

During my Master's degree, I actively collected non-invasive blood pressure (BP) and heart rate (HR) data before conducting terminal invasive haemodynamic evaluations. On termination day, I assisted with acquiring invasive data, including central pressure waveforms, using the PowerLab Lab Chart system. In addition, I gained practical experience observing the acquisition of echocardiographic images used to assess the cardiac structure and function of the various rat models.

For my main methodology, I extracted the raw pressure wave coordinates (BP and HR) from the PowerLab system and aortic flow-velocity images and aortic diameter measurements from the cardiac ultrasound system. The extracted data was used to calculate characteristic impedance (Zc) and aortic flow (Q) using the wave separation analysis technique. This novel approach involved reconstructing 303 aortic velocity wave images from echocardiographic data using a DICOM Viewer, as outlined in Section 2.5. I collected five images per rat for phenylephrine (PE) and four images per rat for ivabradine (IVB) interventions, as detailed in Figure 2.1. In addition, I conducted wave separation analyses using Q and Zc values to derive key haemodynamic parameters, which included forward wave pressures (Pf), backward wave pressures (Pb), the product of flow and characteristic impedance (PQxZc), reflection and re-reflection, and central pulse pressure (PPc).

Furthermore, I performed the statistical analyses described in Section 2.6 to address the study objectives and overall aim of my Master's research.

Abstract

Heart rate-reducing agents such as β-blockers and ivabradine (IVB) are frequently used in the management of patients with cardiovascular disease (CVD). However, reductions in heart rate (HR) are associated with increases in central aortic pulse pressure (PPc), but not peripheral PP. The inverse HR-PPc relationship, and the mechanisms thereof, have been identified primarily in cross-sectional studies. Hence, intervention studies are required to establish causality and the primary mechanisms. The aim of this intervention study in rats, was to assess the effect of changes in HR on PPc, and the mechanisms involved.

Male spontaneously hypertensive rats (SHR) (n=15) and Wistar Kyoto (WKY) (n=12), were studied. To induce changes in blood pressure (BP) and HR, phenylephrine (PE), followed by IVB was administered to anaesthetised rats. Aortic pressures and flow waves were coupled, and wave separation analysis was used to determine the determinants of PPc, including the forward wave pressure (Pf), backward wave pressure (Pb), reflected pressure, re-reflected pressure, aortic flow(Q), aortic characteristic impedance (Zc), and pressure generated by the product of flow and characteristic impedance (PQxZc).

Heart rate was inversely associated with PPc ($p<0.0001$), backward wave pressure (Pb) ($p<0.0001$), forward wave pressure (Pf) ($p<0.0001$), reflected pressure ($p<0.0001$), and re-reflected pressure ($p<0.0001$). On the contrary, Q, Zc, and PQxZc were not related to heart rate. The slope of the relationship between HR and PPc was markedly diminished by adjustments for Pf, Pb, reflected pressure, or re-reflected pressure ($p<0.05$ to $p<0.0001$).

In an intervention study in rats, we confirm the inverse relationship between HR and PPc and show that wave reflection and re-reflection are the primary mechanisms responsible for the HR-PPc relationship. These data suggest that wave reflection and re-reflection should be targeted in individuals who require HR-reducing agents, such as by intensive lowering of BP.

Acknowledgements

I am deeply grateful for the guidance and support of several esteemed individuals. I would like to express my heartfelt thanks to the late Professor Gavin Norton, Professor Angela Woodiwiss, Dr. Vernice Peterson, Dr. Nonhlanhla Mthembu, and Dr. Suraj Yusuf for their invaluable supervision, patience, knowledge, understanding, leadership, and unwavering dedication. My deepest thanks also go to the School of Physiology, the Helen Driver Fund, the National Research Foundation (NRF) Free-standing Master's Scholarship and the NRF Thuthuka Grant for the sponsorship they have given to me to complete my Master's degree.

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List of Abbreviations

β	Beta
AoD	Aortic root diameter
BP	Blood pressure
CAD	Coronary artery disease
CVD	Cardiovascular disease
CCB	Calcium channel blockers
CO	Cardiac output
DBP	Diastolic blood pressure
ED	Ejection duration
ECG	Electrocardiogram
HF	Heart failure
HR	Heart rate
IVB	Ivabradine
LV	Left ventricle/ventricular
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
MI	Myocardial infarction
PE	Phenylephrine
Pb	Backward wave pressure
Pf	Forward wave pressure
PP	Pulse pressure
PPc	Central pulse pressure
PQxZc	Product of flow and characteristic impedance

Q	Aortic flow
SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rats
SVR	Systemic vascular resistance
VTI	Velocity time integral
Vmax	Maximum aortic velocity
WKY	Wistar Kyoto rats
Zc	Characteristic impedance

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CHAPTER 1: INTRODUCTION

1.1 Cardiovascular Risk Factor: Hypertension

Cardiovascular disease (CVD) remains a significant worldwide health concern, with premature mortality accounting for approximately 31% of all deaths (Mensah *et al*., 2019; World Health Organization, 2020). Among the various risk factors for CVD, hypertension is recognised as the major risk factor bearing the highest population-attributable risk due to its high prevalence in most communities globally (Mills *et al*., 2020). Hypertension is responsible for one in every five cardiovascular-related fatalities (Chobanian *et al*., 2003; Hung, 2024). Additionally, it affects more than a billion individuals worldwide (Chockalingam, 2007) and is responsible for 92 million disability-adjusted life years lost (Menus *et al*., 2018). The Global Burden of Disease (GBD) study identifies hypertension as the foremost cause of premature death and disability worldwide (Campbell *et al*., 2015).

Hypertension is a pivotal risk factor for a range of cardiovascular events, including acute myocardial infarction (MI), heart failure (HF), stroke, and renal failure (Chobanian *et al*., 2003; Irazola *et al.*, 2016). Hypertensive individuals exhibit markedly elevated pulse pressure (PP) and increased aortic stiffness, contributing to elevated cardiac afterload. (Staessen *et al*., 1991; Vlachopoulos *et al*., 2011). The resultant increase in cardiac afterload triggers endothelial dysfunction and vascular remodelling (Stanton and Dunn, 2017). These pathological alterations substantially elevate the risk of adverse cardiovascular outcomes (Vlachopoulos *et al*., 2011). Therefore, effective treatment and management of hypertension are essential to prevent these cardiovascular events. In this regard, the most efficient method of lowering hard clinical endpoints is the pharmaceutical treatment of hypertension.

The selection of anti-hypertensive medication in part depends upon concomitant diseases. Although beta (β)-blockers are currently not first line therapy in the treatment of essential hypertension, heart rate (HR)-reducing agents are required in patients with

concomitant angina, HF or tachyarrhythmia. One of the reasons that β-blockers are not recommended as first-line therapy for the treatment of hypertension, is that despite a similar impact on brachial blood pressure (BP), they fail to reduce central BP to the same extent as other anti-hypertensive agents (Williams *et al*., 2006). The failure of β-blockers to adequately reduce central BP has been attributed to the inverse relationship between HR and central aortic pulse pressure (PPc) (Williams *et al*., 2009). Moreover, increases in central aortic BP are associated with increased risk of morbidity and mortality (Avolio *et al*., 2009; Roman *et al*., 2009). Thus, it is important to understand the mechanisms of the inverse relationship between HR and PPc, to limit the adverse effects of a lower HR on PPc in patients where HR-reducing agents are an essential part of management.

Therefore, my study aimed to assess in an intervention study, the haemodynamic mechanisms of the impact of changes in HR on PPc. To support this aim, in this chapter, I will discuss the role of HR-reducing agents, such as β-blockers and ivabradine (IVB), in the treatment and management of CVD. I will explore their effectiveness in lowering HR to mitigate cardiovascular risks and improve patient outcomes. Furthermore, I will explore the limitations of these agents, particularly their ineffectiveness in reducing central BP compared to their impact on brachial BP, and the subsequent implications for cardiovascular health. This discussion will encompass the inverse relationship between HR and PPc, the determinants of PPc, and the potential mechanisms by which HR reduction may lead to adverse cardiovascular outcomes through increases in PPc. By understanding these relationships and mechanisms, we can better assess the therapeutic strategies for managing hypertension and reducing the burden of CVD.

1.2 The Impact of Heart Rate-Reducing Agents in CVD Management

One of the frequent and essential methods of treating and managing CVD is through the use of HR-reducing agents such as β-blocker and IVB. These agents are pivotal in controlling hypertension and preventing cardiovascular-related events. By lowering HR, HR-reducing agents decrease sympathetic nervous system activity, leading to reduced vasoconstriction and peripheral resistance, which in turn lowers systolic BP (Fox *et al*., 2007). This reduction in BP causes a decrease in myocardial oxygen demand, thereby mitigating the risk of ischemia and other heart-related complications (Heusch and Schulz, 2007). Thus, lowering the HR contributes to better brachial BP control, and is crucial in managing hypertension. Moreover, the use of HR-reducing agents is a critical strategy in reducing the burden of CVD and improving patient prognosis.

However, it has been established that β-blockers, while effective in lowering brachial BP, fail to reduce central BP. In this context, central BP serves as a more reliable predictor of potential adverse cardiovascular outcomes compared to brachial BP, as it accurately reflects the pressure exerted on vital organs such as the heart, brain, and kidneys (Wilkinson *et al*., 1999; Kostapanos *et al*., 2016). Thus, elevated central BP is closely associated with increased arterial stiffness and greater left ventricle (LV) workload, both significant risk factors for cardiovascular morbidity and mortality (Westerhof and O'Rourke, 1995; Avolio *et al*., 2009). The distinction between central and brachial BP is crucial as the primary method of diagnosing and monitoring hypertension involves measuring brachial BP (McEniery *et al*., 2014). However, this method does not account for central BP.

Indeed, the Conduit Artery Function Evaluation (CAFÉ) trial indicated that β-blockers are less effective at reducing central BP compared to calcium channel blockers (CCBs), despite both drugs having a comparable impact on brachial BP (Figure 1.1) (Williams *et al*., 2006).

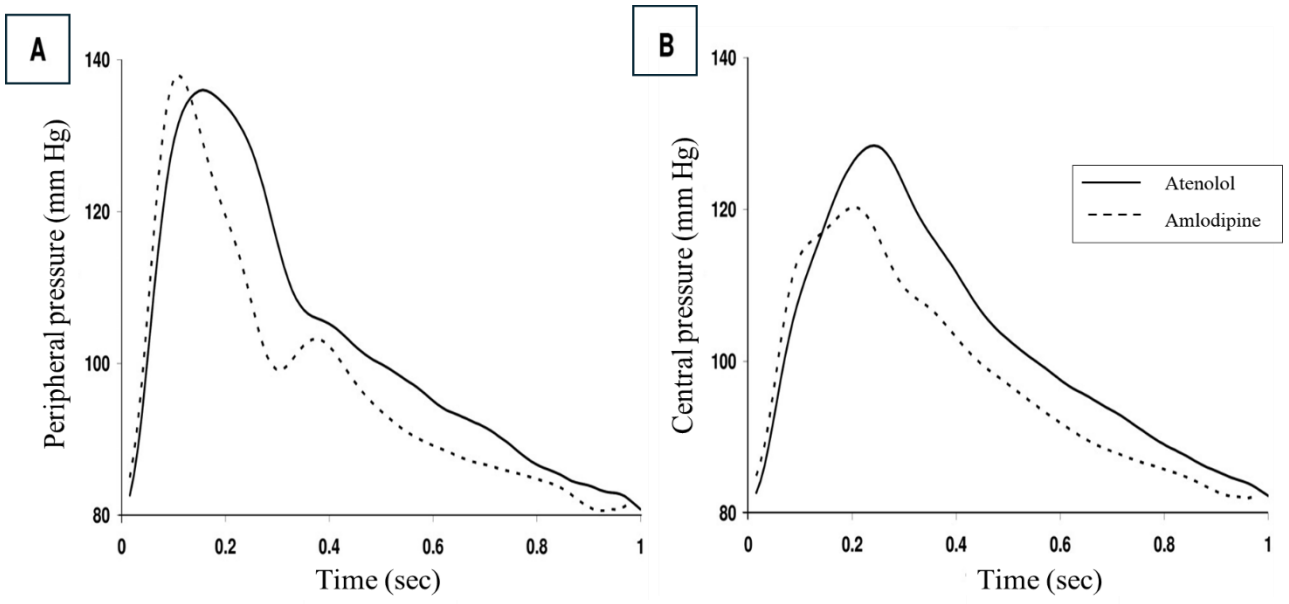


Figure 1.1: The peripheral (brachial) pressure (A) and central aortic pressure (B) waveforms from patients treated with β-blockers - atenolol (*solid line*) or calcium channel blockers - amlodipine (*dashed line*) as monotherapy (modified from Williams *et al*., 2006).

This disparity is due to the impact of HR on PPc, whereby lower HRs are associated with increases in the pulsatile component of central BP, namely PPc (Mthembu *et al*., 2022b). The impact of β-blockers on hypertension outcomes and their effects on central BP raises critical questions regarding the optimal use of HR-reducing agents in CVD without adversely affecting central arterial pressure, which may not be evident peripherally. In HF, β-blockers improve cardiac function by reducing strain on the heart. At the same time, in coronary artery disease (CAD), β-blockers lower the risk of angina and MI by enhancing blood flow to the heart muscle (Ferrari and Fox, 2016). While β-blockers are valuable therapeutic agents for various cardiac conditions, particularly HF and CAD (Steg and De Silva, 2014; Joseph *et al*., 2019), their detrimental effects on central arterial pressure may outweigh their benefits in certain heart conditions often associated with hypertension.

As numerous medications employed in the treatment of CVD lower BP and HR, it is essential to determine and understand the mechanisms through which HR influences PPc. This understanding is important as elevated central BP in patients treated with β-blockers has been associated with the development of left ventricular hypertrophy (LVH) and HF (Deary *et al*., 2002). Furthermore, β-blockers have been linked to higher CVD mortality compared to other antihypertensive drugs, rendering them less effective in reducing CVD-related deaths (Wiysonge *et al*., 2017). Thus, the HR-reducing effects of β-blockers and the associated risks of adverse cardiovascular outcomes due to elevated central BP have become a growing concern in cardiovascular research.

1.3 Inverse Relationship between HR and PPc

The relationship between HR and PPc has been well established (Williams *et al*., 2009; Torjesen *et al*., 2014; Tan *et al*., 2016a; Tan *et al*., 2016b; Rimoldi *et al*., 2016; Alfie *et al*., 2021; Mthembu *et al*., 2022a; Mthembu *et al*., 2022b; Mthembu *et al*., 2022c), demonstrating

a consistent inverse correlation (Figure 1.2). Notably, HR has no significant impact on brachial PP (Figure 1.2) (Williams *et al*., 2009). This inverse relationship between HR and PPc has important implications for cardiovascular health, as it may contribute to the reduced effectiveness of β-blockers in reducing central BP and their association with adverse cardiovascular outcomes (Williams *et al*., 2006; Deary *et al*., 2002; Wiysonge *et al*., 2017).

Previous studies have demonstrated that lowering HR with IVB does not enhance clinical outcomes in individuals with stable CAD (Fox *et al*., 2008; Fox *et al*., 2014). The anticipated advantages of reducing HR in these patients may have been outweighed by an increase in central systolic blood pressure (SBP) (Rimoldi *et al*., 2016). A recent cross-sectional study observed that a lower HR increased PPc in an age-dependent manner, suggesting that age may influence this relationship (Alfie *et al*., 2021). Notably, the study found a steeper increase in PPc among individuals in the lower quartile of HR (<61 beats/min) from midlife (50 years of age) onwards (Alfie *et al*., 2021). Similarly, another cross-sectional study revealed a steeper inverse relationship between HR and PPc in individuals with increased arterial stiffness, including those over 60 years of age (Mthembu *et al*., 2022c).

While various study designs, such as prospective evaluations (Williams *et al*., 2006; Williams *et al*., 2009), randomised controlled trials (Fox *et al*., 2008; Fox *et al*., 2014) computational modelling (Xiao *et al*., 2018), and cross-sectional studies (Tan *et al*., 2016a; Tan *et al*., 2016b; Alfie *et al*., 2021; Mthembu *et al*., 2022a; Mthembu *et al*., 2022b; Mthembu *et al*., 2022c) have provided valuable insights into this relationship, intervention studies are needed to determine the underlying mechanisms of the relationship. There are limited intervention studies which have assessed the impact of HR on PPc. One such study found that HR lowering with IVB, increased central systolic pressure (Rimoldi *et al*., 2016). Another identified a linear inverse relationship between HR and augmentation index (AIx)

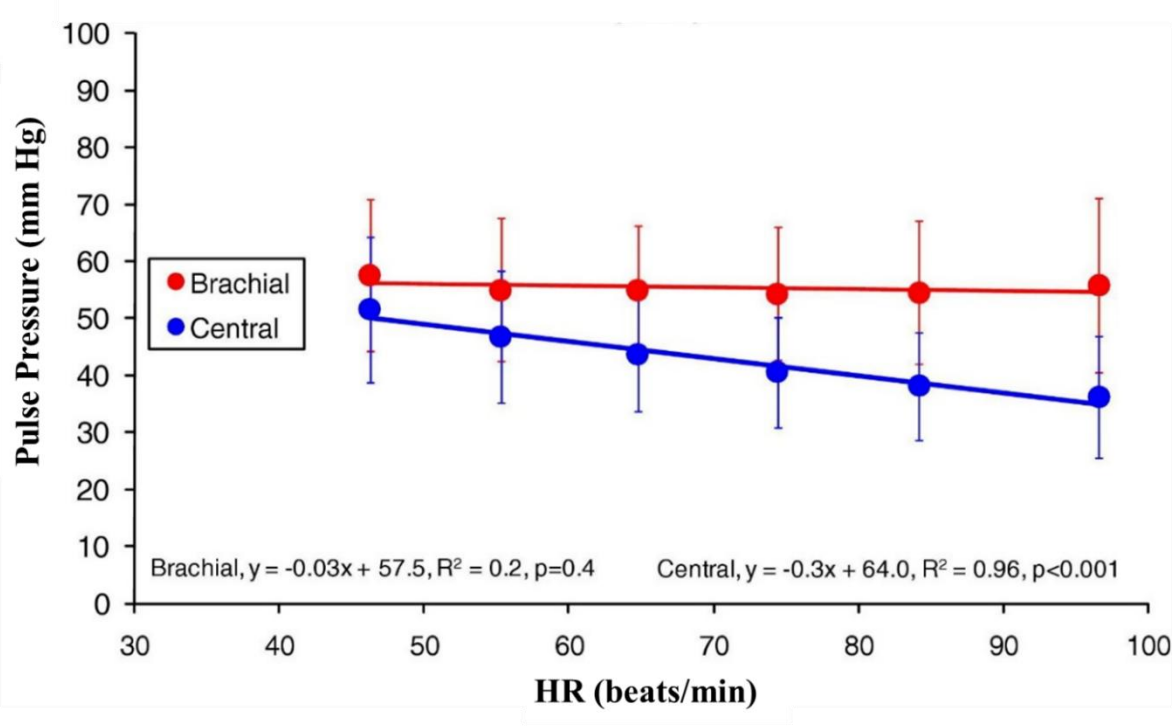


Figure 1.2: Relationship between heart rate, and brachial (*red*) and central (*blue*) pulse pressure (modified from Williams *et al*., 2009).

(Wilkinson *et al*., 2000). Some of the findings from studies that investigated the relationship between HR, brachial PP, and PPc are presented in Table 1.1. Most of these studies indicate an inverse relationship between HR and PPc, with no significant relationship between HR and brachial PP (Table 1.1). However, exceptions are observed in patients with pacemakers, where a direct relationship between HR and brachial PP has been reported (Wilkinson *et al*., 2000; Tan *et al*., 2016a; Tan *et al*., 2016b) (Table 1.1). This difference may be attributed to the artificial regulation of HR by pacemakers, which can modify vascular and haemodynamic responses in ways that differ from the general population. For example, incremental heart pacing in pacemaker patients leads to significant increases in peripheral systolic, diastolic, and mean arterial pressure (MAP) (Giannattasio *et al*., 2003). Additionally, mechanically increasing HR has been shown to reduce arterial distensibility in both large and medium-sized arteries, further contributing to changes in peripheral blood pressure (Wilkinson *et al*., 2000).

Despite these findings, most of these studies do not explore the underlying mechanisms driving the inverse relationship between HR and PPc. Notably, there is limited data on the role of HR reduction in modulating PPc and its determinants. Furthermore, despite the improved capacity to infer causality, compared to cross-sectional studies, there is limited data from previous intervention studies.

1.4 The Role of PPc in Cardiovascular Damage

The inverse relationship between HR and PPc suggests that a reduction in HR results in an elevation of PPc, which can have significant cardiovascular implications. Elevated PPc increases cardiac afterload, placing strain on the LV and potentially leading to LVH (Lorell and Carabello, 2000; Inoue *et al*., 2015). This, in turn, is associated with a higher risk of HF, arrhythmias, and ischemic heart disease (Kahan and Bergfeldt, 2005; Nwabuo and Vasan, 2020). Additionally, higher PPc exacerbates pressure pulsatility, exerting mechanical stress on

Table 1.1: Summary of various studies investigating the relationship between HR, brachial and central PP.

Author	Study Design	Findings	
		HR vs brachial PP	**HR vs central PP**
Alfie *et al*., 2021	Cross-sectional (Patients with stable CAD)	No relationship (P>0.05)	Inverse relationship (P<0.05)
Dillinger *et al*., 2015	Intervention (Patients with stable CAD)	No relationship (P>0.05)	No relationship (P>0.05)
Mthembu *et al*., 2022a	Cross-sectional (Families of black African descent)	No relationship (P>0.05)	Inverse relationship (P<0.05)
Mthembu *et al*., 2022b	Cross-sectional (Families of black African descent)	No relationship (P>0.05)	Inverse relationship (P<0.05)
Mthembu *et al*., 2022c	Cross-sectional (Families of black African descent)	No relationship (P>0.05)	Inverse relationship (P<0.05)
Papaioannou *et al*., 2005	Case-control (Hypertensive and normotensive individuals)	No relationship (P>0.05)	Inverse relationship (P<0.05)
Rimoldi *et al*., 2016	Intervention (Patients with stable CAD)	No relationship (P>0.05)	Inverse relationship (P<0.05)
Tan *et al*., 2016a	Cross-sectional (Patients with implanted pacemakers)	Direct relationship (P<0.05)	Inverse relationship (P<0.05)
Tan *et al*., 2016b	Cross-sectional (Patients with implanted pacemakers)	Direct relationship (P<0.05)	Inverse relationship (P<0.05)
Williams *et al*., 2009	Prospective (Patients with hypertension)	No relationship (P>0.05)	Inverse relationship (P<0.05)
Wilkinson *et al*., 2000	Intervention (Patients with permanent cardiac pacemakers *in situ*)	Direct relationship (P<0.05)	No relationship (P>0.05)
Xiao *et al*., 2018	Computational simulation (Model of the human arterial tree)	-	Inverse relationship (P<0.05)

Abbreviations: HR; heart rate, PP; pulse pressure and CAD; coronary artery disease. Symbol: -; no measurements.

arterial walls, contributing to arterial stiffness and endothelial dysfunction - both pivotal factors in the development of atherosclerosis (Vlachopoulos *et al*., 2011; Nwabuo and Vasan, 2020).

A sustained increase in PPc is associated with target organ damage and an elevated risk of cardiovascular events (Franklin *et al*., 2001). Central systolic blood pressure (SBP) and PPc, in particular, are more accurate predictors of coronary heart disease and a risk for congestive heart failure compared to peripheral (brachial) PP (Franklin *et al*., 2001; Haider *et al*., 2003). Additionally, elevated PPc can impair microcirculation, leading to increased resistance to mean blood flow (Safar and Lacolley, 2007; Laurent *et al*., 2022). The Framingham Heart Study (Mitchell *et al*., 2005) found that increased aortic stiffness and elevated pressure pulsatility are associated with impaired microvascular reactivity and ischemic stress, resulting from higher PPc (Laurent and Boutouyrie, 2007; Laurent *et al*., 2022). Additionally, high PPc is strongly correlated with markers of target organ damage, such as arterial stiffness, intima-media thickness (IMT), and estimated glomerular filtration rate (eGFR) (Wang *et al*., 2009). These findings suggest that elevated PPc may play a significant role in the progression of cardiovascular and kidney damage.

1.5 Central Aortic Pulse Pressure Determinants

Central aortic pulse pressure (PPc) significantly determines cardiovascular damage beyond MAP (Staessen *et al*. 1991). The two principal pressures that influence PPc are the forward travelling pressure wave (Pf) and the backward travelling pressure wave (Pb) (Figure 1.3, panel 1) (Li *et al*., 2017; Tade, 2019). The peak PPc results from the summation of these pressures (Figure 1.3, panel 1) (Li *et al*., 2017; Tade, 2019). The Pf is generated by LV contraction, which ejects blood into an elastic conduit - the aorta (Torjesen *et al*., 2014). The ability of the LV to contract and produce aortic flow (Q), as well as the resistance to Q in the aorta, determine the aortic Pf (Vlachopoulos *et al*., 2011). The aorta, like any other conduit

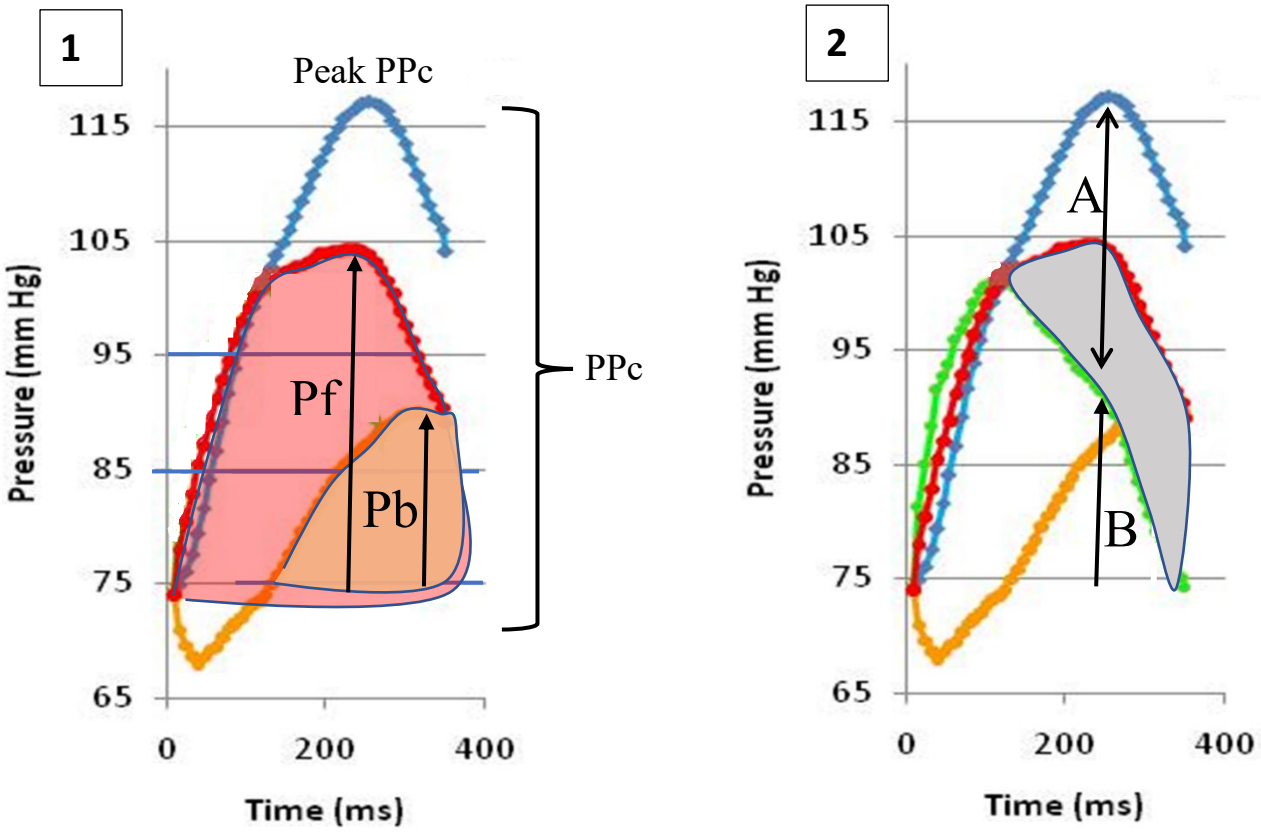


Figure 1.3: PPc (*blue*) and its determinants. Panel 1 shows the sum of the contribution of Pf (*red*) and Pb (*orange*). Panel 2 shows re-reflected wave (*grey shading*) and hence the contribution of reflected + re-reflected wave pressures (A) and the pressure generated by the product of flow and characteristic impedance (PQxZc) (*green*) (B) to PPc. Abbreviations: PPc; central aortic pulse pressure, Pf; forward wave pressure, Pb; backward wave pressure, Q; aortic flow, Zc; characteristic impedance.

carrying pulsatile flow, has a resistance to flow which is referred to as characteristic impedance (Zc) in the absence of wave reflection during blood ejection (Vlachopoulos *et al*., 2011). During left ventricular contraction, the pressure generated drives blood flow into the aorta. This pressure wave interacts with the Zc of the aorta, which is determined primarily by aortic stiffness and diameter (Nichols *et al*., 2011). Increased aortic stiffness, as seen with aging and hypertension, decreases Zc, while reduced aortic diameter can increase it. In a healthy aorta, the Zc is relatively high due to greater elasticity; thus, pressure waves are efficiently transmitted, resulting in optimal flow (Vlachopoulos *et al*., 2011; Goel *et al*., 2017). However, in hypertension, the higher Zc from reduced aortic compliance can lead to increased pulsatile flow and elevated systolic pressure (Nichols *et al*., 2011).

The pressure wave produced by ventricular ejection moves rapidly in a forward direction through the arterial tree, to reach the brachial arteries and other peripheral vessels (Perloff, 2009). The pressure wave generated in the proximal aorta is the same wave seen in all arterial beds downstream; however, pressure attenuates as it moves through the conduit (Mynard and Kondiboyina, 2022). The pulsatile pressure wave that travels to all arterial beds (i.e., Pf), reflects the force of ventricular contraction and the degree to which the aorta alleviates the strain on the cardiovascular system (Mynard and Kondiboyina, 2022).

Additionally, Pf reflects the contribution of elastic recoil to diastolic flow in the arteries, as the aorta stores energy during systole and uses it to maintain blood flow during recoil (Nichols *et al*., 2011). Elastic recoil refers to the ability of the arterial walls, particularly the aorta, to return to their original shape after being stretched by systolic pressure (Saghiv *et al*., 2020; Schaafs and Elgeti, 2024), thus maintaining diastolic blood flow and ~~by~~ ensuring continuous perfusion even when the heart is not actively contracting.

However, in hypertension, the loss of elastic recoil and the subsequent stiffening of the aorta contribute toward elevated Pb. The elevation in Pb results from the reflection of Pf from the stiffer peripheral arterial system, resulting in greater backward wave augmentation and a subsequent increase in PPc (Belz,1995; Nichols *et al*., 2011; Mthembu *et al*., 2022b).

Increases in PPc in hypertension are also attributed to changes in the speed of Pb. In the arterial system, blood flow is transmitted from the aorta to the peripheral arterial tree, while Pb is generated by the return of the Pf from the periphery to the proximal aorta (Torjesen *et al*., 2014). Unlike Pf, which propagates forward, Pb travels in the opposite direction, influencing central pressure. Under normal physiological conditions, the reflected wave returns to the aorta during diastole, aiding coronary perfusion (Torjesen *et al*., 2014). However, with arterial stiffening, as seen in aging and hypertension, the speed of wave reflection increases thus causing the reflected wave to arrive earlier, during systole, when systolic wall stress is elevated (O'Rourke and Nichols, 2005). As a result, the premature return of Pb increases the likelihood of its overlap with Pf, amplifying PPc.

The increase in Pf can be attributed to the re-reflection of backward waves, further compounding central pressure augmentation (Phan *et al*., 2016) (Figure 1.3, panel 2). Pulsatile pressure waves are reflected at points of impedance mismatch, such as vascular bifurcations and areas of tapering (Davies *et al*., 2012). When these reflected waves return to the aorta, they undergo re-reflection at the proximal aorta, merging with the incident Pf (Nichols *et al*., 2011; Davies et al., 2012). This interaction enhances peak Pf, making it less dependent on the direct relationship between flow and characteristic impedance (PQ × Zc) (Figure 1.3, panel 2). The beneficial impact of reducing wave reflections lies in minimizing excessive central pressure, which alleviates cardiac workload and improves ventricular-vascular coupling. However, systemic flow alone may not fully explain the increased PPc observed with HR-lowering therapies. Instead, alterations in wave dynamics, including reduced wave re-reflection and

changes in arterial compliance, are likely to contribute significantly to this phenomenon. Consequently, increased Pb and the reinforcement of backward wave reflections may exacerbate adverse cardiovascular effects, underscoring their role in the inverse relationship between HR and PPc.

The increase in Pf can be attributed to the re-reflection of backward waves (Phan *et al*., 2016) (Figure 1.3, panel 2). It is well established that pulsatile pressure waves are reflected at points where there is an impedance mismatch, such as areas of vascular tapering (Davies *et al*., 2012). When these reflected waves return to the aorta, they are again re-reflected off the proximal aorta (Nichols *et al*., 2011; Davies *et al*., 2012). These re-reflected pressure waves merge with the Pf in the aorta. Hence, as PPc increases, peak Pf may not be solely determined by the pressure generated from the product of flow and characteristic impedance (PQxZc) (Figure 1.3, panel 2). Instead, a significant portion of Pf may be influenced by the re-reflection of the backward wave. As a result, increases in Pb and re-reflected pressure may produce adverse cardiac effects. These determinants are thought to play a significant role in the mechanisms underlying the inverse relationship between HR and PPc.

1.6 Mechanisms Proposed to Explain the HR-PPc Relationship

Theoretical mechanisms proposed to explain the relationship between a reduced HR and increased PPc suggest that a lowered HR extends the diastolic period (Williams *et al*., 2006). This prolonged diastolic phase is believed to enhance left ventricular (LV) filling time and ejection duration (ED), which in turn increases cardiac contraction and stroke volume (SV) (Nieminen *et al*., 2006; Avolio *et al*., 2009; Tan *et al*., 2016a; Sluyter *et al*., 2016). The resulting increase in SV is proposed to augment Q and the Pf component, thereby amplifying PPc (Nieminen *et al*., 2006; Avolio *et al*., 2009). However, the increase in systemic flow appears to

have a beneficial impact, as it enhances organ perfusion and maintains adequate tissue oxygenation rather than imposing an additional cardiovascular burden (Bello *et al*., 2021).

Recent findings suggest that systemic flow alone may not be the primary mechanism driving the increase in PPc observed with HR-lowering therapy (Bello *et al*., 2021; Mthembu *et al*., 2022b). While aortic flow (Q) contributes to PPc, other factors such as arterial wave reflection, vascular compliance, and impedance mismatch play significant roles (Belz,1995; O'Rourke and 2022; Nichols *et al*., 2011; Mthembu *et al*., 2022c). Heart rate reduction alters wave dynamics by increasing the time available for the reflected waves to return to the aorta, predominantly in the diastolic period. Therefore, this prolonged diastole allows more time for reflected waves to return to the aorta, potentially amplifying Pb and thus increasing central pressure independently of changes in systemic flow

An alternative explanation for the HR-PPc relationship involves changes in harmonic effects (Xiao *et al*., 2018). When pressure waves travel through the arterial system, their interactions are governed by wave interference principles, particularly constructive and destructive interference (Schäberle and Schäberle, 2011). Constructive interference occurs when two waves of the same frequency and phase align, reinforcing each other and increasing overall wave amplitude (Quick *et al*., 2001; Schäberle and Schäberle, 2011; Segers and Chirinos, 2022). In contrast, destructive interference occurs when waves of opposite phases overlap, causing partial reduction or complete cancellation of the pressure waveform, thus affecting the wave amplitude (Quick *et al*., 2001; Schäberle and Schäberle, 2011; Segers and Chirinos, 2022).

Indeed, as the heart ejects blood, it generates forward pressure waveforms that travel distally, and form Pf. These forward pressure waves encounter changes in the vasculature, such as branching, stiffening, and narrowing of arteries, and which reflect them back towards the

heart as Pb (Nichols *et al*., 2011). Furthermore, multiple minor reflection sites exist along the aorta, collectively contributing to the overall reflected wave (Davies *et al*., 2012). These sites arise at locations of impedance mismatch, where the degree of impedance mismatch determines the reflection intensity (Nichols *et al*., 2011; Davies *et al*., 2012). A greater mismatch yields stronger reflections, while smaller mismatches result in weaker ones (Davies *et al*., 2012). As forward and backward pressure waves interact in the conduit arteries, waves of the same frequency and amplitude meet and cancel each other out. However, at lower HRs, the reduced frequency of the forward pressure waves leads to decreased cancellation of backward pressure waves (Xiao *et al*., 2018). These backward waves are then re-reflected at the aorta and combine with newly generated forward pressure waves, resulting in increased PPc.

If the inverse HR-PPc relationship were driven primarily by changes in Q, this would be beneficial, as it would reduce the workload and improve haemodynamic efficiency. However, if the mechanism involves an increase in Pf, Pb and re-reflected pressure, this would be detrimental, as it could lead to elevated afterload and increased cardiovascular risk. Therefore, understanding the underlying mechanisms that determine whether HR reduction is beneficial or detrimental in managing central arterial pressure in patients with cardiovascular disease is essential.

1.6.1 Current Understanding About the Mechanisms of the HR-PPc Relationship

Previous studies have investigated the inverse relationship between HR and PPc, indicating various mechanisms that contribute to this association (see Table 1.2). Recent cross-sectional as well as modelling studies have demonstrated that lower HRs correlate with increased Pf, Pb, re-reflected pressure, reflection magnitude (RM), and augmentation index (AIx), all of which contribute to an increase in PPc (Tan *et al*., 2016a; Xiao *et al*., 2018; Mthembu *et al*., 2022a; Mthembu *et al*., 2022b; Mthembu *et al*., 2022c) (see Table 1.2).

Table 1.2: Summary of various studies and mechanisms found to explain the inverse relationship between HR and PPc.

Author	**Study Design**	**Mechanisms**
		Inverse relationship HR vs PPc
Alfie *et al*., 2021	Cross-sectional	-
Avolio *et al*., 2009	Review	-
Dillinger *et al*., 2015	Intervention	-
Mthembu *et al*., 2022a	Cross-sectional	↑ Pb and re-reflected pressure
Mthembu *et al*., 2022b	Cross-sectional	↑ Pf, Pb, and re-reflected pressure
Mthembu *et al*., 2022c	Cross-sectional	↑ Pf and Pb
Nieminen *et al*., 2006	Review	-
Papaioannou *et al*., 2005	Case-control	-
Rimoldi *et al*., 2016	Intervention	-
Sluyter *et al*., 2016	Case-control	↑ EDV, SV and Q
Tan *et al*., 2016a	Cross-sectional	↑ Pf and Pb
Tan *et al*., 2016b	Cross-sectional	-
Torjesen *et al*., 2014	Cross-sectional	↑ AIx
Wilkinson *et al*., 2000	Intervention	↑ AIx and ED
Williams *et al*., 2006	Prospective	↑ AIx and ED
Xiao *et al*., 2018	Computational simulation	↑Pf, Pb, RM, and AIx

Abbreviations: HR; heart rate, PPc; central pulse pressure, AIx; augmentation index, and ED; ejection duration, Pf; forward wave pressure, Pb; backward wave pressure, RM; reflection magnitude, EDV; end-diastolic volume, SV; stroke volume, and Q; aortic flow. Symbols: ↑; increased, -; no measurements.

This occurs because lower HRs extend the travel time of Pf through the central arterial system. As these waves encounter areas of impedance, they generate Pb, which are reflected toward the heart (Nichols *et al*., 2011; Davies *et al*., 2012). In addition, these backward waves may undergo re-reflection at multiple points along the aorta. The interaction between re-reflected backward and newly generated forward waves contributes to an overall increase in PPc (Davies *et al*., 2012; Xiao *et al*., 2018).

In line with these findings, an intervention study identified an inverse relationship between HR, AIx, and ED (Wilkinson *et al*., 2000) (see Table 1.2). While ED, which may affect SV, can influence PPc, the AIx alone does not accurately capture how Pf and Pb contribute to PPc. Another intervention study also found an inverse relationship between HR and PPc (Rimoldi *et al*., 2016) (see Table 1.1); however, this study did not measure the determinants of PPc, similarly with Papaioannou *et al*., (2005) Tan *et al*., (2016b) and Alfie *et al*., (2021) (see Table 1.2).

While computational modelling (Xiao *et al*., 2018) has provided valuable insights into the relationship between HR and PPc, these models often oversimplify the complexities of the human arterial system. Consequently, they may fail to accurately represent the physiological and anatomical variability inherent in human arteries, potentially impacting the reliability of the findings. Moreover, with limited data available from intervention studies (Wilkinson *et al*., 2000; Dillinger *et al*., 2015; Rimoldi *et al*., 2016), much of our understanding of the haemodynamic mechanisms involved in the HR-PPc relationship is derived from cross-sectional studies (Torjesen *et al*., 2014; Tan *et al*., 2016a; Tan *et al*., 2016b; Alfie *et al*., 2021; Mthembu *et al*., 2022a; Mthembu *et al*., 2022b; Mthembu *et al*., 2022c). While these findings seem plausible according to the proposed mechanisms, it is important to recognise their limitations, as cross-sectional studies are subject to numerous confounding factors. The nature

of the design limits the ability to establish causality, highlighting the necessity for intervention studies which may provide a more definitive understanding of causal relationships.

Therefore, this study aimed to investigate the relationship between HR and PPc and the primary mechanisms involved using an animal model to control for confounding factors such as differences in age, gender, lifestyle, disease, and medication. In this regard, clinical studies are no longer possible with HR-reducing therapy such as β-blockers employed as monotherapy. In addition, IVB is generally recommended as an add-on therapy rather than the sole treatment. Thus, only studies in animal models will adequately answer this question.

1.7 Aim

The aim of this study is to assess the effect of changes in heart rate (HR) on central aortic pulse pressure (PPc) and determine the haemodynamic mechanisms involved in the relationship between HR and PPc in spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats.

1.7.1 Objectives

To achieve this aim, my objectives were to:

1. determine the impact of HR on PPc in spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats using non-invasive echocardiography and invasive central aortic pressure measurements.

2. identify and assess the haemodynamic mechanisms by which administration of the vasoconstrictor phenylephrine (PE) and the HR-reducing agent ivabradine (IVB) changes central aortic pressure in SHR and WKY using non-invasive echocardiography and invasive central aortic pressure measurements.

3. identify the determinants of PPc which contribute to the increase in PPc in response to decreases in HR in SHR and WKY rats using non-invasive echocardiography and invasive central aortic pressure measurements.

CHAPTER 2: METHODS

2.1 Study Design and Animal Model

The present intervention study was conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Academies press, 8th edition, 2010). The Animal Ethics Screening Committee of the University of the Witwatersrand reviewed and approved all procedures (approval number: 2021/03/06C).

The study design is presented in Figure 2.1. Fifteen (15) spontaneously hypertensive rats (SHR) and twelve (12) Wistar Kyoto (WKY) rats, all males at 17 months of age, were studied (Figure 2.1). The rats were housed three (3) per cage in standard rat cages and kept in the Wits Research Animal Facility (WRAF) in an ambient temperature-controlled room with a 12-hour light-dark cycle. The rats were allowed access to standard rat chow (Epol, RSA) and water *ad libitum*.

The SHR model was selected for this study due to its well-established physiological and haemodynamic similarities to human essential hypertension. Spontaneously hypertensive rats (SHRs) exhibit progressive arterial stiffness, sustained elevations in blood pressure (BP), and enhanced wave reflection (Schiffrin, 2012; Lerman *et al*., 2019) making them a relevant model for exploring the interplay between heart rate (HR) and central pulse pressure (PPc) in hypertensive conditions. Both WKY and SHR were used in the current study to obtain a wide range of both BP and HR values to adequately assess the relationships between HR and PPc. Due to the higher blood pressures in SHR, in response to PE, greater final BP values can be attained than in WKY. In addition, lower final HR values can be obtained due to the lower HR values (in compensation for the higher blood pressure values) than in WKY.

The selection of 17-month-old rats was based on the understanding that hypertension-associated vascular remodelling and arterial stiffness progress with age. At 17 months, SHRs are expected to exhibit advanced hypertension, characterised by pronounced structural and

functional vascular changes (Chan *et al*., 2011; Al-Gburi *et al*., 2017; Saheera and Krishnamurthy, 2020). The changes have a substantial impact on wave reflection dynamics and PPc augmentation. Investigating haemodynamic mechanisms in aged SHRs enhances the translational relevance of the study, aligning the experimental model with chronic hypertension and vascular dysfunction commonly observed in aging human populations.

Data was collected as described below in sections 2.2, 2.3 and 2.4 for both the hypertensive (SHR) and the normotensive (WKY) rats that were used in this study. The rationale for the use of SHR and WKY was to maximise the range of BP and HR values obtained, and thus to optimise the correlation analyses. Hence, the data from the normotensive and hypertensive rats were combined in the analyses for this dissertation (Figure 2.1). The pressure data collected was extracted and paired with the recreated aortic velocity flow waves as described in sections 2.4 and 2.5.

2.2 Non-invasive Measurements

Non-invasive tail-cuff BP and HR measurements were determined before the terminal invasive haemodynamic evaluations (described in section 2.3 below) were performed. The BP and HR measurements were performed in awake, restrained rats using standard tail cuff techniques (BIOPAC systems, Inc. NIBP250 blood pressure amplifier, Bulgaria, Europe) (Machholz *et al*., 2012; Mosaad *et al*., 2017). The rats were habituated to the tail-cuff measurements by placing them in restrainers for 10 minutes at a time on three separate occasions and allowing them to undergo the BP and HR measurements procedure before the actual BP and HR values were measured and recorded. The habituation process reduced the stress response of the rats during the actual BP and HR measurements.

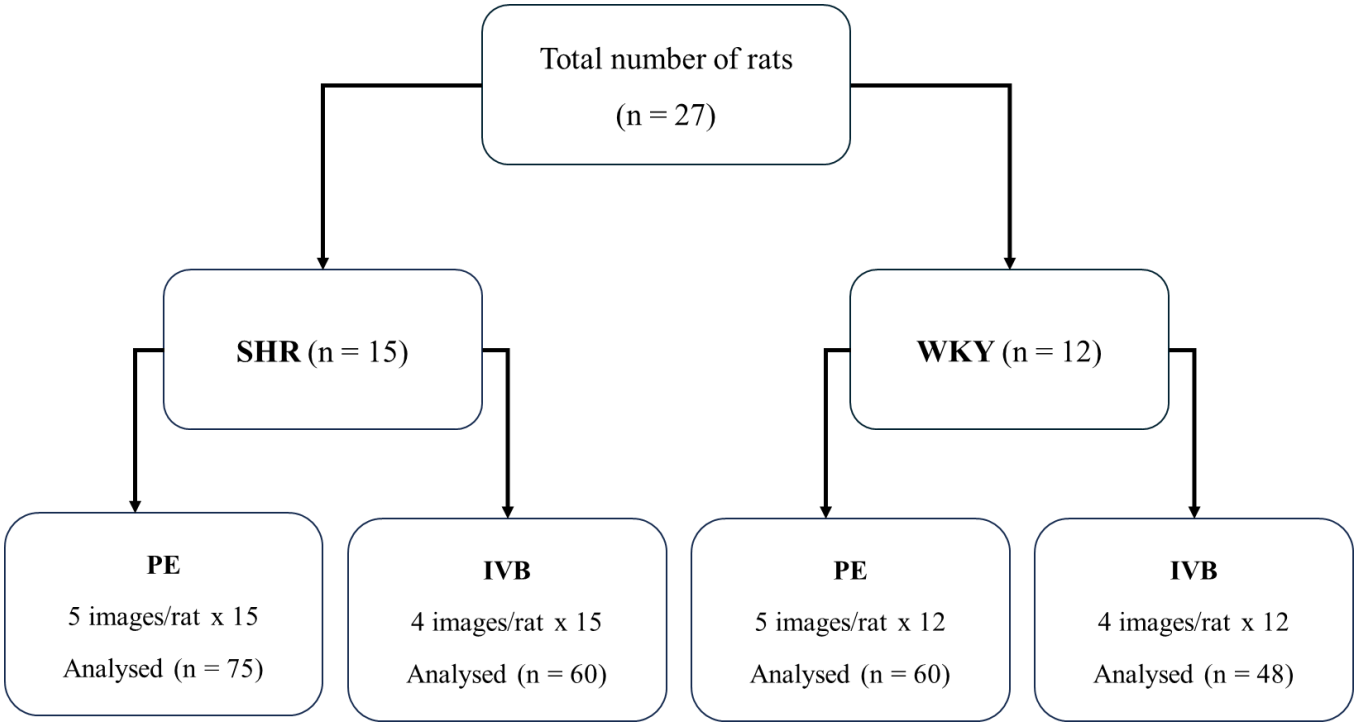


Figure 2.1: Illustration of the process flow: A total sample size of 27 rats, 17 months of age, including 15 SHR and 12 WKY, underwent PE and IVB interventions. Subsequently, echocardiographic aortic flow-velocity images were analysed. Abbreviations: SHR; spontaneously hypertensive rats, WKY; Wistar Kyoto rats, PE; phenylephrine, and IVB; ivabradine.

2.3 Invasive Haemodynamic Evaluation

At the time of the haemodynamic assessments, the rats were placed under anaesthesia induced with 5-8 % isoflurane in oxygen and maintained with 2 % isoflurane. Prior to the surgical procedures, the rats' chest and neck regions were shaved. The left carotid artery segment underwent catheterisation, while the right carotid artery was preserved to ensure blood supply to the brain. The left carotid artery was clamped and then surgically tied off to cease blood flow to the head. Following that, an incision was made between the clamp and the surgical tie, and a 0.9 % heparinised saline filled PP50 polyethylene catheter was inserted into the artery to assess aortic pressure measurements. The catheter was secured in place with suture and then connected to a high-resolution pressure transducer paired with PowerLab Lab Chart 8 system (ADInstruments, Sydney, Australia) to obtain central aortic pressure waveforms.

Echocardiographic measurements were obtained with the rat in a supine position using a Siemens ACUSON SC2000 ultrasound machine (Siemens, Medical Solutions, Germany) (Figure 2.2A). To obtain electrocardiogram (ECG) readings, electrodes with customised reusable small needles (Figure 2.2B) were inserted under the skin on the right and left front limbs, as well as on the left hindlimb of the rat. A high-resolution paediatric cardiac ultrasound probe (10 MHz) (Figure 2.2C) coupled with the Siemens ultrasound system was used to obtain a parasternal long-axis view of the heart (Figure 2.3A). In the parasternal long-axis view, the aortic root diameter (AoD) was measured immediately proximal to the aortic leaflets (Figure 2.3B). The largest diameter recorded in early systole was employed to construct the flow waveform.

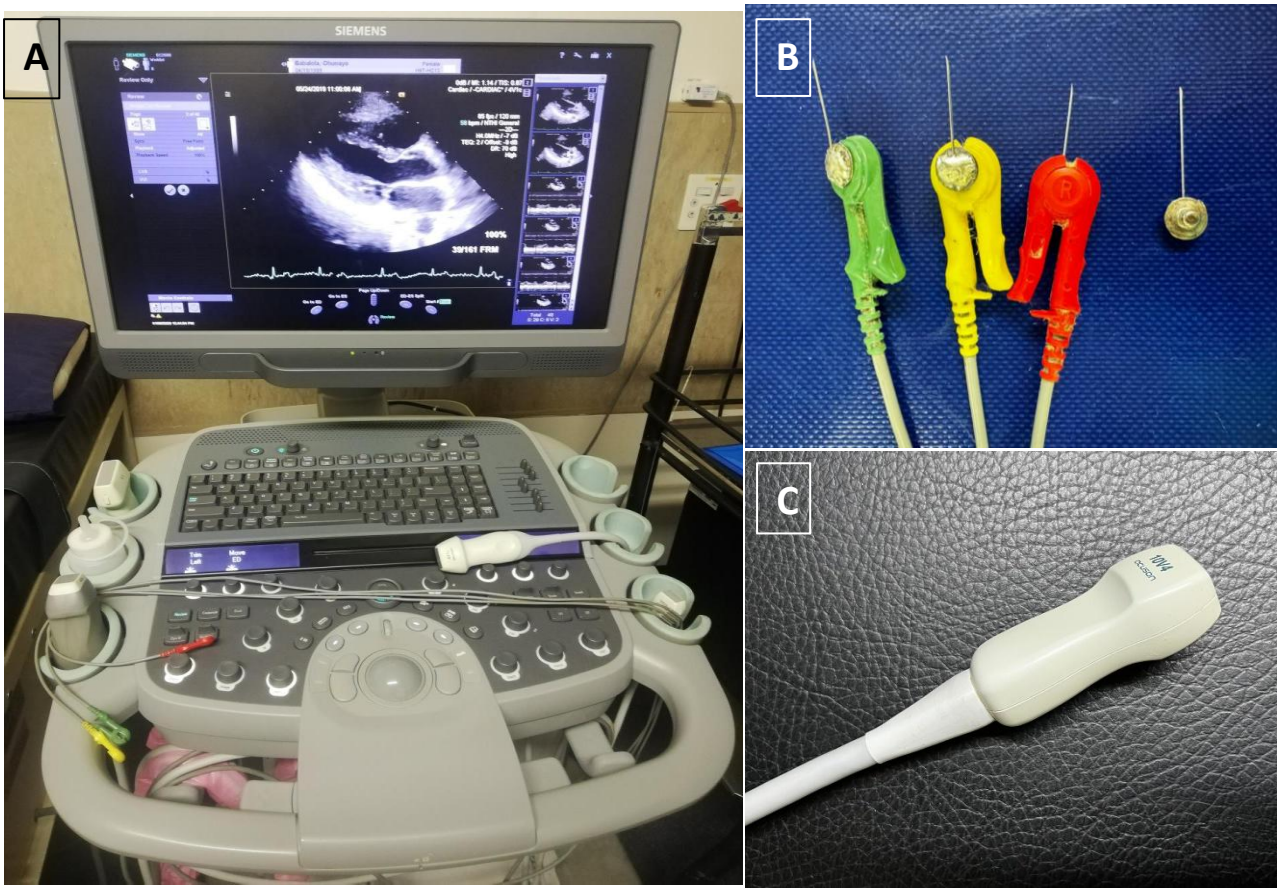


Figure 2.2: Cardiac ultrasound machine and accessories used to obtain aortic velocity waveforms and aortic root diameter (AoD) from the long-axis parasternal view of the heart. A; Siemens ACUSON SC2000 ultrasound machine (Siemens, Medical Solutions, Germany), B; ECG cable with customised reusable needle-like electrodes for small animals and C; paediatric ultrasound probe (10 MHz).

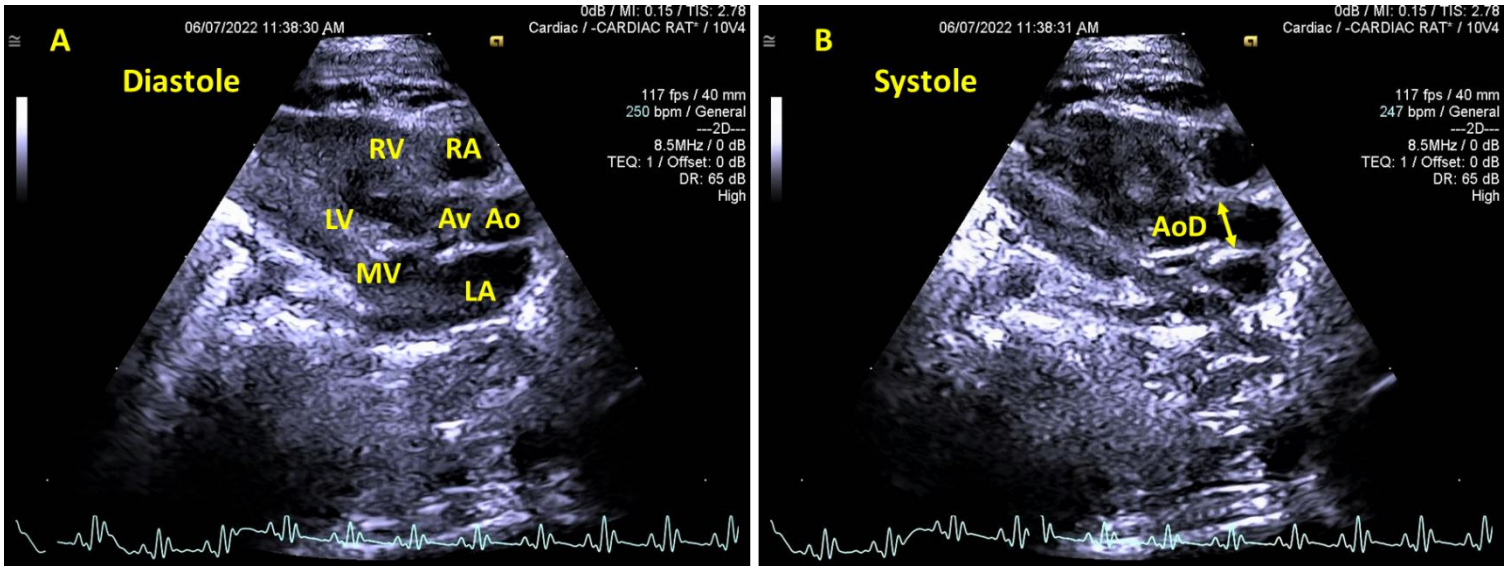


Figure 2.3: The parasternal long axis view with mixed echo dense and echo lucent structures of the RA; right atrium, RV; right ventricle, LV; left ventricle, MV; mitral valve, LA; left atrium, Av; aortic valve, Ao; ascending aorta and AoD; aortic root diameter (proximal to the aortic leaflets). Late diastole is depicted in panel A and early-systole in panel B.

Early systole is the phase of the cardiac cycle when the aortic valve opens, and blood is ejected from the LV into the aorta (Luisada and MacCanon, 1972; Walley, 2016). Throughout this phase, the aortic root is subjected to haemodynamic forces, including pressure and flow changes (Walley, 2016). Therefore, measuring the AoD during the early systolic phase provides valuable information about the size of the aorta under conditions of maximal flow, enabling the assessment of its capacity to accommodate blood flow without excessive stress on the aorta walls (Mitchell *et al*., 2010a; Walley, 2016). Thus, echocardiographic aortic flow-velocity waveforms and diameter images were collected in the ascending aorta using the parasternal long-axis view of the heart.

2.4 Inducing Changes in Blood Pressure and Heart Rate

To obtain a range of BP and HR values, central haemodynamics were recorded and ultrasound images were obtained in each anaesthetised rat, during the administration of the vasoconstrictor phenylephrine (PE, an alpha-1 agonist) followed by the administration of the HR lowering agent ivabradine (IVB, an inhibitor of the cardiac pacemaker current).

Central aortic pressures and echocardiographic images were systematically acquired for each anaesthetised rat at baseline and then during the intraperitoneal administration of approximately seven sequential doses of PE at 0.5 mg/kg (with a total cumulative dosage averaging 3.5 mg/rat) to each rat. The selected dose of PE (0.5 mg/kg) was based on previous studies demonstrating its efficacy in eliciting a controlled and reproducible increase in BP in both normotensive and hypertensive rat models (Rajanathan *et al*., 2022). Studies have indicated that PE within the range of 0.3-0.5 mg/kg reliably stimulates α_1-adrenergic receptors, including a consistent vasoconstrictive response without causing excessive hypertension or reflex bradycardia (Rajanathan *et al*., 2022). By administrating sequential doses of PE, we were able to achieve gradual and controlled increases in BP, which facilitated the acquisition of

detailed haemodynamic data across a physiologically relevant range of mean arterial pressure (MAP) values. This allowed for images to be captured at 10-15 mm Hg intervals during PE administration, and five aortic flow-velocity images per rat for both rat strains (SHR and WKY) were analysed (Figure 2.1).

Additionally, central aortic pressures and echocardiographic images were systematically acquired for each anaesthetised rat following the administration of approximately six sequential doses of ivabradine (IVB) at 0.5 mg/kg administered through the catheter (with a total cumulative dosage averaging 3 mg/rat). The choice of IVB dosage was informed by prior studies indicating its effectiveness in reducing HR without causing significant hypotension or altering myocardial contractility (Silva *et al*., 2016). Sequential dosing of IVB enabled a controlled and stepwise reduction in HR, allowing for the acquisition of images at 15-20 beats/min intervals and the assessment of haemodynamic responses under progressively lower heart rate conditions. Four aortic flow-velocity images per rat for both rat strains (SHR and WKY) were analysed (Figure 2.1).

Selective reduction of HR without causing significant hypotension or directly altering myocardial contractility is crucial, as it allows for the isolation of the effects of HR reduction on wave reflection and PPc. This approach is beneficial because it minimizes potential confounding variables, ensuring that observed changes in PPc can be attributed to HR modulation rather than the direct effects on vascular tone or contractility (Silva *et al*., 2016).

Using these two pharmacological agents a range of BP and HR, a range of BP and HR values were obtained, hence enabling a comparison of central aortic pressures and blood flow over a range of BP and HR values and at similar steady-state pressures (mean arterial pressure, MAP) in normotensive (WKY) and hypertensive (SHR) rats.

After conducting the terminal procedures, tissue samples including heart, lungs, and liver were weighed to identify pathological features associated with hypertension and heart failure (HF) such as hypertrophy or oedema.

2.5 Wave Separation Analysis

Using the echocardiographic aortic flow-velocity images (Figure 2.4A) collected at termination, DICOM graphics software (Sante DICOM Viewer Lite, Aarhus, Denmark) was used to recreate the aortic velocity waveform (Figure 2.4B). The leading (outer) edge or the most dense or brightest portion of the spectral image of the aortic velocity waveform was outlined using the DICOM Viewer software. Care was taken to avoid any overshoot of the waveform. Using the DICOM Viewer program, vertical lines extending from the x-axis to the corresponding points on the waveform were created to ensure accurate recreation of the flow-velocity waveform (Figure 2.4B). The lines were equidistant in pixels (one-pixel apart). Each line provided the vertical distance from the x-axis to its associated point on the waveform, thereby establishing pixel coordinates (Figure 2.4B). The pixel coordinates were converted to velocity coordinates using the maximum aortic velocity (Vmax) measurements as a scale, thereby recreating the velocity flow waveform (as described below).

The Vmax and velocity time integral (VTI) were measured using SyngoDynamics software (Siemens Medical Solutions USA, Inc.). Aortic flow waveforms were created using AoD and velocity measurements using a standard formula, where aortic flow = aortic velocity multiplied by AoD. Aortic flow (Q) was calculated as Vmax, multiplied by AoD. The aortic waveforms were paired with the time and pressure wave coordinates, taken from invasive central pressure measurement, by aligning the start (foot) of the two waveforms (Figure 2.5). Characteristic impedance (Zc) was calculated in the time domain as the ratio of the change in

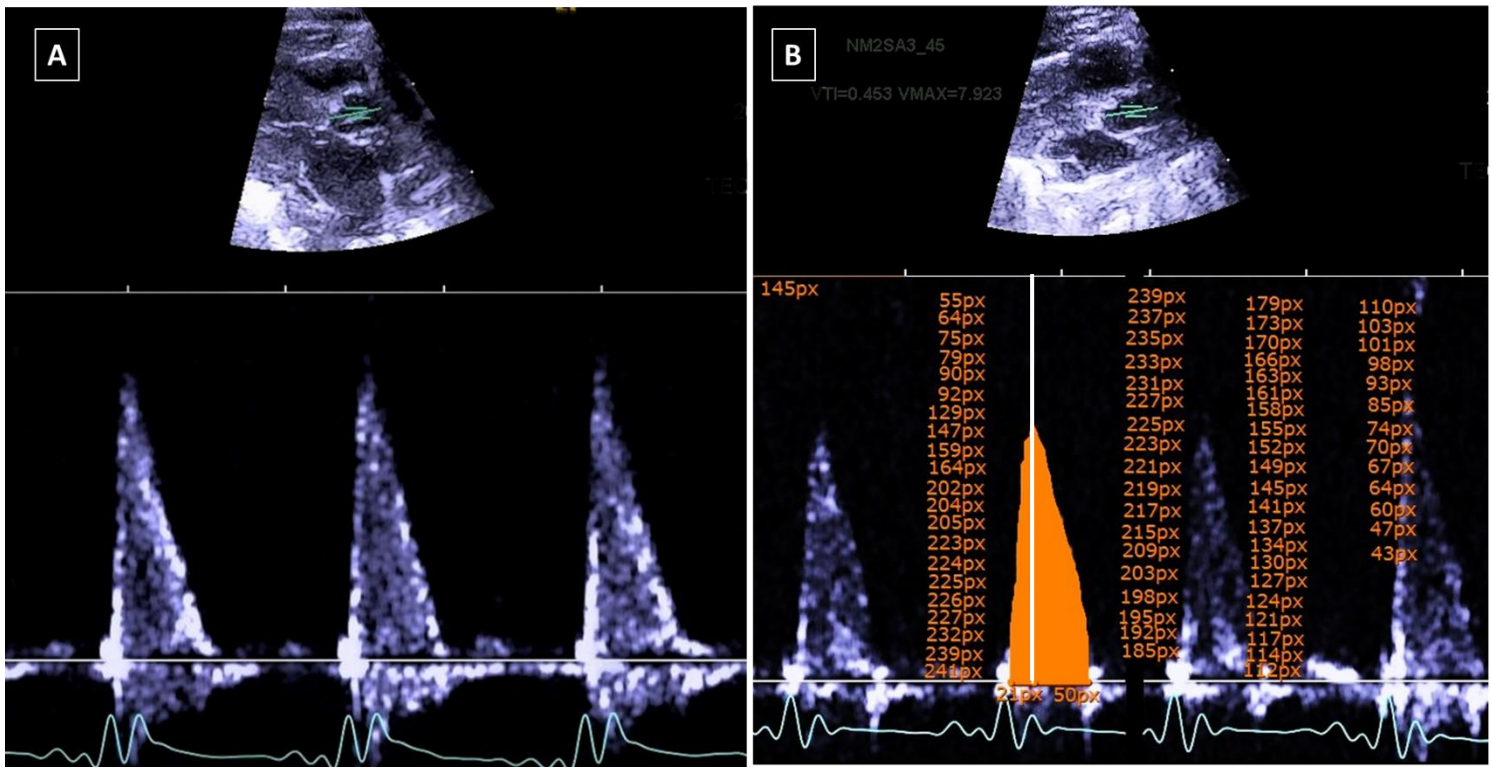


Figure 2.4: Aortic velocity waveform obtained in the parasternal long axis view of the heart. Panel A shows the actual echocardiographic velocity waveform, while Panel B exhibits a recreated version of the aortic velocity waveform with the use of an ultrasound graphics software (Sante DICOM Viewer Lite). Equidistant pixels (px) were determined before the maximum aortic velocity (Vmax) on the left side and after Vmax on the right side of the vertical white line (Panel B).

aortic pressure to the change in aortic flow rate, measured from the onset (foot) of the flow wave to the point where flow reached 95% of its peak value (Figure 2.5) (Mitchell *et al*., 2010a; Segers *et al*., 2017).

Using the Q and pressure waveforms as well as Zc values, wave separation analysis (separation of the aortic pressure waveform into its two component waves) was performed with the use of standard formulae (Murgo *et al.,* 1981; Westerhof *et al*., 1972): Forward wave pressure (Pf) = [Aortic P+(PQxZc)]/2 and backward wave pressure (Pb) = [Aortic P-(PQxZc)]/2. Peak compression pressure waves were calculated by multiplying peak Q by peak Zc (Peak PQxZc). Peak PQxZc was used instead of Pf, because $PQxZc_c$ is the component of Pf generated by aortic stiffness, and thus Zc (Figure 2.5).

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 2.5). The contribution to Pf of pressures determined by an interaction between Q and Zc was identified from the pressures generated by the product of peak aortic Q and Zc (PQxZc) (Mthembu *et al.,* 2022b). We determined peak PQxZc, to identify the component of Pf which excludes pressures generated by wave re-reflection (Figure 2.5).

2.6 Statistical Analysis

Statistical analysis was performed using statistical analysis system (SAS) software, version 9.4 (The SAS Institute Inc., Cary, North Carolina, USA) and Microsoft Excel © (2016). Data are expressed as mean ± standard deviation (SD) or standard error of the mean (SEM). Two sample Student's t-test was performed to compare body and tissue weights, aortic parameters, pulsatile pressures, HR, PPc and the determinants of PPc between SHR and WKY in response to PE and IVB. Bivariate Pearson's correlations were performed to determine the

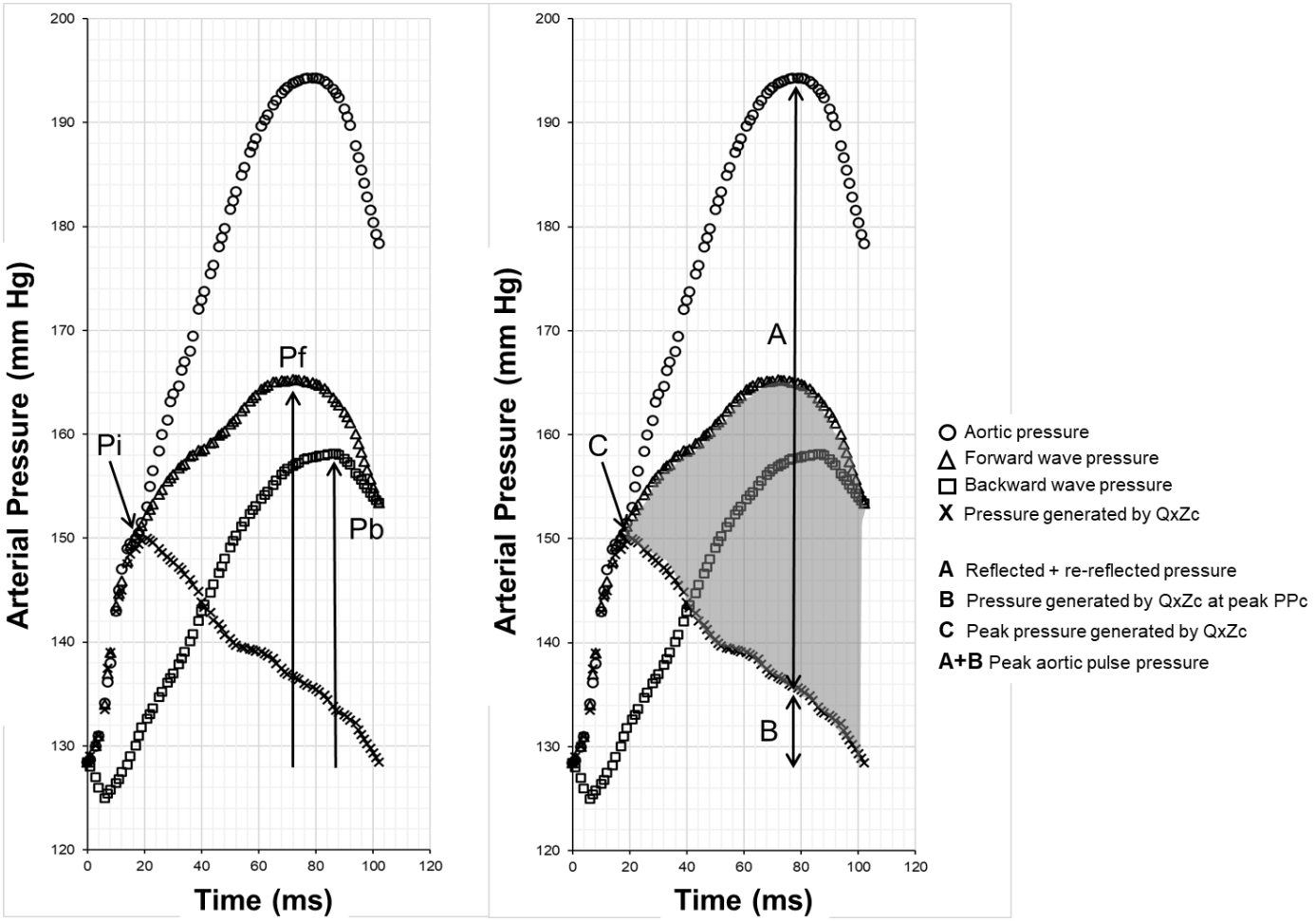


Figure 2.5: The aortic pressure overlaid with central aortic pulse pressure (PPc) wave components. Pi; incident wave pressure, Pf; forward wave pressure. Pb; backward wave pressure, A; reflected + re-reflected wave pressures, B; pressure generated by the product of aortic flow (Q) and characteristic impedance (Zc) at peak PP, and C; Peak pressure generated by the product of Q and Zc (Peak PQxZc). The shaded region represents re-reflected wave.

relationships between HR and PPc, and the determinants of PPc, in response to PE and IVB combined as well as separately. Multivariate analysis was used to determine the impact of adjustments for the determinants of PPc on the relationship between HR and PPc, in response to PE and IVB combined as well as separately. A p-value <0.05 was considered statistically significant.

CHAPTER 3: RESULTS

3.1 Body and Tissue Weights

Table 3.1 compares the weights and weight ratios between spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats. The data was derived from 27 male rats, comprising 12 WKY and 15 SHR. Both WKY and SHR were the same age, 17 months, at the time of the measurements. The SHR had lower body, lung and liver weights compared to the WKY ($p<0.05$) (Table 3.1). When considering the ratios of tissue weights to body weights, it was observed that the SHR group had notably higher heart, left ventricle (LV), right ventricle (RV), and liver weight-to-body weight ratios in comparison to WKY group ($p<0.05$) (Table 3.1). However, the lung weights to body weight were not significantly different between SHR and WKY (Table 3.1).

3.2 Tail-cuff Blood Pressure and Aortic Measurements

Table 3.2 compares the non-invasive tail-cuff BP measurements, which were assessed prior to the terminal invasive haemodynamic evaluations (i.e., in the absence of anaesthetic), and the aortic measurements between SHR and WKY rats. The SHR group exhibited significantly higher non-invasive systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) compared to the WKY rats ($p<0.0001$). No differences in aortic diameter and ejection duration were noted between SHR and WKY rats. Similarly, no significant differences were observed in aortic velocity time integral (VTI) between SHR and WKY rats. However, the SHR group had a higher maximum aortic velocity (Vmax) compared to WKY rats ($p<0.05$).

Table 3.1: Comparison of weights, and weight ratios between the 17-month-old male WKY rats and SHR.

	WKY (n=12)	SHR (n=15)	p-value
Weights (g)			
Body weight	584.13±58.65	382.13±46.93	<**0.0001**
Heart weight	1.58±0.26	1.44±0.16	=0.1744
LV weight	1.19±0.22	1.10±0.15	=0.1032
RV weight	0.27±0.15	0.31±0.17	=0.4945
Lung weight	3.66±0.41	2.45±0.88	<**0.0001**
Liver weight	17.27±4.50	13.57±2.05	=**0.0055**
Ratio (%)			
Heart weight/body weight	0.27±0.05	0.40±0.05	<**0.0001**
LV weight/body weight	0.21±0.04	0.29±0.06	<**0.0001**
RV weight/body weight	0.05±0.02	0.08±0.04	**=0.0055**
Lung weight/body weight	0.63±0.10	0.66±0.27	=0.7260
Liver weight/body weight	2.96±0.74	3.58±0.55	**=0.0109**

Data shown are mean±SD. Abbreviations: WKY; Wistar Kyoto rats, SHR; spontaneously hypertensive rats, LV; left ventricular weight, and RV; right ventricular weight. Significant p-values are shown in bold.

Table 3.2: Comparison of tail-cuff non-invasive blood pressure measurements and aortic measurements between 17-month-old male WKY rats and SHR.

	WKY (n=12)	SHR (n=15)	p-value
SBP tail-cuff (mm Hg)	120±11	191±27	**<0.0001**
DBP tail-cuff (mm Hg)	79±11	140±24	**<0.0001**
MAP tail-cuff (mm Hg)	93±9	157±24	**<0.0001**
Aortic Diameter (cm)	3.51±0.03	3.64±0.03	=0.2807
Aortic Vmax (m/s)	92.12±48.23	108.13±48.64	**=0.0077**
Aortic VTI (cm/stroke)	6.21±3.42	6.33±2.94	=0.7479
Ejection Duration (msec)	105.82±15.70	107.14±14.07	=0.4001

Data shown are mean±SD. Abbreviations: WKY; Wistar Kyoto rats, SHR; spontaneously hypertensive rats, SBP; systolic blood pressure, DBP; diastolic blood pressure, and MAP; mean arterial pressure, Vmax; maximum aortic velocity, and VTI; velocity time integral. Significant p-values are shown in bold.

3.3 Haemodynamic Changes in Response to PE and IVB

Table 3.3 shows comparisons of heart rate (HR) and central pulse pressure (PPc) responses to phenylephrine (PE) and ivabradine (IVB) between SHR and WKY. Spontaneously hypertensive rats (SHR) exhibited a markedly lower HR and concurrently elevated PPc ($p<0.0005$) in response to PE, in comparison to WKY. Similarly, SHR displayed a lower HR in response to IVB, while PPc was greater compared to WKY ($p<0.05$ to $p<0.0001$).

Table 3.4 compares the PPc component responses to PE between SHR and WKY rats. Central systolic blood pressure (SBP), forward wave pressure (Pf), backward wave pressure (Pb), reflected pressure, re-reflected pressure, maximum flow (max Q) and characteristic impedance (Zc) were higher in SHR compared to WKY rats in response to PE ($p<0.05$ to $p<0.0001$). However, in comparison to the pulsatile pressures (PP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) (the steady-state pressures), were not significantly different between SHR and WKY rats in response to PE.

In addition, Table 3.4 indicates that the pressure generated by the product of flow and characteristic impedance at central pulse pressure (PQxZc at PPc) and maximum product of flow and characteristic impedance (max PQxZc) were not significantly different between SHR and WKY rats in response to PE.

Table 3.5 compares the PPc component responses to IVB between SHR and WKY. In response to IVB, similar to the responses to PE, SBP, Pf, Pb, reflected pressure, and re-reflected pressure were all higher in SHR compared to WKY rats ($p<0.0005$), and PQxZc at PPc, max PQxZc, and max Q were not significantly different (Table 3.5). However, steady-state pressures (DBP and MAP) were higher in SHR compared to WKY rats ($p<0.0005$ to $p<0.0001$), whereas Zc did not differ (Table 3.5).

Table 3.3: Comparison of heart rate and central pulse pressure of 17-month-old male WKY rats and SHR, in response to PE and IVB.

	WKY	SHR	p-value
PE			
(n=135 for all)	n=60	n=75	
HR (beats/min)	285.1±23.8	233.3±18.2	<**0.0001**
HR range (beats/min)	201.3 - 329.7	193.5 - 275.2	-
PPc (mm Hg)	52.5±13.2	61.2±13.3	<**0.0005**
PPc range (mm Hg)	27.4 - 84.9	33.6 - 88.5	-
IVB			
(n=108 for all)	n=48	n=60	
HR (beats/min)	230.6±24.6	218.1±19.9	=**0.0034**
HR range (beats/min)	192.3 - 279.1	181.7 - 266.7	-
PPc (mm Hg)	62.0±13.3	82.3±13.5	<**0.0001**
PPc range (mm Hg)	34.3 - 97.8	48.5 - 110.3	-

Data shown are mean±SD and range (min – max). Abbreviations: WKY; Wistar Kyoto rats, SHR; spontaneously hypertensive rats, PE; phenylephrine, HR; heart rate, IVB; ivabradine, and PPc; central pulse pressure. Significant p-values are shown in bold.

Table 3.4: Comparison of central pulse pressure components and steady-state pressures of 17-month-old male WKY rats and SHR, in response to PE.

	WKY	SHR	p-value
PE			
(n=135)	n=60	n=75	
SBP (mm Hg)	172.42±30.84	182.61±40.14	=**0.0273**
DBP (mm Hg)	119.97±19.10	121.43±19.14	=0.3314
MAP (mm Hg)	137.45±22.85	141.83±22.09	=0.1343
Pf (mm Hg)	34.53±6.86	38.24±8.12	=**0.0031**
Pb (mm Hg)	19.26±6.54	23.91±6.23	<**0.0001**
PQxZc at PPc (mm Hg)	14.85±3.77	14.24±6.41	=0.2613
Reflect (mm Hg)	17.92±7.07	22.94±6.72	<**0.0001**
Re-reflect (mm Hg)	19.68±627	23.99±6.07	<**0.0001**
Max PQxZc (mm Hg)	23.61±4.12	24.10±6.89	=0.6250
Max Q (ml/s)	7.94±5.72	10.19±4.06	**=0.0090**
Zc (dynes/cm^5)	3989.62±256.25	4992.10±292.41	**=0.0349**

Data shown are mean±SD. Abbreviations: WKY; Wistar Kyoto rats, SHR; spontaneously hypertensive rats, PE; phenylephrine, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean blood pressure, Pf; forward pressure, Pb; backward pressure, PQxZc at PPc; pressure generated by the product of flow and characteristic impedance at central pulse pressure, Zc; characteristic impedance, max Q; maximum flow, max PQxZc; maximum product of flow and characteristic impedance, reflect; reflected pressure, and re-reflect; re-reflected pressure. Significant p-values are shown in bold.

Table 3.5: Comparison of central pulse pressure components and steady-state pressures of 17-month-old male WKY rats and SHR, in response to IVB.

	WKY	SHR	p-value
IVB			
(n=108)	n=48	n=60	
SBP (mm Hg)	172.05±40.51	215.56±41.79	<**0.0001**
DBP (mm Hg)	110.06±27.68	133.29±31.92	<**0.0005**
MAP (mm Hg)	130.72±31.69	160.71±34.94	<**0.0001**
Pf (mm Hg)	40.33±6.48	49.67±7.33	<**0.0001**
Pb (mm Hg)	23.63±8.79	33.74±7.40	<**0.0001**
PQxZc at PPc (mm Hg)	16.81±5.71	15.82±6.28	=0.2098
Reflect (mm Hg)	21.66±9.87	32.61±8.11	<**0.0001**
Re-reflect (mm Hg)	23.52±9.33	33.85±7.30	<**0.0001**
Max PQxZc (mm Hg)	26.84±4.97	27.89±7.48	=0.4236
Max Q (ml/s)	10.66±5.01	11.10±6.19	=0.2830
Zc (dynes/cm^5)	4404.35±287.95	4516.94±331.20	=0.8593

Data shown are mean±SD. Abbreviations: WKY; Wistar Kyoto rats, SHR; spontaneously hypertensive rats, IVB; ivabradine, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean blood pressure, Pf; forward pressure, Pb; backward pressure, PQxZc at PPc; pressure generated by the product of flow and characteristic impedance at central pulse pressure, Zc; characteristic impedance, max Q; maximum flow, max PQxZc; maximum product of flow and characteristic impedance, reflect; reflected pressure, and re-reflect; re-reflected pressure. Significant p-values are shown in bold.

3.4 Relationship between HR and PPc, and the Determinants of PPc

Table 3.6 and Figure 3.1 show the bivariate relationships and Pearson's correlations between HR and PPc, as well as between HR and the determinants of PPc, when data in response to PE and IVB were analysed in combination (ALL) or separately (PE, IVB,). When data in response to PE and IVB were combined (ALL, Table 3.6), HR was inversely associated with PPc ($p<0.0001$). In addition, Pf, Pb, reflected, re-reflected, reflected + re-reflected, max PQxZc, and max Q were inversely associated with HR ($p<0.05$ to $p<0.0001$). However, PQxZc at PPc and MAP showed no correlation with HR, while Zc was the only determinant of PPc that exhibited a positive relationship with HR.

Similarly, in response to PE alone (PE, Table 3.6), HR was inversely associated with PPc, and its determinants (Pf, Pb, reflected pressure, re-reflected pressure, reflected + re-reflected pressure, and max Q) ($p<0.05$ to $p<0.0001$). However, max PQxZc was not associated with HR in the presence of PE. In the presence of PE, PQxZc at PPc, and MAP similarly showed no relationship with HR, and Zc was the only determinant of PPc demonstrating a positive relationship with the HR.

In response to IVB alone (IVB, Table 3.6), similar to in the presence of PE, HR was inversely associated with PPc, and its determinants (Pf, Pb, reflected pressure, re-reflected pressure, and reflected + re-reflected pressure) ($p<0.05$). Although some of these relationships did not reach significance, the magnitude of the slopes were comparable to those noted in the presence of PE. In the presence of IVB, PQxZc at PPc, and MAP similarly showed no relationship with HR. However, in the presence of IVB, Zc and max Q were not associated with HR.

Table 3.6: Bivariate relationships between HR and PPc, and between HR and the determinants of PPc in response to PE and IVB when combined (ALL), or separately (PE, IVB).

	ALL			PE			IVB		
HR vs	slope±sem	r	p-value	slope±sem	r	p-value	slope±sem	r	p-value
(n)	243			135			108		
PPc (mm Hg)	-0.216±0.032	-0.408	<**0.0001**	-0.144±0.034	-0.342	<**0.0001**	-0.181±0.074	-0.240	=**0.016**
MAP (mm Hg)	-0.083±0.059	-0.093	=0.157	-0.080±0.059	-0.117	=0.179	-0.050±0.159	-0.032	=0.754
Pf (mm Hg)	-0.112±0.017	-0.403	<**0.0001**	-0.064±0.020	-0.271	=**0.0016**	-0.101±0.035	-0.281	=**0.0046**
Pb (mm Hg)	-0.109±0.016	-0.407	<**0.0001**	-0.077±0.016	-0.377	<**0.0001**	-0.097±0.040	-0.238	=**0.017**
Reflect (mm Hg)	-0.104±0.017	-0.364	<**0.0001**	-0.080±0.018	-0.364	<**0.0001**	-0.080±0.045	-0.178	=0.076
Re-reflect (mm Hg)	-0.106±0.016	-0.397	<**0.0001**	-0.073±0.016	-0.373	<**0.0001**	-0.099±0.041	-0.237	=**0.018**
Reflect + re-reflect (mm Hg)	-0.210±0.033	-0.382	<**0.0001**	-0.154±0.034	-0.371	<**0.0001**	-0.179±0.085	-0.208	=**0.038**
PQxZc at PPc (mm Hg)	-0.006±0.011	-0.036	=0.589	-0.010±0.014	0.060	=0.490	-0.002±0.026	-0.007	=0.942
Max PQxZc (mm Hg)	-0.029±0.012	-0.151	=**0.021**	-0.001±0.015	0.004	=0.967	-0.028±0.028	-0.103	=0.309
Max Q (ml/s)	-0.030±0.011	-0.183	=**0.005**	-0.032±0.013	-0.217	=**0.012**	0.018±0.024	0.073	=0.470
Zc (dynes/cm^5)	13.07±5.71	0.149	=**0.023**	20.96±7.03	0.252	=**0.0034**	0.059±13.37	0.0004	=0.996

Data shown are slope±sem, and Pearson's correlation coefficient (r). Abbreviations: ALL; data in presence of either PE or IVB combined, PE; phenylephrine, IVB; ivabradine, HR; heart rate, PPc; central pulse pressure, MAP; mean blood pressure Pf; forward pressure, Pb; backward pressure, reflect; reflected pressure, re-reflect; re-reflected pressure, PQxZc at PPc, product of flow and characteristic impedance at central pulse pressure, max PQxZc, maximum product of flow and characteristic impedance, max Q, maximum flow, and Zc, characteristic impedance. Symbol: r; Pearson's correlation coefficient. Significant p-values are shown in bold.

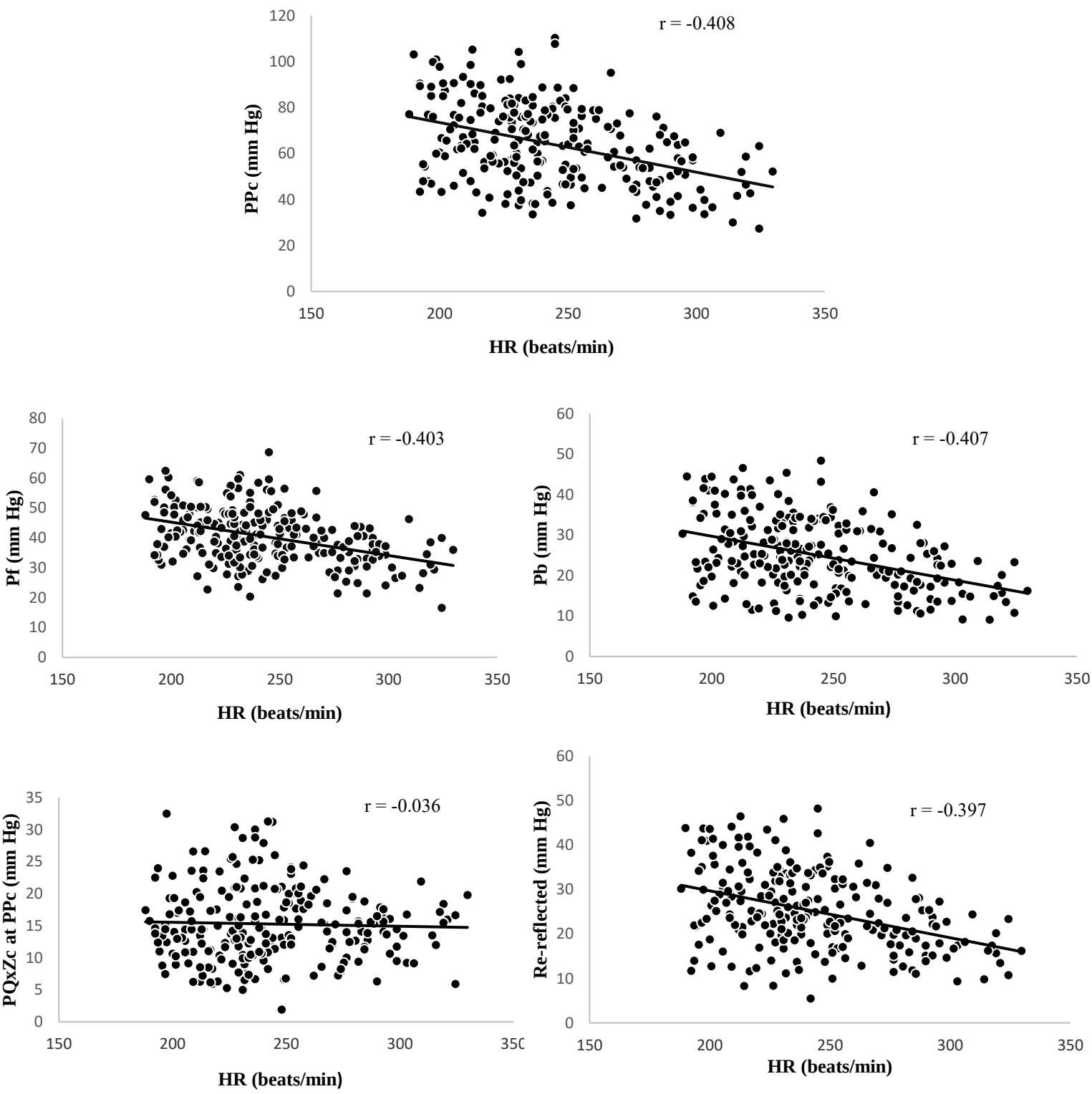


Figure 3.1: Bivariate correlations between HR and PPc, and determinants of PPc in WKY and SHR in response to PE and IVB when combined. Abbreviations: HR; heart rate, PPc; central pulse pressure, Pf; forward pressure, Pb; backward pressure, PQxZc at PPc; product of flow and characteristic impedance at central pulse pressure and re-reflected; re-reflected pressure. Symbol: r; Pearson's correlation coefficient.

3.5 Relationships between PPc and the Determinants of PPc

Table 3.7 illustrates the bivariate relationships between PPc and the determinants of PPc, when data in response to PE and IVB were analysed in combination (ALL) or separately (PE, IVB). When data in response to PE and IVB were combined (ALL, Table 3.7), a positive association was observed between PPc and its determinants, including Pf, Pb, reflected pressure, re-reflected pressure, reflected + re-reflected pressure, max PQxZc max Q and MAP ($p<0.005$ to $p<0.0001$). However, PPc was not significantly associated with either PQxZc at PPc or Zc.

In the presence of either PE or IVB separately (Table 3.7), similar relationships between PPc and the determinants of PPc were noted. However, in the presence of PE alone, PPc was not related to max Q, but was associated with PQxZc at PPc ($p<0.05$). In response to IVB alone, PQxZc at PPc was inversely associated with PPc ($p<0.05$), and max PQxZc was not associated with PPc.

3.6 Impact of the Determinants of PPc on the Inverse Relationship between HR and PPc

Table 3.8 illustrates the relationship between HR and PPc, as well as the impact of each PPc determinant when added separately in multivariate analyses. The analyses were performed on data when responses to PE and IVB were combined (ALL, Table 3.8), as well as separate (PE, IVB, Table 3.8). The addition of Pf, and Pb, either separately or together, eliminated the inverse slope of the relationship between HR and PPc (Table 3.8). The addition of reflected pressure or re-reflected pressure, either separately or together also markedly diminished the relationship between HR and PPc (Table 3.8).

However, MAP, PQxZc at PPc, max PQxZc, max Q, and Zc had no impact on the

Table 3.7: Bivariate relationships between PPc and the determinants of PPc in all WKY and SHR, in response to PE and IVB when combined (ALL), or separately (PE, IVB).

	ALL		PE		IVB	
PPc vs	slope±sem	p-value	slope±sem	p-value	slope±sem	p-value
(n)	243		135		108	
MAP (mm Hg)	0.441±0.026	<**0.0001**	0.453±0.034	<**0.0001**	0.381±0.029	<**0.0001**
Pf (mm Hg)	1.786±0.044	<**0.0001**	1.660±0.058	<**0.0001**	1.930±0.084	<**0.0001**
Pb (mm Hg)	1.889±0.039	<**0.0001**	1.907±0.068	<**0.0001**	1.786±0.051	<**0.0001**
Reflect (mm Hg)	1.738±0.041	<**0.0001**	1.749±0.066	<**0.0001**	1.595±0.054	<**0.0001**
Re-reflect (mm Hg)	1.875±0.043	<**0.0001**	1.957±0.075	<**0.0001**	1.718±0.057	<**0.0001**
Reflect + re-reflect (mm Hg)	0.915±0.020	<**0.0001**	0.940±0.034	<**0.0001**	0.840±0.026	<**0.0001**
PQxZc at PPc (mm Hg)	0.151±0.200	=0.451	0.609±0.220	=**0.0064**	-0.760±0.284	=**0.0088**
Max PQxZc (mm Hg)	0.848±0.174	<**0.0001**	0.968±0.194	<**0.0001**	0.089±0.277	=0.749
Max Q (ml/s)	0.611±0.210	=**0.0040**	0.420±0.291	=0.152	0.569±0.309	=0.069
Zc (dynes/cm^5)	-0.0003±0.0004	=0.459	0.0003±0.0004	=0.453	-0.0008±0.0006	=0.173

Data shown are slope±sem. Abbreviations: ALL, data in presence of either PE or IVB combined, HR; heart rate, PPc; central pulse pressure, MAP; mean blood pressure Pf; forward pressure, Pb; backward pressure, reflect; reflected pressure, re-reflect; re-reflected pressure, PQxZc at PPc; product of flow and characteristic impedance at central pulse pressure, max PQxZc; maximum product of flow and characteristic impedance, max Q; maximum flow, and Zc; characteristic impedance and. Significant p-values are shown in bold.

Table 3.8: Impact of adjustments for determinants of PPc on the relationship between HR and PPc in response to PE and IVB when combined (ALL), or separately (PE, IVB).

	ALL		PE		IVB	
HR vs	slope±sem	p-value	slope±sem	p-value	slope±sem	p-value
(n)	243		135		108	
PPc	-0.216±0.032	<**0.0001**	-0.144±0.032	<**0.0001**	-0.181±0.074	=**0.016**
PPc + MAP	-0.181±0.020	<**0.0001**	-0.107±0.021	<**0.0001**	-0.162±0.044	=**0.0003**
PPc + Pf	-0.020±0.013	=0.143	-0.041±0.014	=**0.0033**	0.015±0.032	=0.641
PPc + Pb	-0.012±0.011	=0.266	-0.004±0.015	=0.810	-0.008±0.021	=0.707
PPc + Pf +Pb	0.011±0.033	=**0.0013**	-0.002±0.004	=0.637	0.023±0.007	=**0.0009**
PPc + Reflect	-0.040±0.012	=**0.0014**	-0.004±0.016	=0.800	-0.055±0.024	=**0.025**
PPc + Re-reflect	-0.021±0.012	=0.089	-0.001±0.016	=0.986	-0.012±0.024	=0.635
PPc + Reflect + re-reflect	-0.028±0.012	**=0.018**	0.001±0.015	=0.968	-0.032±0.023	=0.160
PPc + PQxZc at PPc	-0.215±0.032	<**0.0001**	-0.151±0.033	<**0.0001**	-0.183±0.072	=**0.0125**
PPc + Max PQxZc	-0.215±0.032	<**0.0001**	-0.145±0.031	<**0.0001**	-0.181±0.075	=**0.0178**
PPc + Max Q	-0.205±0.032	<**0.0001**	-0.148±0.035	<**0.0001**	-0.192±0.073	=**0.0099**
PPc + Zc	-0.217±0.032	<**0.0001**	-0.161±0.035	<**0.0001**	-0.181±0.074	=**0.0159**

Data shown are slope±sem. Abbreviations ALL, data in presence of either PE or IVB combined, HR; heart rate, PPc; central pulse pressure, MAP; mean blood pressure, Pf; forward pressure, Pb; backward pressure, reflect; reflected pressure, re-reflect; re-reflected pressure, PQxZc at PPc; product of flow and characteristic impedance at central pulse pressure, max PQxZc; maximum product of flow and characteristic impedance, max Q; maximum flow, and Zc; characteristic impedance. Significant p-values are shown in bold

relationship between HR and PPc (Table 3.8). The impact of Pf, Pb, reflected pressure, and re-reflected pressure on the relationship between HR and PPc was noted in the presence of either PE or IVB (Table 3.8). Similarly, the lack of impact of MAP, PQxZc at PPc, max PQxZc, max Q, and Zc on the relationship between HR and PPc, was noted in the presence of either PE or IVB (Table 3.8).

Figures 3.2, 3.3 and 3.4 show the impact on the relationship between HR and PPc, of one standard deviation change in each of the determinants of PPc, when data in response to PE and IVB were analysed in combination (Figure 3.2) or separately PE, (Figure 3.3), and IVB, (Figure 3.4). When data in response to PE and IVB were combined (Figure 3.2) or separated, PE (Figure 3.3) and IVB (Figure 3.4), the slope of the relationship between HR and PPc was markedly diminished by Pf, Pb, reflected pressure, and re-reflected pressure ($p<0.05$ to $p<0.0001$). Indeed, in the presence of Pf, Pb, reflected pressure, or re-reflected pressure, HR was no longer significantly associated with PPc. However, PQxZc at PPc, max PQxZc, max Q, Zc, and MAP had no impact on the slope of the relationship between HR and PPc. The slope of the relationship between HR and PPc when either PQxZc at PPc, max PQxZc, max Q, Zc, or MAP, were added separately in the multivariate model did not differ from the slope of this relationship prior to the addition of these determinants of PPc.

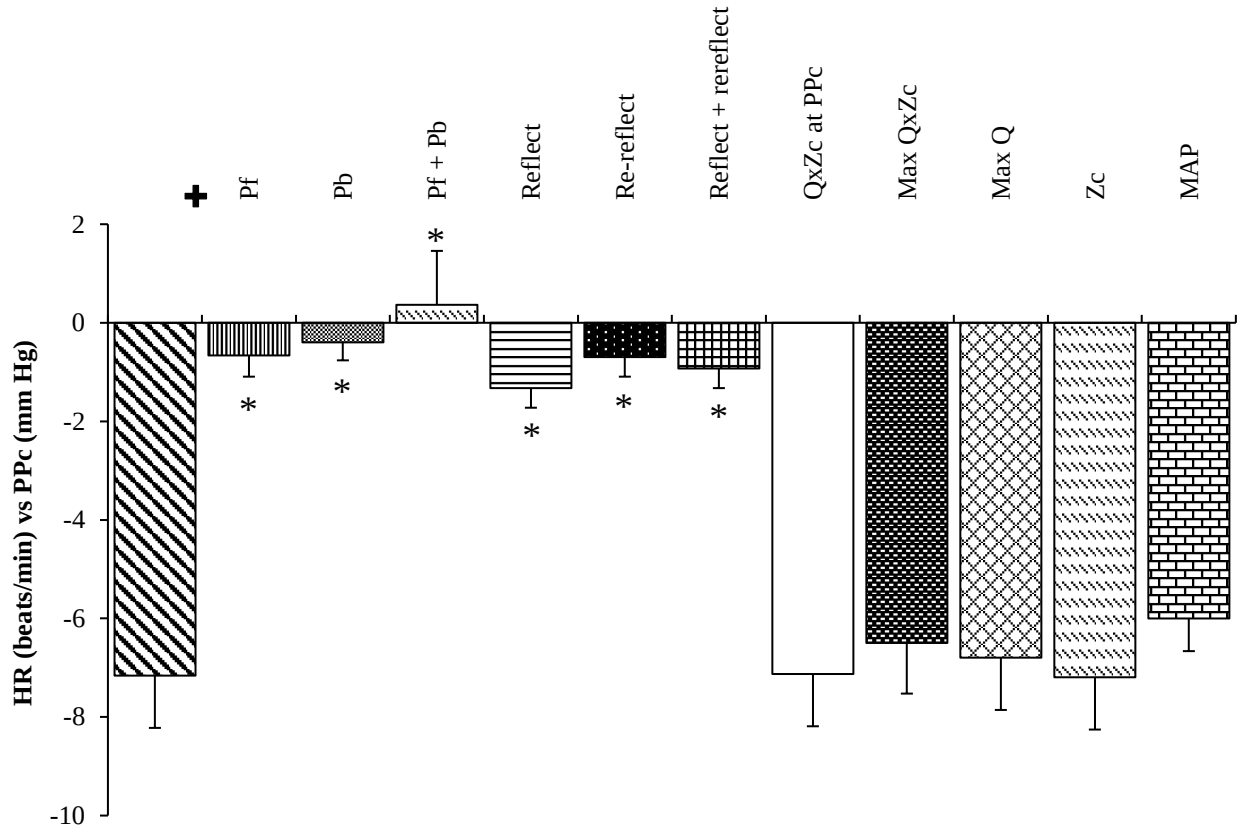


Figure 3.2: The slopes of the relationships between HR and PPc, and the impact of each of the determinants of PPc on the relationship between HR and PPc in the presence of either PE or IVB (combined). A positive (✚) symbol indicates the addition of each of the determinants of PPc separately in the multivariate model – the relationship between HR and PPc. * $p<0.0001$ compared to the relationship between HR and PPc before adjustments. Abbreviations, HR; heart rate, PPc; central pulse pressure, Pf; forward pressure, Pb; backward pressure, reflect; reflected pressure, re-reflect; re-reflected pressure, PQxZc at PPc; product of flow and characteristic impedance at central pulse pressure, max PQxZc; maximum product of flow and characteristic impedance, max Q; maximum flow, MAP; mean blood pressure, and Zc; characteristic impedance.

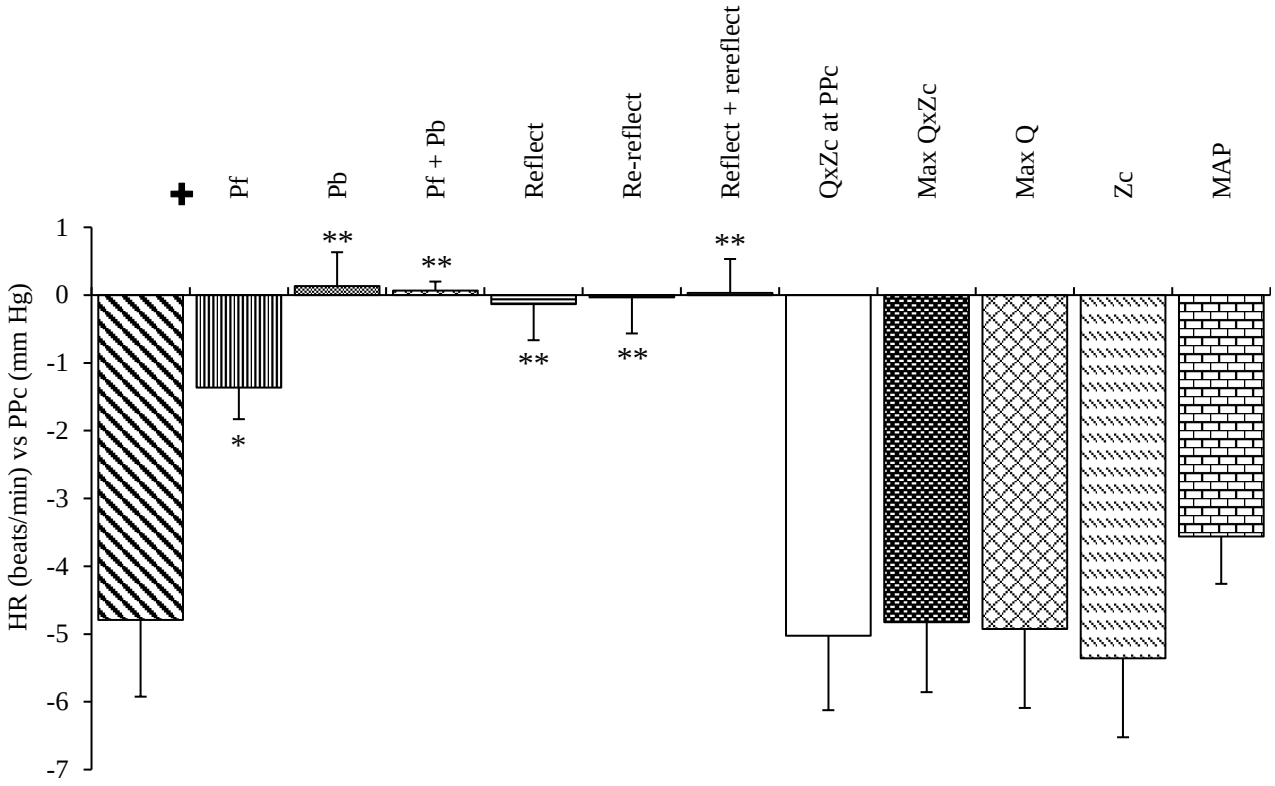


Figure 3.3: The slope of the relationship between HR and PPc, and the impact of each of the determinants of PPc on the relationship between HR and PPc in response to PE. A positive (**✚**) symbol indicates the addition of each of the determinants of PPc separately in the multivariate model – the relationship between HR and PPc. * $p<0.005$, ** $p<0.0001$ compared to the relationship between HR and PPc before adjustments. Abbreviations, HR; heart rate, PPc; central pulse pressure, Pf; forward pressure, Pb; backward pressure, reflect; reflected pressure, re-reflect; re-reflected pressure, PQxZc at PPc; product of flow and characteristic impedance at central pulse pressure, max PQxZc; maximum product of flow and characteristic impedance, max Q; maximum flow, MAP; mean blood pressure, and Zc; characteristic impedance.

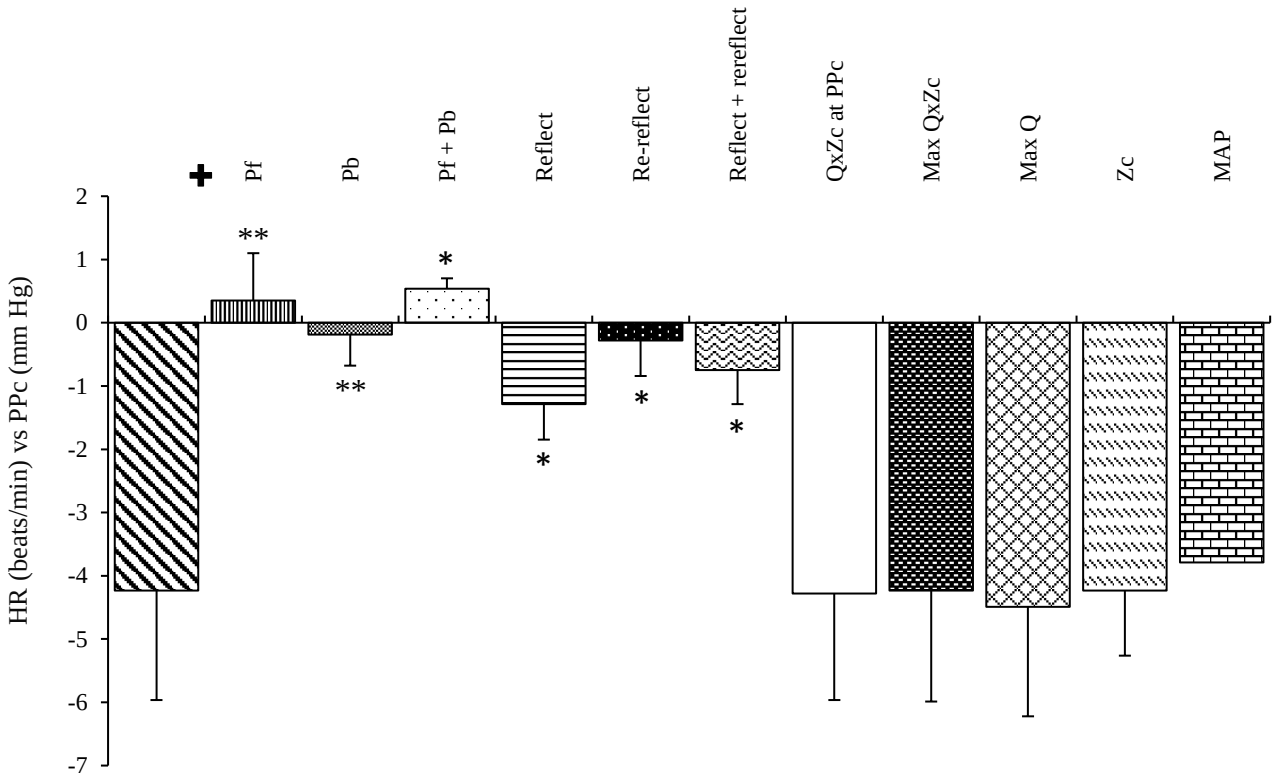


Figure 3.4: The slope of the relationship between HR and PPc, and the impact of each of the determinants of PPc on the relationship between HR and PPc in response to IVB. A positive (**+**) symbol indicates the addition of each of the determinants of PPc separately in the multivariate model – the relationship between HR and PPc. * $p<0.05$ ** $p<0.005$, compared to the relationship between HR and PPc before adjustments. Abbreviations, HR; heart rate, PPc; central pulse pressure, Pf; forward pressure, Pb; backward pressure, reflect; reflected pressure, re-reflect; re-reflected pressure, PQxZc at PPc; product of flow and characteristic impedance at central pulse pressure, max PQxZc; maximum product of flow and characteristic impedance, max Q; maximum flow, MAP; mean blood pressure, and Zc; characteristic impedance.

CHAPTER 4: DISCUSSION

4.1 Summary of Main Findings

In the present intervention study, we assessed the effect of heart rate (HR) on central pulse pressure (PPc), and how the determinants of PPc affect the relationship between HR and PPc in two rat models. We found an inverse relationship between HR and PPc in anaesthetised spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, in response to phenylephrine (PE) and ivabradine (IVB). Although aortic flow (Q) was inversely associated with HR, adjustments for Q did not alter the inverse relationship between HR and PPc. Similarly, the maximum pressure produced by the product of flow and characteristic impedance (max PQxZc) was inversely associated with HR, however, it did not affect the relationship between HR and PPc. Characteristic impedance (Zc) showed a positive relationship with HR and similar to max PQxZc and Q, Zc had no impact on the relationship between HR and PPc. In contrast, forward wave pressure (Pf), backward wave pressure (Pb), reflected pressure, and re-reflected pressure were inversely associated with HR. In addition, adjustments for Pf, Pb, reflected pressure, and re-reflected pressure in separate models, abolished the inverse relationship between PPc and HR. Hence, forward and reflected wave pressures, and not Q and Zc, are the determinants of the inverse association between HR and PPc.

4.2 Inverse Relationship between HR and PPc

The prevailing body of research corroborates the notion of an inverse correlation between HR and PPc, a relationship predominantly observed in cross-sectional investigations (Mthembu *et al*., 2022a), case-control studies (Papaioannou *et al*., 2005), and intervention-based research. For example, the CAFÉ study found that β-blockers increased central BP more than amlodipine despite similar brachial BP, with lower HR linked to higher central BP (Williams *et al*., 2006; Williams *et al*.,2009). This inverse relationship was also observed in healthy individuals (Torjesen *et al*., 2014; Mthembu *et al*., 2022b; Mthembu *et al*., 2022c) and

patient populations (Tan *et al*., 2016a; Tan *et al*., 2016b; Alfie *et al*., 2021). Furthermore, an intervention study on IVB in CAD patients (Rimoldi *et al*., 2016) supported this trend, suggesting that reduced heart rate may impact central blood pressure.

The present findings maintain and expand on previous studies, demonstrating a negative correlation between HR and PPc, whether through the administration of the vasoconstrictor (PE) or the inhibitor of the cardiac pacemaker current (IVB) interventions. Consistent with this notion, our investigation revealed that HR was reduced in response to PE and IVB administration, which is associated with an elevated PPc, observed in hypertensive and normotensive rats.

However, our findings and those of others (Williams *et al*., 2006; Torjesen *et al*., 2014; Tan *et al*., 2016a; Tan *et al*., 2016b; Rimoldi *et al*., 2016; Xiao *et al*., 2018; Alfie *et al*., 2021; Mthembu *et al*., 2022a; Mthembu *et al*., 2022b; Mthembu *et al*., 2022c) appear to be inconsistent with those of Dillinger *et al*., (2015), who conducted an intervention study involving normotensive patients with stable CAD. This study did not observe an increase in central arterial pressure despite reducing HR with IVB. Although it is commonly posited that the HR-PPc relationship strengthens with advancing age and arterial stiffness, neither of these parameters' accounts for their findings (Dillinger *et al*., 2015). The Dillinger study employed a crossover design with a cohort consisting of 12 individuals above 60 years of age (Dillinger *et al*., 2015). It is important to highlight that this study did not directly investigate the relationship between HR and PPc (Dillinger *et al*., 2015). Instead, the primary focus was on the response to IVB, revealing a significant decrease in HR but no change in PPc (Dillinger *et al*., 2015). The observed differences in PPc (baseline and intervention follow-up) between the IVB and placebo groups were 3.8 mm Hg and 2.9 mm Hg, respectively (Dillinger *et al*., 2015). The apparent lack of relationship between HR and PPc in this study (Dillinger *et al*., 2015),

may be attributed to the small sample size (n=12), which might have limited the capacity to detect the significant difference in PPc in response to IVB.

4.3 Mechanisms Explaining the Inverse Relationship between HR and PPc

The inverse relationship between HR and PPc has been a topic of considerable discussion, with multiple mechanisms suggested to explain this phenomenon. Among these, Pf, Pb, reflected pressure, and re-reflected pressure, arterial stiffness and aortic Q are key determinants. The impact of each of these determinants on the relationship between HR and PPc, in the present study in comparison to previous studies, will be discussed below.

4.3.1 Impact of Pf, Pb and Reflected Waves on the HR-PPc Relationship

Recent cross-sectional studies indicate that HR may be related to backward (reflected, Pb) wave pressures (Tan *et al*., 2016a; Mthembu *et al*., 2022a; Mthembu *et al*., 2022b; Mthembu *et al*., 2022c), whereby a lower HR may be associated with an increase in Pb. The pressures generated by Pb, and their subsequent re-reflections explain the inverse relationship between HR, Pf, and PPc. Furthermore, recent cross-sectional and modelling studies have indicated that the inverse relationship between HR and PPc is influenced by the frequency-dependent nature of wave reflection (Xiao *et al*., 2018; Mthembu *et al*., 2022b). This phenomenon may explain the increases in wave reflection, re-reflection and, consequently, the augmentation of Pf, as discussed below.

In the present intervention study, we observed an inverse correlation between HR and PPc, as well as between HR and several determinants of PPc including Pf, Pb, reflected pressure, and re-reflected pressure. Moreover, statistical adjustment for these variables appeared to diminish or abolish the relationship between HR and PPc, suggesting that these wave-related components may mediate this association.

These effects may be explained by harmonic interactions between forward and backward waves. At lower HRs (corresponding to lower harmonic frequencies), the frequency range is narrower, and waveforms are more likely to interact constructively—meaning they align in phase, thereby amplifying overall wave amplitude and central pressure. In contrast, at higher HRs, the wider frequency range may increase the likelihood of destructive interference, where out-of-phase wave components attenuate each other (Xiao *et al*., 2018; Segers and Chirinos, 2022). Constructive interference refers to the phenomenon where pressure waves travelling in the same direction and phase reinforce each other, increasing pressure amplitude. Destructive interference occurs when these waves are out of phase and reduce overall amplitude. Additionally, backward pressure waves that undergo re-reflection may further increase Pf by superimposing on the forward wave (Davies *et al*., 2012; Westerhof *et al*., 2015). Together, these dynamics may contribute to elevated PPc at lower HRs.

It remains an open question whether the inverse HR–PPc relationship differs between WKY and SHR. Spontaneously hypertensive rats (SHRs) displayed lower HR and higher PPc than WKY, potentially suggesting strain-specific variations in wave reflection. However, the current study was not designed or powered to directly test for these differences.

4.3.2 Impact of Arterial Stiffness on HR-PPc Relationship

Previous studies have shown that changes in arterial stiffness impact wave reflections, consequently influencing PPc (Wang *et al*., 2010; Mthembu *et al*., 2022c). Similarly, several other studies highlight the significance of wave reflections in predicting cardiovascular outcomes across various patient cohorts, particularly in association with arterial stiffness (Wang *et al*., 2010; Tsao *et al*., 2014; Mthembu *et al*., 2022c). Indeed, in cross-sectional studies, the slope of the relationship between HR and PPc was steeper in individuals with increased

arterial stiffness, including those over 60 years of age and patients with critical limb ischemia (CLI) or stroke (Alfie *et al*., 2021; Mthembu *et al*., 2022c).

In the present study, we found that SHR exhibited increased Zc in response to PE administration compared to WKY. Notably, there were no differences in baseline aortic root diameter (AoD) between the strains, but PE also caused a greater increase in peak aortic flow (Q) in SHR. Since Zc is calculated from both pressure and flow, this elevation in Zc may reflect the combined effects of PE-induced changes in both vascular tone and aortic flow rather than baseline stiffness alone. Importantly, during IVB administration, no significant differences in Zc or Q were observed between WKY and SHR.

These findings highlight the complexity of interpreting Zc in different haemodynamic contexts. The increase in Zc during PE suggests a transient elevation in impedance that may amplify wave reflections and Pb, particularly in SHR. However, without a consistent difference in Zc across interventions and given the lack of baseline measurements, we cannot definitively attribute observed PPc changes solely to differences in aortic stiffness.

Nonetheless, increased arterial stiffness has been shown to reduce harmonic frequencies and enhance wave reflection, particularly at lower HRs (Wang *et al*., 2009; Mthembu *et al*., 2022b; 2022c). These changes may increase the magnitude of both Pb and Pf, thus contributing to elevated PPc. The reflection coefficient and pulse wave harmonics are also believed to mediate this relationship (Xiao *et al*., 2018). Although our study observed a rise in Zc in SHR during PE, it remains speculative whether these changes reflect inherent strain differences or are purely pharmacologically induced. Future studies should include baseline haemodynamic recordings and be adequately powered to assess potential differences between normotensive and hypertensive models.

4.3.3 Impact of Flow on HR-PPc Relationship

In contrast to the contributions of Pf, Pb and re-reflected pressure as discussed in the aforementioned sections, some of the studies have indicated that increased Q and stroke volume (SV) are the primary mechanisms responsible for the HR-PPc relationship. Prior research by Nieminen *et al*., (2006) and Avolio *et al*., (2009) has posited that alterations in LV filling volumes and SV are key determinants influencing the relationship between HR and PPc. They proposed that a lower HR allows for extended cardiac filling time, resulting in increased LV filling volumes, consequently leading to an increase in SV and subsequent Q augmentation, thereby elevating PPc (Nieminen *et al*., 2006; Williams *et al*., 2006; Avolio *et al*., 2009; Sluyter *et al*., 2016). However, in all of these studies, the authors focused on pressure wave analysis alone and assumed that late systolic pressure augmentation is an adequate index of Pb. However, more recent studies have demonstrated that HR effects on PPc are in fact accounted for by increases in reflected pressure waves rather than increases in systemic flow (Bello *et al*., 2021; Mthembu *et al.,* 2022b).

Our findings indicate a lack of significant correlation between Q and HR. This finding aligns with recent cross-sectional studies which have demonstrated that although there is an increase in LV filling volumes and hence Q at lower HR, these factors fail to account for the relationship between HR and PPc (Mthembu *et al*., 2022a; Mthembu *et al*., 2022b). Thus, the present study suggests that the determinants of PPc, including Pf, Pb, reflected pressures and re-reflected pressures rather than Q, all contribute to the elevation of PPc when HR is reduced.

4.3.4 Consequences of Increased PPc

In the present study, we observed that the increase in PPc was primarily driven by the combined effects of reflected and re-reflected pressure waves, as well as heightened arterial stiffness. Our findings indicate that at lower HRs, augmented wave reflections contributed

significantly to the elevation in PPc, particularly in SHR, where increased arterial stiffness amplified wave reflection pressures, leading to greater central arterial load. The increased Zc in SHR following PE administration further supports the role of arterial stiffness in modulating PPc; greater impedance results in earlier wave reflections and higher central pressures.

Moreover, our data suggest that the exaggerated wave reflections in SHR likely impose a greater afterload on the LV compared to WKY. This increased afterload may contribute to ventricular remodeling, myocardial strain, and impaired cardiac efficiency, independent of changes in brachial PP. These findings align with previous studies demonstrating that higher PPc is a stronger predictor of LV hypertrophy and cardiovascular risk than brachial PP (Phan *et al*., 2016; Bello *et al*., 2020).

The elevated PPc observed in SHR also suggests a potential link between increased wave reflections and cardiac dysfunction. A stiffer aorta, as reflected in the increased Zc, leads to earlier wave reflections that coincide with systole. Thus, increasing myocardial oxygen demand and reducing coronary perfusion efficiency; potentially elevating the risk of LV dysfunction, ischaemic heart disease and heart failure (Kahan and Bergfeldt, 2005; Chirinos *et al*., 2012; Nwabuo and Vasan, 2020).

4.4 Physiological Effects of IVB and PE

Ivabradine (IVB) is a specific HR-lowering agent that selectively acts on pacemaker activity in the sinoatrial node of the heart (Ferrari *et al*., 2006). Ivabradine, similar to β-blockers, decreases HR and myocardial oxygen consumption through its pharmacological action (Bangalore *et al*., 2008; Guglin, 2013), which benefits and hence is used to manage chronic stable angina in patients with a normal sinus rhythm (Li *et al*., 2014). These benefits are believed to improve diastolic filling time and hence potentially enhance SV and cardiac output (CO) (Oliver *et al*., 2019), indicating a potential role for improved cardiac remodelling

(Rehsia and Dhalla, 2010; Bain, 2018; Oliver *et al*., 2019). In the SHIFT trial, it was observed that combining β-blockers with IVB to reduce HR when it exceeded 70 beats/min resulted in fewer negative cardiovascular outcomes (Swedberg *et al*., 2010). However, the current study has demonstrated that while IVB effectively lowers HR, it also increases PPc, which may exert deleterious effects on the cardiovascular system. Furthermore, this impact may be related to the well-established limited ability of β-blockers reduce PPc when employed as first-line therapy for hypertension (Wiysonge *et al*., 2017; Dézsi and Szentes, 2017; Wright *et al*., 2018; Whelton *et al*., 2018).

Phenylephrine (PE) primarily functions as an alpha (α)-1 adrenergic receptor agonist, causing vasoconstriction and increasing systemic vascular resistance (SVR) and cardiac afterload (Kalmar *et al*., 2018). In the present study, we observed that the administration of PE resulted in increased BP and decreased HR in both SHR and WKY rats. This response is attributed to a reflex mechanism (Kalmar *et al*., 2018). Notably, our findings revealed that Zc was higher in SHR compared to WKY rats in response to PE. Additionally, Zc was positively correlated with HR in the presence of PE, indicating vasoconstriction. This vasoconstrictive effect is believed to significantly impact SV and CO, leading to increased workload and afterload which may impair ventricular function and exacerbate LVH over time (Anusorntanawat *et al*., 2016; Swenson *et al*., 2018).

Moreover, previous studies have shown that older (≥60 years) normotensive and hypertensive individuals respond to PE similarly, characterised by more substantial increases in systolic BP and less pronounced reductions in HR and LV function compared to younger normotensives (White *et al*., 1999). In hypertensive individuals, who often present with persistently high BP and left ventricular remodelling or hypertrophy, the cardiac responses to increased afterload are comparable to those of normotensives in the presence of PE (White *et al*., 1999; Nwabuo and Vasan, 2020). This similarity suggests that hypertension does not

significantly alter these responses once remodelling has occurred (Nadruz, 2015). Thus, the reduction of HR with PE mimics hypertension in older normotensive individuals, and the inverse relationship between HR and PPc may explain this effect.

4.5 Clinical Relevance and Implications

Patients with hypertension and cardiac conditions often necessitate HR-reducing agents such as β-blockers and IVB to lower BP (and HR) to physiologically safe levels, mitigating the risks of HF, stroke, and kidney damage (Perret-Guillaume *et al*., 2009). If the underlying cause of the inverse correlation between HR and PPc were solely an increase in Q due to prolonged filling time, it would be advantageous for patients prescribed HR-reducing medications (such as IVB or β-blockers) for HF. The present study reveals that wave reflection pressures, rather than increased filling time or Q, serve as the mechanism augmenting PPc. Understanding these mechanisms is critical, as it guides to a better and safer use of IVB, β-blockers and alternative HR-reducing agents in patients compelled to use these drugs.

In the present study, the observed increase in Zc in response to PE in SHR indicates resistance to Q, likely stemming from hypertension-related complications such as proximal aortic stiffness. This resistance increases LV afterload (Mthembu *et al*., 2022b), with increases in Pb and wave re-reflection further contributing to heightened cardiac afterload. This rise in cardiac afterload increases the risk of LVH, exacerbating the pre-existing cardiac conditions (high central BP) and increases the likelihood of cardiac dysfunction. Therefore, reducing cardiac afterload is crucial for effectively managing patients with cardiac conditions. Thus, patients with hypertension and additional cardiac conditions requiring HR-reducing treatment necessitate more intensive medication to effectively manage hypertension thus lowering central BP.

In cases where HR-lowering medications are required, their administration should be accompanied by strategies aimed at mitigating these cardiovascular adverse effects. One prospective strategy to alleviate the detrimental effects of HR on central arterial pressure involves reducing Zc by lowering aortic distending pressures (Mitchell *et al*., 2010b). This can be achieved using various antihypertensive agents, particularly those with vasodilatory properties (Agnoletti *et al*., 2013; Manisty and Hughes, 2013), and thus, intensive brachial BP reduction, potentially to levels recommended by the current American guidelines (systolic BP <130 mmHg) (Whelton *et al*., 2018) are warranted to diminish Zc. This reduction would, in turn reduce the influence of HR on central arterial pressures in individuals with increased aortic stiffness. By lowering distending pressures and thereby reducing aortic stiffness and Pf, the magnitude of backward waves would reduce, as Pb depends on Pf due to inertial effects. As a result, a lower PPc would be achieved at normal HR range. Thus, the present study findings may in-part be used to explain why intensive brachial BP lowering, as seen in the SPRINT trial (SPRINT Research Group, 2021) may have led to improved outcomes for HF patients.

In this study, we demonstrate the impact of each of the determinants of PPc on the relationship between HR and PPc, revealing their direct and independent influences. Our examinations yield insights into the causative mechanisms by which HR influences PPc, harnessing an understanding of this dynamic relationship. Understanding the relationships between HR, PPc, and haemodynamic determinants can help develop targeted interventions aimed at persons with hypertension and associated cardiovascular risks.

4.6 Possible Limitations

My study has several potential limitations. Firstly, the study was carried out with animals under anaesthesia, which is acknowledged to impact cardiovascular function and, hence, absolute pressure and HR values. However, the study aimed to assess relationships

between HR and pressures rather than specific BP and HR values. Furthermore, when extrapolating these findings to human physiology, it is important to proceed with caution as animal models do not perfectly replicate the complexity of the human cardiovascular system. In this regard, rodents have much higher (approximately 5 times) HR than humans. Nevertheless, the use of animal models is vital to uncovering the mechanisms of the HR-PPc relationship, as ethical issues preclude placebo-controlled intervention studies in humans. However, an ethical human study design to investigate the relationship between HR and PPc, as well as the determinants of PPc, would involve patients with artificial pacemakers. In such studies, patients with pacemakers could serve as their own controls, as the pacing allows for HR to be altered within the same individual. Such a study is currently ongoing within our research unit.

This is the first study to demonstrate this relationship in animals under experimental conditions. However, it is important to determine whether this relationship differs between SHR and WKY rats, as strain-specific variations may exist. While trends were consistent across both strains, some relationships differed in magnitude, reinforcing the need for separate analyses. Nonetheless, due to the limited sample size in each strain, this was not the study's primary focus.

4.7 Possible Future Studies

Future studies may explore the HR-PPc relationship in models of increased arterial stiffness and compare hypertensive versus normotensive rats to better understand how these conditions affect the determinants of PPc, such as Pf, Pb, and wave reflection. Investigating models with increased arterial stiffness (e.g., hypertensive rats) could provide insights into whether the inverse relationship between HR and PPc is enhanced under heightened arterial stiffness, consistent with previous cross-sectional studies. Such insights are important as

increases in arterial stiffness are common in many cardiovascular diseases. Comparative studies between hypertensive and normotensive rats would help determine how elevated BP and associated vascular changes modify the HR-PPc relationship and whether specific management strategies are required for hypertensive individuals.

Additionally, future research may include a broader range of BP and HR-modulating agents beyond PE and IVB in order to further clarify the mechanisms by which HR influences PPc. Examining different classes of HR-reducing agents, especially those with diverse effects on arterial tone, could identify optimal therapeutic approaches for managing conditions with increased PPc.

Previous studies have primarily utilised wave separation analysis (WSA) in human subjects to assess arterial stiffness and wave reflection dynamics. The application of WSA in rodent models has been limited, necessitating validation for preclinical research. Recent advancements have adapted WSA for use in rats, specifically in the femoral and carotid arteries, enabling the computation of key haemodynamic indices such as backward wave amplitude, Zc, and the reflection index (Bohaychuk, 2016). The validity of this approach has been supported by the expected alterations in backward wave amplitude following acute administration of norepinephrine and sodium nitroprusside, and by the consistency of Zc values with those previously reported for the aorta and carotid artery, demonstrating WSA's capacity to detect physiologically relevant haemodynamic changes in rats (Bohaychuk, 2016).

While these findings provide a foundation for using WSA in rodent studies, we acknowledge the need for further validation of WSA in rodent models. Future research should focus on comparing central (ascending aorta) and peripheral (femoral and carotid) arterial sites in rats to ensure the consistency of WSA indices across different vascular regions. Moreover, studies investigating the impact of BP and HR variations on WSA parameters in rodents would

strengthen its reliability. Furthermore, future research should also explore the utility of WSA in chronic arterial stiffening models, such as hypertension, atherosclerosis, and diabetes, to assess its applicability in preclinical research.

4.8 Conclusion

The findings from this intervention study confirm the inverse relationship between HR and PPc. Through the administration of PE and IVB, we observed a consistent inverse correlation between HR and PPc, and identified the determinants of the inverse relationship between HR and PPc (Pf, Pb, reflected pressure, and re-reflected pressure). Notably, even after making adjustments for Q and Zc, the inverse relationship between HR and PPc remained unchanged, indicating that these factors are not the mechanisms behind this relationship. However, the present study, effectively demonstrated that the determinants of PPc, particularly Pf, Pb, reflected pressure, and re-reflected pressure, are the mechanisms by which PPc increases in association with decreases in HR. These findings offer valuable insights into the inverse relationship between HR and PPc, as well as the determinants of PPc, which can potentially guide the management of patients requiring HR-reducing agents. One potential therapeutic approach highlighted by this study would involve more aggressive BP reduction in these patients, leading to reductions in Pf, Pb, and wave reflection. This approach could effectively lower PPc, cardiac afterload, and pressure pulsatility, thereby offering new avenues for patient management.

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Appendix I: Animal Ethical Clearance Certificate

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

CLEARANCE CERTIFICATE NUMBER: 2021/03/06/C

APPLICANT: Ms N Mthembu
School: School of Physiology; Department: N/A; Location: WRAF

PROJECT TITLE: Aortic Functional Changes with Heart Rate Reduction Following ß-Adrenoreceptor Blocker Use in the Aging Spontaneously Hypertensive Rat
Category: C; Species and Numbers involved: *15X 4-7 months and 15X 14 months, male WYK Rats, 30X 4-7 months and 30X 14 months, male SHR Rats and 15X 4-7 months and 15X 14 months, male Wistar Rats*

Approval is hereby given for the use of animals for the research project named above and described in the application reviewed by a quorate meeting of the AREC held on 30 Mar 2021. This approval remains valid until 9 May 2021 and is conditional to the following (if blank there are no special conditions):

Condition 1	Condition 2	Condition 3	Condition 4

All material changes to the approved research must be reported to the AREC before they are implemented. Failure to do so will invalidate this clearance certificate.

An annual progress report must be provided to the AREC.

The use of these animals is subject to AREC guidelines on the use and care of laboratory animals, is limited to the procedures described in the application and is subject to additional conditions listed below:

I, the Chair of the AREC (or my designated representative) am satisfied that the proposed research is ethical as judged by local law, international standards and University policy.

Signed: _____ _____ Date: ___10/05/2021____

(Chairperson of the AREC)

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

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Wits Research Animal Facility (WRAF): Dr Kim Jardine

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Signed: _ _____ Date: _11 May 2021_____

(Registered Veterinarian)

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL RESEARCH ETHICS COMMITTEE

MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Ms Nonhlanhla Mthembu and Mr Muhammed Suraj Yusuf

b. School and email address: School of Physiology, nonhlanhla.mthembu@yahoo.com

yusufmsuraj@kasu.edu.ng

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

Original AESC number	**2021**	**03**	**06C**
Other M&Es :			Nil

d. Project Title: Aortic Functional Changes with Heart Rate Reduction Following ß-Adrenoreceptor Blocker Use in the Aging Spontaneously Hypertensive Rat.

		No.	**Species**
e.	Number and species of animals originally approved:	120	*Rats*
f.	Number of additional animals previously allocated on M&Es:	0	N/A
g.	Total number of animals allocated to the experiment to date:	120	Rats
h.	Number of animals used to date:	46	Rats

i. Specific modification / extension requested: Nil

j. Motivation for modification / extension I (Ms Nonhlanhla Mthembu and Mr Suraj Yusuf) would like to add PIs: Katleho Khanye (student number: 1474838) and Ntapo Marcus Lebelo (student number 1607905) to be involved in the rat study as CPGRU postgraduate students. Soon we will start terminations. We require more hands during surgery and they will be assisting in looking after the animals every day of the week especially as the animals are growing older.

Date: 18/03/2022

Signature:

RECOMMENDATIONS

Date:

Signature:

Chairman, AREC

Appendix II: Change of title

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Ms Sandra Munesar
E-mail: Sandra.munesar@wits.ac.za

11 October 2024
Person No: 1474838
TAA

Mr KN Khanye
5006 Blackwood Ave
Ratanda
Heidelberg
1441
South Africa

Dear Mr Katleho Khanye

Master of Science in Medicine: Change of title of research

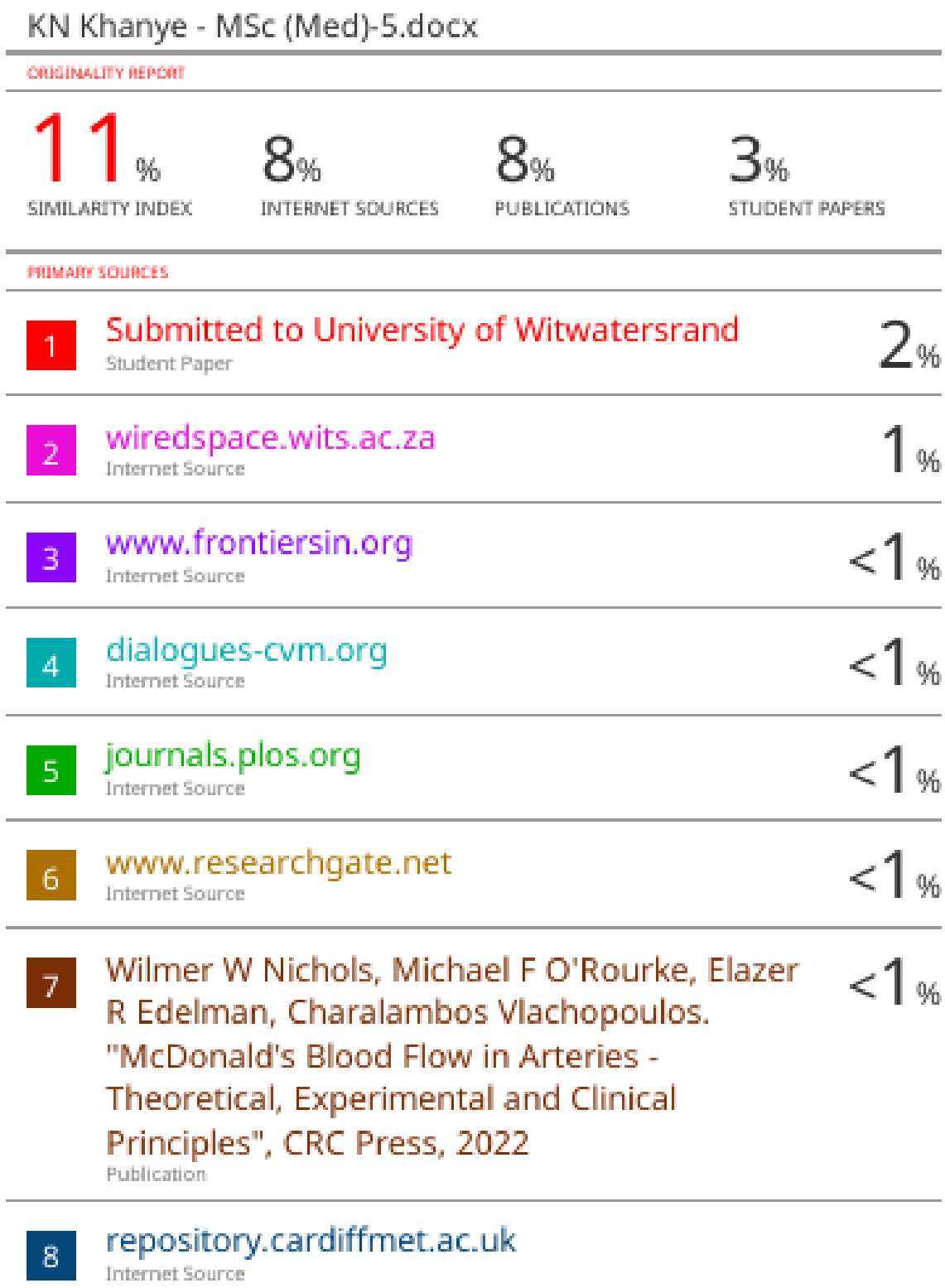
I am pleased to inform you that the following change in the title of your Dissertation for the degree of **Master of Science in Medicine** has been approved:

From:	**The impact of ß-adrenoreceptor blocker therapy on aortic functional changes and central pressure in Spontaneously Hypertensive Rats (SHR)**
To:	**Impact of aortic pulse pressure determinants on the heart rate-aortic pulse pressure relationship: an intervention study in rats**

Yours sincerely

Ms Sandra Munesar
Faculty Registrar
Faculty of Health Sciences

Appendix III: Turn-it-in Plagiarism Report



KN Khanye - MSc (Med)-5.docx

ORIGINALITY REPORT

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