Effects of heart rate on arterial stiffness

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Declaration

I certify that the work in this thesis entitled "Effects of heart rate on arterial stiffness" has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis was approved by both the Macquarie University Animal Ethics Committee and the Macquarie University Human Research Ethics Committee, with reference numbers and approval dates listed below:

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List of publications

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Articles:

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2. **Tan I**, Spronck B, Kiat H, Barin E, Reesink KD, Delhaas T, Avolio AP, Butlin M. Heart Rate Dependency of Large Artery Stiffness. *Hypertension*. 2016;68(1):236-242. doi:10.1161/HYPERTENSIONAHA.116.07462.

3. **Tan I**, Kiat H, Barin H, Butlin M, Avolio AP. Effects of pacing modality on non-invasive assessment of heart rate dependency of indices of large artery function. *J Appl Physiol*. 2016;121:771-780.

Book chapters:

4. Avolio, AP, Butlin, M, **Tan, I**. Heart rate, synchrony and arterial hemodynamics. In: *Blood Pressure and Arterial Wall Mechanics in Cardiovascular Diseases*. 2014:267-279. doi:10.1007/978-3-319-02904-7.

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5. **Tan I**, Ng K, Butlin M, Liu YY, Xu K, Avolio AP. Effects of arterial pressure mediated baroreflex heart rate changes on pulse wave velocity in the rat aorta. *Heart, Lung and Circulation*. 2009;18:S274. (57th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, Sydney, August 2009)

6. **Tan I**, Butlin M, Liu YY, Lindesay G, Ng K, Avolio AP. Heart rate-dependence of aortic pulse wave velocity in the rat. *Hypertension.* 2010;55:1511. (31st Annual Scientific Meeting of the High Blood Pressure Research Council of Australia, Sydney, December 2009)

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8. **Tan I**, Butlin M, Kiat H, Barin E, Avolio AP. Effects of heart rate on arterial stiffness, central haemodynamics, cardiac output and total peripheral resistance. *Hypretension.* 2014;63:e149. (35th Annual Scientific Meeting of the High Blood Pressure Research Council of Australia, Melbourne, December 2013)

9. **Tan I**, Kiat H, Barin E, Butlin M, Avolio AP. Effects of acute, sympathetic-independent increases in heart rate on arterial stiffness, central haemodynamics and cardiac function. *Journal of Hypertension.* 2014;32:e96. (Joint Meeting of the European Society of Hypertension and International Society of Hypertension, Athens, June 2014).

10. **Tan I**, Kiat H, Barin E, Butlin M, Avolio AP. Acute, sympathetic-independent increases in heart rate by way of cardiac pacing raises aortic and brachial blood pressure with increased cardiac output and arterial stiffness. *Artery Research.* 2014;8:166. (ARTERY 2014, Maastricht, October 2014).

11. **Tan I**, Butlin M, Kiat H, Barin E, Avolio AP. Augmentation index differs in paced subjects with different pacing modalities, independent of heart rate and blood pressure. *Hypertension.* 2015;8:166. (36th Annual Scientific Meeting of the High Blood Pressure Research Council of Australia, Adelaide, December 2014).

12. Butlin M, Lindesay G, Viegas KD, **Tan I**, Avolio AP. Cardiovascular parameters other than mean arterial pressure are predictive of dynamics changes in aortic stiffness in the rat. *Artery Research.* 2011;5:160. (ARTERY 2011, Paris, October 2011)

13. Lindesay G, **Tan I**, Butlin M, Avolio AP. Large artery stiffness increases with local neurogenic blockade in rats. *Hypertension.* 2010;55:1503. (31st Annual Scientific Meeting of the High Blood Pressure Research Council of Australia, Sydney, December 2009)

14. Liu YY, **Tan I**, Butlin M, Ng K, Xu K, Avolio AP. Effect of high dose gamma irradiation on aortic pulse wave velocity in rats. *Heart, Lung and Circulation.* 2009;18:S273?S274. (57th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, Sydney, August 2009)

15. Ng K, Butlin M, Liu YY, **Tan I**, Xu K, Avolio AP. Pressure-dependent amplification of the pressure pulse along the aorta in normotensive and spontaneously hypertensive rats. *Heart, Lung and Circulation.* 2009;18:S281. (57th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, Sydney, August 2009)

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20. **Tan I**, Butlin M, Liu YY, Ng K, Avolio AP. Heart rate dependency of pulse wave velocity in the rat aorta. (23rd Scientific Meeting of the International Society of Hypertension Meeting, Vancouver, 2010)

21. **Tan I**, Xu K, Butlin M, Avolio AP, Pressure-independent effects of heart rate on aortic pulse wave velocity. (22nd European Meeting on Hypertension and Cardiovascular Protection, London, 2012)

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Abstract

Both elevated heart rate (HR) and increased arterial wall stiffness are independent predictors for cardiovascular disease and mortality. Whilst it is well established that arteries stiffen with age and increased blood pressure (BP), research findings to date have failed to converge on the relationship between HR and arterial stiffness. This thesis explores the HR-arterial stiffness relationship through the study of pulse wave velocity (PWV) changes, a surrogate measure of arterial stiffness, with changes in HR induced by artificial cardiac pacing in both rodents and humans. In both the rodent study and human study, HR was shown to exert an independent effect on PWV, with PWV increasing as HR increased, and the intrinsic HR dependency of PWV was quantified in the human study as 0.17 m/s per 10 bpm increase in HR. As external cardiac pacing is often used as a means to induce HR changes, as it was in the studies in this thesis, the effect of different pacing modalities on indices of pulse wave analysis and arterial stiffness was also explored. Practical applications of the associations between HR, BP and PWV were also demonstrated through the estimation of systolic times that utilised pulse wave analysis and PWV. To investigate a possible mechanism by which HR exerts an influence on arterial stiffness, a computerised transmission line model of the human arterial tree was utilised to simulate effects of HR on PWV at different scenarios where arterial wall elasticity was modelled with varying frequency dependence. Model simulations showed that frequency dependency of arterial wall elasticity, beyond a critical point, could partly explain the observed increases in PWV with increasing HR. The findings in this thesis not only lend further evidence to an independent HR effect on arterial stiffness, but also provide an insight into the mechanisms behind this relationship. Quantification of the intrinsic HR dependency on PWV will allow for practical application of this established relationship in future cardiovascular studies.

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List of Acronymns

AIx	augmentation index
AP	augmentation pressure
BP	blood pressure
DBP	diastolic blood pressure
ECG	electrocardiogram
ED	ejection duration
HR	heart rate
LVET	left ventricular ejection time
MAP	mean arterial pressure
PEP	pre-ejection period
PP	pulse pressure
PWV	pulse wave velocity
RI	reflection index
RM	reflection magnitude
SBP	systolic blood pressure
TLM	transmission line model

CHAPTER 1

Introduction

A beating heart is the most basic evidence of life, and arteries are the link between a beating heart and the rest of the body for sustaining life. The intricate interaction between the heart and the vasculature has fascinated physicians and scientists alike for centuries, and particular attention has been paid to the arterial pulse as the window through which information about the heart, the arteries, and their interaction could be obtained.

With each beat of the heart, the contraction of the heart's ventricles generates a force that propels blood forward into the pulmonary circulation for replenishment of oxygen, and into the systemic circulation for transporting oxygen and nutrients to the rest of the body. As the heart relaxes, there is temporary relief before the next cycle begins again. This rhythmic contraction and relaxation of the heart results in a pulsatile outflow of blood, and is the source of the arterial pulse. Whilst the heart's output is intermittent, the organs and tissues require a continuous, steady supply of oxygen, and the large elastic arteries, in particular the aorta, play the important function of cushioning and buffering the pulsatile output from the left ventricle such that smooth, laminar flow can be achieved at the level of the arterioles and capillaries (London and Guerin, 1999; Nichols et al., 2011). This function is achieved by the arteries distending due to the increased arterial blood pressure and storing a portion of the stroke volume during systole, then returning to their steady state and thus releasing the stored volume of blood during diastole. Such action can only be made possible by a viscoelastic arterial wall that

has the capacity to stretch and recoil in a nonlinear fashion, where stiffness is matched to the arterial compliance such that an optimal portion of stroke volume is stored with each heartbeat (Avolio, 2013). An increase in arterial wall stiffness beyond the optimal state can significantly impact on the cushioning reservoir function of the large arteries, resulting in an increase in central aortic systolic pressure and increased stroke work for the left ventricle (Nichols et al., 2011). These consequences can further lead to left ventricular hypertrophy (Nitta et al., 2004; Roman et al., 2000), reducing the heart's mechanical efficiency and increasing myocardial oxygen demand (Kelly et al., 1992). Furthermore, the impact of arterial stiffening has been shown to extend much further from the heart to the brain and kidneys, where increased pulsations in the microcirculation resulting from the diminished buffering function of the large arteries can lead to damage in these organs (Mitchell, 2008). The importance of arterial stiffness has been emphasised with the recent guidelines on the management of hypertension, which added arterial stiffening as a factor influencing the prognosis of hypertension (Mancia et al., 2007). This came in the midst of a wealth of studies over the past two decades indicating that increased arterial stiffness is an independent risk factor for cardiovascular diseases and events (Mattace-Raso et al., 2006; Willum-Hansen et al., 2006), as well as an independent predictor for cardiovascular and all-cause mortality in both healthy (Shokawa et al., 2005; Sutton-Tyrrell et al., 2005; Willum-Hansen et al., 2006) and diseased populations (Blacher et al., 1999; Cruickshank et al., 2002; Laurent et al., 2001, 2003).

Arteries, in particular large arteries such as the aorta, are subject to continuous and repetitive strain throughout life due to the pulsatile action of the heart. Whilst the total number of cardiac cycles throughout life has been shown to be associated with the loss of elasticity in the aortic wall, hence increased arterial stiffness (Avolio et al., 1998), the relationship between heart rate and arterial stiffness has remained controversial. Studies investigating the association of heart rate with arterial stiffness have been few and far between, and conclusions drawn from existing studies in both human and animal models have failed to converge. *Ex-vivo* studies of canine

arterial segments found that stiffness was independent of the rate of pulsations at frequencies above an equivalent heart rate of 60 bpm (Callaghan et al., 1984) or 120 bpm (Bergel, 1961a), but significant decreases in arterial distensibility with an increase in heart rate were observed in *in vivo* animal studies (Mangoni et al., 1996; Mircoli et al., 1999). Results from acute pacing studies on the effects of heart rate on arterial stiffness in humans were often confounded by a concomitant change in blood pressure with heart rate (Liang et al., 1999; Millasseau et al., 2005), while cross-sectional studies were evenly split in their conclusions, with just over half finding a significant association between heart rate and arterial stiffness (Cecelja and Chowienczyk, 2009). Taken together, current evidence seems to indicate that arterial wall stiffness can indeed be affected by the frequency of pulsatile strain an artery undergoes, but the effects may be relatively small compared to other major determinants of arterial stiffness like blood pressure and age (Reference Values for Arterial Stiffness' Collaboration, 2010). The implications of an association between heart rate and arterial stiffness are two-fold. Firstly, with the inclusion of arterial stiffening as a factor affecting the prognosis of hypertension (Mancia et al., 2007), measurement of arterial stiffness is increasingly being included in routine clinical assessments as well as large-scale clinical studies (Reference Values for Arterial Stiffness' Collaboration, 2010). This cements the need to identify any factors that may influence arterial stiffness measurements, such that appropriate adjustments can be made in order for accurate interpretation of the results. If heart rate, one of the most readily measured haemodynamic parameter as well as being highly variable, is found to influence arterial stiffness, it must be accounted for when arterial stiffness is measured. Secondly, elevated heart rate, like increased arterial stiffness, is also an independent risk factor for cardiovascular diseases and events (Benetos et al., 1999; Palatini and Julius, 1997), but there is no evidence as yet as to whether the presence of both risk factors would compound the cardiovascular risk. To further investigate the relationship between heart rate and arterial stiffness and the possible mechanisms underlying this relationship, in particular for acute heart rate changes, this thesis focuses on the effects of acute changes in heart rate on arterial stiffness,

using the propagation velocity of the arterial pulse, otherwise known as pulse wave velocity (PWV), as a surrogate measure of arterial stiffness.

The first investigation concerns the heart rate dependency of aortic stiffness in the rat at different mean arterial pressures (Chapter 3). Heart rate changes were achieved by *in situ* cardiac pacing at the right atrium, and PWV was measured in the aorta (details of how PWV measurements were achieved are described in Chapter 3). As blood pressure is known to influence PWV (Avolio et al., 1983; Bramwell and Hill, 1922a; Cecelja and Chowienczyk, 2009; Pruett et al., 1988; Spronck et al., 2015b), the study pharmacologically controlled for blood pressure such that beat-to-beat PWV could be measured over a wide physiological range of mean arterial pressures (60 mmHg to 150 mmHg) at paced heart rates of 300 bpm to 450 bpm. This allowed for PWV measured at different heart rates to be compared at the same arterial pressure at both low and high blood pressure, making this study the first *in-vivo* study to investigate whether the heart rate dependency of aortic stiffness would depend on the level of distending pressure.

Several animal studies in the past have shown an association between heart rate and arterial stiffness (Bergel, 1961a; Mangoni et al., 1996; Mircoli et al., 1999), but whilst aortic wall structure is similar in all mammals, including humans (Wolinsky and Glagov, 1967), conclusions drawn from animal studies cannot be directly applied to humans for many reasons. In addition, limited studies in humans have failed to converge in their findings, with results in acute studies often confounded by concomitant changes in blood pressure with heart rate (Liang et al., 1999; Millasseau et al., 2005), and study sizes were relatively small (Haesler et al., 2004; Lantelme et al., 2002b). To explore the relationship between heart rate and arterial stiffness in humans further, PWV was measured in a sample of 52 subjects with implanted pacemakers (Chapter 4), the largest cohort in acute studies to-date, at paced heart rates between 60 bpm and 100 bpm. By applying several blood pressure correction methods, this was the first study to quantify the intrinsic heart rate dependency of arterial stiffness in humans.

Although inducing changes in heart rate by way of cardiac pacing can minimise confounding effects on systemic circulation, different pacing modalities which stimulate the heart from different cardiac chambers can incur varying haemodynamic consequences. Whether or not these consequences can affect arterial stiffness measurements in acute pacing studies has not been investigated in detail. Thus, a sub-analysis of the pacing study (Chapter 5) was performed to determine the effects of pacing modality on heart rate dependency of arterial stiffness, as well as wave reflection, which is also related to arterial stiffness (Chirinos, 2012; Nichols et al., 2011).

To investigate a possible mechanism by which changes in heart rate can exert influence on changes in arterial stiffness, a transmission line model (Xiao et al., 2016) was used to assess the degree of frequency dependence of the elastic modulus necessary to simulate the effects of heart rate changes on PWV (Chapter 6). The aim of this study was to determine whether the arterial wall's frequency dependent elastic modulus could explain PWV differences with changing heart rates, and if so, propose a plausible frequency dependency of the elastic modulus.

Finally, the validity of using PWV and its associations with heart rate and blood pressure, together with the pulse wave analysis of the carotid pressure waveform, for estimating pre-ejection period, a cardiac related parameter, was investigated (Chapter 7).

CHAPTER 2

Literature Review

In an average human lifespan of 70 years (as per statistics reported by the World Health Organisation in 2015), the heart undergoes a total of some 2 billion contraction and relaxation cycles, assuming an average heart rate of 60 beats per minute. Correspondingly, the large arteries, which act as both conduits and cushioning buffers for the pulsatile outflow from the heart, experience repeated pulsatile strain for the same number of times. Arteries fulfil their cushioning function through the viscoelastic characteristics of the arterial wall (London and Guerin, 1999), which stretches under pressure to allow arteries to accommodate and store a portion of the stroke volume during systole, then recoils such that the stored portion is released during diastole, thus buffering the pulsatile output from the heart and resulting in an effectively smooth and constant flow at the arteriolar and capillaries level (London and Guerin, 1999; Nichols et al., 2011). The design of the arterial system is such that the stiffness of the arterial wall is matched to give arteries a compliance that maximises this blood storage, without being detrimental to blood flow to the peripheral tissues as would be the case if arteries were too compliant (Avolio, 2013). However, increased stiffening of the arterial wall can disrupt the cushioning function of the large arteries and result in increased systolic but decreased diastolic pressure, further leading to increased load on the left ventricle of the heart (Nichols et al., 2011) and other undesirable consequences (Kelly et al., 1992; Mitchell, 2008; Nitta et al., 2004; O'Rourke and Safar, 2005).

Evidently, an increase in heart rate would result in an increase in the number of times the

arteries undergo cyclic strain. Not only so, but the reduction in cycle duration may lead to the arterial wall reaching peak strain in a shorter period of time, with less time to recoil back to its resting state (Armentano et al., 1995; Mangoni et al., 1996). Although this may explain the apparent increased arterial wall stiffness with an increase in heart rate observed in some studies (Lantelme et al., 2002b; Mangoni et al., 1996; Salvucci et al., 2007), the relationship between heart rate and arterial stiffness has yet to be firmly established. Not only have studies been divergent in their findings, but majority of the studies in humans that have found an effect of heart rate on arterial stiffness were either confounded by a concomitant change in arterial blood pressure (Liang et al., 1999; Millasseau et al., 2005), or the effect of heart rate was much less than that of other determinants (Mitchell et al., 2004; Reference Values for Arterial Stiffness' Collaboration, 2010). Notwithstanding, the association between heart rate and arterial stiffness cannot be dismissed, and, given the intimate interaction between the heart and the arterial system, a change in heart rate may directly influence arterial wall stiffness through changes in wall viscoelasticity, or indirectly through blood pressure or other haemodynamic parameters.

2.1 Heart rate, cardiac function and arterial haemodynamics

Without the pumping of the heart, there can be no blood flow. Yet not only is the action of the heart pumping important, but the frequency at which the heart pumps also has an intricate relationship with the whole cardiovascular system. As a self-regulating pump, the heart imparts kinetic energy through the force of its contractions, generating pressure that causes blood to flow from the heart to the rest of the circulatory system. If the heart pumps at too fast a rate, as in the case of tachycardia, it can lead to a fall in both blood pressure and cardiac output (Samet, 1973), which can result in insufficient circulation to the vital organs, including the brain (Corday et al., 1953) and kidneys (Leithe et al., 1984). Similarly, a heart rate that is too slow, as in the case of severe bradycardia, can also lead to circulatory insufficiency (Dreifus et al., 1983).

Long before the origin of the pulse and of heart rate was described by William Harvey (1578 - 1657 AD) to be integral to the circulation of blood in early 1600s, the rate of the arterial pulse had already been widely used in ancient medicine as an indicator of health or illness (Ghasemzadeh and Zafari, 2011). The Greek physician, Herophilus (335 - 280 BC), was the first in recognising the importance of measuring the pulse rate in his patients (Ghasemzadeh and Zafari, 2011). However, there was no record of it being measured since his time until the 15th century, during which Santorio Sanctorius (1561 - 1636 AD) invented a device for measuring the pulse rate based on a pendulum (Moore, 1897). It wasn't until a century later that heart rate, as it is known today in modern medicine and measured in beats per minute (bpm), was first determined by the physician John Floyer (1649 - 1734 AD) using a pulse watch that ran for sixty seconds (Floyer, 1710; Fye, 1993). The further advancement of medical instruments allowed heart rate to be measured objectively rather than just by a physician's touch, and this haemodynamic parameter continues to play an important role in clinical assessment, though, somewhat surprisingly, its importance may have been diminished in modern medicine (Avolio et al., 2014; Palatini, 2009).

The concept that "blood moves in a closed circle" dates back to around 2650 BC, when Huangdi, the then emperor of China, introduced it through the writing of his book Huangdi Neijing (The Emperor's Inner Canon) (Li, 2004). With Harvey's reintroduction of the concept that the circulatory system was a closed system (McMullen, 1995), it became evident that blood was pumped around the body by the heart in two loops: one loop pumped from the right ventricle into the lungs to be replenished with oxygen (the pulmonary circulation), and the other loop pumped from the left ventricle into the rest of the body to provide oxygen (the systemic circulation). Whilst the amount of blood pumped by the right and left ventricles with any one heartbeat is balanced (Franklin et al., 1962), the amount of work for pumping blood is vastly different in the two ventricles. An increase in heart rate will increase the amount of blood being pumped around the body in a given time period (e.g. 1 minute), but at the expense of increased left ventricular work (Schmidt-Nielsen, 1984). Furthermore, there is an intricate interplay in the

ventricular-vascular coupling between the left ventricle and the vasculature, resulting in changes in cardiac function and arterial haemodynamics with changes in heart rate.

2.1.1 Heart rate of mice, rats and man

The function of the heart, or rather, the pumping heart, is to deliver blood, and hence oxygen and nutrients, to the rest of the body to ensure survival. The rate at which the heart pumps is highly correlated to the rate of oxygen consumption (Boothby, 1915; Fick, 1870), as it is with metabolic rate (Green, 2011). Both heart rate and metabolic rate in humans and animals of the mammalian kingdom are dependent on body-size, and, like many other physiological parameters, scale with body mass according to the allometric equation (Schmidt-Nielsen, 1984):

$$x = aM^b, \tag{2.1}$$

where x is the physiological parameter, a and b are constants, and M is body mass in kilograms. For metabolic rate P_{met} in kcal/day, the relation is (Kleiber, 1961)

$$P_{\text{met}} = 70M^{0.75}. \tag{2.2}$$

When expressed as the metabolic rate per unit mass, also known as the specific metabolic rate (P^*_{met}), the relation becomes

$$P^*_{\text{met}} = 70M^{-0.25}. \tag{2.3}$$

For heart rate (HR) in beats per minute (bpm), the relation to body mass is very similar to P^*_{met}, with the exponent being precisely the same (Stahl, 1967):

$$HR = 241M^{-0.25}. \tag{2.4}$$

Taken together, these allometric relations imply that the specific metabolic rate, hence oxygen consumption for energy production, is related to body mass in much the same way as heart rate, and that the decrease in specific metabolic rate with increasing body mass can be totally accounted for by the rate at which the heart pumps (Schmidt-Nielsen, 1984). In fact, the size of the heart (M_{h}) in mammals of all sizes remains constant at around 0.6% of the body mass, as expressed in the following allometric equation (Prothero, 1979):

$$M_{\mathrm{h}} = 0.0058 M^{0.98}. \tag{2.5}$$

Whether it is in mice, rats, or humans, the decreased relative demand for oxygen as the body mass increases is not taken care of by a change in the size of the heart, but rather by a decrease in the heart's pumping rate (Schmidt-Nielsen, 1984). Rodents are often used as animal models for arterial stiffness (Lacolley et al., 2014) and other cardiovascular studies (Byrom et al., 2010; Zaragoza et al., 2011), and even though mice have a resting heart rate in the order of 600 bpm, rats have a heart pumping at around 300 bpm, and humans at around 80 bpm, given the similarities in arterial blood pressure (Schmidt-Nielsen, 1984) and arterial wall structure in all mammalian species (Wolinsky and Glagov, 1967), they also make an appropriate animal model for the study of heart rate effects on arterial stiffness. The animal model presented in this thesis for the investigation on effects of heart rate on arterial stiffness was a rodent model using rats (Chapter 3).

2.1.2 Heart rate and cardiac function

In order for the total blood volume to continuously circulate the whole body, the heart must pump at a rate that delivers a sufficient amount of blood with each pump. The relationship between the volume of blood the heart ejects in one minute (cardiac output, in L/min), the volume ejected with each pump (stroke volume, in L), and the rate at which the volume is ejected

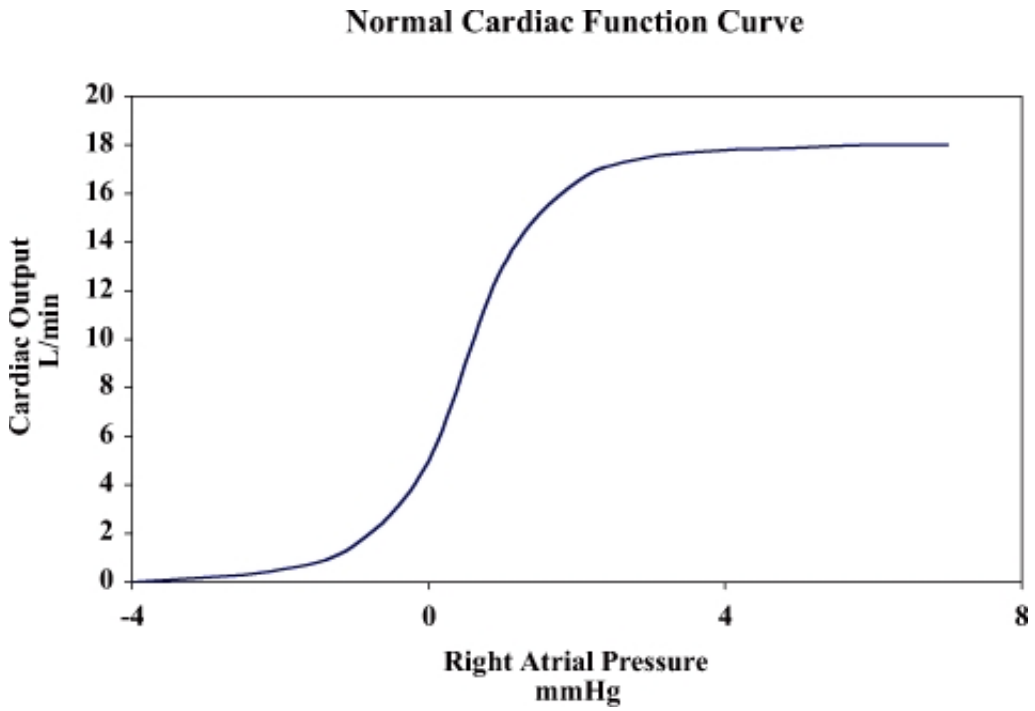

Figure 2.1: Cardiac function curve, with cardiac output values scaled to normal human levels. Reproduced from Young (2010) with permission.

(heart rate, in bpm), can be described with the following simple equation:

$$CO = SV \times HR, \tag{2.6}$$

where CO is the cardiac output, SV is the stroke volume, and HR is the heart rate. That is, changes in stroke volume, heart rate, or both, affect the output of the left ventricle. As observed by Patterson and Starling over a century ago (Patterson and Starling, 1914), stroke volume is dependent upon the pressure in the heart, which in turn is influenced by the amount of blood in the heart chambers. An increase in blood volume, such as in the case of increased venous return, increases the length of the cardiac muscle fibres, leading to an increase in chamber pressure. This results in increased contractility, thus resulting in an increased stroke volume and cardiac output. Figure 2.1 shows the cardiac function curve expressed as cardiac output change with right atrial pressure.

On the other hand, a change in heart rate alone, even with other factors such as right atrial

pressure remaining constant, can also affect the effectiveness of the heart's pumping ability and cause the heart to operate on a different cardiac function curve (Young, 2010). Changes in heart rate have direct effects on both systolic and diastolic durations, with the change in systolic duration being linear with heart rate (Boudoulas et al., 2015; Weissler et al., 1961), and the change in diastolic duration being nonlinear with heart rate (Boudoulas et al., 2015). Hence, the influence of heart rate on diastolic duration is much greater (Weissler et al., 1961). As heart rate increases, the decrease in diastolic time leads to decreased ventricular filling, hence decreased left ventricular pressure and stroke volume through the Frank-Starling law of the heart (Noble et al., 1966; Suga and Sagawa, 1974; Weissler et al., 1961). However, cardiac output increases despite the decrease in stroke volume, until such time when the stroke volume is so low that the cardiac output decreases as heart rate increases past a certain point (Noble et al., 1966; Young, 2010). This can be understood as a change in the slope and plateau of the cardiac function curve at different heart rates (Figure 2.2). Notwithstanding, a normal increase in heart rate would result in increased cardiac output, which can lead to changes in the steady component of arterial pressure (mean arterial pressure) given an unchanged resistance in the peripheral circulation. Furthermore, a change in heart rate can also impact on the oscillatory component of arterial pressure (pulse pressure), which plays an important role in ventricular-vascular interaction.

2.1.3 Heart rate and arterial haemodynamics

For effective perfusion of organs, not only must there be sufficient pressure from the heart to propel blood forward into the systemic circulation, but continuous blood flow must be maintained within the vasculature. In particular, the interaction between the heart's left ventricle and the large conduit arteries is especially important for optimal cardiac output and transformation of pulsatile outflow from the heart into continuous, steady flow in arterioles and capillaries. As discussed in the earlier section, changes in heart rate can effect the heart's pump function

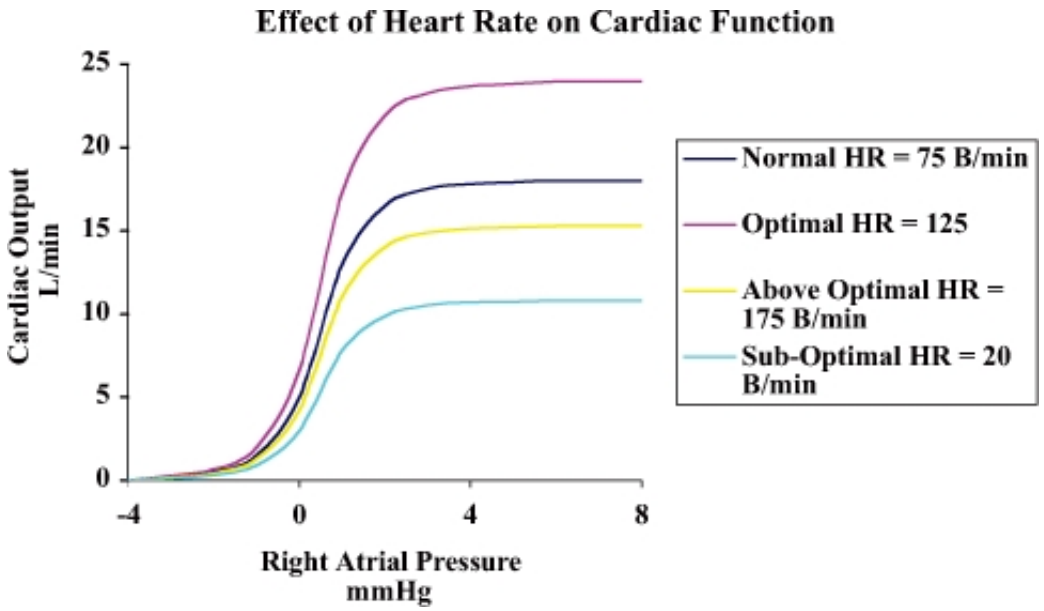

Figure 2.2: Cardiac function curves obtained at different fixed heart rates. Reproduced from Young (2010) with permission.

and thus affect the left ventricle's performance and output, which in turn can affect arterial haemodynamics.

Whilst heart rate in mammals changes with body size, mean arterial blood pressure and arterial pulse pressure (systolic pressure minus diastolic pressure) in mammals are essentially similar over the whole range of animal body size and weight (Schmidt-Nielsen, 1984). One possible explanatory mechanism was elegantly illustrated by Westerhof and Elzinga (Westerhof and Elzinga, 1991, 1993). In their study of vascular parameters in relation to heart rate and animal size, they demonstrated that both heart period (T) and exponential diastolic decay (τ) of the arterial pressure pulse (defined as the product of peripheral resistance R and aortic compliance (C) increase with body mass with an exponent of 0.27 and 0.29, respectively (Westerhof and Elzinga, 1991). As the exponents were not significantly different at the 5% level, the ratio T/τ is essentially constant regardless of body size, and may explain the reason for the inverse relationship of heart rate to body size in order to main similar pulse pressure across animal species of different sizes (Westerhof and Elzinga, 1991). As such, the ratio T/τ can be considered the basic coupling parameter between the heart and the arterial system (Avolio et al., 2014).

During any one cardiac cycle, arterial pressure undergoes instantaneous variation with time. As the heart beats continuously in a periodic manner, arterial pressure essentially becomes a composition of a steady component, as indicated by mean arterial pressure, and a periodic oscillatory component, as indicated by pulse pressure (Nichols et al., 2011). Although large epidemiological studies have shown that a high resting heart rate is often associated with high arterial blood pressure (Benetos et al., 2003; Julius, 1988; Koskela et al., 2013; Morcet et al., 1999; Palatini and Julius, 1997), acute changes in heart rate do not necessarily lead to a change in mean arterial pressure due to the baroreflex mechanism. However, large acute changes in heart rate, such as those that are induced in acute pacing studies, can lead to changes in both the steady and oscillatory components of the arterial pulse. At any one point in the arterial tree, the arterial pressure pulse contour is determined by the pressure wave generated by the contracting heart (Westerhof et al., 1972), as well as reflected waves that occur at sites where there is a physical change in the arterial properties or at branching points (Nichols et al., 2011). The opposite travelling reflected waves, which together can be viewed as a single composite reflected wave, augment the composite forward propagating wave and change its amplitude and contour (Westerhof et al., 1972). Any change to the forward wave contour caused by wave reflection is determined by the timing and magnitude of the reflected wave (Hashimoto and Ito, 2009; Nichols et al., 2011; Townsend et al., 2015) (see also Section 2.2.3), as shown in Figure 2.3, upper left panel. Whilst a change in heart rate may not affect the time of arrival or magnitude of the reflected wave (Wilkinson et al., 2000, 2002), due to the change in duration for both systole and diastole, the relative timing of the reflected wave to the forward wave can result in a change in the arterial pulse in particular at the aortic level close to the heart, which is furthest away from peripheral reflection sites. A decrease in heart rate lengthens systolic and diastolic durations, hence increasing τ, resulting in the reflected wave coinciding with the forward wave in systole (Avolio et al., 2009; Dart and Kingwell, 2001). This boosts systolic pressure and thus increases pulse pressure (Figure 2.3, lower panel).

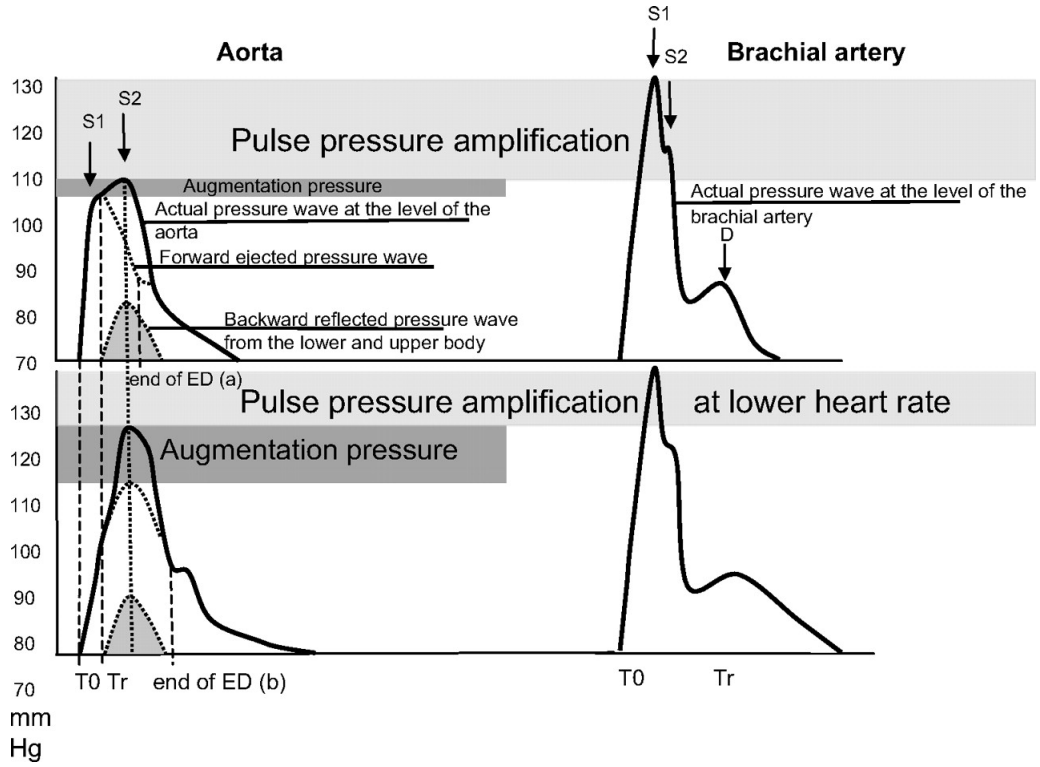

Figure 2.3: (A) Schematic representation of the morphological differences in the aortic and brachial arterial pressure pulse wave (upper panel). Note the backward reflected pressure wave augmenting the forward pressure wave, resulting in the actual aortic pressure wave. (B) Effect of heart rate on aortic systolic pressure augmentation and pulse pressure amplification (upper vs lower panel). At lower heart rate, given unchanged time of return of backward pressure wave (T_r, see Section 2.2.3), and similar pulse height of both forward and backward pressure waves as compared to upper panel, lower heart rate would result in increased augmentation pressure and decreased pulse pressure amplification. S1, first systolic peak; S2, 2nd late systolic peak attributable to augmentation by reflected pressure wave; D, accentuated diastolic wave attributable to the delayed arrival of the reflected wave from the lower body; ED, ejection duration; T_0, onset of forward pressure wave. Reproduced from Avolio et al. (2009) with permission.

On the contrary, an increase in heart rate reduces the duration of diastole, thus reducing τ and shifting the reflected wave into diastole, resulting in a lower systolic pressure and a decrease in pulse pressure (Wilkinson et al., 2000, 2002). Thus, heart rate is a particularly relevant haemodynamic parameter in the study of wave reflection (Lieber et al., 2010) and assessment of blood pressure lowering drugs that also affect heart rate (Avolio et al., 2014).

Due to the presence of wave reflections, transmission of arterial pressure from the ascending aorta to the periphery is accompanied by amplification of pulse pressure (Dart and Kingwell, 2001; Nichols et al., 2011) (Figure 2.4), meaning that sphygmomanometric blood pressure values, in particular systolic pressure, measured at peripheral sites such as the brachial artery do not

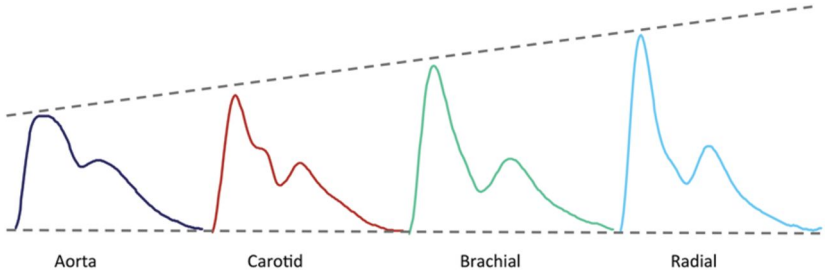

Figure 2.4: Amplification of the blood pressure pulse waveform from the aorta to the radial artery. Reproduced from McEniery et al. (2014) with permission.

necessarily reflect the pressure at the aorta. Due to the effect of heart rate on wave reflection timing, amplification of pulse pressure from the aorta to the peripheral artery can be significantly different at different heart rates (Avolio et al., 2009; Dart and Kingwell, 2001; Wilkinson et al., 2000). An increase in heart rate would lead to a decrease in pulse pressure at the ascending aorta, thus increasing pulse pressure amplification; a decrease in heart rate would produce a relative increase of pulse pressure at the aorta and thus decrease pulse pressure amplification (Figure 2.3).

As standard practice for blood pressure measurement is still based on measurements at the brachial site, this relationship between heart rate and pulse pressure amplification can have significant implications on investigative studies that assess left ventricular load based on brachial systolic pressure for conditions where there may be substantial changes in heart rate, such as exercise (Rowell et al., 1968), as well as those in antihypertensive treatments which also affect heart rate (Avolio et al., 2014). Large-scale studies investigating the effects of different blood pressure lowering drugs in the past have demonstrated the importance of taking heart rate and pulse pressure amplification into consideration, or the lack thereof. The Losartan Intervention For Endpoint reduction in hypertension study (LIFE) found that losartan, an angiotensin receptor blocking agent, reduced brachial blood pressure to an almost identical extent as atenolol, a β-blocking agent which also reduces heart rate, in over 9,000 hypertensive subjects over the duration of 4 years (Dahlöf et al., 2002). However, losartan produced additional beneficial effects such as regression of left ventricular hypertrophy, whereas atenolol, whilst reducing heart

rate in the order of 6 bpm, did not produce additional cardioprotective effects (Dahlöf et al., 2002; Devereux et al., 2002; Lindholm et al., 2002). Indeed, a meta-analysis on the studies that compared atenolol to other antihypertensive drugs found that, whilst there were no significant differences in brachial blood pressure reduction, a significantly higher all-cause and cardiovascular mortality was associated with atenolol treatment (Carlberg et al., 2004). Subsequent studies identified the potential cause of the adverse cardiovascular outcomes: a higher sustained central aortic pressure, resulting from reduced pulse pressure amplification due to reduced heart rate (De Luca et al., 2004; Williams et al., 2006). Omission of the consideration of heart rate related changes in pulse pressure amplification can also have implications in the calculation of parameters of arterial stiffness (Avolio et al., 2014) (see Section 2.3.4).

2.1.4 Heart rate and cardiovascular risk

Despite heart rate being the most readily measurable haemodynamic parameter, its apparent relationship with life expectancy (Benetos et al., 2003; Boudoulas et al., 2015; Levine, 1997), and its obvious association with the heart and circulation, modern medicine has often, surprisingly, neglected heart rate both in clinical practice and in hypertension research (Palatini and Julius, 1997, 1999). Although heart rate is closely associated with many other cardiovascular risk factors (Inoue et al., 2001; Palatini, 1999b; Palatini and Julius, 1997), which may partly be the reason why the clinical and scientific communities have been slow to take up heart rate as an important parameter to consider, research in the past 7 decades has demonstrated time and time again the value of heart rate as an independent prognostic factor. The first pioneering study to report that elevated heart rate was related to cardiovascular disease, specifically hypertension, was conducted by Levy *et al* in 1945 (Levy et al., 1945), but it wasn't until 1980 that the first study to report an association between resting heart rate and cardiovascular mortality was published (Dyer et al., 1980). Since then, numerous epidemiological studies have been conducted to investigate heart

rate as a cardiovascular risk factor and its association with cardiovascular and all-cause mortality (Caetano and Delgado Alves, 2015; Cook et al., 2006; Custodis et al., 2010; Fox et al., 2007; Habib, 2001; Palatini, 1999b, 2009; Palatini and Julius, 1997; Perret-Guillaume et al., 2009). There is now surmounting evidence that indicates elevated heart rate is an independent risk factor for coronary heart disease (Medalie et al., 1973; Schroll and Hagerup, 1977), heart failure (Hjalmarson et al., 1990), myocardial infarction (Casolo et al., 1992; Dyer et al., 1980), hypertension (Levy et al., 1945; Palatini, 2011; Stamler et al., 1975) and atherosclerosis (Beere et al., 1984; Kaplan et al., 1987; Palatini, 1999a). Furthermore, all but a few longitudinal epidemiological studies have shown an association of elevated heart rate with cardiovascular and all-cause mortality (Palatini, 2011; Palatini et al., 2006a; Palatini and Julius, 2009; Perret-Guillaume et al., 2009), including large scale studies such as the Framingham Heart Study (Kannel et al., 1987), Paris Prospective Study (Jouven et al., 1999), the Italian CASTEL study (Palatini et al., 1999), and the Israeli CORDIS study (Kristal-Boneh et al., 2000). The association between heart rate and both cardiovascular and all-cause mortality has gender differences, with the association stronger in men (Benetos et al., 1999, 2003; Goldberg et al., 1996).

It has been estimated that an increase in heart rate by 10 bpm is associated with at least a 20% increase in cardiac death (Perret-Guillaume et al., 2009). This is equivalent to the increase in risk with an increase in systolic blood pressure by 10 mmHg (Benetos et al., 1999). However, the difficulty in identifying a threshold level above which heart rate is considered "too high" has been one of the main reasons heart rate has yet to be included in management guidelines for cardiovascular diseases, such as hypertension, for risk stratification (Palatini and Julius, 2009; Palatini et al., 2016), although a heart rate of 80 - 85 bpm or above was considered elevated in most epidemiological studies (Palatini et al., 1997). Heart rate is variable depending on an individual's physical and mental states, as well as circulating hormones and autonomic nervous system activity, amongst others (Verrier and Tan, 2009). Furthermore, whilst heart rate is on average between 3 to 7 bpm higher in women than in men (Palatini and Julius, 1997), the

higher heart rate in women does not translate to shorter life expectancy nor increased risk of cardiovascular disease (Palatini and Julius, 1997). However, evidence stands to show that elevated heart rate is equally detrimental in both men and women, and that reduction of heart rate would be beneficial for both (Palatini, 2001).

Heart rate and hypertension

An elevated resting heart rate is often observed in hypertensive individuals (Palatini et al., 2006a), and several studies in the past have shown that the presence of a high heart rate in conjunction with hypertension can have an additive effect on the risk of cardiovascular disease and all-cause mortality (Palatini, 2011). Furthermore, both baseline heart rate and the progressive increase in heart rate over time predict subsequent development of hypertension (Levy et al., 1945; Palatini et al., 2006b). Elevated heart rate in hypertension is a strong marker of increased sympathetic and decreased parasympathetic tone (Palatini and Julius, 1997), and the abnormality in autonomic control, in particular sympathetic overactivity, can lead to a number of other cardiovascular risk factors, such as insulin resistance-dyslipidemia (Deibert and DeFronzo, 1980), increased haematocrit (Julius, 1993) and cardiac hypertrophy (Palatini and Julius, 1997). Although the case for heart rate being an important parameter in the prognosis for hypertension is strong, to date there has been no clinical trial that specifically studied the effects of lowering heart rate on morbidity and mortality in the hypertensive population, as opposed to retrospective analysis of existing studies (Palatini et al., 2016). This is complicated by the paradox that, as aforementioned, when compared to other antihypertensives or placebos that do not alter heart rate, β-blockers were associated with an increase in cardiovascular and all-cause mortality, myocardial infarction, stroke and heart failure despite lowering peripheral blood pressure to similar levels, as shown by a recent meta-analysis (Bangalore et al., 2008). Notwithstanding that this may be explained by the heart rate effects on pulse pressure amplification (De Luca et al.,

2004; Williams et al., 2006), whereby a lower heart rate would result in a higher central aortic systolic pressure with the same level of peripheral systolic blood pressure, individually based analysis of each separate study included in the meta-analysis showed that persistence of elevated heart rate, regardless of the type of antihypertensives taken, still showed an increased risk of all-cause mortality, future myocardial infarction and fatal coronary outcome (Palatini et al., 2016). Taken together, current evidence supports that heart rate is an important cardiovascular risk factor that needs to be considered, and even more so in the presence of hypertension, and should be included the assessment of hypertensive individuals (Palatini et al., 2016).

2.2 Arterial stiffness

Between the heart and the rest of the body, therein lies a complex network of arteries that allow blood and nutrients to be delivered to the organs and tissues to ensure survival. At the forefront of this transit system are the large conduit arteries, which, as aforementioned, function both as passageways for blood flow and as cushions for taking the brunt of the pulsatile ejection from the heart such that continuous, steady flow could be maintained downstream of the vasculature (London and Guerin, 1999; Nichols et al., 2011). The design of the arterial system is such that the stiffness of large arteries optimises damping of the pulsatile blood flow without the expense of ineffective circulation to the periphery (Avolio, 2013), and as such is achieved by the unique structure and composition of the arterial wall. Undesirable increases in arterial stiffness, whether by natural degradation of arterial wall components due to aging (Avolio et al., 1998; Lee and Oh, 2010) or by external factors, can have a significant impact on arterial function (London and Guerin, 1999) and as a result lead to target organ damage (Mitchell, 2008).

Stiffness is a purely mechanical property that reflects the amount of deformation, or strain, a material undergoes when stress (force per unit area) is applied, and can be expressed as the elastance, or elastic modulus of the material (see Section 2.2.2). The higher the elastic modulus,

the stiffer the material. The term "arterial stiffness" refers specifically to the mechanical property of the arterial wall, where stress is imposed by the intra-arterial pressure and strain is the resulting change in circumference (Peterson et al., 1960). Whilst arteries of different caliber have varying degrees of wall stiffness, with stiffness gradually increasing from the proximal to distal vessels (Mohiaddin et al., 1989), it is the stiffness of large conduit arteries, in particular the aorta, that has the most influence on cardiac and arterial function in relation to the pulsatile outflow of blood with each cardiac cycle.

2.2.1 Arterial wall and viscoelasticity

The arterial wall is composed of three layers: the innermost layer, the tunica intima, comprises of a single layer of endothelial cells and an underlying basal elastin layer; the middle layer, the tunica media, consists of smooth muscle cells and interconnected elastin and collagen fibres; the outermost layer, the tunica adventitia, contains a network of elastin and predominantly collagen fibres (Shadwick, 1999). Each of these layers contributes to the varying stiffness of arteries throughout the vasculature through either passive mechanisms, such as that caused by an increase in distending pressure or degradation of elastin with age; or active mechanisms, such as modulation of smooth muscle tone or alterations in the vessel wall's extracellular matrix (Townsend et al., 2015). However, it is the media and adventitia layers that lend most to the stiffness of the arterial wall (Roach and Burton, 1957; Wolinsky and Glagov, 1964).

Throughout lifespan, arteries are subjected to constant stress arising from distending pressure generated by the outflow of blood from the heart. The large arteries proximal to the heart, in particular, are subjected to large variations in distending pressure with each heartbeat. Under stable conditions and the assumption that wall thickness is relatively small compared to the radius (R), the force exerted radially on the arterial wall is balanced by the wall's circumferential

tension (T), as determined by the law of Laplace:

$$T = PR \tag{2.7}$$

The key to arteries being able to withstand high arterial pressures without rupturing, and at the same time maintaining sufficient stretch without collapsing at low distending pressures, is the non-linear change in arterial wall stiffness with pressure (Shadwick, 1999). This non-linearity is a characteristic owing to the layout of the components in the tunica media and tunica adventitia (Roach and Burton, 1957), though the collagen-rich adventitia is only involved at very high distending pressures (Wolinsky and Glagov, 1964). The tunica media contains precisely oriented elastin, collagen, smooth muscle cells and a non-fibrous matrix in concentric layers, termed lamellar units (Wolinsky and Glagov, 1964). Whilst smooth muscle cells contribute to arterial stiffness through active contraction or relaxation, thus changing vessel diameter (Cox, 1975; Gow, 2011), elastin and collagen act passively as the main load bearing components, particularly for conduit arteries where smooth muscle content is only about 20% of the dry weight in the media (Wolinsky and Glagov, 1967). At low distending pressures, elastin, a highly elastic protein, acts as the major load bearing component, resulting in large changes in circumference for small changes in pressure; as distending pressure increases, collagen, which is more than 1000 times stiffer than elastin (Burton and Matsumoto, 1954; Shadwick, 1999), gradually and increasingly becomes the major load bearing component, resulting in small changes in circumference even with large increases in pressure (Dobrin, 1978; Roach and Burton, 1957). As such, stiffness of the arterial wall is intrinsically pressure dependent (Cox, 1975), with elastance increasing as pressure increases.

Due to the elasticity of elastin and collagen in the arterial wall, arteries do not remain at the peak distension reached during systole, but recoil as distending pressure is decreased during diastole, an effect that is predominantly governed by the elastin fibres (Armentano et al., 1995).

If the arterial wall were purely elastic and blood were inviscid, energy stored in the wall during distension would be fully returned into the circulation as the artery recoils, which could result in resonance in the arterial system from the reflected pressure waves (Shadwick, 1999). In reality, the arterial wall exhibits a viscous behaviour, such that at each cardiac cycle, approximately 15-20% of the stored energy is dissipated within the arterial wall (Bertram, 1980; Bodley, 1976; Shadwick, 1999). This is an important aspect of arterial wall property as it aids in dampening the travelling pressure wave (Shadwick, 1999), which is essential to the buffering function of the large arteries. Viscosity of the arterial wall has been attributed to vascular smooth muscle (Armentano et al., 1995, 2006; Bergel, 1961a), and the viscous effect is mainly developed during systole (Armentano et al., 1995) (Figure 2.5). One property of viscoelastic materials is that they become stiffer when stress is applied more rapidly than when the stress is applied slowly (Özkaya et al., 2012), resulting in a frequency dependence of the stress-strain relationship that characterises the elastic modulus. However, the extent to which a material is dependent on the rate of applied stress differs. As such, the arterial wall may become stiffer at higher heart rates, but to what extent arterial stiffness is dependent on heart rate is unknown, and thus is what this thesis set out to investigate.

2.2.2 Indices of arterial stiffness

The assessment of arterial stiffness can be grouped into three categories: systemic, regional or local (Laurent et al., 2006). Due to the proportional changes in the arterial wall constituents along the arterial tree, no single arterial segment has identical viscoelastic properties, and as such systemic arterial stiffness can only be estimated from models of the circulation (Laurent et al., 2006). However, regional and local arterial stiffness can be measured directly both invasively and non-invasively. It is beyond the scope of this thesis to detail every index used in practice to characterise arterial stiffness. Table 2.1 summarises the various indices of arterial stiffness

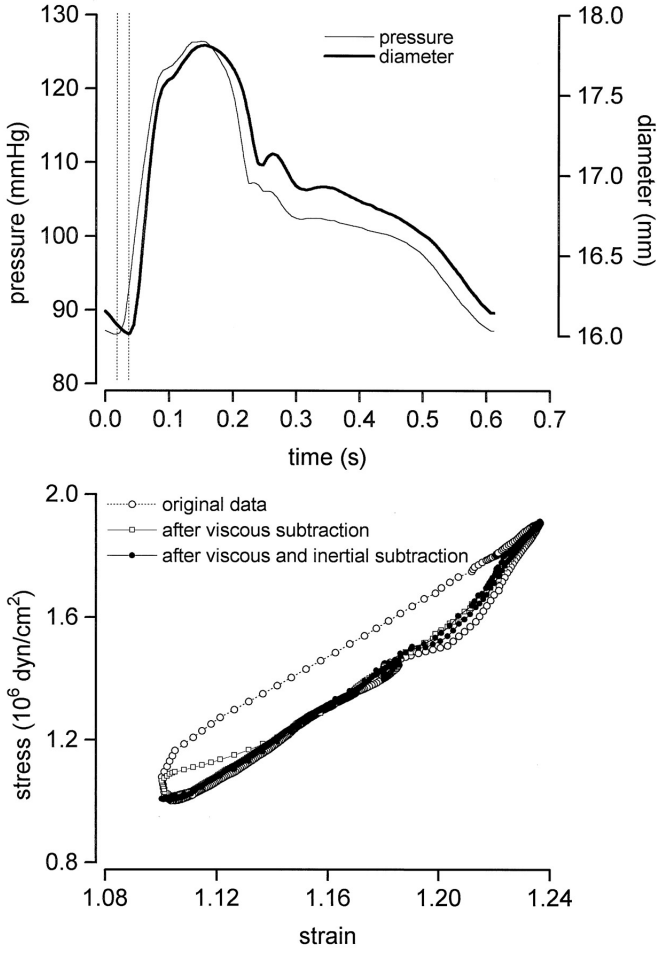

Figure 2.5: Top, aortic diameter and pressure waveforms measured in steady state by Armentano et al. (1995). Bottom, the aortic stress-strain relation is denoted by open circles. A hysteresis loop is formed due to the viscous and inertial properties of the arterial wall. Reproduced from Armentano et al. (1995) with permission.

commonly used, and the indices most commonly adopted in the determination of heart rate effects on arterial stiffness are detailed below.

Elastic modulus

As briefly mentioned in Section 2.2.1, stiffness of a material can be expressed as elastance, or elastic modulus. Young's modulus (E) describes the uniaxial strain under stress imposed on the same axis, and for arterial stiffness, can be defined as the ratio of intra-atrial pressure over diameter change. However, due to the non-linear elasticity of the arterial wall, E increases as distending pressure increases, thus incremental elastic modulus (E_{inc}), defined as the ratio of the

Table 2.1: Common indices of arterial stiffness (Laurent et al., 2006; Mackenzie et al., 2002; Nichols et al., 2011).

Indices	Formula	Description
Local stiffness		
Arterial distensibility	$\frac{\Delta D/D}{\Delta P}$	Relative diameter (or area) change for a given pressure change ($mmHg^{-1}$)
Arterial compliance	$\frac{\Delta D}{\Delta P}$	Absolute diameter (or area) change for a given pressure change (m.mmHg or m^2/mmHg)
Young's elastic modulus	$\frac{\Delta P \cdot D}{\Delta D \cdot h}$	Pressure change per unit area required for a theoretical 100% stretch from the original length (mmHg/m)
Peterson's elastic modulus	$\frac{\Delta P \cdot D}{\Delta D}$	Pressure change required for a theoretical 100% stretch from the original length (mmHg/m)
Stiffness index β	$\frac{ln(P_s/P_d)}{(D_s - D_d)/D_d}$	Ratio of the logarithm of systolic/-diastolic pressures to the relative change in diameter
Regional stiffness		
PWV	$L/\Delta t$	Velocity of travel of the arterial pulse along length L of artery (m/s)
Systemic stiffness		
Systemic arterial compliance (area method)	$A_d/[R(P_s - P_d)]$	Ratio of area under the blood pressure diastolic decay curve A_d to the product of total peripheral resistance R and difference in systolic P_s and diastolic P_d pressures

P, pressure; D, diameter; A, area; h, wall thickness; t, time; R, total peripheral resistance; subscript s, systolic; subscript d, diastolic

change in pressure over the relative change in diameter, is the more appropriate representation of arterial wall stiffness (Cox et al., 1975). In practice, measurement of E_{inc}, which can be obtained using ultrasound (Laurent et al., 2006), takes into account arterial wall thickness and is calculated as (Nichols et al., 2011):

$$E_{inc} = \frac{1.5 \Delta P R_i^2 R_o}{(R_o^2 - R_i^2)\Delta R} \tag{2.8}$$

where R_o is the outer radius and R_i is the inner radius.

Although E_{inc} gives a good representation of elasticity of the arterial wall, viscosity is not represented in the measure. Viscosity in the arterial wall has been shown to be highly frequency dependent (Armentano et al., 1995; Gow and Taylor, 1968) and hence related to heart rate. In

order to describe the dynamic viscoelasticity of the arterial wall, investigators have used the dynamic elastic modulus (E'), defined in complex form with one elastic (E_{dyn}) and one viscous (μ) component (Nichols et al., 2011):

$$E' = E_{\text{dyn}} + \text{j}\mu\omega \tag{2.9}$$

where ω is the angular frequency and $\text{j}\mu\omega$ is the imaginary part of E'. The magnitude of the dynamic elastic modulus is thus given by:

$$|E'| = \sqrt{E_{\text{dyn}}^2 + (\mu\omega)^2} \tag{2.10}$$

When $\mu\omega$ is small relative to E_{dyn}, the dynamic elastic modulus E' can be approximated by E_{dyn} alone (Bergel, 1961a). The dynamic elastic modulus was used in the seminal study by Bergel (1961a) on the frequency dependency of arterial stiffness (see Section 2.3).

Distensibility and compliance

As with elastic modulus, distensibility and compliance are indices of local arterial stiffness, and are in fact its inverse. Compliance (C) is defined as the change in diameter (D) (or lumen area or volume) with a change in pressure (P):

$$C = \frac{\Delta D}{\Delta P} \tag{2.11}$$

Distensibility (D) is defined as the *relative* change in diameter (or lumen area or volume) with a change in pressure:

$$C = \frac{\Delta D/D}{\Delta P} \tag{2.12}$$

In practice, distensibility and compliance can be measured from any superficial artery using echotracking techniques in ultrasound, and are mostly measured at the carotid, brachial or femoral arteries (Laurent et al., 2006), and were the indices used in the animal studies on the heart rate dependence of arterial stiffness in intact and sympathectomised rats (Mangoni et al., 1996; Mircoli et al., 1999) (see Section 2.3.2). It should be noted that for both of these measurements, it is essential that arterial pressure is measured at the same site as diameter, due to the presence of pulse pressure amplification (Nichols et al., 2011). Failure to do so can have a severe impact on the interpretation of results. A recent large epidemiological study (Whelton et al., 2013) investigating the effects of heart rate on arterial stiffness measured carotid diameter using ultrasound and aortic diameter using magnetic resonance imaging (MRI), but calculated carotid and aortic distensibility using pressure measured at the brachial artery. Although the study found a significant negative association between heart rate and both carotid and aortic distensibility, once heart rate dependent pulse pressure amplification was accounted for, the association between heart rate and carotid distensibility was lost, and even reversed for aortic distensibility (Avolio et al., 2014).

Pulse wave velocity

Left ventricular ejection generates flow velocity, pressure and distension diameter waves, which travel along the arterial wall (Nichols et al., 2011). The velocity of wave travel, or pulse wave velocity (PWV), is directly related to the elastance of the arterial wall and increases with increasing stiffness, as described by the Moens-Korteweg equation (Moens, 1878):

$$\mathrm{PWV} = \sqrt{\frac{Eh}{2R\rho}} \tag{2.13}$$

where E is the Young's modulus, h is wall thickness, R is internal radius and ρ is the density of blood. This relationship implies the assumption that the artery is thin-walled and thus $h/2R$ is

small (Nichols et al., 2011). As such, PWV is considered the 'gold standard' for assessment of regional arterial stiffness (Laurent et al., 2006; Nichols et al., 2011), and has been used extensively in cardiovascular research in the determination of risk association with increased arterial stiffness (see Section 2.2.5) as well as investigative studies on heart rate effects on arterial stiffness (see Section 2.3). Furthermore, PWV can be measured without the direct measurement of E, h or R, and can be obtained readily using various methods.

By definition, velocity of wave travel is the distance travelled by the wave divided by the transit time over said distance. For determination of PWV, this requires measurement of flow velocity or pressure waves at two distinct locations in the body some distance (Δz) apart. Transit time (Δt) of the wave can then be determined as the time delay of a common point of identity, or characteristic point, between the two waves. PWV is then simply calculated as:

$$\mathrm{PWV} = \frac{\Delta z}{\Delta t} \tag{2.14}$$

Although conceptually simple, accurate determination of path length can be particularly challenging in practice for non-invasive human studies (Sugawara et al., 2010), as is the determination of a characteristic point on the wave (Chiu et al., 1991; Nichols et al., 2011) (see Section 2.2.4).

Another method for PWV determination is to utilise the Bramwell-Hill relationship (Bramwell and Hill, 1922a), which relates PWV to arterial distensibility and is derived from the Moens-Korteweg equation (Eq. 2.13):

$$\mathrm{PWV} = \sqrt{\frac{V\delta P}{\rho\delta V}} \tag{2.15}$$

or, if expressed in terms of vessel cross-sectional area:

$$\mathrm{PWV} = \sqrt{\frac{A\delta P}{\rho\delta A}} \tag{2.16}$$

This methodology allows PWV to be calculated from distensibility or compliance measurements using echotracking techniques in ultrasound (Townsend et al., 2015) and has been used to quantify PWV at different blood pressure points of the cardiac cycle (Hermeling et al., 2010, 2012; Spronck et al., 2015b).

2.2.3 Arterial stiffness and arterial function

As previously discussed, viscoelasticity of the arterial wall is fundamental to the buffering function of large conduit arteries (London and Guerin, 1999). The design of the arterial system is such that stiffness of the arterial wall is optimised to give arteries sufficient recoil capability so that the blood volume stored during systole is returned to the circulation during diastole, as opposed to maximal damping of the pulsatile oscillations resulting in insufficient circulation (Avolio, 2013). This achieves the effect of buffering the pulsatile output from the heart to steady, laminar flow in the periphery (London and Guerin, 1999; Nichols et al., 2011). Under normal conditions, large conduit arteries store approximately 60% of stroke volume during systole whilst 40% is forwarded to the peripheral circulation; during diastole, the recoil of the arterial wall propels the stored volume forward into the peripheral circulation (Figure 2.6). However, when arterial stiffness is increased, the amount of stroke volume stored during systole is reduced, meaning a greater amount is forwarded into the peripheral circulation, leading to an increase in systolic blood pressure. Increase in arterial stiffness also causes a greater fall in diastolic blood pressure, resulting in an increase in pulse pressure (Figure 2.7). Increased pressure pulsatility in the large arteries would be transmitted downstream to the systemic circulation, and although vasoconstricted small arteries and arterioles upstream from most organs help dampen the pulsatility (O'Rourke and Safar, 2005), increased pulsations in pressure and flow extend well into the brain and kidney (O'Rourke and Safar, 2005), and can lead to damage in these organs (Mitchell, 2008).

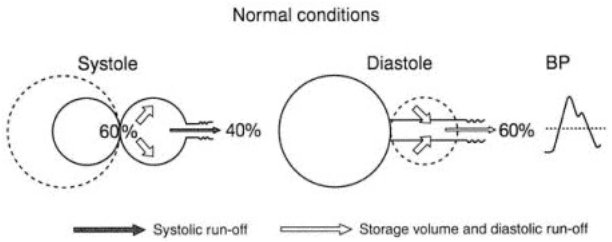

Figure 2.6: Schematic representation of normal large artery function as a cushioning buffer in terms of storage volume, systolic run-off and the resulting arterial pulse wave. Reproduced from London and Guerin (1999) with permission.

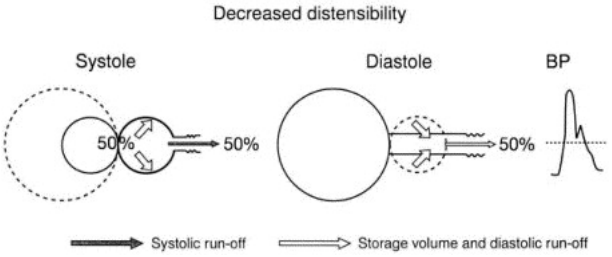

Figure 2.7: Schematic representation of disrupted large artery function as a cushioning buffer due to increased wall stiffness in terms of storage volume, systolic run-off and the resulting arterial pulse wave. Reproduced from London and Guerin (1999) with permission.

Arterial stiffness and wave reflection

The contour of the arterial pressure pulse waveform, as aforementioned, is determined by the forward travelling pressure wave generated by ventricular ejection (Westerhof et al., 1972), as well as the backward travelling reflected wave that is a composite of waves reflected at sites where there is a physical change in the arterial properties or at branching points (Nichols et al., 2011). Alterations to the pressure wave amplitude and contour are dependent on the changes in both amplitude and timing of wave reflections from downstream reflection sites (Hashimoto and Ito, 2009; Nichols et al., 2011; Townsend et al., 2015), and changes in arterial stiffness can influence both of these factors (Murgo et al., 1980; Nichols et al., 2011). Increased arterial stiffness causes faster wave travel, i.e. increased PWV, and results in decreased damping of the pulsatile force, leading to an increased reflected wave amplitude (Nichols et al., 2011). Furthermore, increased PWV causes the reflected wave to return earlier during systole, thus, along with increased reflection wave magnitude, further augments the forward wave amplitude and results in increased systolic pressure. Wave reflection effects on the central aortic pressure waveform is a major

determinant of left ventricular afterload (Nichols et al., 2011), and increased wave reflection can lead to left ventricular hypertrophy and arterial wall damage (Townsend et al., 2015).

Wave reflection at the central aortic waveform can be quantified with several indices, including augmentation index (AIx), reflection index (RI) and reflection magnitude (RM). Briefly, AIx is calculated as the ratio of the augmentation pressure (AP) from the reflected wave and the central aortic pulse pressure (PP) (Figures 2.3 and 5.1):

$$\mathrm{AIx} = \frac{\mathrm{AP}}{\mathrm{PP}} \tag{2.17}$$

Although central aortic AIx has been used as a surrogate measure of arterial stiffness in various studies in the past (Stefanadis et al., 1998; Wilkinson et al., 1998), it is now accepted that this index is in fact not an accurate measure of stiffness (Gurovich et al., 2009) due to its dependence on heart rate (Wilkinson et al., 2000, 2002) , aortic taper (Cecelja and Chowienczyk, 2009), pattern of ventricular ejection (Westerhof and O'Rourke, 1995) and body length (Langenberg et al., 2003).

Using measured pressure and flow waves, or an approximation of flow wave, forward travelling pressure wave (P_f) and reflected backward pressure wave (P_b) can be derived (Westerhof et al., 2006, 1972). The indices RM and RI can then be calculated from the magnitudes of P_f and P_b (Murgo et al., 1981), where

$$\mathrm{RM} = \frac{|P_\mathrm{b}|}{|P_\mathrm{f}|} \tag{2.18}$$

and

$$\mathrm{RI} = \frac{|P_\mathrm{b}|}{|P_\mathrm{f}| + |P_\mathrm{b}|} \tag{2.19}$$

These wave reflection indices were adopted in the investigative study on the influence of pacing modality on heart rate dependency of arterial stiffness and wave reflection (Chapter 5).

It should be noted that, although the concept of wave reflections in the arterial system is a traditionally accepted concept upon which a vast amount of cardiovascular and haemodynamic theories and research have been based (Chirinos et al., 2012; Mitchell et al., 2004; Murgo et al., 1981; Nichols et al., 2011; Wang et al., 2010), a recent alternative paradigm has been developed to explain the phenomenon of wave morphology changes (Davies et al., 2007; Wang et al., 2003). The latter methodology utilises the concept of the aortic reservoir pressure to account for the storage of blood in the arteries during systole, and recent research findings have shown that when reservoir pressure was accounted for, contribution of the backward travelling reflected wave on the pressure and flow waveform morphology was minimal (Davies et al., 2010, 2007; Wang et al., 2003). However, the methodology for calculating aortic reservoir pressure has also been disputed (Mynard, 2013; Mynard and Smolich, 2014; Segers et al., 2015). The fundamental issues with the reservoir theory are discussed in greater detail in the discussion section of Chapter 5, where the explanation behind pacing modality differences in arterial parameters relates to wave reflection parameters. As such, although the reservoir wave approach is being used in a subset of cardiovascular research studies (Hametner et al., 2014; Schultz et al., 2014; Sharman et al., 2009), concepts relating to wave reflections is used throughout this thesis.

2.2.4 Factors affecting arterial stiffness and its measurement

It has now been firmly established that the dominant determinants of arterial stiffness, as measured by PWV, are age and blood pressure in both healthy and diseased populations (Cecelja and Chowienczyk, 2009; Reference Values for Arterial Stiffness' Collaboration, 2010). Other factors that have been found to influence PWV include heart rate (Haesler et al., 2004; Lantelme et al., 2002b; Mitchell et al., 2004; Tan et al., 2012, 2016), presence of diabetes mellitus (Kim et al., 2007; van Trijp et al., 2004), gender (Amar et al., 2001; Park et al., 2007), smoking (Sutton-Tyrrell et al., 2001), lipid status (Lebrun et al., 2002) and body mass index (Benetos

et al., 2002). However, it has been shown that even in studies where a significant association was detected, the associations between PWV and diabetes mellitus, gender, smoking and lipid status were weak (Cecelja and Chowienczyk, 2009), and that these factors had no independent influence on PWV after correction for age and blood pressure (Reference Values for Arterial Stiffness' Collaboration, 2010).

Methodology used in obtaining PWV can influence interpretation of results. As aforementioned in Section 2.2.2, determination of arterial path length and characteristic point on the wave are required for the calculation of PWV. Differences in the way path length is determined can lead to differences in PWV of up to 30% (Rajzer et al., 2008; Salvi et al., 2008), and different timing algorithms for determining the characteristic point can lead to differences in PWV of 5-15% (Millasseau et al., 2005). As such, method of path length and timing determination needs to be explicitly stated (Townsend et al., 2015), and comparisons of PWV values across studies should be standardised accordingly (Reference Values for Arterial Stiffness' Collaboration, 2010; Van Bortel et al., 2012).

In human studies, accurate non-invasive measurement of arterial path length can only be obtained using MRI (Sugawara et al., 2010). In most human studies where PWV is measured, arterial path length is estimated by measuring the distance between the recording sites over the body surface. The most common region where PWV is measured is the aorta, for which the non-invasive measurement sites are commonly at the carotid artery and femoral artery. The path length between the carotid and femoral sites can either be measured as the straight distance between the two sites (Blacher et al., 1999; Laurent et al., 2001), or as length obtained by subtracting the distance between the carotid site to the suprasternal notch from the distance between the femoral to the suprasternal notch (Avolio et al., 1985; McEniery et al., 2005a; Mitchell et al., 2004; Sutton-Tyrrell et al., 2001). Another commonly measured regional PWV is the brachial-ankle PWV, which requires blood pressure to be measured on both sides of the body at the brachium

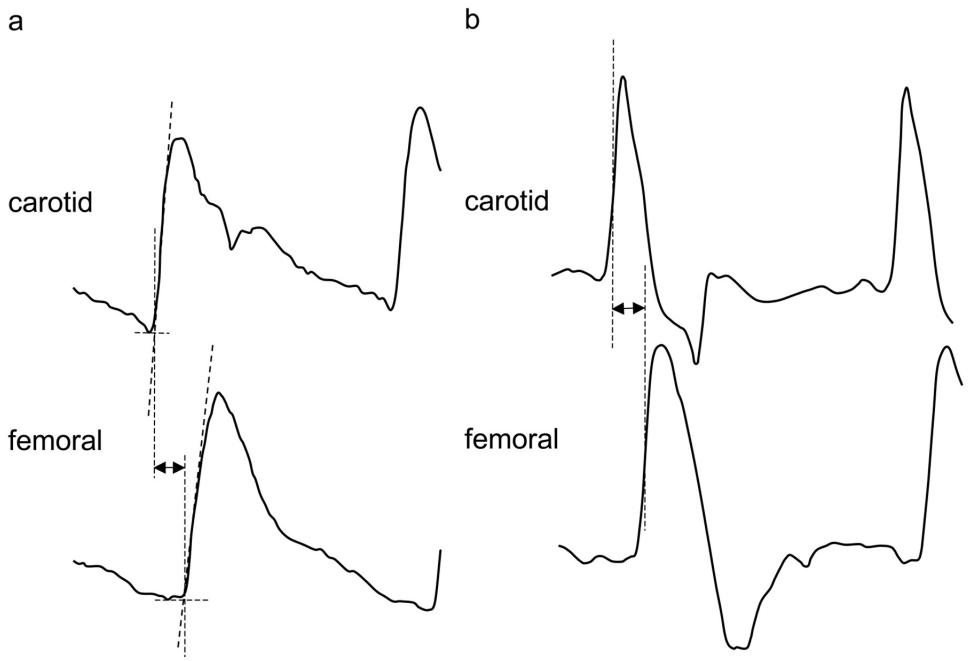

Figure 2.8: Methods of measurement of characteristic point used for calculation of PWV. (A) Foot of the pressure waveform is identified by the intersection of the tangent to the maximum systolic upstroke and the horizontal line through the minimum of the waveform. (B) The point of maximum systolic upstroke. (Millasseau et al., 2005) Used with permission.

and ankle, and arterial path length is determined from a calculation using distances measured from the suprasternal notch to the brachium, suprasternal notch to the ankle, and the height of the subject (Yamashina et al., 2002). Aortic PWV was the index used for the evaluation of arterial stiffness throughout the studies presented in this thesis. Distance between invasive measurement sites (thoracic and abdominal aorta) was obtained invasively in the rat postmortem (Chapter 3), and subtraction method was used for determining the arterial path length in all human studies (Chapters 4, 5 and 6).

Although various points on the arterial pressure wave can be used as a characteristic point for the calculation of PWV, the most common points of choice are (i) the foot, or the nadir, or the wave, as it is little affected by arterial wave reflections (Nichols et al., 2011); and (ii) the point of maximal systolic upstroke (Reference Values for Arterial Stiffness' Collaboration, 2010) (see Figure 2.8). For both animal and human studies presented in this thesis, the foot of the measured arterial pressure waves were used as the characteristic point in the calculation of PWV.

Due to the influence of blood pressure on arterial stiffness, the blood pressure at which PWV was measured needs to be accounted for. The steady component of arterial pressure, mean arterial pressure (MAP), is highly correlated with PWV (Giannattasio et al., 1996; Kim et al., 2007; Nichols et al., 2011; Stewart et al., 2003) and is the most common blood pressure component used in studies of arterial stiffness next to systolic blood pressure (Cecelja and Chowienczyk, 2009). However, it has recently been shown that PWV, as determined by compliance measurements obtained from ultrasound and utilising the Bramwell-Hill equation (Eq. 2.16), may vary about 0.8-4.4 m/s within individuals over the cardiac cycle (Hermeling et al., 2012), and thus PWV measurements should discriminate between systolic and diastolic pressure ranges (Hermeling et al., 2010). In the animal study presented in this thesis (Chapter 3), blood pressure was manipulated pharmacologically and PWV values were compared at the same MAP. For the human study presented in Chapter 4, diastolic blood pressure was used for correcting PWV values for blood pressure.

2.2.5 Arterial stiffness and cardiovascular risk

Before the first study to demonstrate that arterial stiffness, as measured by aortic PWV, was a marker of cardiovascular risk in hypertensive patients (Blacher et al., 1999) was published, prior studies had already shown that arterial stiffness increased with age and blood pressure (Avolio et al., 1983), as well as in the presence of diabetes mellitus (Lehmann et al., 1992), atherosclerosis (Wada et al., 1994) and end-stage renal disease (London et al., 1990). In the two decades that have passed since then, a vast amount of research has been dedicated to study of the association of arterial stiffness, and more specifically PWV, with cardiovascular risk. Arterial stiffness has now emerged as an independent predictor of major cardiovascular events such as stroke (Laurent et al., 2003; Mattace-Raso et al., 2006; Mitchell et al., 2010; Sutton-Tyrrell et al., 2005) and coronary heart disease (Boutouyrie et al., 2002; Mattace-Raso et al., 2006;

Sutton-Tyrrell et al., 2005; Willum-Hansen et al., 2006), as well as cardiovascular and all-cause mortality in both general (Mitchell et al., 2010; Sutton-Tyrrell et al., 2005; Willum-Hansen et al., 2006), and diseased populations such as those with hypertension (Laurent et al., 2001), coronary heart disease (Sakuragi et al., 2005) and end-stage renal disease (Blacher et al., 1999; Shoji et al., 2001). Furthermore, it has recently been shown that consideration of aortic PWV can improve overall 10-year risk classification for cardiovascular disease in individuals at intermediate risk by some 13% beyond that with standard risk factors alone (Ben-Shlomo et al., 2014). Recently, it has also been suggested that aortic stiffening may contribute to the pathogenesis of, and therefore precedes, hypertension (Weisbrod et al., 2013).

The clinical importance of arterial stiffness was recognised when, in the 2007 Guidelines for the Management of Arterial Hypertension (Mancia et al., 2007), PWV was added to the list of factors influencing prognosis of hypertension as an early index of large artery stiffening. The threshold value of carotid-femoral PWV, above which the risk for cardiovascular events would increase, was set at 12 m/s. However, this was later revised to 10 m/s (Mancia et al., 2013) further to the recommendation for standardising PWV arterial path length measurement (Van Bortel et al., 2012).

2.3 Heart rate and arterial stiffness

Unlike arterial blood pressure, which is determined by the ventricular-vascular coupling between the heart and the vasculature, arterial stiffness is entirely determined by vascular characteristics (Nichols et al., 2011). As such, it may seem counterintuitive to suggest that the rate at which the heart contracts has any independent influence on the stiffness of arteries save for the effects of heart rate on blood pressure and other haemodynamic parameters. However, various epidemiological studies on arterial stiffness, both large and small, have found an independent association between PWV and heart rate (Cecelja and Chowienczyk, 2009). Furthermore, though only a limited

number of studies have studied the acute effects of heart rate on arterial stiffness, some have shown that acute increases in heart rate led to increase in PWV (Haesler et al., 2004; Lantelme et al., 2002b; Millasseau et al., 2005), with investigators speculating that the effect of heart rate was manifested through the frequency-dependent viscoelasticity (Armentano et al., 1995) of the arterial wall (Lantelme et al., 2002b). Notwithstanding, though evidence suggests that there is at least a modest relationship between heart rate and arterial stiffness (Mitchell et al., 2004), the strength of the evidence is not yet strong enough to establish a relationship between heart rate and arterial stiffness due to the lack of convergence in study results.

2.3.1 Heart rate and the viscoelasticity of the arterial wall

In the seminal studies performed by Bergel (1961a,b), the frequency dependency of the dynamic elastic modulus (E') of the arterial wall (see Section 2.2.2) was elegantly demonstrated in isolated canine arterial segments held at a pressure of 100 mmHg. Given that the product of viscosity and angular frequency, or viscous moduli ($\mu\omega$), was small relative to the elastic component of the elastic modulus (E_{dyn}), E' was taken to be equivalent to E_{dyn}, and results showed that E_{dyn} was dependent on frequency up to 2 Hz (Bergel, 1961a) (Figure 2.9).

Linear extrapolation of Bergel's data from 0 to 2 Hz shows that the increase of E_{dyn} from 60 bpm to 120 bpm was 30% in the carotid artery, 16.5% in the femoral artery, 9.5% in the abdominal aorta and 3.5% in the thoracic aorta. By implication of the relationship between PWV and elastic modulus through the Moens-Korteweg equation (Eq. 2.13), this thus demonstrated a frequency dependency of PWV, as shown in Bergel's calculation of the term $\frac{E_{\text{dyn}}.h}{2R}$ (Bergel, 1960) (Figure 2.10).

By taking blood density as 1.06 g.cm^{-3}, the PWV values at 1 Hz and 2 Hz can then be calculated using Moens-Korteweg equation (Eq. 2.13), again by linear extrapolation (Table 2.2), with correction terms applied to the calculated velocities to account for Poisson's ratio of blood and

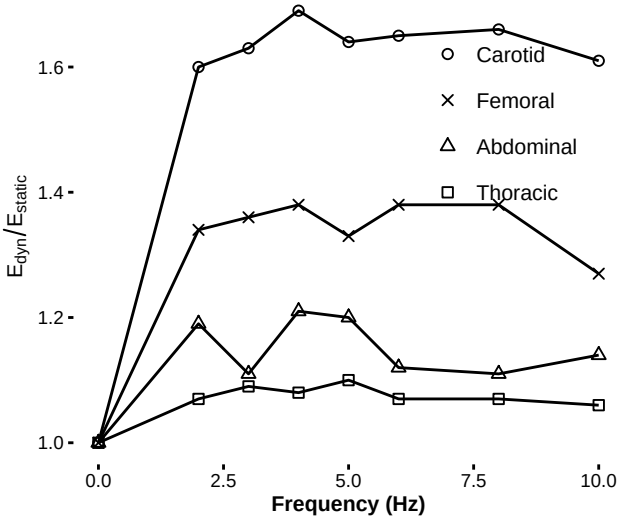

Figure 2.9: Ratio of dynamic elastic modulus E_{dyn} to static elastic modulus E_{static} in various canine arterial segments when measured across frequencies of 2 Hz to 20 Hz. There is a steep increase in the ratio between 0 to 2 Hz, but it becomes essentially constant above 2 Hz. Data from Bergel (1961a).

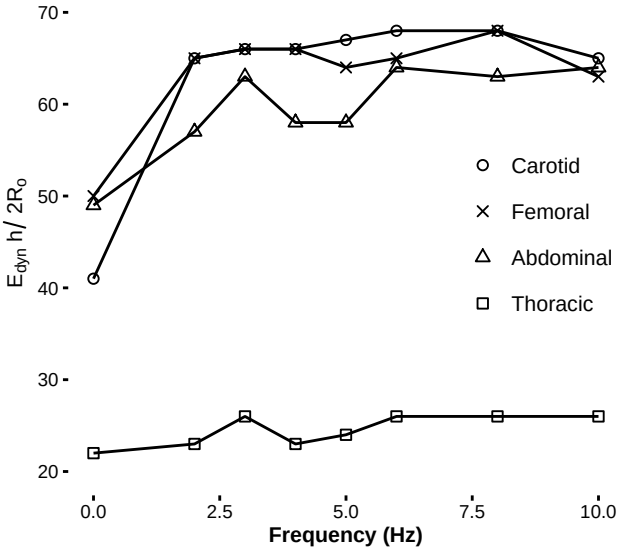

Figure 2.10: Frequency dependency of the Moens-Korteweg related term, $\frac{E_{\text{dyn}}h}{R_o}$, at various canine arteries. This term is related to PWV by the Moens-Korteweg equation (Eq. 2.13), and calculated frequency dependency of PWV in these arteries are presented in Table 2.2. Data from Bergel (1960).

Table 2.2: PWV calculated at static and sinusoidal stretch at 1 Hz and 2 Hz in canine arterial segments distended at 100 mmHg. Data from Bergel (1960).

Frequency (Hz)	PWV (m/s)			
	Carotid	Femoral	Abdominal	Thoracic
0	7.0	7.7	7.7	5.1
1	7.9	8.3	8.0	5.2
2	8.8	8.8	8.4	5.3

The greatest increase in PWV from static to 2 Hz was observed in the canine carotid artery, with a 26% increase. Regression on the PWV values between static to 2 Hz resulted in a heart rate dependency of PWV of 0.13 m/s per 10 bpm increase in heart rate. When compared to 1 Hz, PWV increased by 1% in the thoracic aorta, 4% in the abdominal aorta, 6% in the femoral artery and 10% in the carotid artery. PWV was calculated with Moens-Korteweg equation (Eq. 2.13), with the following correction terms applied to account for Poisson's ratio of blood and the relative wall thickness in the different arterial segments: thoracic and abdominal aorta: 1.124; femoral artery: 1.121; carotid artery: 1.116 (Bergel, 1960).

relative wall thickness (Bergel, 1960). Thus, frequency dependency of arterial stiffness was shown to be more pronounced with increased muscularity in the arteries, with Bergel noting that the *relative* change in elastic modulus with frequency was independent of the mean pressure at which the arteries were held (Bergel, 1961a).

Contrary to the results obtained by Bergel (1961a), other investigators demonstrated that PWV was independent of frequency in isolated canine common carotid arteries (Callaghan et al., 1984). Whilst Bergel's experiments were limited to a minimum frequency of 2 Hz due to the limitations of equipment (Avolio and Benetos, 2006), the study by Callaghan et al. (1984) showed that PWV was independent of frequency down to 1 Hz, i.e. 60 bpm, and that this, too, was independent of mean pressure at which the arteries were distended (Figure 2.11). However, when examining the PWV values at 1 Hz and 2 Hz only, it can be observed that the change in PWV was of similar order to the change in PWV observed in the canine abdominal aorta as measured by Bergel (1961a) (Table 2.3).

Another important aspect of the arterial wall, other than its elasticity, is its viscosity. By definition, viscosity of a material includes units of time ($N \cdot s \cdot m^{-2}$) and so related to frequency

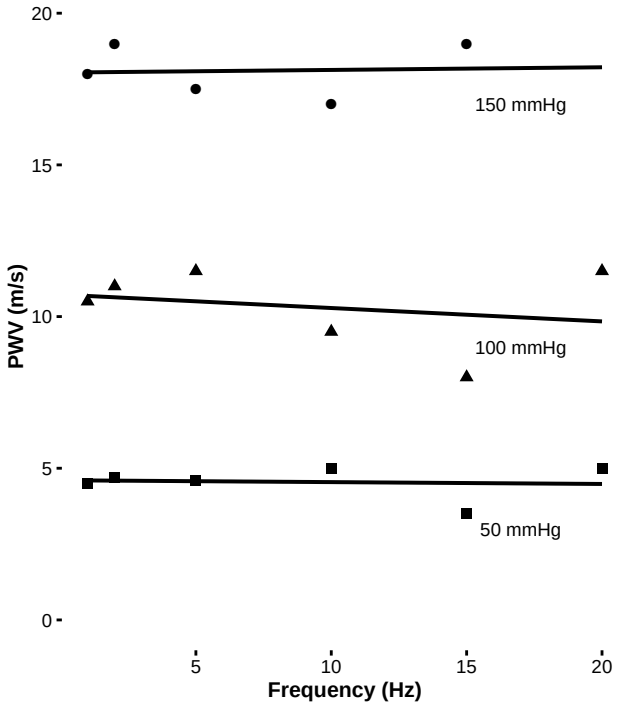

Figure 2.11: Frequency dependency of PWV calculated in the canine carotid artery at distending pressures of 50 mmHg, 100 mmHg and 150 mmHg. PWV was shown to be independent of frequency across frequencies of 1 Hz to 20 Hz. Data from Callaghan et al. (1984).

Table 2.3: PWV calculated at sinusoidal stretch of 1 Hz and 2 Hz in the canine carotid artery at different distending pressures. Data from Callaghan et al. (1984).

Frequency (Hz)	PWV (m/s)		
	50 mmHg	100 mmHg	150 mmHg
1	4.5	10.5	18.0
2	4.7	11.0	19.0

When compared to 1 Hz, PWV in the carotid artery increased by 4% at 50 mmHg, 5% at 100 mmHg, 6% at 150 mmHg. These increases were of a similar order as compared to the observations by Bergel (1960) in the canine abdominal aorta (Table 2.2).

(Nichols et al., 2011). Both *in vitro* and *in vivo* studies in arteries of canines (Bergel, 1961a; Gow and Taylor, 1968), rats (Armentano et al., 1995; Boutouyrie et al., 1997), sheep (Salvucci et al., 2007), as well as humans (Imura et al., 1990; Learoyd and Taylor, 1966) have all shown a frequency dependency of arterial wall viscosity. This indicates that changes in heart rate would lead to changes in wall viscosity, although arterial stiffness would be unaffected if E_{dyn} is unchanged and the viscous moduli is relatively much smaller ($< 10\%$) (Bergel, 1961a). Furthermore, most

of these studies have shown that the viscous moduli remains relatively constant (Salvucci et al., 2007) or increases slightly (Bergel, 1961a) with increasing frequency, with *in vivo* viscosity being three-fold lower than *in vitro* in rats(Armentano et al., 1995), but with no significant difference in humans (Imura et al., 1990). It is generally accepted that changes in viscosity in the arterial wall are mediated by smooth muscle activation (Armentano et al., 1995, 2006; Bergel, 1961a; Gow and Taylor, 1968), and increases in heart rate may affect arterial stiffness via altered vascular muscle tone due to changes in sympathetic activity (Mitchell et al., 2004). As such, although studies in which an acute increase in heart rate by means of cardiac pacing led to changes in arterial stiffness may have attributed the observation to viscoelasticity of the arterial wall (Lantelme et al., 2002b; Mangoni et al., 1996), the precise mechanism by which heart rate can affect arterial stiffness is still largely unknown.

2.3.2 Acute effects of heart rate on arterial stiffness

Only a limited number of studies have been performed in the past in either animals or humans to determine the effects of acute heart rate changes on arterial stiffness. In both intact (Mangoni et al., 1996) and sympathectomised rats (Mircoli et al., 1999), the same group of investigators found that increases in heart rate by way of cardiac pacing resulted in a decrease in common carotid distensibility. Furthermore, although no changes were observed in femoral distensibility with increasing heart rate in the intact rat (Mangoni et al., 1996; Mircoli et al., 1999), the effect of heart rate was unmasked in sympathectomised rats (Mircoli et al., 1999). Sympathectomised rats showed higher distensibility in the common carotid artery as compared to intact rats, whether during sinus rhythm or pacing, whereas femoral distensibility was only higher than intact rats during sinus rhythm (Mircoli et al., 1999). Together, these studies provided the first evidence of heart rate influence on arterial stiffness *in vivo* (Mangoni et al., 1996), and demonstrated the effect of heart rate on the stiffness is independent of sympathetic tone in the arteries (Mircoli et al.,

1999). The latter study by Mircoli et al. (1999) also demonstrated that sympathetic tone did in fact contribute to the stiffness in both elastic-type (common carotid) artery and muscular-type (femoral) arteries, and that once the influence of sympathetic tone was removed, the stiffening effect of heart rate was more prominent in the muscular-type artery. The investigators attributed the decrease in distensibility with increasing heart rate to viscosity of the arterial wall, whereby reduced time for the artery to recoil would lead to arterial stiffening (Mangoni et al., 1996), and that viscosity was influenced by mechanical load rather than by smooth muscle tone (Boutouyrie et al., 1997; Mircoli et al., 1999). These findings also concur with the *in vitro* observations made by Bergel (1961a), who found that arterial wall stiffness was more sensitive to frequency changes in more muscular arteries (although in canines the carotid artery is more muscular than the femoral artery). On the contrary, another study by Albaladejo et al. (2004) observed no change in isobaric carotid distensibility in either normotensive or hypertensive rats when heart rate was reduced pharmacologically. Interestingly, an earlier study led by the same investigator found that chronic reduction in heart rate with the same pharmacological agent resulted in increased carotid distensibility in normotensive but not hypertensive rats (Albaladejo et al., 2003a).

Findings from human studies of acute heart rate effects on arterial stiffness have also failed to converge. Factors which may have contributed to the different findings are likely i) the index for arterial stiffness used, and ii) the presence or absence of concomitant blood pressure changes. Of the studies that have been conducted to date, most studies adopted carotid-femoral PWV as the measure for arterial stiffness (Albaladejo et al., 2001, 2003b; Haesler et al., 2004; Lantelme et al., 2002b; Liang et al., 1999; Millasseau et al., 2005; Rhee et al., 2004); two studies used time of the first inflection point in the central aortic pressure wave, or time of return of the reflected wave (T_{r}), as an estimate of PWV (Wilkinson et al., 2000, 2002); two studies used distensibility measurements (Giannattasio et al., 2003; Stefanadis et al., 1998). Only one study found an improvement in arterial distensibility with an increase in heart rate (Stefanadis et al., 1998); other studies either found no change in stiffness (Albaladejo et al., 2001, 2003b; Rhee et al., 2004;

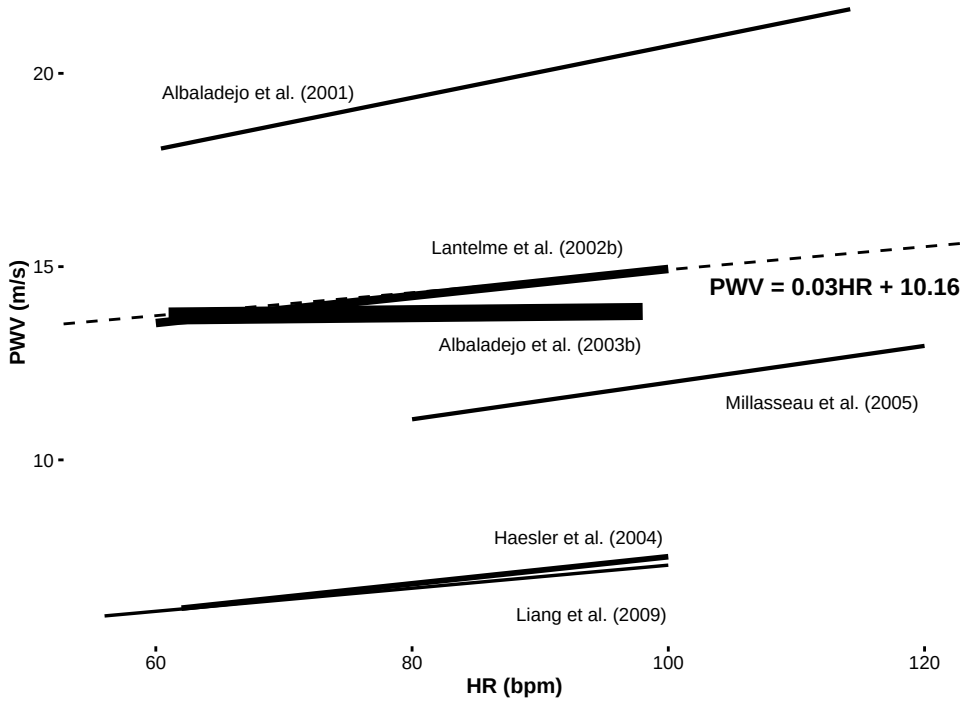

Figure 2.12: A summary of acute pacing studies investigating the effects of heart rate on carotid-femoral PWV. All studies except the one by Albaladejo et al. (2003b) observed an increase in PWV with increasing HR, but the increase was only significant in the studies by Haesler et al. (2004); Lantelme et al. (2002b); Millasseau et al. (2005) and Liang et al. (1999). Regression was performed on the mean PWV values presented in the individual studies. The average heart rate dependency of PWV as calculated from the presented studies, weighted by study sample size (see Table 2.5), was 0.03 m/s/bpm, and is denoted by the dashed line. When including only the males from the study by Albaladejo et al. (2003b), the average heart rate dependency of PWV was 0.037 m/s/bpm. With the exception of the studies by Lantelme et al. (2002b) and Haesler et al. (2004), there was a concomitant increase in blood pressure with increasing heart rate.

Wilkinson et al., 2000, 2002) or increased stiffness with increasing heart rate (Giannattasio et al., 2003; Haesler et al., 2004; Lantelme et al., 2002b; Liang et al., 1999; Millasseau et al., 2005) (Table 2.4).

In most of the studies that investigated the effects of acute heart rate changes on PWV, increases in heart rate were induced via either cardiac or transoesophageal pacing, with one study also reducing heart rate with intravenous β-blocker prior to pacing (Liang et al., 1999). All these studies observed an increase in PWV with increasing heart rate (Figure 2.12), and the average for the change in PWV with heart rate, when weighted on precision of the regression on the sample size of these studies, was 0.3 m/s per 10 bpm increase in heart rate. However, not all PWV differences reached statistical significance, and all but two studies (Haesler et al., 2004; Lantelme

Table 2.4: Studies on acute heart rate effects on arterial stiffness in humans.

Reference	Sample, n	Males, n(%)	Age, years	Measure	HR range, bpm	MAP range, mmHg	Association
Albaladejo et al. (2001)	PM, 11	6(55)	69 ± 14	cfPWV	60 - 120	93 - 111	NS
Albaladejo et al. (2003b)	PM, 30	15(50)	77 ± 9	cfPWV	60 - 100	96 - 99	NS
Giannattasio et al. (2003)	PM, 20	14(70)	62 ± 13	carotid/radial distensibility	63 - 110	96 - 97	∗
Haesler et al. (2004)	PM,14	9(64)	68 ± 8	cfPWV	62 - 100	102 - 104	∗ (+ve)
Lantelme et al. (2002b)	PM, 22	13(59)	78 ± 8	cfPWV	60 - 100	88 - 89	∗ (+ve)
Liang et al. (1999)	healthy, 9	9(100)	26(20-30)	cfPWV	56 - 100	78 - 102	∗ (+ve)
Millasseau et al. (2005)	PM, 11	6(55)	62 ± 17	cfPWV	80 - 100	90 - 96	∗ (+ve)
Rhee et al. (2004)	HT, 17	3(18)	59 ± 9	cfPWV	76 - 122	N/A	NS
Stefanadis et al. (1998)	Angina, 14	7(50)	55 ± 2	stiffness constant	67 - 160	99 - 96	∗ (-ve)
Wilkinson et al. (2000)	PM, 22	13(59)	63 ± 17	T_r	60 - 110	98 - 112	NS
Wilkinson et al. (2002)	Agina, 20	14(70)	47 ± 16	T_r	65 - 120	97 - 98	NS

With the exception of Rhee et al. (2004), which induced HR changes pharmacologically with isoproterenol, all studies induced HR changes via cardiac pacing, with only Liang et al. (1999) using transoesophageal pacing. Stiffness constant in the study by Stefanadis et al. (1998) was calculated from aortic pressure and diameter. HR, heart rate; MAP, mean arterial pressure; PM: pacemaker patients; cfPWV, carotid-femoral pulse wave velocity; NS, not statistically significant; N/A, not reported. ∗ significant association between HR and stiffness measure reported.

Table 2.5: Heart rate (bpm) dependency of carotid-femoral PWV (m/s) from various acute studies in humans.

Reference	Sample size	Pacing mode (n)	Regression
Albaladejo et al. (2001)	11	Ap(11)	PWV = $6.7x10^{-2}$HR + 14.0
Albaladejo et al. (2003b)	30	Ap(3), ApVp(18), Vp(9)	PWV = $3.2x10^{-3}$HR + 13.5
Males	15		PWV = $1.7x10^{-2}$HR + 13.3
Females	15		PWV = $-1.3x10^{-2}$HR + 14.1
Haesler et al. (2004)	14	Ap(11)	PWV = $3.5x10^{-2}$HR + 4.0
Lantelme et al. (2002b)	22	Ap(2), Vp(20)	PWV = $3.5x10^{-2}$HR + 11.4
Liang et al. (1999)	9	TE	PWV = $3.0x10^{-2}$HR + 4.3
Millasseau et al. (2005)	11	ApVp(8), Vp(3)	PWV = $4.8x10^{-2}$HR + 7.3

Regression was performed on the mean data obtained from each of the referenced studies. HR, heart rate; PWV, pulse wave velocity; Ap, atrial pacing; ApVp, atrioventricular pacing; Vp, ventricular pacing; TE, transoesophageal pacing.

et al., 2002b) observed a concomitant significant increase in blood pressure with increasing heart rate (Table 2.5). In addition, it has been argued that, due to the high frequency components ($>$ 10 Hz) that make up the foot of the pulse wave (Nichols et al., 2011), and the fact that the dynamic elastic modulus is essentially independent of frequency above 2 Hz, theoretically it would be unlikely that changes in heart rate would influence PWV (Hayward et al., 2002). Furthermore, the study by Albaladejo et al. (2003b) found that the relationship between acute heart rate changes and PWV differed between men and women, with PWV increasing significantly with increasing heart rate in men, but only regression from the combined data was used in calculation of the average heart rate dependency of PWV.

2.3.3 Association between arterial stiffness and heart rate in epidemiological studies

Both elevated heart rate and arterial stiffness are established risk factors for cardiovascular disease and mortality (see Sections 2.1.4 and 2.2.5). However, possibly due to the many confounding factors on both, the association between the two cardiovascular risk factors remains to be firmly established. Furthermore, it is unknown whether the presence of both high heart rate and increased arterial stiffness would be additive to the risk than either factor on its own.

In the review on the association of PWV with cardiovascular risk factors by Cecelja and Chowienczyk (2009), it was shown that, up until the year of 2008, approximately half (26 out of 51) of the epidemiological studies that included heart rate in a multiple regression model on the determinants of PWV found a significant association between heart rate and PWV (Figure 2.13), although the authors noted that the association was weak. In the years since the review, there have been other epidemiological studies, both small and large, that have also found a significant association between heart rate with PWV even after adjustment for other confounding factors. Table 2.6 lists these studies, in addition to the studies included in the review by Cecelja and Chowienczyk (2009).

Recently, it has also been shown that left ventricular ejection duration, rather than heart rate, was a determinant of PWV (Nürnberger et al., 2003; Salvi and Parati, 2013).

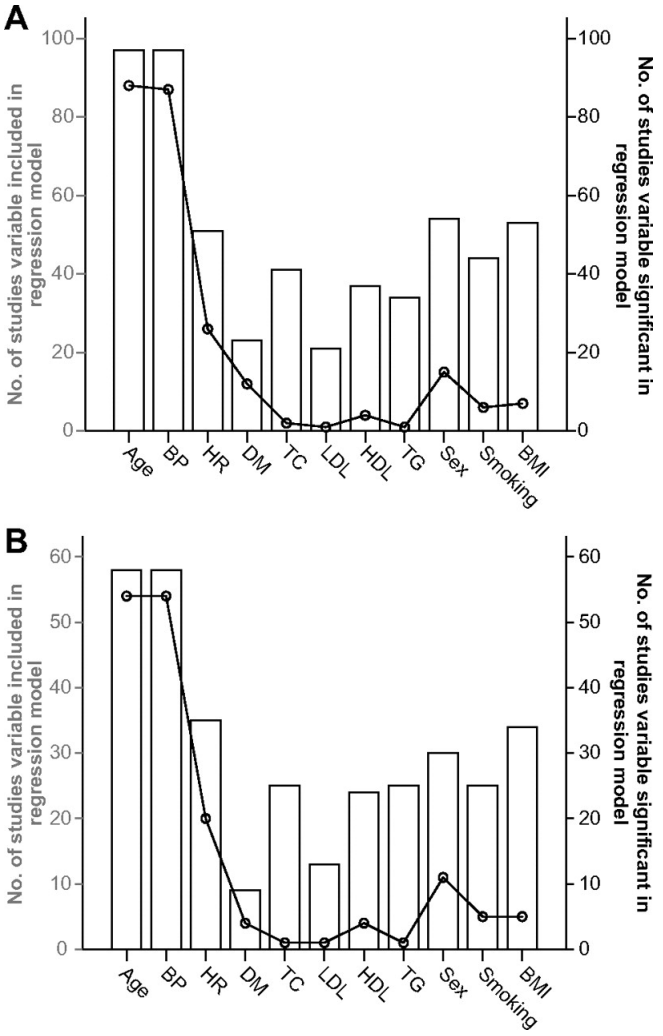

Figure 2.13: A. Approximately half of the studies that included heart rate (HR) in the regression analysis for predictors of PWV showed a significant association. B. In studies including only healthy, hypertensive or population cohorts, a little over half of the studies found a significant association between HR and PWV. Reproduced from Cecelja and Chowienczyk (2009) with permission.

Table 2.6: List of epidemiological studies, by publication year, in which heart rate was found to have a significant association with PWV in multiple regression models

Reference	Sample, n	Men, n (%)	Age, y	HR, bpm	BP, mmHg	PWV, m/s	PWV, type	Comments
Taquet et al. (1993)	Healthy, 429	0 (0)	49 ± 3	71 ± 11	130/82 ± 13/8	...	cfPWV, t	
Sa Cunha et al. (1997)	Men, 153	153 (100)	45 ± 19	66 ± 10	145/85 ± 25/14	9.3 ± 2.3	cfPWV, d	
	Women, 100	0 (0)	45 ± 17	69 ± 11	134/79 ± 22/11	8.7 ± 2.0	cfPWV, d	
Lacolley et al. (1998)	NT, 123	78 (63)	44 ± 21	...	124/76 ± 21/16	9.2 ± 3.7	cfPWV, t	
	HT, 309	212 (69)	49 ± 21	...	157/96 ± 26/21	12.5 ± 6.4	cfPWV, t	
Albaladejo et al. (2000)	HT, 213	130 (61)	50 ± 8	76 ± 10	140/89 ± 16/11	12.1 ± 2.9	cfPWV, c	24-hour ambulatory HR and BP
Amar et al. (2001)	Untreated, 746	393 (53)	48 ± 8	67 ± 9	127/79 ± 16/10	8.8 ± 1.6	cfPWV, c	
	Treated HT, DM, HC, 247	128 (52)	54 ± 8	68 ± 9	142/83 ± 19/11	9.8 ± 1.8	cfPWV, c	
Asmar et al. (2001)	HT, 1087	616 (57)	58 ± 14	68 ± 10	144/82 ± 19/11	12.6 ± 3	cfPWV, c	
Meaume et al. (2001)	Elderly, 124	36 (29)	87 ± 7	76 ± 11	136/73 ± 16/11	14.1 ± 3.1	cfPWV, c	
Sutton-Tyrrell et al. (2001)	Population, 2488	1186(48)	74	...	...	9.0	cfPWV, d	SD values not reported
Achimastos et al. (2002)	HT, 162	162 (100)	52 ± 10	74 ± 14	150/97 ± 19/13	12.2 ± 2.5	cfPWV, c	
Benetos et al. (2002)	Treated HT, 187	118 (63)	57 ± 10	68 ± 12	145/88 ± 14/10	11.4 ± 2.6	cfPWV, c	HR was associated with increase in cfPWV over a 6 year period
Lantelme et al. (2002a)	HT,193 of 271	148(55)	53 ± 13	74 ± 11	149/92 ± 22/14	12.7 ± 3.1	cfPWV, c	24-hour ambulatory BP and HR
Lebrun et al. (2002)	Postmenopausal ♀, 385	0(0)	66 ± 4	69 ± 10	147/76 ± 21/14	9.2 ± 2.1	cfPWV, t	
Mackey et al. (2002)	Men, 166	166(0)	79 ± 4	62 ± 10	129/73 ± 19/11	8.7 ± 2.4	cfPWV, d	
	Women, 190	0 (0)	78 ± 4	66 ± 10	131/70±20/9	8.4 ± 2.4	cfPWV, d	
Albaladejo et al. (2003b)	NT, 558	558 (0)	45 ± 11	67 ± 10	132/81 ± 12/9	8.8 ± 1.6	cfPWV, c	NT women also included but no significant association between HR and PWV was found
	HT, 323	323 (0)	51 ± 10	76 ± 13	164/102 ± 13/9	10.6 ± 2.4	cfPWV, c	HT women also included but no significant association between HR and PWV was found
Hansen et al. (2004)	Healthy, 2420	1207 (50)	54 ± 11	66 ± 11	129/82 ± 19/11	11.3 (6.0-41.9)	cfPWV, t	PWV presented as median (range)
Lacy et al. (2004)	DM and controls, 132	90 (68)	55 ± 20	67 ± 15	134/83 ± 21/14	8.5 ± 3.4	cfPWV, t	
Mitchell et al. (2004)	Healthy, 521	188 (36)	57 ± 9	62 ± 9	115/69 ± 11/9	8.5 ± 2.0	cfPWV, t	Framingham Offspring Study cohort
Kullo et al. (2005)	Community, 214	114 (53)	60 ± 10	62 ± 9	126/73 ± 16/9	9.8 ± 2.9	cfPWV, t	
McEniery et al. (2005a)	Healthy, 998 of 4001	1969 (49)	18-90	67 ± 11	123/75 ± 10	5.6-11.9	cfPWV, t	ACCT study cohort
McEniery et al. (2005b)	Young adults, 1008	438 (51)	20 ± 3	71 ± 11	134/81 ± 8/7	6.0 ± 0.4	cfPWV, t	Age, HR and BP excluded subjects with high-normal BP (n=151); ENIGMA study cohort
Briet et al. (2006)	CKD, 95; CKD and HT, 121; HT, 57	175 (64)	58 ± 11	66 ± 10	128/74 ± 17/9	10.5 ± 1.9	cfPWV, c	
Strain et al. (2006)	European T2DM, 51	25 (49)	57 ± 7	70 ± 14	147/84 ± 21/7	14 (11, 16)	cfPWV, c	PWV log transformed and shown as median(25th, 75th percentile); study also included European without T2DM and African Caribbean with and without T2DM, but no signification association between HR and PWV was found in all these groups
van Trijp et al. (2006)	Healthy, 299	299 (100)	59 ± 11	69 ± 11	143/81 ± 22/10	9.3 ± 2.8	cfPWV, t	
Achimastos et al. (2007)	Men, 298	298 (100)	46 ± 15	77 ± 11	128/78 ± 16/11	8.8 ± 3.1	cfPWV, c	
	Women, 126	0(0)	43 ± 15	76 ± 10	128/78 ± 16/10	7.7 ± 2.9	cfPWV, c	
Park et al. (2007)	HT, 438	246 (56)	57 ± 10	64 ± 10	133/81 ± 17/11	10.1 ± 1.9	hfPWV, h	
Protogerou et al. (2007)	HT females, 249	0 (0)	60 ± 6	68 ±8	145/81 ± 6/6	11.9 ± 6.3	cfPWV, c	HT males also included in study but no significant association between HR and PWV was found
Tan et al. (2007)	HT, 202	159 (79)	60 ± 12	67 ± 12	144/89 ± 20/13	11.4 ± 2.1	cfPWV, c	

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Table 2.6: (continued)

Reference	Sample, n	Men, n (%)	Age, y	HR, bpm	BP, mmHg	PWV, m/s	PWV, type	Comments
Zhou et al. (2007)	Untreated HT, 215	108 (50)	46 ± 13	70 ± 10	159/93 ± 17/10	10.5 ± 1.9	cfPWV, c	
Alecu et al. (2008)	Population, 455	245 (54)	66 ± 4	68 ± 11	133/73 ± 18/10	9.5 ± 2.5	cfPWV, t	
Chen et al. (2008)	Black, 255	97 (38)	36 ± 5	70 ± 10	122/82 *pm* 16/11	5.4 ± 1.1	cfPWV, d	White young adults also included in study but no significant association between HR and PWV was found; Bogalusa Heart Study cohort
Delles et al. (2008)	CAD and controls, 163	106 (65)	56 ± 11	61 ± 10	134/75 ± 17/10	7.5 ± 2.7	cfPWV, t	
Matsumae et al. (2008)	Haemodialysis without DM, 184	88 (60)	64 ± 11	69 ± 11	146/89 ± 26/16	9.2 ± 2.0	cfPWV, h	Haemodialysis patients with DM were also included in the study, but no significant association between HR and PWV was found
Cardoso et al. (2009)	T2DM, 482	180 (37)	63 (47–76)	72 (53–96)	127/72 (109–158/59–89)	11.1 ± 2.4	cfPWV, c	24-hour ambulatory BP; haemodynamic variables presented as median (5–95 percentile range)
Odaira et al. (2009)	Japanese, 2904	2439 (84)	42 (37–53)	...	120/74 (110–132/68–82)	12.9 (12.8–12.9)	baPWV, h	Data presented as median (5 - 95 percentile range); baPWV presented as mean (95% CI)
Song et al. (2009)	HT, 438	246 (56)	57 ± 10	64 ± 10	133/81 ± 17/10	10.1 *pm* 1.9	hfPWV, h	hfPWV was logged transformed for regression analysis
McEniery et al. (2010b)	Population, 4421	2529 (51)	46 ± 23	67 ± 11	131/78 ± 20/11	7.9 ± 3.0	cfPWV, t	ACCT study cohort, included untreated population
	Untreated HT, HC, DM, 3613	1991 (55)	41 ± 22	67 ± 11	128/78 ± 19/11	7.3 ± 2.7	cfPWV, t	ACCT study cohort
McEniery et al. (2010a)	Population, 825	825 (100)	56 ± 5	66 ± 10	142/87 ± 20/11	11.5 ± 2.8	cfPWV, t	Caerphilly Prospective Study cohort at baseline; PWV measured at 20-year followup (see below)
	Population, 825	825 (100)	74 ± 4	64 ± 11	141/74 ± 20/11	11.5 ±2.8	cfPWV, t	Caerphilly Prospective Study cohort at 20-year follow up
Park et al. (2010)	Korean, 641	366 (57)	48 ± 12	Q1 $\leq$ 56, Q4 $\geq$ 69	120/73 ± 15/12	13.3 ± 3.9	baPWV, h	Association between HR and age-adjusted baPWV shown with ANCOVA, univariate correlation and multivariate logistic regression determining odds ratio for high baPWV according to HR quartiles
Sengstock et al. (2010)	CKD, 264	139 (53)	58 ± 14	64 ± 10	138/74 ± 25/13	8.4 ± 2.9	cfPWV, t	
Tomiyama et al. (2010)	Healthy, 1795	1404 (78)	40 ± 8	64 ± 5	120/73 ± 13/10	12.4 ± 6.5	baPWV, h	both baseline HR and change in HR was significantly associated with change in baPWV over 5-6 year period
Cecelja et al. (2011)	Women, 900	0 (0)	58 ± 9	63 ± 9	128/79 ± 16/10	8.9 ± 1.7	cfPWV, t	Twins UK cohort
Wang et al. (2011)	Chinese, 2375	1142 (48)	58 ± 8	72 ± 9	128/76 ± 8/10	11.1 ± 2.0	cfPWV, c	
Bulzico et al. (2012)	GD and control, 51	0 (0)	30 ± 6.4	84 ± 12	117/71 ± 8/7	7.3 ± 1.1	cfPWV, h	Significant association between HR and PWV when categorised according to median value of PWV
Cooper et al. (2012)	Moderately overweight, obese, 344	78 (23)	38 ± 6	64 ± 9	113/73 ± 10/9	7.8 (7.1, 8.8)	hfPWV, h	PWV presented as median (interquartile range)
Johansen et al. (2012)	Men,2857	2857 (100)	49 ± 6	63 ± 11	121/80 ± 13/9	8.5 ± 2.0	cfPWV, t	Whitehall II study cohort; PWV measured at 16-year follow up
	Women,912	0 (0)	49 ± 6	66 ± 10	115/75 ± 13/9	8.1 ± 2.0	cfPWV, t	Whitehall II study cohort; PWV measured at 16-year follow up
Rodrigues et al. (2012)	Population, 1608	740 (46)	45 ± 11	70 ± 11	128/84 ± 22/14	9.9 ± 2.2	cfPWV, c	

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Table 2.6: (continued)

Reference	Sample, n	Men, n (%)	Age, y	HR, bpm	BP, mmHg	PWV, m/s	PWV, type	Comments
Koskela et al. (2013)	Finnish, 522	261 (50)	46 (44–47)	64 (63–64)	135/79 (132–138/77–81)	8.5 (8.3–8.7)	PWV_{imp}	HR was divided into tertiles, with PWV significantly higher in tertiles 2 and 3; whole body impedance was used to measure PWV, and radial BP was measured; data presented as mean (95% CI)
Gottsäter et al. (2015)	Population, 2679	1007 (38)	72 ± 6	...	143/83 ± 19/10	10.1 (8.8–11.8)	cfPWV, t	PWV was measured at follow-up only and presented as median (1st–3rd quartile); follow-up data presented as only follow-up HR was significantly associated with PWV
Masaki et al. (2015)	Elective cardiac catheterisation, 74	62 (84)	67 ±10	68 ± 13	131/77 ± 19/11	17.6 ± 3.6	baPWV, h	
Pivin et al. (2015)	Population, 1001	475 (47)	47 ± 17	66 ± 11	117/75 ± 16/10	7.9 ± 2.1	cfPWV, t	
Sheng et al. (2015)	Men, 709	709 (100)	62 ± 11	75 ± 10	135/79 ± 19/11	11.9 ± 3.2	cfPWV, c	
	Women, 971	0 (0)	61 ± 11	76 ± 10	129/75 ± 18/10	11.3 ± 2.9	cfPWV, c	

Data presented as mean ± standard deviation unless specified. HR, heart rate; BP, blood pressure; PWV, pulse wave velocity; cfPWV, carotid-femoral PWV; baPWV, brachial-ankle PWV; hfPWV, heart femoral PWV; suffix c, cuff-based; suffix t, tonometry-based; suffix h, Hagesawa method (Hasegawa, 1970); suffix d, Doppler; NT, normotensive; HT, hypertensive; DM, diabetes mellitus; T2DM, Type II DM; HC, hypercholesterolaemia

2.3.4 Heart rate, arterial pressure and arterial stiffness

Experimental and epidemiological evidence suggests that heart rate and arterial stiffness both appear have an inseparable relationship with arterial pressure. In acute studies on the effects of heart rate on PWV, an increase in heart rate was often accompanied by an increase in blood pressure, which by implication contributed to the increase in PWV due to the intrinsic pressure-dependency of arterial wall stiffness. As shown in numerous epidemiological studies, elevated heart rate and increased arterial stiffness are both independently associated with hypertension. Results from existing studies are inconclusive regarding the effect of heart rate on arterial stiffness due to both conflicting results in acute studies, and the lack of consensus in epidemiological studies. As such, two questions remain:

1. In the presence of blood pressure changes, can changes in heart rate further contribute to changes in arterial stiffness beyond that of blood pressure; and

2. Could there exist a synergistic effect between heart rate, arterial stiffness and hypertension, such that the presence of both heart rate and arterial stiffness would have a significant prognosis on hypertension?

The possibility of a synergistic effect of the presence of both increased heart rate and arterial stiffening was shown by Benetos et al. (2002), whereby individuals with an elevated HR of > 80 bpm showed a markedly more pronounced increase in PWV compared to those with a heart rate of < 60 bpm in a 6-year longitudinal study. Furthermore, in the study by Ong et al. (2011), it was shown that in subjects treated with antihypertensives, regardless of drug class, the change in heart rate over the study period (1 - 6 months) was significantly associated with the change in PWV even after adjustment for the change in pressure, whereby the change in heart rate explained 2% of the variance, suggesting that heart rate exerts influence on PWV beyond that of blood pressure.

In light of the findings from existing literature, there still remains the need to pursue and improve on the existing understanding of the relationship between heart rate and arterial stiffness. This thesis aimed to determine the effects of heart rate on arterial stiffness beyond that of blood pressure, firstly through controlling for blood pressure pharmacologically in an animal model (Chapter 3), then through determining the heart rate dependency of PWV in humans after correcting for blood pressure effects using three different methods (Chapter 4). The effects of artificial cardiac pacing, which is a commonly used method for inducing heart rate changes in acute studies, on PWV was also investigated (Chapter 5). Finally, in order to determine possible mechanisms by which heart rate exerts an influence on arterial wall stiffness, a transmission line model of the human arterial tree (Xiao et al., 2016) was used to simulate increases in heart rate and different frequency dependency of the elastic modulus (Chapter 6).

Chapter 3 (pp. of this thesis ha ee removed as t contain published material under copyright. Removed contents published as:

Isabella Tan, Mark Butlin, Ying Yi Liu, Keith Ng, & Alberto P. Avolio (2012), Heart Rate Dependence of Aortic Pulse Wave Velocity at Different Arterial Pressures in Rats. *Hypertension*, vol. 60, no. 2, pp. 528-533. https://doi.org/10.1161/HYPERTENSIONAHA.112.194225

CHAPTER 3 Heart Rate Dependency of Aortic Pulse Wave Velocity at Different Mean Arterial Pressures in Rats

This following chapter presents the study on the effects of heart rate on aortic stiffness at different blood pressures in the rat, with contents based on the publication:

> **Tan I**, Butlin M, Liu YY, Ng K, Avolio AP. Heart rate dependence of aortic pulse wave velocity at different arterial pressures in rats. *Hypertension.* 2012;60(2):528-533.

Author contributions for the published manuscript were as follows:

- Study concept and design: **Tan**, Butlin, Avolio
- Acquisition of data: **Tan** (with guidance from Butlin, Liu, Ng)
- Analysis and interpretation of data: **Tan**
- Drafting of manuscript: **Tan**
- Critical revision: **Tan**, Butlin, Avolio, Liu, Ng

Chapter 4 (pp. 71-94) of this thesis has been removed as it contains published material under copyright. Removed contents published as:

Isabella Tan, Bart Spronck, Hosen Kiat, Edward Barin, Koen D. Reesink, Tammo Delhaas, Alberto P. Avolio, & Mark Butlin (2016), Heart Rate Dependency of Large Artery Stiffness. *Hypertension*, vol. 68, no. 1, pp. 236-242. https://doi.org/10.1161/HYPERTENSIONAHA.116.07462

CHAPTER 4 Heart rate dependency of large artery stiffness in humans

This following chapter presents the study on the effects of heart rate on large artery stiffness in humans, with contents based on the publication:

> **Tan I**, Spronck B, Kiat H, Barin E, Reesink KD, Delhaas T, Avolio AP, Butlin M. Heart Rate Dependency of Large Artery Stiffness. *Hypertension*. 2016;68(1):236-242.

Author contributions for the published manuscript were as follows:

- Study concept and design: **Tan**, Butlin, Avolio
- Acquisition of data: **Tan**, Butlin
- Analysis and interpretation of data: **Tan** (all analysis and interpretation), Spronck (developed advanced methods of blood pressure correction)
- Drafting of manuscript: **Tan** (all except: Spronck, parts of Methods, Discussion relating to advanced methods of blood pressure correction)
- Critical revision: **Tan**, Spronck, Kiat, Barin, Reesink, Delhaas, Avolio, Butlin

CHAPTER 5

Effects of pacing modality on non-invasive assessment of heart rate dependency of indices of large artery function

This following chapter presents the study on the influence of pacing modality in acute HR studies on indices of large artery function, with contents based on the publication:

> **Tan I**, Kiat H, Barin E, Butlin, M, Avolio AP. Effects of pacing modality on non-invasive assessment of heart rate dependency of indices of large artery function. *J Appl Physiol.* 2016:121:771-780.

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- Study concept and design: **Tan**, Butlin, Avolio
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- Analysis and interpretation of data: **Tan**
- Drafting of manuscript: **Tan**
- Critical revision: **Tan**, Kiat, Barin, Butlin, Avolio

Summary—Studies investigating the relationship between heart rate (HR) and arterial stiffness or wave reflections have commonly induced acute HR changes through *in situ* cardiac pacing, as have the animal and human studies presented in previous chapters of this thesis. Although pacing produces consistent HR changes, haemodynamics can vary with different pacing modalities. As such, further analysis was performed on a subset of the study cohort presented in Chapter 4 in order to investigate the influence of pacing modality on the HR relationship with arterial stiffness and wave reflection indices. Subjects were paced at 60, 70, 80, 90 and 100 bpm under atrial (n=14), atrioventricular (n=21) or ventricular pacing (n=13). At each paced heart rate, brachial cuff-based pulse wave analysis was used to determine central haemodynamic parameters, including ejection duration (ED) and augmentation index (AIx). Wave separation analysis was used to determine wave reflection magnitude (RM) and reflection index (RI). Arterial stiffness was assessed by carotid-femoral pulse wave velocity (cfPWV). Pacing modality was found to have significant effects on the HR relationship with ED (P=0.01), central aortic pulse pressure (P=0.01), augmentation pressure (P<0.0001), and magnitudes of both forward and reflected waves (P=0.05 and P=0.003, respectively), but not cfPWV (P=0.57) or AIx (P=0.38). However, at a fixed HR, significant differences in pulse pressure amplification (P<0.001), AIx (P<0.0001), RM (P=0.03) and RI (P=0.03) were observed with different pacing modalities. These results demonstrate that, whilst the HR relationships with arterial stiffness and wave reflection, as measured by cfPWV and AIx, were unaffected by pacing modality, it should still be taken into account for studies where mixed pacing modalities are present, in particular for wave reflection studies.

5.1 Introduction

Arterial stiffness and arterial wave reflections have both been shown to be independent predictors and risk factors for all-cause and cardiovascular events and mortality (Chirinos et al., 2012; Laurent et al., 2001; London et al., 2001; Nürnberger et al., 2002). Measures of arterial stiffness, such as pulse wave velocity (PWV), and arterial wave reflections, such as augmentation index (AIx), are now readily used in research, and there is an increased interest to include such indices in routine clinical procedures due to their prognostic value (Mancia et al., 2007, 2013). As such, large population studies have been conducted in order to establish reference values for these indices (Herbert et al., 2014; Janner et al., 2010; Reference Values for Arterial Stiffness'

Collaboration, 2010). Although blood pressure (BP) is associated with both PWV (Avolio et al., 1983; Bramwell and Hill, 1922a; Kim et al., 2007; Pruett et al., 1988) and AIx (van Trijp et al., 2004; Wilkinson et al., 2001), the effect of heart rate (HR), another haemodynamic parameter that can be highly variable between and within individuals, is less well-established. Whilst AIx has been shown to decrease linearly with HR in both cross-sectional (Janner et al., 2010; Koskela et al., 2013; Mitchell et al., 2004) and acute studies (Stefanadis et al., 1998; Wilkinson et al., 2000, 2002), a consensus has yet to be reached on the relationship between PWV and HR, particularly due to concomitant changes in BP with HR observed in most acute studies (Albaladejo et al., 2001; Liang et al., 1999; Millasseau et al., 2005), as previously discussed.

Earlier investigations on the acute effects of HR on PWV or AIx commonly induced changes in HR by way of cardiac pacing (Albaladejo et al., 2001, 2003b; Haesler et al., 2004; Lantelme et al., 2002b; Millasseau et al., 2005; Stefanadis et al., 1998; Wilkinson et al., 2000, 2002), as was the study presented earlier in Chapter 4. Where the study cohort consisted of subjects with *in situ* cardiac pacemakers, subjects could be paced from the right atrium (atrial pacing, Ap) (Albaladejo et al., 2001; Haesler et al., 2004; Lantelme et al., 2002b; Wilkinson et al., 2000), right ventricle (ventricular pacing, Vp) (Albaladejo et al., 2003b; Lantelme et al., 2002b; Millasseau et al., 2005; Stefanadis et al., 1998), or both the right atrium and the right ventricle (sequential atrioventricular pacing, ApVp) (Albaladejo et al., 2003b; Millasseau et al., 2005; Wilkinson et al., 2000). Although haemodynamic consequences of pacing from different chambers in the heart have been well investigated (Fujiyama et al., 1984; Geddes and Wessale, 1991; Wessale et al., 1990; Whiting et al., 1983), whether or not these consequences affect the HR relationship with arterial stiffness or arterial wave reflections have not been studied in detail. Past studies have found temporal changes relating to HR, such as ejection duration (ED), had a stronger association with the resultant changes in PWV (Salvi and Parati, 2013) and AIx (Sharman et al., 2009) than HR itself. Furthermore, increased HR can lead to changes in left ventricular ejection, which can affect the forward travelling pressure wave (Westerhof et al., 1972) and in turn lead to changes in

AIx (Torjesen et al., 2014). Since pacing at different cardiac sites have been shown to influence ED (Buch, 1987; Ferro et al., 1980; Whiting et al., 1983) and left ventricular function (Rosenqvist et al., 1991), conclusions drawn from pacing studies regarding HR effects on arterial stiffness and wave reflections may need to take into account the pacing modalities that were undertaken. For example, Ap typically results in a greater ED than ApVp or Vp for the same HR (Buch, 1987; Ferro et al., 1980; Whiting et al., 1983), theoretically resulting in a later occurrence of the systolic peak, with greater augmentation due the reflected wave, and, depending on the timing of the peak in that wave, a higher AIx. Furthermore, Ap is also associated with a greater cardiac output (CO) than ApVp or Vp for the same HR (Leclercq et al., 1995). For the same conditions in the vasculature, this would result in a higher pulse pressure (PP) and an increase in AIx. In order to investigate whether different pacing modalities would impact on the relationship between HR and indices of arterial stiffness and arterial wave reflections, further analysis was performed on the data obtained from the study presented in Chapter 4.

5.2 Methods

As described in Section 4.1, a total of 52 subjects entered the study. However, 4 subjects were excluded from this additional analysis due to biventricular pacing (n=2) or suboptimal electrocardiogram (ECG) recording (n=2), with the latter resulting in the inability to identify their pacing modality at the time of measurement. Overall, 48 subjects (age 40-93, mean 78±10 (mean±SD) years, 9 female) were included in the analysis.

5.2.1 Pulse Wave Analysis

Brachial and central aortic BP were obtained as previously described (Section 4.1.1). In addition, augmented pressure (AP) of the central aortic BP, augmentation index (AIx), time of return for

the reflected wave (Tr) and ED were determined from the same brachial cuff-based pulse wave analysis (SphymoCor XCEL, AtCor, Sydney, Australia). AIx was defined as the ratio of AP to the central aortic PP, expressed as a percentage. PP amplification (PPA) was expressed as the ratio of brachial PP to central aortic PP. ED was defined as the duration between end diastolic time to the incisura, and T_r was defined as the time from end diastole to the inflection point (Figure 5.1).

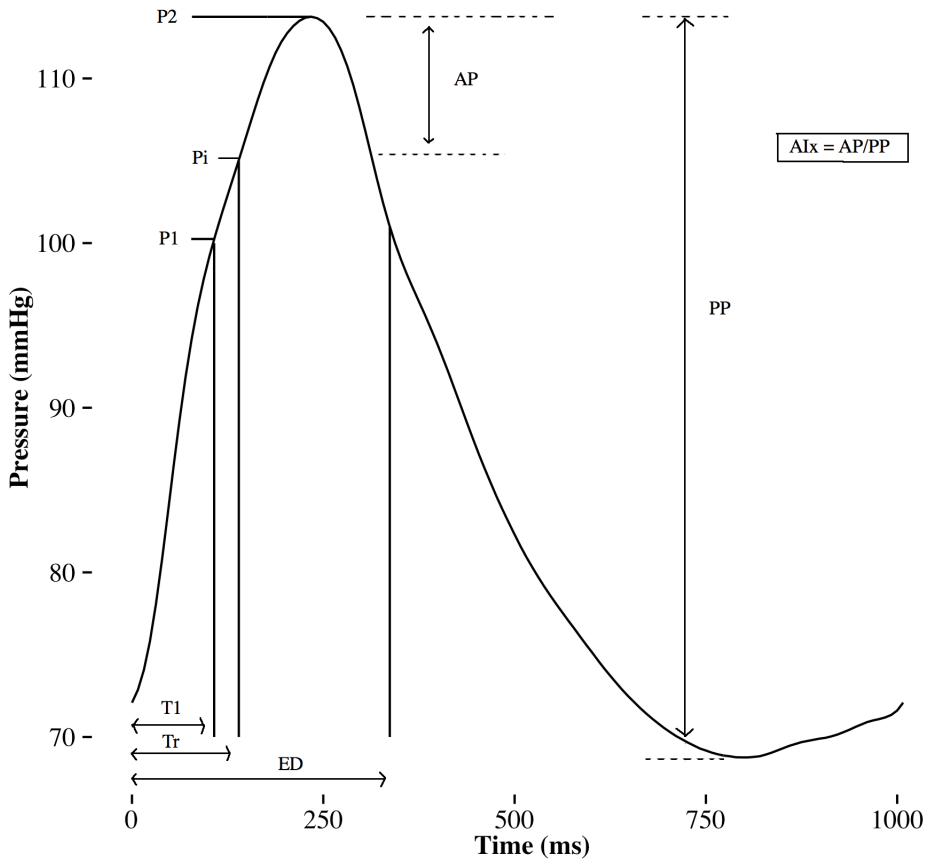


Figure 5.1: Parameters obtained using pulse wave analysis. P1 is the first systolic shoulder on the central aortic waveform, which occurs at the time of peak blood flow velocity T_1 (Nichols et al., 2011), and P2 is the systolic peak; P_i is the pressure at the first inflection point, which denotes the initial upstroke of the reflected pressure wave, occurring at time T_r (Nichols et al., 2011); augmentation pressure (AP) is defined as $P2 - P_i$, and AIx is defined as the ratio of AP and pulse pressure (PP); ejection duration (ED) is defined as the duration between end diastolic time to the incisura.

5.2.2 Arterial Stiffness and Wave Reflection Indices

Arterial stiffness, as assessed by means of carotid-femoral PWV (cfPWV), was obtained as previously described (Section 4.1.1). In addition to the wave reflection related parameters (AIx, Tr and PPA) determined from pulse wave analysis, the magnitude of wave reflection was also investigated. Magnitudes of the forward (P_f) and backward pressure (P_b) waves, reflection magnitude (RM) and reflection index (RI) were determined with wave separation analysis using the triangulation method as described by Westerhof et al. (2006) and equations as described by Murgo et al. (1981). Briefly, aortic blood flow was approximated using a triangle, with the peak set at the first systolic shoulder (T_1, Figure 5.1) of the derived aortic pressure. The start and end of the triangle were set at the end diastolic time and the time at incisura, respectively. As calibration of the flow wave is not required (Wessale et al., 1990), the peak of the triangle was set at unity. The forward (P_f) and backward pressure (P_b) waves were then calculated accordingly using the following equations:

$$P_f(t) = \frac{[P_m(t) + Z_c \cdot Q(t)]}{2} \tag{5.1}$$

$$P_b(t) = \frac{[P_m(t) - Z_c \cdot Q(t)]}{2} \tag{5.2}$$

where $P_m(t)$ was the averaged central aortic pressure waveform, $Q(t)$ was the constructed triangular blood flow, and Z_c was the characteristic impedance. Z_c was derived as the average of the 4th to 7th harmonic of the input impedance, which in turn was determined from the Fourier transform of $P_m(t)$ and $Q(t)$. A smoothing function was applied to the inverse of the modulus of $Q(t)$'s Fourier transform in order to remove extreme peaks in the harmonics resulting from the sharp peak in the constructed flow wave. Reflection magnitude (RM) was defined as a ratio of

the magnitudes (peak - trough) of the forward and reflected pressure waves, and allows for an estimation on the amount of wave reflection (71). Reflection index (RI) is defined as the ratio of the magnitudes of the reflected and measured pressure waves. In this study, RM and RI were calculated with the following equations:

$$\mathrm{RM} = \frac{|\mathrm{P_b}|}{|\mathrm{P_f}|} \tag{5.3}$$

$$\mathrm{RI} = \frac{|\mathrm{P_b}|}{|\mathrm{P_f}| + |\mathrm{P_b}|} \tag{5.4}$$

5.2.3 Study Protocol

Study protocol was as previously described in Section 4.1.2. Briefly, after 10 minutes of seated rest, seated brachial BP was measured in duplicate (SphygmoCor XCEL). After a further 10 minute supine rest, SV, CO and TPR measurements were derived from the finger arterial pressure waveform (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands), and brachial and central aortic BP, as well as cfPWV were thereafter obtained (SphygmoCor XCEL). Subjects were then paced in a randomised sequence of 60, 70, 80, 90 and 100 bpm, with measurements repeated at each pacing step. In most instances, only the pacemaker's pacing rate setting was changed, and the prescribed pacemaker algorithm would adjust the atrioventricular (AV) delay accordingly. However, in some patients the AV delay had to be changed to ensure that pacing was triggered. ECG was also acquired continuously for the duration of the study for monitoring of HR (PowerLab acquisition system, LabChart software, ADInstruments, Dunedin, New Zealand), and SV, CO and TPR from the Finometer PRO device were also recorded via PowerLab and LabChart. The average duration for study protocol completion was 60 minutes.

5.2.4 Data Analysis

Brachial and central aortic BP waveforms were averaged over 5 seconds, and cfPWV was averaged over 10 seconds. SV and TPR values were averaged across 10 cardiac cycles, and CO was calculated as the product of SV and HR, where HR was determined from the ECG. The pacing modality at each pacing step was identified from the ECG (see Figure 5.2). Observations with measured HR differing from the paced rate by more than 5 bpm were excluded from the analysis. Pacing rates of 60 and 70 bpm were not achievable in some subjects due to a higher unpaced resting heart rate. Optimal finger arterial pressure waveform, and consequently SV, TPR and CO measurements, could not be obtained in 5 subjects. For subjects who had pacemakers in DDD mode (dual chamber sensed and dual chamber paced), some were under Ap at the lower HRs but switched to ApVp at the higher HRs. Only data from a single pacing modality were included for analysis for these subjects.

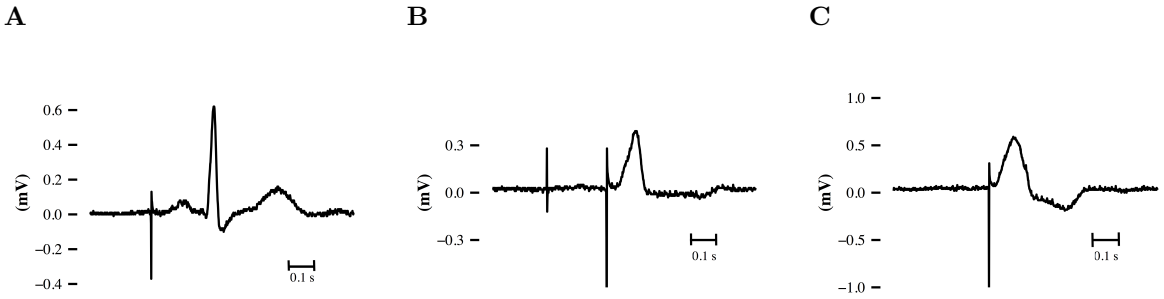


Figure 5.2: Identification of pacing modality from ECG. (A) Atrial pacing (Ap); (B) Atrioventricular pacing (ApVp); (C) Ventricular pacing (Vp).

5.2.5 Statistical analysis

A linear mixed model with maximum likelihood estimation was fitted to all measured parameters, with HR, pacing modality and their interaction term modelled as fixed effects, and each subject's individual intercepts modelled as random effects (Equation 5.5):

$$Y_{ij} = \beta_0 + \beta_1 \cdot \mathrm{HR} + \beta_2 \cdot \mathrm{PM} + \beta_3 \cdot \mathrm{HR} \cdot \mathrm{PM} + \varepsilon_{ij} + u_j \tag{5.5}$$

where HR is the paced HR at each pacing step (modelled as a continuous predictor), PM is the pacing modality (modelled as a categorical predictor), and HR·PM is the interaction term; ε_{ij} denotes the residual of variances and u_j denotes the random effect due to individual subject variances, i.e. variance of the random intercepts. Treatment contrasts were used for pacing modality comparisons, with Ap being the reference group that ApVp and Vp were compared against, thus resulting in the following model (Equation 5.6):

$$\begin{aligned} Y_{ij} =& \beta_0 + \beta_1 \cdot \mathrm{HR} + \beta_2 \cdot \mathrm{ApVp} + \beta_3 \cdot \mathrm{Vp} + \\ & \beta_4 \cdot \mathrm{HR} \cdot \mathrm{ApVp} + \beta_5 \cdot \mathrm{HR} \cdot \mathrm{Vp} + \varepsilon_{ij} + u_j \end{aligned} \tag{5.6}$$

where β_0 denotes the intercept of the HR trajectory for the Ap group; β_1 denotes the estimated effect of HR for the Ap group; β_2 and β_3 denote the difference in intercepts between Ap and ApVp, and Ap and Vp groups, respectively; β_4 and β_5 denote the differences in estimated HR effect between Ap and ApVp, and Ap and Vp groups, respectively. Thus, the estimated HR effect for ApVp can be calculated as $\beta_1 + \beta_4$, and the estimated HR effect for Vp can be calculated as $\beta_1 + \beta_5$. The correlation between repeated measures from the same subject is accounted for via the random effect. For parameters where the HR·PM interaction was not significant, the model was refitted without the interaction term to determine the main effect of pacing modality, where β_1 denotes the overall average effect of HR; β_2 and β_3 denote the differences in the measured parameter, as compared to Ap, at a fixed HR with ApVp and Vp, respectively. One-way analysis of variance (ANOVA) was used to compare differences in baseline haemodynamics across pacing modalities, and post-hoc Student's t-tests with Bonferroni correction were performed as appropriate. Categorical variables were compared by Pearson's Chi-squared tests. Descriptive statistics are presented as mean±standard deviation (SD) unless otherwise stated, and model estimates are presented as mean [95% confidence intervals (CI)]. A P value of less than 0.05 was considered as statistically significant. Data analysis was performed using the software R (R Core Team, 2014) and mixed modeling was performed using R's nlme

package (Pinheiro et al., 2014).

5.3 Results

Subject clinical characteristics as grouped by pacing modality are outlined in Table 5.1. At baseline, all but 4 subjects were actively paced, and there were no significant haemodynamic differences amongst the three pacing modality groups except for AP and AIx (Table 5.2). Some subjects in the ApVp group were under Ap at baseline due to their pacemakers being in DDD mode.

Table 5.1: Clinical characteristics of the study cohort grouped by pacing modality.

Parameters	Ap	ApVp	Vp	*P*
n	14	21	13	-
Male (Female)	9 (5)	17 (4)	13 (0)	0.06
Age, yrs	78 ± 6	77 ± 12	80 ± 8	0.70
Height, m	1.69 ± 0.1	1.72 ± 0.1	1.73 ± 0.8	0.32
Weight, kg	69 ± 12	79 ± 15	82 ± 13	0.05
Implant Indications, *n*				
SSS	5	3	1	0.14
Bradycardia	8	2	6	0.01
Irregular HR	1	2	1	0.97
Heart Block	0	7	3	0.06
Syncope	1	0	1	0.44
Atrial Fibrillation	2	0	9	<0.001
Ventricular Tachycardia	0	1	0	0.52
Cardiomyopathy	1	2	0	0.53
Other	1	3	1	0.74
Pacemaker Mode, *n*				
AAI/DDD	3	3	0	0.23
DDD	11	18	1	<0.001
VVI	0	0	11	<0.001
VVD	0	0	1	0.25
Medications, *n*				
α-blocker	1	2	0	0.53
β-blocker	8	8	6	0.54
Calcium antagonists	3	6	1	0.35
Nitrates	0	2	3	0.14
ACE-inhibitors	2	6	4	0.54
AngII-blockers	3	7	5	0.61
Diuretic	1	4	6	0.05
Antiarrhythmics	6	4	4	0.31
Anticoagulants	7	5	10	0.01
Antiplatelets	2	4	3	0.84
Statins	7	13	8	0.75
Aspirin	2	7	0	0.05

SSS, sick sinus syndrome; AAI, atrial pacing and sensing; DDD, dual chamber (atrium and ventricle) pacing and sensing; VVI, ventricular sensing and pacing; VVD, ventricular pacing and atrial tracking; ACE, angiotensin-converting enzyme; AngII, angiotensin II.

Table 5.2: Baseline haemodynamic measurements of the study cohort grouped by pacing modality.

Parameters	Ap	ApVp	Vp	*P*
HR, bpm	64 ± 6	63 ± 6	64 ± 10	0.70
Brachial SBP, mmHg	129 ± 15	126 ± 15	122 ± 15	0.51
Brachial DBP, mmHg	74 ± 11	71 ± 9	71 ± 3	0.55
Brachial PP, mmHg	55 ± 11	55 ± 13	52 ± 15	0.70
Aortic SBP, mmHg	119 ± 13	115 ± 13	111 ± 12	0.25
Aortic DBP, mmHg	74 ± 10	72 ± 9	71 ± 3	0.70
Aortic PP, mmHg	45 ± 7	43 ± 10	39 ± 12	0.32
MAP, mmHg	91 ± 11	87 ± 10	85 ± 5	0.30
AP, mmHg	16 ± 6	13 ± 4	9 ± 5*	0.004
AIx, %	36 ± 12	30 ± 6	24 ± 8*	0.004
cfPWV, m/s	9.0 ± 1.6	9.8 ± 1.5	10.0 ± 1.6	0.21

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; AP, augmentation pressure; AIx, augmentation index; cfPWV, carotid-femoral pulse wave velocity. * $P<0.01$ compared to Ap

The estimated values for the effect of HR on measured haemodynamic parameters for each pacing modality group are shown in Table 5.3. Significant differences in the HR trajectories between pacing modality groups were observed for ED ($\chi^2(6)=8.63$, $P=0.01$), central aortic PP ($\chi^2(6)=9.20$, $P=0.01$), AP ($\chi^2(6)=22.02$, $P<0.0001$), $|P_f|$ ($\chi^2(6)=6.05$, $P=0.05$), $|P_b|$ ($\chi^2(6)=11.99$, $P=0.003$) and TPR ($\chi^2(6)=7.67$, $P=0.02$) (Figure 5.3), but not for other haemodynamic measures (Table 5.3). Pacing modality was shown to have a main effect on PPA ($\chi^2(6)=14.73$, $P<0.001$), AIx ($\chi^2(6)=19.31$, $P=0.0001$), RM ($\chi^2(6)=7.33$, $P=0.03$) and RI ($\chi^2(6)=7.10$, $P=0.03$), with contrasts showing the Ap group having lower PPA, but higher AIx, RM and RI than both ApVp and Vp groups at constant HR (Table 5.4). No significant differences in other haemodynamic parameters were observed (Table 5.4).

5.4 Discussion

This is the first study to demonstrate the relevance of pacing modality when analyzing data in acute HR studies that employ cardiac pacing for inducing HR changes, in particular for investigations on wave reflection characteristics.

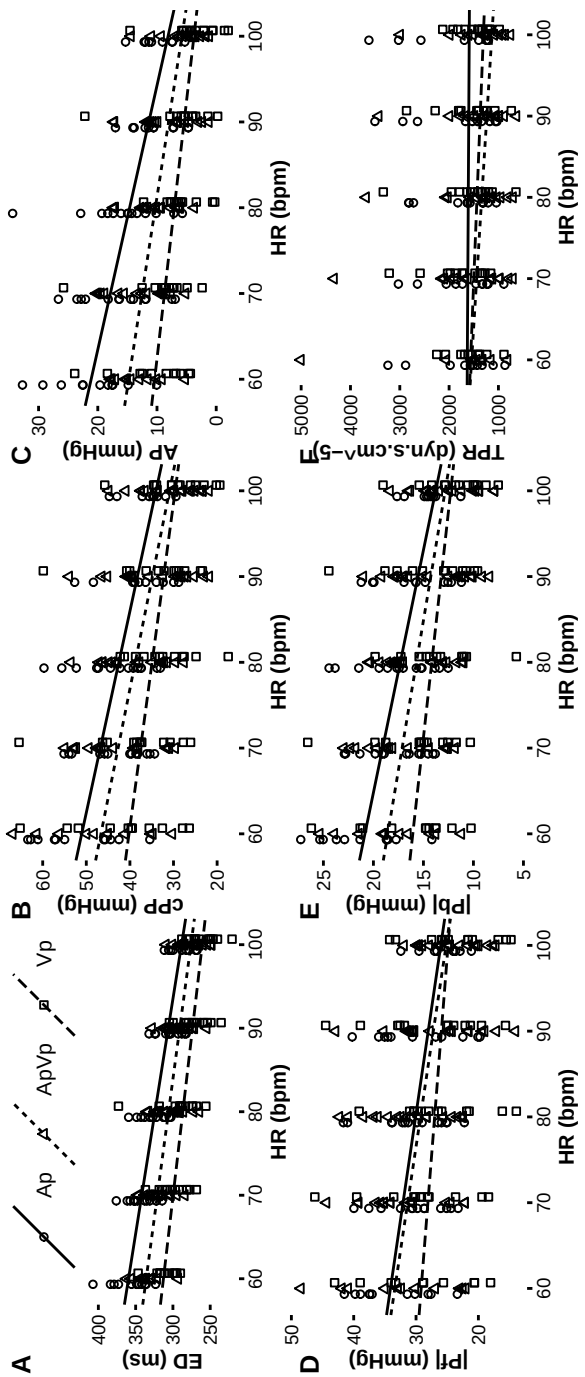


Figure 5.3: Scatter plots for parameters that had significantly different HR trajectories with different pacing modalities. (A) Ejection duration (ED); (B) Central aortic pulse pressure (cPP); (C) Central aortic augmentation pressure (AP); (D) Magnitude of forward pressure wave ($|P_f|$); (E) Magnitude of backward reflected wave ($|P_b|$); (F) Total peripheral resistance (TPR). Values of the slopes can be found in Table 5.3

Table 5.3: Estimated values for effect of HR per 10 bpm increase on measured haemodynamic parameters for each pacing modality group

	Ap		ApVp	Vp
Parameters	Estimate	P^1	Estimate[2]	Estimate[2]
cfPWV, m/s	0.4 [0.3,0.5]	<0.0001	0.3 [0.2,0.4]	0.4 [0.3, 0.5]
T_r, ms	-2.1 [-2.8,-1.4]	<0.0001	-3.2 [-3.7,-2.6]	-2.8 [-3.5,-2.2]
AP, mmHg	-3.3 [-3.7,-2.8]	<0.0001	-2.3 [-2.6,-1.9]†	-1.7 [-2.1,-1.2]‡
AIx, %	-4.0 [-4.8,-3.2]	<0.0001	-3.6 [-4.3,-2.9]	-3.2 [-4.0,-2.4]
$\lvert P_f \rvert$, mmHg	-2.0 [-2.7,-1.3]	<0.0001	-2.1 [-2.6,-1.5]	-1.1 [-1.7,-0.5]
$\lvert P_b \rvert$, mmHg	-1.8 [-2.1,-1.4]	<0.0001	-1.4 [-1.7,-1.2]	-0.9 [-1.3,-0.6]‡
RM, x10^{-3}	-1.79 [-2.55,-1.04]	<0.0001	-1.33 [-1.94,-0.71]	-1.32 [-2.00,-0.65]
RI, x10^{-3}	-0.72 [-1.04,-0.40]	<0.0001	-0.57 [-0.83,-0.31]	-0.57 [-0.86,-0.28]
ED, ms	-17.8 [-20.1,-15.4]	<0.0001	-15.0 [-16.9,-13.1]	-13.0 [-15.1,-10.9]†
SV, ml	-6.9 [-8.4,-3.3]	<0.0001	-5.7 [-7.1,-4.3]	-5.1 [-6.5,-3.8]
CO, L/min	0.1 [0.0,0.2]	0.05	0.3 [0.2,0.4]	0.2 [0.1,0.3]
TPR, dyn.s.cm^{-5}	9.9[-39.2,59.0]	0.70	-83.0 [-125.4,-40.5]†	-45.1 [-86.7,-3.5]
bSBP, mmHg	1.0 [0.1,2.0]	0.04	0.4 [-0.4,1.1]	1.3 [0.4,2.1]
bDBP, mmHg	4.1 [3.3,4.9]	<0.0001	3.5 [2.9,4.2]	3.1 [2.4,3.8]
bPP, mmHg	-3.1 [-4.1,-2.0]	<0.0001	-3.2 [-4.0,-2.3]	-1.9 [-2.8,-0.9]
cSBP, mmHg	0.1 [-0.8,0.9]	0.89	-0.1 [-0.8,0.6]	0.5 [-0.2,1.3]
cDBP, mmHg	4.4 [3.6,5.2]	<0.0001	3.8 [3.1,4.5]	3.2 [2.4,3.9]
cPP, mmHg	-4.3 [-5.1,-3.4]	<0.0001	-3.9 [-4.6,-3.3]	-2.7 [-3.4,-1.9]†
bPP:cPP	0.06 [0.05,0,07]	<0.0001	0.06 [0.05,0.07]	0.05 [0.04,0.06]
MAP, mmHg	4.1 [3.3,4.9]	<0.0001	3.6 [2.9,4.2]	3.3 [2.6,4.0]

HR, heart rate; cfPWV, carotid-femoral pulse wave velocity; Tr, time of arrival of reflected wave; AP, augmentation pressure; AIx, augmentation index; $\lvert P_f \rvert$, magnitude of forward pressure wave; $\lvert P_b \rvert$, magnitude of backward reflected pressure wave; RM, reflection magnitude; RI, reflection index; ED, ejection duration; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; bSBP and bDBP, brachial systolic and diastolic blood pressure, respectively; cSBP and cDBP, carotid systolic and diastolic blood pressure, respectively; bPP and cPP, brachial and central aortic pulse pressure, respectively, MAP, mean arterial pressure. Significant differences in HR trajectories as compared to Ap denoted by † $P < 0.01$ when compared to Ap; ‡ $P < 0.001$ when compared to Ap.

[1] P value indicates statistical significance of β_1, the estimated HR effect for Ap.

[2] Estimates calculated as $\beta_1 + \beta_4$ for ApVp, and $\beta_1 + \beta_5$ for Vp, from the coefficients of the fitted linear mixed model (see Equation 5.6)

Table 5.4: Estimated average differences in measured haemodynamic parameters between pacing modalities at a fixed HR level.

	ApVp vs. Ap		Vp vs. Ap	
Parameters	Estimates[1]	*P*	Estimates[1]	*P*
cfPWV, m/s	0.8 [-0.4,2.1]	0.19	0.8 [-0.6,2.3]	0.24
Tr, ms	-0.7 [-4.2,2.8]	0.69	-2.4 [-6.1,1.6]	0.24
AIx, %	-7.8 [-13.5,-2.2]	0.01	-14.6 [-20.9,-8.4]	<0.0001
P_f|, mmHg	-0.9 [-4.9,3.1]	0.65	-3.0 [-7.5,1.4]	0.18
RM	-0.04 [-0.08,-0.00]	0.03	-0.05 [-0.09,-0.01]	0.01
RI	-0.02 [-0.03,-0.00]	0.03	-0.02 [-0.04,-0.01]	0.01
SV,ml	8.9 [-5.6,23.3]	0.23	-1.0 [-16.3,14.4]	0.90
CO, L/min	0.7 [-0.4,1.9]	0.20	-0.1 [-1.3,1.1]	0.91
bSBP, mmHg	-3.8 [-14.9,7.3]	0.50	-11.8 [-24.1,0.6]	0.06
bDBP, mmHg	-0.7 [-7.1,5.8]	0.84	-5.0 [-12.2, 2.2]	0.17
bPP, mmHg	-3.2 [-9.9,3.6]	0.36	-6.7 [-14.3,0.8]	0.08
cSBP, mmHg	-4.6 [-14.5,5.3]	0.36	-13.0 [-24.0,-1.9]	0.02[2]
cDBP, mmHg	-0.7 [-7.2,5.8]	0.83	-5.3 [-12.6,1.9]	0.15
bPP:cPP	0.05 [0.01,0.09]	0.01	0.09 [0.04,0.13]	<0.001
MAP, mmHg	-2.8 [-10.6,5.1]	0.49	-9.4 [-18.2,-0.7]	0.04[2]

bPP, brachial pulse pressure; cPP, central aortic pulse pressure; AIx, augmentation index; RM, reflection magnitude; RI, reflection index. Estimates denote the difference in measured parameters as compared to Ap at a fixed HR. Negative values indicate a lower value than Ap.

[1]Estimates were denoted by β_2 for ApVp vs Ap, and by β_3 for Vp vs Ap from the fitted linear mixed model without the HR · PM interaction term in Equation 5.6.

[2]Although the contrasts were statistically significant, pacing modality did not improve the model with HR as the sole predictor ($\chi^2(6)$=5.41, P=0.07 for cSBP; $\chi^2(6)$=4.76, P=0.09 for MAP)

Previous studies investigating the effects of acute changes in HR on arterial stiffness and wave reflections have mostly employed cardiac pacing as the means to induce HR changes (Albaladejo et al., 2001, 2003b; Haesler et al., 2004; Lantelme et al., 2002b; Millasseau et al., 2005; Stefanadis et al., 1998). Unlike pharmacological or exercise induced heart changes that enact through systemic mechanisms such as the sympathetic and parasympathetic system, heart rate pacing is likely to have fewer confounding effects on the systemic circulation. What is unknown is whether different pacing modalities can lead to different changes in arterial stiffness or wave reflections in response to acute HR changes. In this analysis, it was shown that, whilst the HR relationship with cfPWV and AIx were not significantly different between pacing modalities, the changes in the HR relationship with ED, magnitudes of forward and reflected waves, and thus central aortic PP and AP were. Furthermore, at constant HR, there were significant differences observed between pacing modalities in PPA, AIx, RM and RI.

5.4.1 Pacing modality and arterial stiffness

With all three modes of pacing, cfPWV increased with increasing HR, with no significant differences in slope observed between the pacing modalities (Figure 5.4). Correspondingly, the time of arrival of the reflected wave, T_r, also decreased with decreasing HR. At constant HR, cfPWV was on average 0.8 ms/s lower with Ap than with ApVp and Vp (Table 5.4), though the differences did not reach statistical significance. The increase in cfPWV with increasing HR has been discussed in detail in Chapter 4. When compared at baseline and at the same heart rate, however, those with Ap had lower cfPWV but higher brachial and central aortic pressures than ApVp and Vp (Table 5.4). These differences were not statistically different, possibly due to the small sample size in each pacing modality group and thus there was not enough statistical power, but the trend was consistent with other studies that have shown Vp led to lower arterial pressures than Ap (Buch, 1987; Gold et al., 2000) and ApVp (Millasseau et al., 2005; Taylor et al., 1996). Furthermore, although there was no difference in the average cfPWV at constant HR between ApVp and Vp, an earlier study demonstrated the apparent paradoxical increase in cfPWV with a decrease in BP with Vp as compared to ApVp (Altun et al., 2004). As proposed by the investigators, a possible explanation to this observation could be the increased muscle sympathetic nerve activity (SNA) associated with Vp (Taylor et al., 1996) in response to lower BP due to lower SV (Altun et al., 2004), resulting in lower arterial distensibility. Although TPR, which has been shown to strongly correlate with muscle SNA (Charkoudian et al., 2005), was measured in this study, there was no significant association with pacing modality, thus it was not possible to ascertain whether SNA levels indeed differed between pacing groups. Furthermore, whilst there were negligible differences in TPR between the pacing groups at 60 bpm, the differences increased as HR increased, with TPR decreasing with HR in ApVp and Vp groups, but relatively unchanged with Ap.

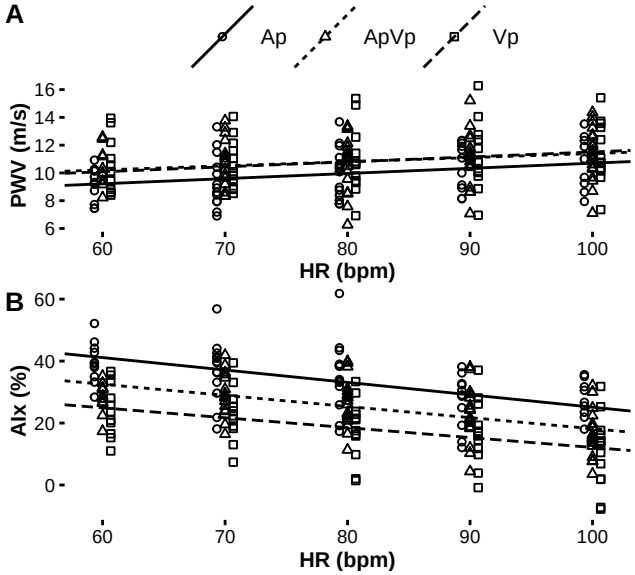


Figure 5.4: Scatter plots of (A) cfPWV and (B) central aortic AIx with HR for different pacing modalities.

5.4.2 Pacing modality and wave reflections

With increasing HR, diastolic filling time would be shortened, leading to a reduction in SV (Noble et al., 1966; Weissler et al., 1961) and a decrease in ED (Weissler et al., 1961), as observed at all pacing modalities in the present study. Due to the large increases in HR induced in this study, CO increased despite the decrease in SV. The decline in ED with HR was steeper with Ap than with ApVp and Vp, with the difference being significant between Vp and Ap. As with previous studies, when compared at the same HR, subjects in the Ap group had longer ED than those with ApVp (Whiting et al., 1983) and Vp (Buch, 1987; Ferro et al., 1980), with significant differences observed between Ap and Vp at all HRs, and between Ap and ApVp at lower HRs. Although ED has been shown to relate to SV (Reant et al., 2010; Weissler et al., 1968), and Vp has been shown to result in lower CO compared to Ap and ApVp due to disruption of ventricular activation sequence and atrioventricular synchrony (Leclercq et al., 1995), the differences in SV and CO between pacing modality groups did not reach statistical significance in the present study, whether at the same HR or with changes in HR. This may be due to variability in the measurements, as it was previously shown that Finapres' model flow method for determining CO

was not entirely reliable without invasive calibration (Remmen et al., 2002), thus comparison of absolute CO values determined from this method across individuals, and by implication SV, was not recommended.

The decrease in AP with HR in the present study followed a similar pattern to the decrease in ED at all three pacing modalities (Figure 5.3C), with the steepest decrease in those with Ap, followed by those with ApVp and Vp. Significant differences in the HR trajectories were observed with ApVp and Vp as compared to Ap. This was likely due to the reflected pressure wave augmenting the forward pressure wave during late systole to diastole as a result of reduced ED with HR (Avolio et al., 2009). Similarly, central aortic PP also decreased due to an increase in central aortic diastolic pressure with no change in systolic pressure (Figure 5.3B), leading to a decrease in AIx with HR at all pacing modalities (Figure 5.4B). This is consistent with other HR studies that have shown a linear decrease in AIx with HR (Stefanadis et al., 1998; Wilkinson et al., 2000, 2002). The decrease in AIx with HR, whilst slightly higher in Ap, then followed by ApVp and Vp (Figure 5.4B), was not significantly between the pacing modalities. As such, the HR relationship with AIx would not change when paced HR changes are achieved with a single pacing modality.

Although pacing modality did not impact on the relationship between HR and AIx, when compared at the same HR, AIx was significantly higher in the Ap group than both ApVp and Vp, as was ED. Moreover, the differences in ED between pacing modalities, in particular at lower HRs (at 60 bpm, ED difference was 23 ms between Ap and ApVp, and 46 ms between Ap and Vp), were much larger compared to the decrease in ED with HR (13 ms/10 bpm with Vp to 18 ms/10 bpm with Ap). With similar heart period and time of arrival of the reflected wave, a reduced ED would result in the reflected wave augmenting the forward wave in late systole to early diastole to a much larger extent than a change in both HR and ED. This resulted in a much larger decrease in AP and AIx. Figure 5.5 shows the result of altered forward and backward

wave interaction due to changes in ED with different pacing modalities at 60 and 100 bpm.

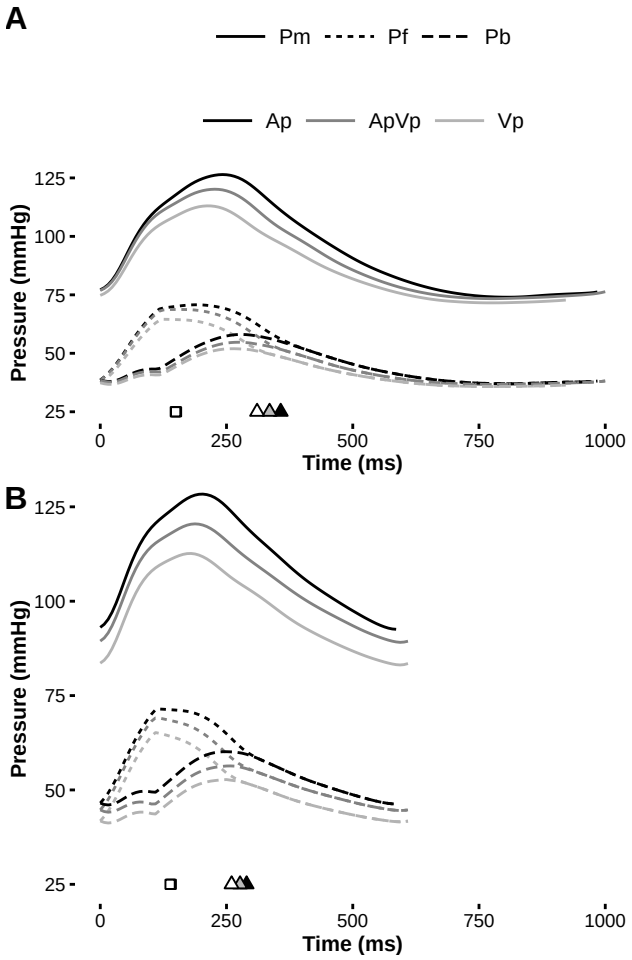


Figure 5.5: Averaged aortic waves with calculated forward and backward waves for the three pacing modalities at A. 60 bpm and B. 100 bpm. Due to the differences in ED, the backward waves augment the forward waves later in systole as ED decreased, resulting in a smaller AIx. Squares represent T_r, and triangles represent ED (black = Ap, grey = ApVp and white = Vp). T_r remained unchanged with all three pacing modalities but ED was significantly shorter in Vp compared to Ap

Given the large differences in ED and AIx between pacing modalities at the same HR, particularly at lower HRs, studies concerning the association between HR and AIx should either design the study with HR changes induced by a single pacing modality, or account for the different pacing modalities in their analysis. Previous studies have demonstrated that ED had a higher correlation with AIx than HR (Gatzka et al., 2001; Salvi and Parati, 2013; Sharman et al., 2009; Weber et al., 2007), and although fitting AIx with ED in place of HR did not improve the model in the present study (AIC=1330.17 with ED, AIC=1296.88 with HR), our results further support that the reduction in wave reflection with HR is related to the change in ED. This effect becomes

more evident with large changes in ED in the presence of relatively small changes in HR. It should be noted that the averaged change in AIx with HR across the three pacing modalities observed in this study was -3.6%/10 bpm, slightly lower than the commonly used correction for AIx at 75 bpm (-4.8%/10bpm), as implemented in the AtCor Medical SphygmoCor software. This correction was derived from two studies: one in subjects with *in situ* cardiac pacemakers (-3.9%/10 bpm), with both ApVp and Vp (Wilkinson et al., 2000), and another study in subjects temporarily paced with Ap (-5.6%/10 bpm) (Wilkinson et al., 2002). Although the difference in slopes did not reach statistical significance (Wilkinson et al., 2002), possibly due to the small sample size of both studies (n=22 and 20, respectively), this nonetheless demonstrated that individuals with permanent pacing may exhibit different changes in AIx with HR as compared to those without, indicating that the relationship can be highly variable and a uniform correction cannot be applied to all populations. As such, it has been suggested that a more appropriate method for correcting AIx for HR would be to include HR as an independent predictor or covariate (Stoner et al., 2014). Furthermore, it has previously been shown that the correlation between AIx and HR was stronger in those with greater cfPWV (Papaioannou et al., 2008), but the trend observed in the present study was that those with greater cfPWV, those with Vp, showed smaller changes in AIx with HR.

Reduced ED would result in an increase in central to peripheral PP amplification (London and Pannier, 2010; Wilkinson et al., 2000) as shown in our results with increased HR and Vp. This is also consistent with the reduction in wave reflection due to a shift in the timing of the reflected wave relative to the forward wave (Avolio et al., 2009). However, our results showed that the reduction in wave reflection with the increase in HR is not solely due to a change in timing, but also in the magnitudes of the forward and reflected pressure waves. Both $|P_f|$ and $|P_b|$ decreased with HR at all pacing modalities (Figures 5.3D and 5.3E), and may also have contributed to the decrease in AIx with HR (Torjesen et al., 2014). Similar to AIx, both RM and RI decreased with increasing HR regardless of pacing modality. Magnitudes of the forward and

reflected waves are not affected by confounding effects of timing (Westerhof et al., 2006), thus the decrease in RM and RI with HR may be a frequency-related effect, since RM is comparable to the frequency-dependent reflection coefficient (Westerhof et al., 1972). Furthermore, when compared at the same HR, despite there being no significant differences in $|P_f|$, and minimal significant differences in $|P_b|$, the magnitude ratios were significantly higher with Ap than with ApVp and Vp, meaning wave reflection was significantly reduced in the latter groups. These observations may in part be due to the different spectral pattern of cardiac frequency harmonics arising from the differences in ED, and in part due to possible attenuation of the reflected waves resulting from decreased impedance mismatch (Davies et al., 2012), which, in the present study, may have resulted from the trend of increased cfPWV with ApVp and Vp. In a recent study by Parragh et al. (2015), subjects with reduced ejection fraction (EF) were shown to have significantly lower RM compared to subjects with normal EF (Parragh et al., 2015). However, statistical significance was lost after correction for HR and ED, indicating that RM was likely influenced by both these parameters, as observed in our study. Reduced wave reflection due to impedance matching can result in transmission of excessive pulsatile energy to the microvasculature, which can be especially damaging in low impedance organs such as the brain and kidney (Mitchell et al., 2005).

Whilst it was not the purpose of this study to investigate the functional source of the waveform features leading to the parameters AIx and T_r, throughout this thesis these parameters have been discussed as parameters associated with wave reflections occurring at sites of discontinuities in impedance, based on the conventional understanding of systemic haemodynamics (Nichols et al., 2011). However, a recent theoretical proposal involving the empirical fitting and subtracting of a pressure component named the reservoir pressure results in a model of the arterial system that is largely free from wave reflection (Wang et al., 2003). This theory has been applied to the understanding of the coupling of AIx and left ventricular dynamics, with AIx reducing with dobutamine induced stress, but not associated with wave reflection as evaluated using the

reservoir pressure based analysis (Sharman et al., 2009). However, the various permutations of the proposed reservoir pressure, a theoretically zero dimensional phenomena, behaves as a wave itself, posing a conceptual anomaly in the theory (Alastruey, 2010; Mynard, 2013; Mynard et al., 2012; Westerhof et al., 2015). The fitted reservoir pressure also has no correlation with intra-aortic volume (Segers et al., 2015), confounding the intrinsic assumption behind the theory. Given the current fundamental and practical problems that form the basis of the reservoir pressure theory, reviewed elsewhere more thoroughly (Mynard and Smolich, 2014; Mynard et al., 2015), the conventional understanding of AIx and T_r as parameters associated with wave propagation have been used in this thesis.

The present study has several limitations. Firstly, this study was a post hoc analysis of the data obtained from the study presented in Chapter 4. As such, no a priori power analysis was performed, and statistical power may have been insufficient to detect true differences in the HR trajectories between pacing modality groups. However, significant differences in the relationship between ED and HR were still detected. Furthermore, consistent trends in the data warrant further investigation in a larger cohort of individuals with different pacing modalities, and the present study can provide data estimates required for power analysis in future studies. When compared at a fixed HR and using the data obtained from the present study, it was determined that a sample size of 150 would be required to detect a moderate effect of pacing modality ($f = 0.25$) on cfPWV with a statistical power of 80% at an α level of 0.05 when using a one-way ANOVA test. On the contrary, a total sample size of 42 would be sufficient to detect a large effect of pacing modality on AIx ($f = 0.50$), and thus there was sufficient power in the present study to detect significant differences in AIx with different pacing modalities.

As previously mentioned in Chapter 4, the cohort in this study consisted mainly of elderly male subjects, thus age and gender differences of haemodynamic and arterial responses to acute HR changes could not be investigated. In addition, subjects were heterogeneous in their cardiac

function and indication for pacemaker implantation, and the influence of potentially clinical pertinent factors, such as left ventricular ejection fraction and diastolic dysfunction, were not stratified in the study. Indeed, it has previously been shown that AIx is higher in females (McEniery et al., 2005a; Mitchell et al., 2004; Segers et al., 2007), and thus could contribute to the results observed in the present study where Vp resulted in the lowest AIx, Vp not having any females in the group. However, an analysis excluding females still showed a significant difference in AIx between Ap and both ApVp and Vp groups for the average HR (Ap vs ApVp, -8.2 [-15.0,-1.4], P=0.02; Ap vs Vp, -15.4 [-22.5,-8.4], P=0.0001). Furthermore, all patients with Vp presented with atrial fibrillation (AF), which can significantly impact haemodynamics (Samet, 1973) and has been shown to be correlated with higher PWV (Lee et al., 2008). Thus, it cannot be discounted that AF may be a contributing factor to the Vp group differences. As such, conclusions drawn from this study cannot be directly extrapolated to the general population nor beyond the studied HR range. Furthermore, responses to acute changes in HR by means of cardiac pacing may not be reflective of long-term changes caused by tachycardia.

Thirdly, wave separation analysis for determination of wave reflection parameters were based on an estimate of the flow waveform using a triangular wave shape, rather than with a measured flow wave. However, this method of estimation has been shown to correlate highly with parameters derived from direct measurements of pressure and flow (Westerhof et al., 2006).

Changes in haemodynamics and wave reflection parameters in response to HR changes for each pacing group may not be representative of the long term effects of cardiac pacing with different pacemaker modes. In this study cohort, subjects in the Ap and ApVp groups had their pacemaker modes set to either AAI/DDD (AAI = atrial sensed, atrial paced pacemaker) or DDD, and proprietary algorithms were implemented in the pacemakers to reduce occurrence of Vp. Some subjects who had Ap at baseline switched to ApVp at higher HRs, thus changes observed in this study may be a combination of both long-term effects of pacing as well as acute effects

dependent on the cardiac site(s) being paced. AV delays were not taken into account for subjects with DDD, which has been shown to affect haemodynamic responses to pacing, in particular BP (Whinnett et al., 2006a,b). Although long term Vp in the right ventricular apex is known to result in increased incidence of myocardial perfusion and dysfunction (Tse and Lau, 1997), whether these adverse effects contributed to the increasing trend of arterial stiffness and reduced wave reflection as observed in the present study could not be determined. However, differences in various parameters already observed at baseline may indicate, at least in part, long term effects of different modalities of cardiac pacing.

Finally, factors other than ED that could influence wave reflections were not investigated. Thus, whilst the changes in wave reflections observed in the present study were consistent with reduced ED, there may be other haemodynamic differences between pacing modalities that influenced the wave reflection parameters.

5.5 Conclusions

In conclusion, the present study demonstrates that pacing from different cardiac sites exhibited varying effects on central aortic haemodynamics, arterial stiffness and wave reflection with acute changes in HR. Whilst the changes with HR in cfPWV and AIx, the common indices for large artery stiffness and wave reflections, respectively, were unaffected by pacing modality, it significantly influenced wave reflection indices at a fixed HR level. The main driving factor behind differences in wave reflection parameters between pacing modality groups can be attributed to differences in ED. Thus, further studies that employ cardiac pacing as means to induce HR changes should either design the study to induce HR changes with a single pacing modality, or account for it in the analysis. In particular, for wave reflection studies where large changes in ED are present, correction for wave reflection parameters may need to be made for ED rather than HR. Although cfPWV was lower in subjects with Ap compared to those with Vp at the

same HR, the differences did not reach statistical significance, presumably due to the lack of statistical power in the small sample size. Thus, further studies on the effects of pacing modality on the relationship between HR and cfPWV in larger cohorts would be needed.

CHAPTER 6

Heart rate dependency of arterial stiffness: underlying mechanisms related to frequency dependency of arterial elasticity

This chapter presents results of simulations conducted on the multi-branched transmission line model of the human systemic vasculature developed by Dr Hanguang (Simen) Xiao (Xiao et al., 2016), who developed the transmission line model (TLM) used in this investigation and implemented the different investigative scenarios.

Summary—The earlier studies presented in this thesis provided further evidence that heart rate does indeed have an independent effect on arterial stiffness. However, possible mechanisms behind heart rate (HR) dependency of arterial stiffness are as yet unknown. To explore a plausible mechanism by which heart rate exerts an influence on arterial stiffness, a computerised transmission line model of the human arterial tree was utilised to simulate effects of HR on heart to femoral pulse wave velocity (hfPWV) with elasticity of the arterial segments modelled with varying degrees of frequency dependence. hfPWV was calculated in a similar manner to that used for PWV measurement in humans and compared to the PWV calculated from the model using propagation characteristics. In the scenarios where the arterial wall elasticity had low to zero frequency dependence, hfPWV was shown to decrease with HR. However, as the degree of frequency dependence increased, an increase in hfPWV with increasing HR could be observed. The critical frequency, defined as the frequency where arterial wall elasticity reaches 80% of the static elastic modulus and above which HR could be shown to positively influence hfPWV, was around 3 Hz. The change in hfPWV with increasing HR plateaued at around 0.06 m/s per 10 bpm increase in HR as the degree of frequency dependence was increased to above 9 Hz. As such, a frequency dependency of arterial wall elasticity can be a part mechanism through which PWV changes with changing HR. Further physiological studies are required to confirm these results.

6.1 Introduction

It has been shown in the studies presented in previous chapters of this thesis that the stiffness of the arterial wall, in both rats and humans, indeed exhibits an intrinsic dependency on heart rate. However, possible mechanisms behind heart rate (HR) dependency of arterial stiffness have not been fully investigated. To date, there have only been a few *ex vivo* studies demonstrating the frequency dependency of arterial stiffness in canine arteries (Bergel, 1960, 1961a) and in human arterial specimens (Learoyd and Taylor, 1966). Furthermore, whilst viscoelasticity of the arterial wall has often been attributed as the contributing factor for the increased wall stiffness observed with increasing HR (Albaladejo et al., 2004; Antonov et al., 2008; Armentano et al., 1995; Lantelme et al., 2002b; Mangoni et al., 1996), where elevated HR would result in reduced time for the artery to recoil and thus become stiffer, experimental studies have yet to confirm this explanation.

In vivo studies on heart rate effects on arterial stiffness, particularly in humans, can be confounded by various physiological parameters such as blood pressure. Whilst an independent association between heart rate and arterial stiffness have been found through statistical means, mechanisms through which changes in heart rate lead to changes in stiffness cannot be determined. On the contrary, *ex vivo* studies, such as those performed by Bergel (1960, 1961a) and Callaghan et al. (1984), can specifically address the frequency aspects of arterial wall stiffness, but require extensive experimental setup and still do not permit control over every physiological element. Computerised models of the human arterial tree, such as those developed by Noordergraaf et al. (1963), Avolio (1980) and Stergiopulos et al. (1992), allow for manipulations of model elements to simulate arterial flow and pressure wave propagation (O'Rourke and Avolio, 1980), and have been widely used in literature in the investigations of haemodynamics in both physiological and pathological conditions (Karamanoglu et al., 1995; O'Rourke and Avolio, 1980; Stergiopulos et al., 1992; Taylor and Draney, 2004). As such, a computerised multibranched transmission line model (TLM) of the human arterial tree (Xiao et al., 2016) was used in the present study to simulate the effects of heart rate changes on arterial stiffness as measured by pulse wave velocity (PWV). Based on the findings of Bergel (1960, 1961a), it was hypothesised that varying frequency dependency of the arterial wall's elasticity would result in varying degrees of HR dependency of PWV.

6.2 Methods

The model used in the present study was a 55 segment multibranched TLM developed by Xiao et al. (2016), based upon the model by Stergiopulos et al. (1992). Each arterial segment was modelled as a uniform thin-walled cylindrical tube with varying radii and lengths, with internal inertial, viscous and elastic properties (Avolio, 1980). Values for arterial dimensions and elastic constants were based on the study by He et al. (2012). With arterial flow wave as input, arterial

pressure and flow waveforms could be generated in each modelled arterial segment, and wave propagation was modelled using Womersley's principles on fluid flow in elastic tubes (Womersley, 1957). This involved the calculations of various impedances, PWV (using Moens-Korteweg equation, Equation 2.13), propagation constant and reflection coefficient for each individual segment (Xiao et al., 2016), which then allow for pressure waveforms to be generated (He et al., 2012). Transit times between blood pressure waveforms generated at different arterial segments, and subsequently regional PWV, can then be determined in a similar manner to that used for *in vivo* PWV measurements in humans. This measured PWV was then compared with the calculated PWV from the model using propagation characteristics.

6.2.1 Modelling different frequency dependency of the arterial wall's elasticity

The static elastic modulus (E_{static}) of each arterial wall segment in the TLM was fixed at a particular value according to arterial type (He et al., 2012). As such, stiffness of the arterial wall was not dependent on blood pressure. To simulate varying degrees of frequency dependency of the arterial wall's elastic modulus (E) between 0 and 20 Hz, one constant (E_0) and five exponential functions (E_1, E_2, E_3, E_4, E_5) were generated in the following general form:

$$y = -e^{-cx} + 1 \tag{6.1}$$

where c was a constant of 0, 0.1, 0.5, 1, 2 and 5, respectively, and x was a value between 0 and 3, with intervals determined by the number of frequency harmonics resulting from the Fourier transform of the blood flow and pressure waveforms. y values were normalised to be between 0.5 and 1 to produce the final functions (Figure 6.1), which essentially denoted the ratio of the

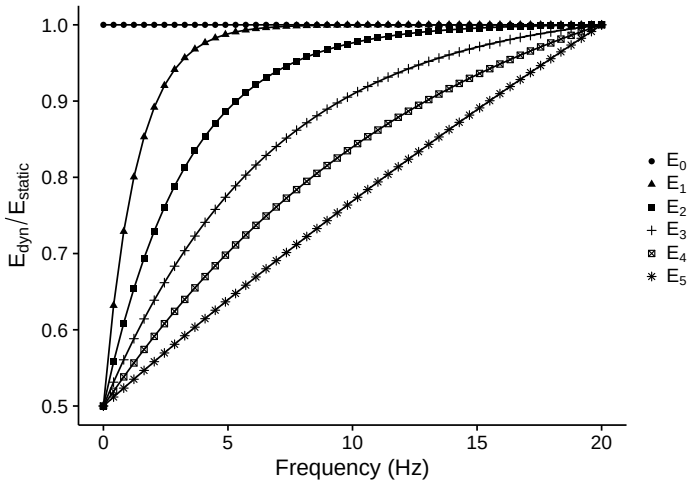


Figure 6.1: Relative elastic modulus ($E_{\text{dyn}}/E_{\text{static}}$) with varying frequency dependence as simulated in the transmission line model

dynamic elastic modulus (E_{dyn}) to E_{static} at different frequencies:

$$E_{\text{dyn}} = E_{\text{static}} \cdot E_n \tag{6.2}$$

It should be noted that the viscosity term of the dynamic elastic modulus (Equation 2.9) was not included in this calculation, as it has previously been shown that the term remains relatively constant with increasing frequency, and is small relative to E_{dyn} (Bergel, 1961a).

6.2.2 Simulation of the effects of heart rate on pulse wave velocity

Different HRs were simulated in the TLM as inputs of different flow waveforms (Figure 6.2), where heart period (HP, s), diastolic time (DT, s) and left ventricular ejection time (LVET, s) were adjusted to HR accordingly:

$$\text{HP} = 1/\text{HR} \tag{6.3}$$

$$\text{LVET} = -0.0017 \cdot \text{HR} + 0.413 \tag{6.4}$$

$$\text{DT} = \text{HP} - \text{LVET} \tag{6.5}$$

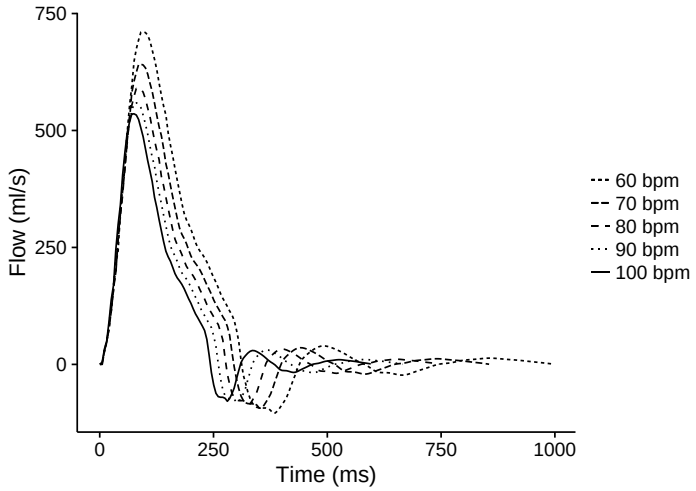


Figure 6.2: Flow wave input to the transmission line model at different heart rates

where LVET was adjusted according to the relationship between LVET and HR as previously determined by Weissler et al. (1968). Furthermore, the input flow waves were scaled to ensure consistent mean arterial pressures and peripheral resistance throughout the arterial tree at all HRs.

Heart to femoral PWV (hfPWV) was determined from the pressure pulse propagation from the ascending aortic segment to the femoral arterial segment with two methods. Firstly, foot to foot transit time (t_{hf}, ms) of the arterial pulse waveform generated at the ascending aorta and at the femoral artery, respectively, was determined. Foot of the arterial pulse was obtained using the intersecting tangent method (Chiu et al., 1991), and arterial path length (l) from the ascending aorta and femoral artery was calculated as the total length of each arterial segment. Foot to foot hfPWV ($hfPWV_{foot\text{-}foot}$, m/s) was thus calculated as:

$$hfPWV_{foot-foot} = l/t_{hf} \tag{6.6}$$

Secondly, hfPWV was calculated from the phase velocity ($hfPWV_{phase\text{-}v}$, m/s) in the high frequency harmonics, determined from the Fourier analysis of the pressure waveforms between the two arterial segments (Nichols et al., 2011).

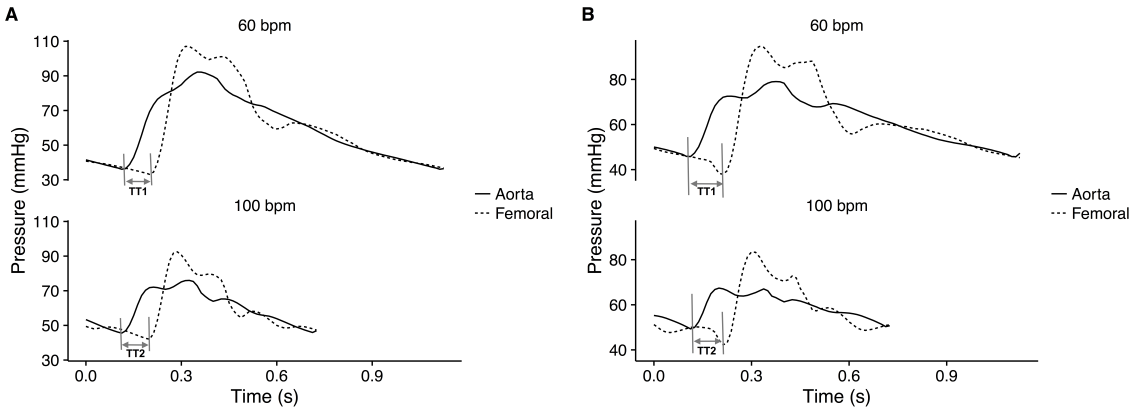


Figure 6.3: Aortic and femoral blood pressure waveforms generated at 60 bpm and 100 bpm for (A) static elastic modulus (E_0) and (B) dynamic elastic modulus with the highest frequency dependence (E_5). TT1: transit time at 60 bpm; TT2: transit time at 100 bpm. Note that for E_0, TT2 > TT1, but for E_5, TT2 < TT1.

At each static and dynamic elastic modulus, hfPWV was determined at HRs of 60 to 100 bpm, in 5 bpm increments, resulting in 54 simulation scenarios in total. Figure 6.3 shows an example of the resulting pressure waveforms generated at the ascending aorta and femoral artery segments at E_0 (i.e. E_{static}) and E_5 (i.e. elastic modulus with the highest frequency dependence).

6.2.3 Data analysis

Linear regression analysis with least-squares was performed on HR and hfPWV data to determine whether or not a significant HR dependency of hfPWV was observed at different arterial elasticities. Multiple linear regression was used to determine the difference in HR dependencies of hfPWV between different methods of PWV determination. Data analysis was performed using the software R (R Core Team, 2014) and $P<0.05$ was considered statistically significant. Data presented as mean [95% CI].

6.3 Results

At static elastic modulus and at a dynamic elastic modulus with low dependence on frequency, hfPWV decreased with increasing HR; but as frequency dependency of dynamic elastic modulus

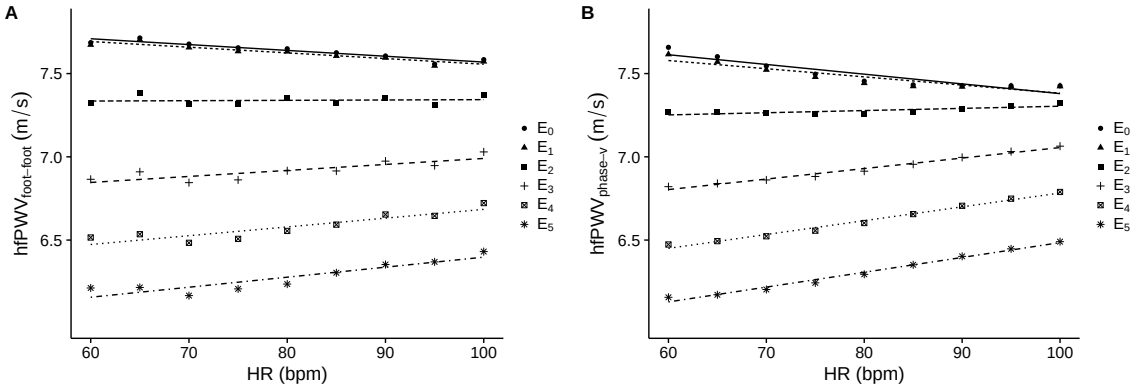


Figure 6.4: Relationship between PWV and HR at varying frequency dependency of arterial wall elasticity, as simulated by the TLM. (A) hfPWV measured with the foot-to-foot method; (B) hfPWV determined from phase velocity.

increased, an increase in hfPWV was observed with increasing HR (Figure 6.4). Although the same trend was observed regardless of whether hfPWV was calculated with the foot-to-foot method or with phase velocity, the resulting HR dependency of hfPWV differed significantly between the two methods (Table 6.1). Frequency dependency of the elastic modulus was denoted by the frequency at which E_n reached 80% of the magnitude of E_{static} (critical frequency, f(0.8)), and the higher the value, the higher the frequency dependency of E_n (Figure 6.5).

Table 6.1: Comparisons of HR dependency of PWV between methods of PWV determination at different frequency dependency of elastic modulus.

		HR dependency of hfPWV (m/s/10 bpm)		
E_n	f(0.8) (Hz)	hfPWV$_{\text{foot-foot}}$	hfPWV$_{\text{phase-v}}$	P
E_0	0	-0.035 [-0.046, -0.024]‡	-0.058 [-0.080, -0.037]‡	0.039
E_1	1.2	-0.034 [-0.045, -0.023]‡	-0.049 [-0.068, -0.030]‡	0.128
E_2	3.3	0.002 [-0.015, 0.002]	0.013 [0.003, 0.023]*	0.209
E_3	5.7	0.036 [0.015, 0.057]†	0.063 [0.056, 0.071]‡	0.013
E_4	8.5	0.053 [0.030, 0.075]‡	0.083 [0.075,0.091]‡	0.010
E_5	11.4	0.060 [0.036, 0.085]‡	0.089 [0.080, 0.098]‡	0.020

f(0.8) denote the critical frequency at which E_n reached 80% of the magnitude of E_{static}; P values shown on table denote significance of difference in heart rate (HR) dependency of pulse wave velocity (PWV); for comparisons of HR dependency to zero dependency, *, $P<0.05$; †, $P<0.01$; ‡, $P<0.001$

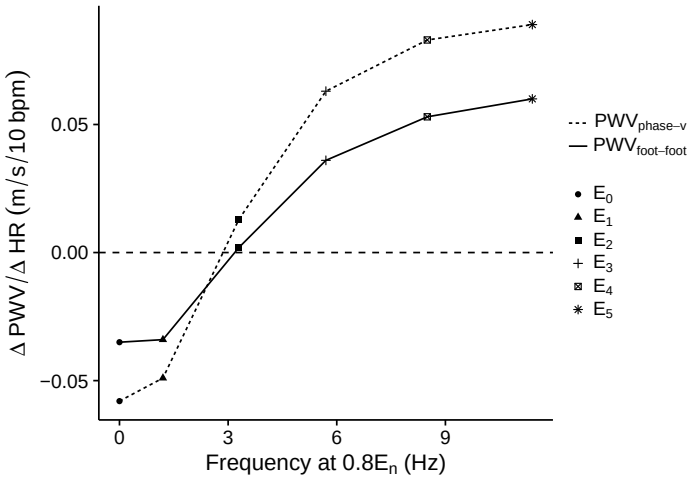


Figure 6.5: HR dependency of PWV at varying degrees of frequency dependency of E_n. Note that positive changes in PWV with increasing HR occurs at around 3.3 Hz.

6.4 Discussion

Using a multibranched TLM of the human arterial tree, the present study demonstrated that HR dependency of arterial stiffness could be explained, in principle, by the frequency dependency of arterial wall elasticity.

It has previously been shown in the elegant studies by Bergel (1960, 1961a) that in the canine artery, arterial wall elasticity was sensitive to frequency changes of up to about 2 Hz, after which arterial stiffness remained relatively constant. It was also shown in the same studies that the more muscular the arterial segment, the greater the change in stiffness with frequency, with the greatest change observed in the canine carotid artery. On the contrary, Callaghan et al. (1984), who also studied the canine carotid artery, observed that stiffness was independent of frequency. In the present study, a significant increase in arterial stiffness was only observed when the frequency dependency of the dynamic elastic modulus exceeded 3 Hz (Figure 6.5), as modelled by the functions E_2, E_3, E_4 and E_5. Whilst this may not reflect existing experimental observations, it is a proof of concept that demonstrated how changes in PWV with changing HR could be associated with the dynamic elasticity of the arterial wall.

The HRs simulated in this study were much lower than the critical frequency of 3 Hz (equivalent to a HR of 180 bpm). The observation that frequency dependency of hfPWV increased as the critical frequency increased may be due to the fact that the foot of the pulse wave is determined by the high frequency harmonics of the wave (Nichols et al., 2011). It has previously been argued that, since contributions of the high frequency components to the foot of the arterial pressure wave do not change with HR, and that there is no appreciable change in the arterial wall's dynamic elastic properties at high frequencies, theoretically a mere change in HR alone would not have an effect on PWV (Hayward et al., 2002). However, if, as shown with the simulation, the arterial wall's dynamic elastic modulus has substantial frequency dependence, then changes in PWV would be observed with changes in HR. Interestingly, the higher frequency components of the generated pressure waves at different E_n were slightly higher at a great frequency dependence than when compared to zero frequency dependence (Figure 6.6). Furthermore, the HR dependencies for each E_n were of similar order and followed the same trend regardless of whether hfPWV was calculated with the foot-to-foot method or from phase velocity (Figure 6.5), though the latter calculation produced significantly greater change in hfPWV with a change in HR. This could be due to the shape of the generated pressure waveforms (Figure 6.3), where the feet of the waves may be too rounded and thus making precise location of the foot difficult.

The HR dependencies of hfPWV observed with the simulation data (0.02 m/s to 0.06 m/s per 10 bpm increase in HR when PWV was measured using the foot-to-foot method) were more than one third lower than the intrinsic heart rate dependency of cfPWV (0.17 m/s/10 bpm) as determined from the human study presented in Chapter 4 of this thesis. As such, frequency dependency of the arterial wall elasticity may explain up to 30% of the change in cfPWV with HR. Furthermore, it was observed that heart rate dependency of hfPWV was negative at static and low frequency dependency of the dynamic elastic modulus, and positive changes in hfPWV with increasing HR was only observed when the frequency dependence extended beyond 3 Hz (Figure 6.5). This suggests that, if different arterial segments in the arterial tree have wall

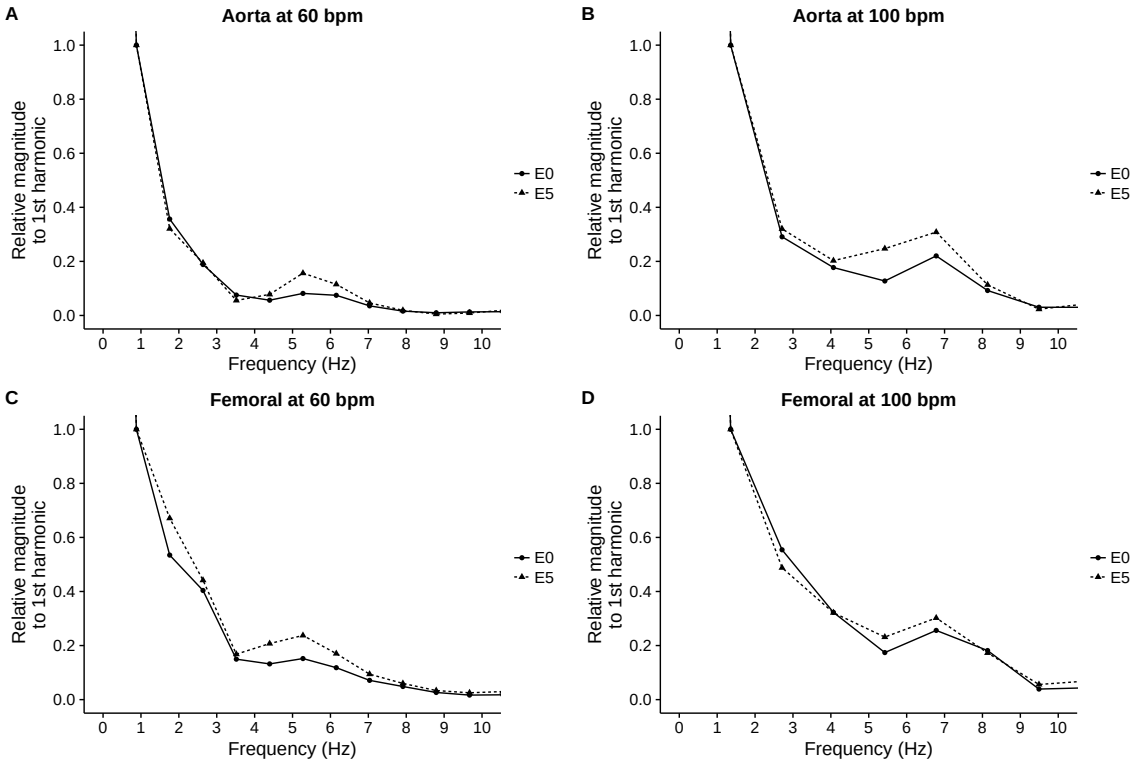


Figure 6.6: Frequency spectrums of generated aortic pressure waves at E_0 (no frequency dependence) and E_5 (maximum frequency dependence) at different heart rates.

elasticity with varying degrees of frequency dependence, measured HR dependency of PWV over an arterial path that consists of more than one arterial segment would be the total effect of the various frequency dependencies. Thus, if the elasticity of one or more arterial segments does not have a high frequency dependence, the positive effect of HR could be diminished or even cancelled out. This could partially be the reason why existing studies, many conducted in a variety of subjects of different ages and gender, have failed to converge in their findings on the effects of HR on arterial stiffness.

There are several limitations in the present study. Firstly, the simulation of the varying frequency dependencies of arterial wall elasticity was not based on real experimental data, and thus can only serve as a proof of concept for a possible mechanism through which HR effects on arterial stiffness could be observed. Future experimental studies will be required to determine whether or not the arterial wall elasticity exhibits a frequency dependent, and if so, to what extent the dependence is. Secondly, the dynamic elastic modulus was modelled to be lower than the static elastic modulus,

which is contrary to the observations made by other investigators (Bergel, 1960, 1961a; Learoyd and Taylor, 1966). However, this would likely only have affected the absolute magnitude of hfPWV, but not the relative changes in hfPWV with HR. Thirdly, as aforementioned, hfPWV values calculated with the foot-to-foot method were significantly different to those obtained from phase velocities, and this may be due to the shape of the generated pressure waveforms. Furthermore, the magnitudes of the generated pressure waveforms were much lower than normal physiological values. However, since pressure dependence of arterial stiffness was not implemented in this model, this would not have affected the relative changes in hfPWV calculations. Finally, whilst the static elastic modulus was different for different arterial types in the model (e.g. the femoral arterial segment having a higher E_{static} than the aortic segment) (He et al., 2012), the same dynamic elastic modulus was applied to all arterial segments. As shown by Bergel (1961a), different arterial types exhibit varying dynamic elastic properties, and thus further simulations should be performed with different arterial segments having varying dynamic wall elasticity.

6.5 Conclusions

In conclusion, the present study demonstrated a possible mechanism by which heart rate changes can exert an influence on arterial wall stiffness: the frequency dependency of arterial wall elasticity. The higher the frequency dependence, the greater the change in PWV with changing HR. Further physiological studies are needed to determine the degree of frequency dependence of the arterial wall's elastic modulus, and whether elasticity of different arterial segments in the arterial tree would have varying degrees of frequency dependence on HR.

CHAPTER 7

Non-invasive estimation of systolic time intervals: application of relationships between heart rate, pulse wave velocity and blood pressure

All data presented in this chapter were collected at Macquarie University by I. Tan, with the assistance from an experienced sonographer, Mr Kyle Tan, as part of a multi-centre study led by Dr Paolo Salvi from the Italian Institute for Auxology, Milan, Italy (Salvi et al., 2015). Whilst related to the multi-centre study, the analysis and results presented in this chapter, except where stated, were solely performed by I. Tan.

Summary—Systolic time intervals, which comprise the pre ejection period (PEP) and left ventricular ejection time (LVET), are important parameters in the assessment of left ventricular function. Whilst LVET can be non-invasively determined from pulse wave analysis of the arterial pressure waveform, non-invasive measurement of PEP requires the technical expertise, such as that of a sonographer for echocardiographic measurements, and is less readily available. This study aimed to determine the validity of estimating PEP using pulse wave analysis of the carotid pressure waveform and carotid-femoral pulse wave velocity (cfPWV) as compared to the measured PEP with echocardiography. This study also aimed to demonstrate the application of both heart rate and blood pressure dependency of cfPWV in studies with non-synchronous measurements of cardiovascular parameters. Simultaneous acquisition of the carotid pressure waveform with applanation tonometry and PEP measurement with echocardiography (PEP_{echo}) were performed in 29 subjects (aged 20 - 66, 12 female). cfPWV was measured separately and used to estimate the transit time from the aortic valve to the carotid measurement site (t_{hc}). PEP was estimated (PEP_{est}) as the the transit time from the Q wave of the ECG to the foot of the carotid subtracted by t_{hc}. cfPWV was then further adjusted for heart rate (HR) and mean arterial pressure (MAP). Finally, the ascending aorta was taken into account in arterial path length and cfPWV adjustments. All adjustments resulted in new estimates of t_{hc} and thus PEP. PEP_{est} showed a strong correlation with PEP_{echo} (118±17 ms vs 88±13 ms, R=0.89, P<0.0001). Adjustments for HR and MAP did not improve either the estimated PEP or the correlation, but consideration of the ascending aorta segment significantly improved the estimated PEP with similar correlation to PEP_{echo} (103±18, P<0.0001 compared to PEP_{est}; R=0.88 with P<0.0001 compared to PEP_{echo}). Thus, non-invasive pulse wave analysis, with appropriate adjustments to cfPWV and arterial path length, can be used to estimate PEP.

7.1 Introduction

A change in heart rate (HR) not only changes the overall cardiac cycle time, but also affects both systolic and diastolic durations. Systolic time intervals, which comprise pre-ejection period (PEP) and left ventricular ejection time (LVET), are important parameters for assessing left ventricular function (Ahmed et al., 1972; Weissler et al., 1968, 1969, 1961). In particular, PEP is a useful measure for myocardial contractility (Newlin and Levenson, 1979; Talley et al., 1971), and the ratio of PEP to LVET (PEP/LVET) is widely used in the diagnosis of heart disease such as heart failure (Ahmed et al., 1972; Reant et al., 2010; Weissler et al., 1969). Furthermore, it

has recently been suggested that inclusion of PEP's isovolumetric contraction time could improve the assessment of stroke work and myocardial oxygen demand and supply (Salvi et al., 2015). It is well established that LVET is strongly associated with HR (Weissler et al., 1968, 1961), but the relationship between PEP and HR is much weaker. Although the study by Weissler et al. (1968) found a signification association between PEP and HR by linear regression analysis in a cohort of 211 healthy individuals, studies in smaller cohorts did not always observe changes in PEP with changes in HR (Cokkinos et al., 1976; Ferro et al., 1980; Mertens et al., 1981).

Non-invasive pulse wave analysis of the arterial pressure pulse can determine LVET, but determination of PEP requires the measurement of the time of aortic valve opening, such as through the use of echocardiography (Hirschfeld et al., 1975), phonocardiography (Weissler et al., 1968) or impedance cardiography (Willemsen et al., 1996). Given that propagation velocity of the arterial pulse wave (pulse wave velocity, PWV) can be measured, and the distance between the heart and the carotid artery can be approximated by body surface measurement, propagation time from the aortic opening to the carotid artery can be estimated. As such, an estimation of PEP could be obtained by subtracting this propagation time from the time delay between start of left ventricular depolarisation, as determined from ECG, and the carotid pressure pulse of the corresponding cardiac cycle. This study aimed to determine the validity of estimating PEP using pulse wave analysis of the carotid pressure waveform and carotid-femoral pulse wave velocity (cfPWV) by comparing estimated PEP values to the PEP values measured by echocardiography. Furthermore, this study also aimed to demonstrate a possible application of the intrinsic HR dependency of PWV by correcting cfPWV for HR differences between the time of cfPWV and PEP measurements and determining whether estimation of PEP could be improved.

7.2 Methods

Healthy volunteers were recruited from Macquarie University's staff and student communities. Exclusion criteria included heart failure, cardiac arrhythmia, uncontrolled hypertension, kidney or liver disease, respiratory failure, and morbid obesity. A total of 29 subjects (aged 20 - 66, 12 female) entered the study. The study protocol was approved by Macquarie University's Human Research Ethics Committee and written consent to participate in the study was obtained from all volunteers.

7.2.1 Study Protocol

Participants first underwent an echocardiography examination, performed by an experienced sonographer using a Philips S5-1 transducer with a Philips iE33 ultrasound machine (Philips Medical Systems). During pulsed wave Doppler acquisition of the aortic outflow for systolic times measurement, simultaneous acquisition of the carotid pressure waveform was obtained by applanation tonometry on the skin above the right carotid artery with a high fidelity manometer (Millar SPT-301, Millar Instruments; Houston, Texas, USA) at a sampling rate of 1 kHz through the PowerLab acquisition system and software (PowerLab acquisition system, LabChart software, ADInstruments; Dunedin, New Zealand). Two sets of ECG signals were acquired in order to time-match the carotid pressure waveform to the aortic outflow waveform: one through the PowerLab system, and the other through the ultrasound system. ECG electrodes from both systems were placed at the same locations, adjacent to each other, to ensure acquisition of the same signal. A repeat measurement of 4 to 5 cardiac cycles was obtained, and supine brachial blood pressure was measured on the left arm immediately before or after carotid pulse waveform acquisition using a validated oscillometric device (MicroLife BP A100 Plus, MicroLife AG; Widnau, Switzerland).

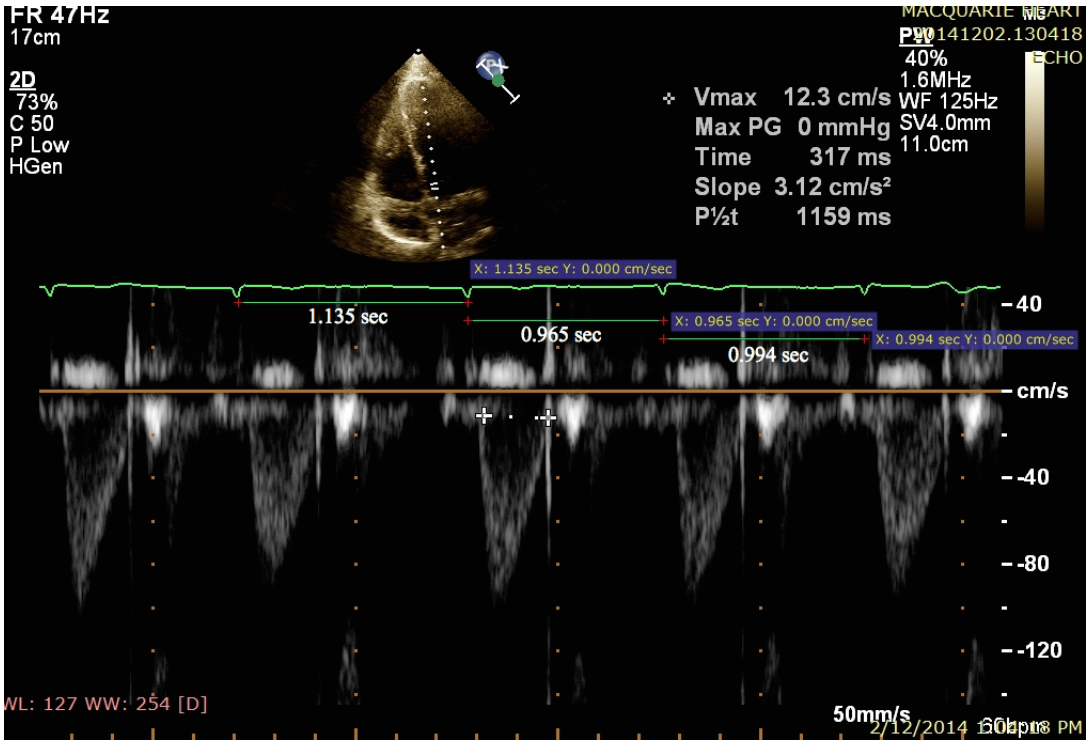
At the conclusion of the echocardiography examination, subjects were asked to move to another room for PWV measurement. Subjects then rested in the supine position for 5-10 minutes, after which their brachial blood pressure was obtained on the left arm (MicroLife BP A100 Plus). For measurement of cfPWV, carotid and femoral pressure waveforms were obtained simultaneously using the SphygmoCor XCEL system (AtCor, Sydney, Australia) as previously described in Chapter 4. cfPWV was measured in duplicate, with brachial blood pressure again obtained at the time of PWV measurement, and the average of the two measurements was used in the analysis.

7.2.2 Data analysis

In order to match the cardiac cycles on the carotid pressure waveform to the aortic outflow waveform, consecutive R-R intervals were measured from the ECG signal acquired with PowerLab using LabChart software's ECG analysis module (ADInstruments, Dunedin, New Zealand), as well as the ECG signal from the ultrasound DICOM image file using the RadiAnt software (Mexiant, Poznan, Poland) for each subject (Figure 7.1). The data were then passed to the investigators at the Italian Institute of Auxology for verification and further analysis to obtain the following for each subject: 1) PEP obtained from echocardiography (PEP_{echo}); 2) interval between the Q wave of the ECG to the foot of the carotid pressure pulse (Figure 7.2). Values were averaged over the range of 2 to 4 matched consecutive cardiac cycles. 3 subjects were subsequently excluded from the analysis due to the lack of matched R-R intervals between the two ECG measurements. As a result, only 26 subjects were included in the present analysis.

Various estimations of PEP were then obtained using an established relationship between HR and PEP (Weissler et al., 1968) and using pulse wave analysis of the carotid pressure waveform as described below.

A



B

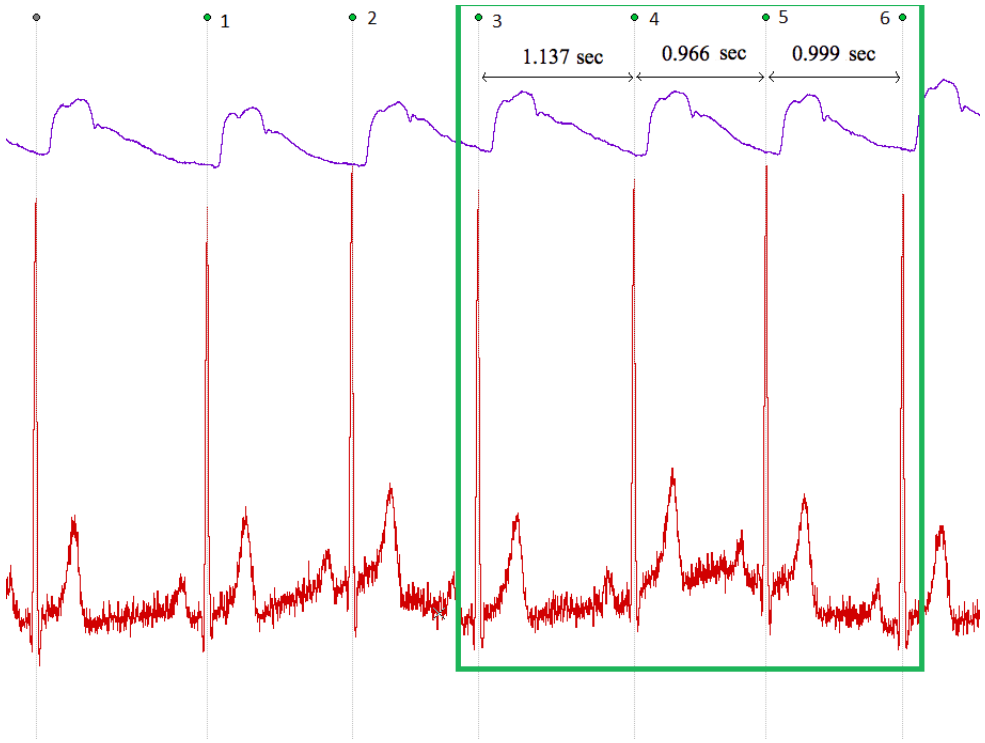


Figure 7.1: Example of RR intervals match between ECG signals measured during echocardiography (A) and applanation tonometry (B). The RR intervals measured in (A) were 1.135 s, 0.965 s, and 0.994 s, respectively; the RR intervals measured in (B) were 1.137 s, 0.966 s, and 0.999 s, respectively.

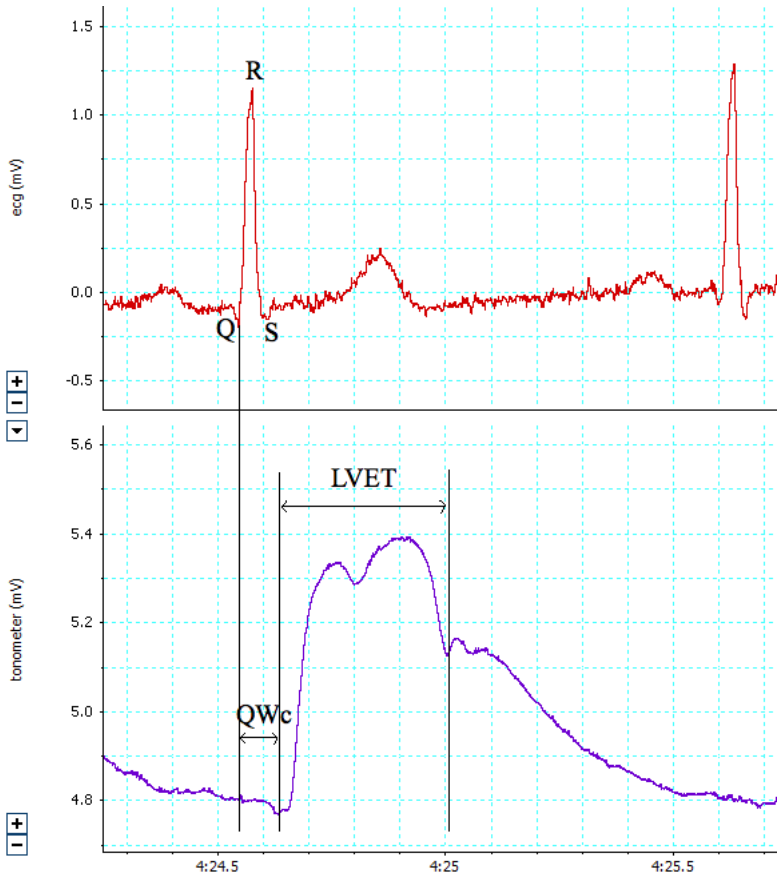


Figure 7.2: Measurements of left ventricular ejection time (LVET) and transit time from Q wave of the ECG signal to foot of the carotid pressure pulse (QW_c). QW_c is equivalent to the sum of the pre ejection period (PEP) and transit time from the aortic valve to the carotid artery (t_{hc}) (Eq 7.3).

1. Estimation of PEP using a previously established relationship between PEP and HR

As a first estimation, PEP (in ms) was calculated using the regression relationship between PEP and HR as established by Weissler et al. (1968):

$$\mathrm{PEP}_{\mathrm{Weissler}} = -0.4 \cdot \mathrm{HR}_{\mathrm{echo}} + 131 \tag{7.1}$$

where $\mathrm{HR}_{\mathrm{echo}}$ denotes the HR (in bpm) at the time of echocardiographic PEP measurement.

2. Estimation of PEP by carotid pulse wave analysis

Due to the additional propagation time travelled by the pressure pulse wave from the aortic opening to the carotid artery (t_{hc}), PEP cannot simply be taken as the interval between the Q wave of the ECG, which represents the depolarisation of the left ventricle, to the foot of the carotid pressure pulse (t_{QWc}). Assuming that the arterial pressure pulse travels at the same velocity from the aortic opening to the carotid artery as that measured by cfPWV (in m/s), an estimation of $_{hc}$ (in ms) was obtained by:

$$t_{hc} = \frac{d_{sc}}{cfPWV} \tag{7.2}$$

where d_{sc} denotes the measured distance (in mm) between the suprasternal notch and the measurement site of the carotid pressure, an estimation of the distance between the aortic arch and carotid artery. Thus, PEP as estimated from the carotid pressure pulse (PEP_{est}) was calculated as:

$$PEP_{est} = t_{QWc} - t_{hc} \tag{7.3}$$

Using HR corrected PWV for estimation of PEP

To account for possible differences in cfPWV between the time of PEP_{echo} measurement and PWV measurement due to HR differences, cfPWV was adjusted for HR using the intrinsic HR dependency of cfPWV as calculated in Chapter 5 (0.017 m/s/bpm):

$$cfPWV_{adj\_HR} = cfPWV + (0.017 \cdot (HR_{echo} - HR_{PWV})) \tag{7.4}$$

where HR_{echo} denotes the HR at the time of echocardiographic PEP measurement, and HR_{PWV} denotes the HR at time of PWV measurement.

The adjusted propagation time from the aortic opening to the carotid artery was thus

$$t_{hc,adj\_HR} = \frac{d_{sc}}{cfPWV_{adj\_HR}} \tag{7.5}$$

and a third estimation for PEP was calculated as

$$PEP_{est,adj\_HR} = t_{QWc} - t_{hc,adj\_HR} \tag{7.6}$$

Using blood pressure corrected PWV for estimation of PEP

A separate adjusted cfPWV was calculated to account for the differences in mean arterial pressure (MAP) at the time of PEP_{echo} measurement and cfPWV measurement. The MAP dependency of cfPWV was taken as 0.0423 m/s/mmHg as per the age specific regression equation for PWV vs MAP for the age category of 30-39 years (Reference Values for Arterial Stiffness' Collaboration, 2010):

$$cfPWV_{adj,MAP} = cfPWV + (0.0423 \cdot (MAP_{echo} - MAP_{PWV})) \tag{7.7}$$

where MAP_{echo} denotes MAP measured at time of echocardiography, and MAP_{PWV} denotes MAP measured at time of PWV measurement.

The estimated propagation time for the aortic opening to the carotid artery was adjusted accordingly,

$$t_{hc,adj\_MAP} = \frac{d_{sc}}{cfPWV_{adj\_MAP}} \tag{7.8}$$

and a fourth estimation for PEP was calculated as

$$PEP_{est,adj\_MAP} = t_{QWc} - t_{hc,adj\_MAP} \tag{7.9}$$

Accounting for aortic arch length and regional PWV in estimation of PEP

As the body surface measurement of the distance between the suprasternal notch and site of carotid pressure measurement does not take into account the length of the ascending aorta, which has been shown to increase with age (Sugawara et al., 2008), it would likely underestimate the true distance travelled by the arterial pressure wave from the aortic opening to the carotid artery. Furthermore, it has been shown that PWV increases progressively in the aorta, and that PWV in the aortic arch is lower than that measured by cfPWV (Hickson et al., 2010). To account for both these factors, length of the aortic arch (d_{AscAo}, in mm) was estimated using the regression in the study by Sugawara et al. (2008):

$$d_{AscAo} = 0.9 \cdot Age + 26.1 \tag{7.10}$$

and regional PWV in the aortic arch (PWV_{AscAo}) was estimated from the relationship between PWV_{AscAo} and age, as well as the relationship between PWV_{AscAo} and cfPWV, as per the study by Hickson et al. (2010):

$$PWV_{AscAo} = cfPWV \cdot (-0.0034 \cdot Age + 0.9627) \tag{7.11}$$

Thus, the adjusted propagation time from the aortic opening to the carotid artery would equate to:

$$t_{hc,adj\_AscAo} = \frac{(d_{sc} + d_{AscAo})}{PWV_{AscAo}} \tag{7.12}$$

and a fifth estimation for PEP was calculated as:

$$PEP_{est,adj\_AscAo} = t_{QWc} - t_{hc,adj\_AscAo} \tag{7.13}$$

7.2.3 Statistical analysis

Paired Student's t test was used to compare the means of haemodynamic variables obtained during echocardiography and during PWV measurement, as well as to compare the measured and estimated PEP values. Pearson product moment coefficient of correlation (r) was used to assess the correlation between PEP_{echo} and PEP_{est}, PEP_{est,adj_HR}, PEP_{est,adj_MAP} and PEP_{est,adj_AscAo}, respecitvely. Comparisons between the correlations were made using Steiger's test for dependent correlations. Statistical analysis was performed using the software R (R Core Team, 2014), and $P<0.05$ was considered statistically significant. Data are presented as mean ± SD unless otherwise stated.

7.3 Results

Table 7.1: Characteristics of the study cohort ($n = 26$).

Parameters	Mean ± SD
Age, years	38 ± 13
Males, n (%)	15 (58)
Height, cm	174 ± 12
Weight, kg	70 ± 12
BMI, kg/m^2	23.2 ± 3.3

BMI, body mass index.

Table 7.2: Haemodynamic measurements during echocardiography and PWV measurement.

Parameters	PEP_{echo} Measurement	PWV Measurement	P
HR, bpm	64 ± 9	61 ± 8	0.106
Systolic BP, mmHg	122 ± 15	116 ± 12	0.070
Diastolic BP, mmHg	78 ± 10	73 ± 8	0.017
MAP, mmHg	93 ± 11	87 ± 9	0.027
cfPWV, m/s	-	6.3 ± 1.2	-

PEP, pre ejection period; PWV, pulse wave velocity; HR, heart rate; BP, blood pressure; MAP, mean arterial pressure; cfPWV, carotid-femoral pulse wave velocity

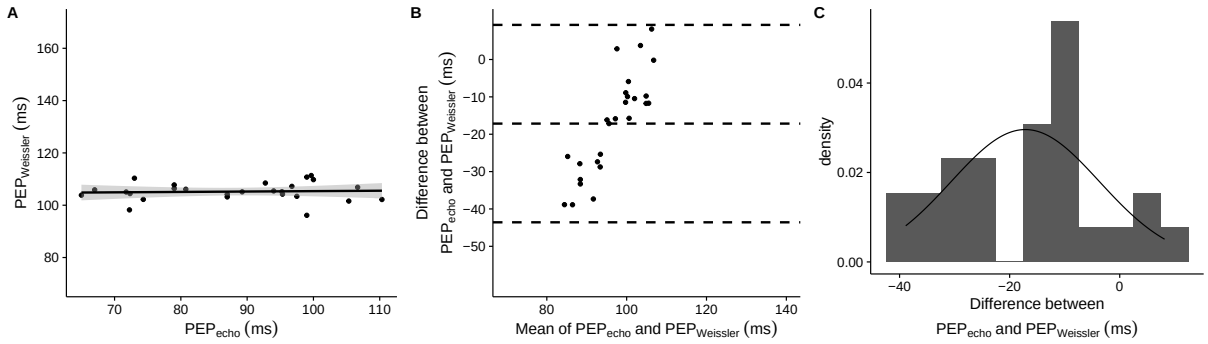


Figure 7.3: Comparisons of measured PEP (PEP_{echo}) and estimated PEP values based on Weissler's regression (Weissler et al., 1968) ($PEP_{Weissler}$). Bland-Altman plot shows the mean difference between estimated and measured PEP with 1.96SD below and above the mean. Equation of the linear regression fitted to the measured PEP against estimated PEP values is presented in Table 7.4.

Characteristics of the study cohort are presented in Table 7.1. Diastolic and mean blood pressures, but neither HR nor systolic blood pressure, were significantly different at the time of PWV measurement as compared to that at PEP_{echo} measurement (Table 7.2). The mean values for measured and estimated PEP, as well as their correlations, are presented in Table 7.3.

As shown in Figures 7.3 and 7.4, all estimated PEP values, except for $PEP_{Weissler}$, showed a high correlation with PEP_{echo} (Table 7.3). Excluding $PEP_{Weissler}$, which did not have a significant correlation to measured PEP, correlations between measured and various estimated PEP were not significantly different from each other (P<0.74 for correlations between PEP_{est} and PEP_{est,adj_HR}; P<0.78 for correlations between PEP_{est} and PEP_{est,adj_MAP}; P<0.61 for correlations between PEP_{est} and PEP_{est,adj_AscAo}).

All methods for estimating PEP showed a significant negative bias compared to PEP measured using echocardiography. Only the adjustment for arterial path length and regional PWV to take into account the ascending aorta (PEP_{est,adj_AscAo}) significantly improved the mean PEP estimate from pulse wave analysis with no adjustments (PEP_{est}) ($\Delta PEP = 15.1$ ms, P<0.0001), as shown in the lower panel of Figure 7.4.

Difference between PEP_{echo} and $PEP_{Weissler}$ showed a significant positive trend with the difference decreasing as PEP values increased, likely due to the lack of change $PEP_{Weissler}$ values with HR

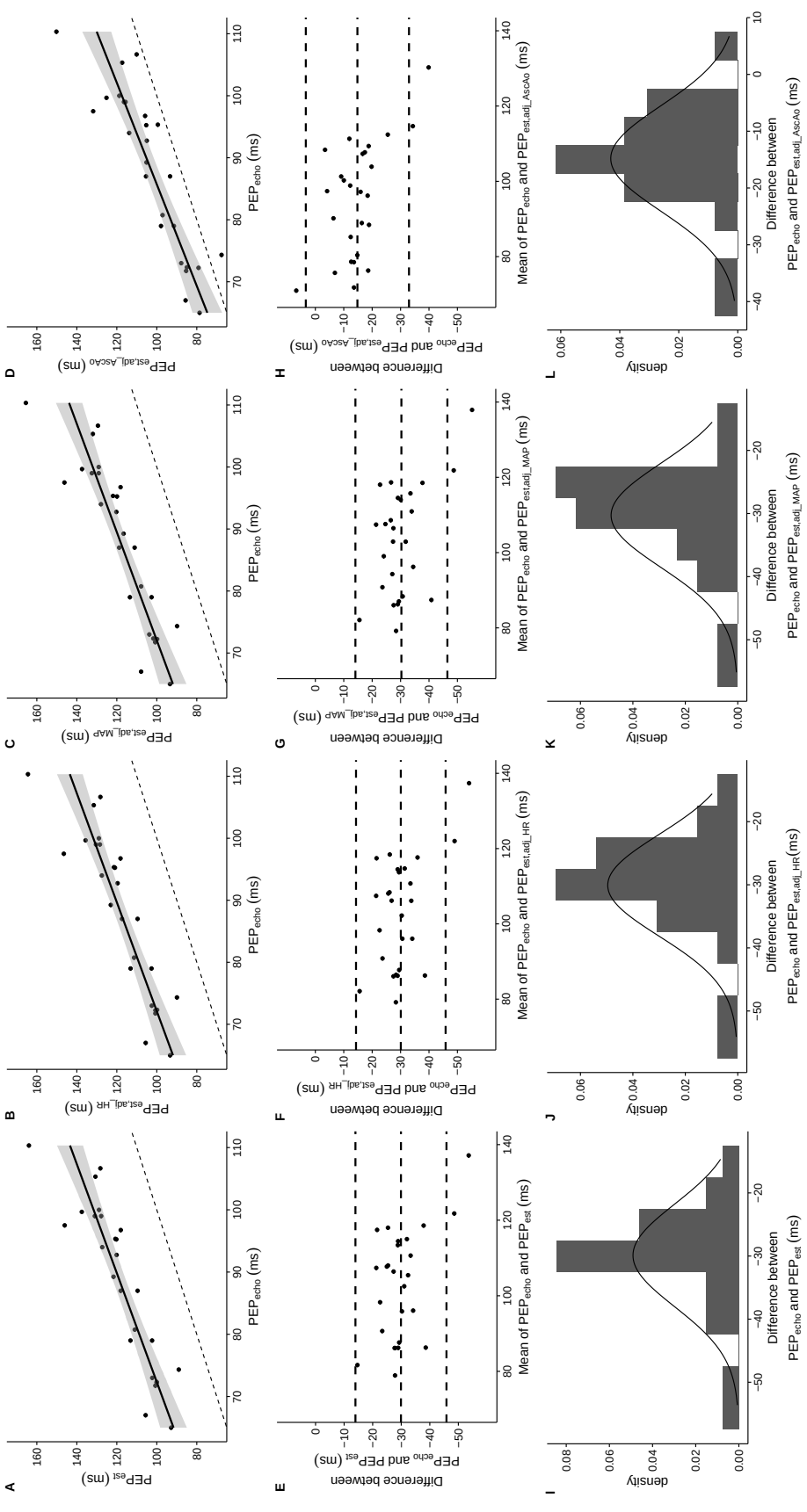


Figure 7.4: Comparisons of measured PEP ($\mathrm{PEP_{echo}}$) and estimated PEP values using pulse wave analysis of carotid waveform and measured cfPWV ($\mathrm{PEP_{est}}$, panels A and E), PEP estimated with HR adjusted cfPWV ($\mathrm{PEP_{est,\,adj\_HR}}$, panels B and F), PEP estimated with blood pressure adjusted cfPWV ($\mathrm{PEP_{est,\,adj\_MAP}}$, panels C and G), and PEP estimated with. Bland-Altman plots show the mean difference between measured and estimated PEP with 1.96SD below and above the mean. Equations of the linear regression fitted to the measured against estimated PEP values are presented in Table 7.4. Panels J to L show the distribution of the differences between measured and estimated PEP values.

Table 7.3: Values of measured PEP and estimated PEP

Parameters	Mean ± SD	*R*	*P*
Measured PEP			
PEP_{echo}, ms	88.0 ± 13.2	–	–
Estimated PEP			
$PEP_{Weissler}$, ms	105.2 ± 3.6‡	0.057	0.782
PEP_{est}, ms	117.9 ± 17.0‡	0.886	<0.0001
PEP_{est,adj_HR}, ms	118.1 ± 17.0‡	0.887	<0.0001
PEP_{est,adj_MAP}, ms	118.3 ± 17.1‡	0.883	<0.0001
PEP_{est,adj_AscAo}, ms	102.9 ± 18.3‡	0.878	<0.0001

R denotes Pearson's correlations between PEP_{echo} and each estimated PEP, with *P* denoting the statistical significance of these correlations; PEP, pre-ejection period; $PEP_{Weissler}$, PEP estimated with Weissler's regression Weissler et al. (1968); PEP_{est}, PEP estimated from carotid pulse wave analysis; $PEP_{est,\ adj\_HR}$, PEP estimated from carotid pulse wave analysis adjusted for heart rate (HR); $PEP_{est,\ adj\_MAP}$, PEP estimated from carotid pulse wave analysis adjusted for mean arterial pressure (MAP); $PEP_{est,\ adj\_AscAo}$, PEP estimated from carotid pulse wave analysis with ascending aortic length and PWV taken into consideration. ‡all estimated PEP values were significantly different to measured PEP with $P<0.0001$

(Figure 7.3). Other estimation methods resulted in an averaged negative bias with consistent variability around the bias, but with the presence of outliers (Figure 7.4). As shown in the accompanying histograms, the differences between measured and estimated PEP values with all methods followed a roughly normal distribution. Shapiro-Wilk tests on the differences showed that only differences between measured PEP and $PEP_{Weissler}$ and PEP_{est,adj_AscAo} were truly normal (W = 0.96, P=0.34 for $PEP_{Weissler}$; W = 0.90, P=0.02 for PEP_{est}; W = 0.89, P=0.01 for PEP_{est,adj_HR}; W = 0.88, P=0.01 for PEP_{est,adj_MAP}; W = 0.93, P=0.09 for PEP_{est,adj_AscAo}). However, exclusion of outliers revealed that all estimation methods resulted in a normal distribution of differences (W = 0.98, P=0.87 for PEP_{est}; W = 0.98, P=0.94 for PEP_{est,adj_HR}; W = 0.98, P=0.86 for PEP_{est,adj_MAP}; W = 0.97, P=0.71 for PEP_{est,adj_AscAo}).

Table 7.4: Relationship between measured PEP and estimated PEP values as determined by linear regression fitted to measured PEP against estimated PEP values.

PEP estimate	β	Intercept	R^2	P
$PEP_{Weissler}$	0.21 [-1.35, 1.77]	65.80 [-98.43, 230.03]	0.003	0.782
PEP_{est}	0.69 [0.53, 0.84]*	7.07 [-11.00, 25.14]	0.78	<0.0001
$PEP_{est,\ adj\_HR}$	0.69 [0.54, 0.84]*	6.44 [-11.65, 24.54]	0.78	<0.0001
$PEP_{est,\ adj\_MAP}$	0.68 [0.53, 0.83]*	7.46 [-10.74, 25.67]	0.77	<0.0001
$PEP_{est,\ adj\_AscAo}$	0.63 [0.49, 0.78]*	17.28 [7.96, 38.28]*	0.76	<0.0001

β and intercept presented as mean [95% CI]; PEP, pre-ejection period; $PEP_{Weissler}$, PEP estimated with Weissler's regression Weissler et al. (1968); PEP_{est}, PEP estimated from carotid pulse wave analysis; $PEP_{est,\ adj\_HR}$, PEP estimated from carotid pulse wave analysis adjusted for heart rate (HR); $PEP_{est,\ adj\_MAP}$, PEP estimated from carotid pulse wave analysis adjusted for mean arterial pressure (MAP); $PEP_{est,\ adj\_AscAo}$, PEP estimated from carotid pulse wave analysis with ascending aortic length and PWV taken into consideration; * denotes $P<0.05$

7.4 Discussion

This study demonstrated the validity of estimating PEP through the use of pulse wave analysis of the carotid pressure pulse waveform together with measurement of cfPWV, and how the intrinsic HR dependency of PWV could be applicable in studies such as this where there are non-synchronous measurements of cardiovascular parameters.

PEP is a useful parameter for the assessment of left ventricular function (Ahmed et al., 1972; Weissler et al., 1968), particularly in the assessment of myocardial contractility (Newlin and Levenson, 1979; Talley et al., 1971) and diagnosis of cardiovascular disease such as heart failure, where PEP would be prolonged (Weissler et al., 1969). Although it has been shown to be related to HR by Weissler et al. (1968), the changes in PEP with HR is generally small and often negligible (Cokkinos et al., 1976; Mertens et al., 1981). Thus, PEP values cannot be estimated by a simple regression alone, as demonstrated in the poor correlation between the estimated PEP values using Weissler's regression (Weissler et al., 1968) and measured PEP in the present study. In fact, the present study observed no relationship between HR_{echo} and PEP_{echo} (by linear regression analysis, PEP_{echo} = -0.08·HR + 93.5 ms, R^2=0.003, P=0.782).

This study demonstrated that a good estimate of PEP could be obtained using simple pulse wave analysis and measurement of cfPWV. By estimating the propagation time of the arterial pulse wave from the aortic valve to the carotid artery, with the assumption that the pulse wave travels at the same velocity over the distance as that measured by cfPWV, a reasonable estimation for PEP could be obtained. Although the correlation between the estimated and measured PEP values was high, PEP was overestimated by an average of 30 ms. Overestimation of PEP was likely the result of underestimation of the propagation time between the aortic opening to the site of carotid pressure measurement, which could be due to the underestimation of the distance between the aortic valve and the site of carotid pressure measurement, as the distance between the suprasternal notch to the carotid do not take into account the length of the ascending aorta. Furthermore, timing could also be underestimated due to not accounting for the fact that the arterial pressure pulse wave travels at lower velocities in the ascending aorta as compared to the rest of the aorta (Hickson et al., 2010). This was confirmed by the significant improvement of the PEP estimate once these two factors were accounted for in the present study. However, regardless of the improvement of the PEP estimate, the bias is still clinically significant and must be corrected for, as the average measured PEP in the present cohort of healthy individuals was only 88±13 ms (mean±SD), and individuals with congestive heart failure are known to have prolonged PEP, in the order of 120 - 170 ms (Weissler et al., 1968). Usage of these PEP estimates without correction could erroneously implicate the presence of heart failure and lead to a misdiagnosis.

Neither correction of cfPWV for HR nor blood pressure at the time of PEP and cfPWV measurements made a difference to the estimated PEP values. This was likely due to the negligible changes in HR, and although mean arterial pressure was significantly higher at the time of PEP measurement, the changes were still relatively small (≈5 mmHg, equivalent to a PWV increase of 0.2 m/s), resulting in only a small change in PWV and therefore transit time. However, in similar studies where correlations of non-synchronous measurements relating to

cfPWV need to be determined, such corrections would still be relevant and useful to account for the haemodynamic differences between the separate times of measurement.

The validity of using pulse wave analysis and cfPWV to estimate PEP, with appropriate corrections, can expand the utility of non-invasive pulse wave analysis. As LVET can already be determined with pulse wave analysis, provision for PEP measurement allows for the determination of the PEP/LVET ratio, which is a useful parameter for overall left ventricular performance (Lewis et al., 1977; Weissler et al., 1968). Furthermore, LVET is used in the determination of subendocardial viability ratio (SEVR), which provides information on the degree of myocardial oxygen perfusion relative to myocardial oxygen demand (Buckberg et al., 1972) and can already be obtained from pulse wave analysis. Inclusion of the isovolumetric contraction time within the PEP in determining SEVR can improve the evaluation of left ventricular workload (Salvi et al., 2015).

As the present study was performed in a relatively small cohort of healthy individuals, the applicability of the proposed method of PEP estimation in individuals with conditions that severely impact PEP, such as heart failure and left ventricular dysfunction, remains to be determined. Furthermore, the study cohort consisted mainly of individuals under 40 years of age, but PEP has been shown to increase with age (Montoye et al., 1971), thus a study in a cohort with larger age range would be needed to determine whether the estimation deviates with age. Such are the aims of the multi-centre study led by Dr Paolo Salvi from the Italian Institute for Auxology, Milan, Italy (Salvi et al., 2015), to which the data presented in this chapter will contribute. Despite the aforementioned limitations, if the change in PEP is linear, then the presented method of PEP estimation with pulse wave analysis and cfPWV would not be affected.

7.5 Conclusions

This studied confirmed the validity of estimating PEP in healthy individuals through the use of simple pulse wave analysis of the carotid pressure waveform and cfPWV measurement, albeit with a 30% overestimation. Whilst neither HR nor blood pressure correction of cfPWV improved estimation of PEP, most likely due to the small differences between the time of PEP and cfPWV measurements, accounting for ascending aorta in the arterial path length as well as using regional PWV in the ascending aorta significantly improved the PEP estimate. Notwithstanding, corrections for the HR and blood pressure dependency of cfPWV would be applicable in similar studies where non-synchronous measurements are undertaken.

CHAPTER 8

Conclusions

Arteries are subjected to constant stress arising from the distending pressure generated by the pulsatile outflow of the heart at every moment in the life of any mammal. In particular, the large arteries proximal to the heart, which act as cushioning buffers and conduits to the pulsatile blood flow, are subjected to large variations in distending pressure with each heartbeat. The viscoelastic design of the arterial wall, with its unique structure and composition, gives large arteries the stiffness that optimises damping of the pulsatile blood flow without the expense of ineffective circulation (Avolio, 2013). It has been well established that the arterial wall stiffens with age and increased blood pressure (Cecelja and Chowienczyk, 2009; Reference Values for Arterial Stiffness' Collaboration, 2010). Although viscoelastic materials, by nature, have a frequency dependent stress-strain relationship (Özkaya et al., 2012), the effects of heart rate on arterial stiffness have been disputed (Hayward et al., 2002), and results from previous studies on the influence of heart rate on arterial stiffness in both animal models and humans have failed to converge. Notwithstanding, there exists evidence that arterial wall stiffness can indeed be acutely affected by the frequency of the pulsatile strain an artery undergoes (Bergel, 1961a; Lantelme et al., 2002b; Mangoni et al., 1996; Mircoli et al., 1999), and several epidemiological studies have found an independent association between heart rate and pulse wave velocity (PWV), a surrogate measure of arterial stiffness (Cecelja and Chowienczyk, 2009). Furthermore, both elevated heart rate and increased arterial stiffness are associated with hypertension (Mancia et al., 2007; Palatini et al., 2006b), and both are in and of themselves cardiovascular risk factors for cardiovascular

disease (Blacher et al., 1999; Palatini and Julius, 1997) as well as independent predictors of cardiovascular and all-cause mortality (Laurent et al., 2003; Palatini, 2011). However, whether or not the presence of both elevated heart rate and increased arterial stiffness would compound cardiovascular risk is unknown. In addition, there is a lack of understanding on the possible mechanisms by which heart rate can influence arterial wall stiffness. As such, this thesis set out to further investigate the relationship between heart rate and arterial stiffness through 1) studying the acute effects of heart rate on aortic stiffness in the rat model at fixed levels of blood pressure, with heart rate changes induced by cardiac pacing; 2) determining the intrinsic heart rate dependency of aortic PWV in humans, again by means of cardiac pacing; and 3) using a transmission line model (TLM) of the human arterial tree (Xiao et al., 2016) to assess the degree of frequency dependence of the arterial wall elasticity necessary to simulate heart rate effects on PWV as a possible mechanism through which heart rate can influence arterial stiffness.

The animal study presented in this thesis (Chapter 3) was the first *in vivo* animal study to show the heart rate dependency of aortic PWV at different levels of mean arterial pressures, and that arterial wall stiffness was more sensitive to heart rate changes at greater distending pressures. When data were analysed with blood pressure correction and heart rate normalised to the lowest paced heart rate in the study, the changes in PWV with increasing heart rate were of similar order compared to that observed in humans when the same heart rate normalisation was applied (Lantelme et al., 2002b).

Through the use of three different methods of correcting blood pressure effects on carotid-femoral PWV (cfPWV) in a cohort of 52 subjects with *in situ* cardiac pacing and heart rate changes from 60 bpm to 100 bpm, the intrinsic heart rate dependency of cfPWV in humans was found to be 0.17 m/s per 10 bpm increase in heart rate (Chapter 4). This was the first study to quantify the blood pressure independent heart rate dependency of arterial stiffness in humans. The established relationship between heart rate and arterial stiffness can be applicable in correcting for heart

rate effects on cfPWV in studies where non-synchronous measurements relating to cfPWV and heart rate are made, as demonstrated in the study on estimating pre-ejection period through the use of PWV and pulse wave analysis of the carotid pressure waveform (Chapter 7). This heart rate dependency of PWV can also also be a useful parameter in studies or clinical measurements where there are large variations in heart rate, such as exercise studies.

As cardiac pacing is often the method of choice for inducing heart rate changes in studies investigating acute effects of heart rate on arterial stiffness and other indices of large artery function, the effects of pacing from different cardiac sites, that is, pacing modality, was investigated (Chapter 5). It was found that, whilst pacing modality did not influence the heart rate dependency of PWV or other indices of large artery function such as wave reflection indices, when compared at the same heart rate level, there was a significant difference in wave reflection indices that should be taken into account. Furthermore, there were slight differences in PWV between different pacing modalities when compared at the same heart rate, but the differences did not reach statistical significance, and a larger study would be able to provide more statistical power to determine whether or not there are true differences in PWV with different pacing modalities.

Finally, a possible mechanism by which heart rate changes could influence arterial stiffness was proposed through the use of a TLM of the human arterial tree (Xiao et al., 2016), demonstrating that if arterial wall elasticity has sufficient frequency dependence, changes in PWV could be observed with changes in heart rate (Chapter 6). This frequency dependence of arterial wall elasticity could explain up to one third of the intrinsic change in PWV for every 10 bpm increase in heart rate.

To summarise, there were three major findings and conclusions presented in this thesis:

(i) There is an independent heart rate effect on arterial stiffness in both the rat and humans.

(ii) The intrinsic heart rate dependency of aortic PWV in humans is 0.17 m/s per 10 bpm increase in heart rate.

(iii) A possible mechanism through which heart rate exerts an influence on arterial stiffness is the frequency dependency of the arterial wall's elastic modulus. This mechanism can explain up to one third of the intrinsic changes in PWV observed with increasing heart rate.

As a whole, the findings presented in this thesis strengthened the evidence that heart rate influences arterial stiffness independent of blood pressure, and provided insight on the mechanism by which heart rate changes can lead to changes in arterial stiffness. Quantification of the intrinsic heart rate dependency on PWV will allow for practical application of the relationship between heart rate and arterial stiffness in future cardiovascular research studies.

CHAPTER 9

Future resesarch

The research presented in this thesis is but a snapshot of the relationship between acute heart rate changes and arterial stiffness, as measured by pulse wave velocity (PWV), and a window into the part mechanism through which heart rate exerts an effect on stiffness of the arterial wall. There is still much to be explored in order to fully understand the synergistic effects of heart rate, blood pressure and arterial stiffness in cardiovascular health, the mechanisms involved, and in particular the long term implications of an elevated heart rate on arterial wall stiffening.

The synergistic effects of heart rate, blood pressure and arterial stiffness could be further explored through extending the animal study presented in Chapter 3 to investigate the effects of heart rate on aortic stiffness in hypertensive rats as compared to normotensive rats. Similarly, the pacing study in Chapter 4 could be repeated in a cohort of untreated hypertensive, treated hypertensive and normotensive individuals to determine whether differences exist in the heart rate dependency of arterial stiffness in these groups. Furthermore, the influence of age, the other major determinant of aortic stiffness besides blood pressure (Cecelja and Chowienczyk, 2009; Reference Values for Arterial Stiffness' Collaboration, 2010), on the heart rate dependency of arterial stiffness was not explored in this thesis, and thus warrants further research.

Just as arterial wall stiffness increases gradually with distance away from the heart, the heart rate dependency of arterial stiffness may vary in different regions of the aorta and other arterial segments. The transmission line model of the human arterial tree utilised in Chapter 6 (Xiao

et al., 2016) can thus be further modified to model varying degrees of frequency dependence of the elastic modulus of the arterial wall in different arterial segments, and the changes in measured PWV with changing heart rate can then be determined from the model simulations. Further *ex vivo* experiments, similar to those performed by Bergel (1960, 1961a); Callaghan et al. (1984), could be performed in animal and human arterial specimens to determine the frequency dependency of elastic modulus and validate the results from model simulations.

The influence of pacing modality on arterial stiffness should be further investigated given the non-significant trend of higher PWV and significantly higher augmentation index in subjects with ventricular pacing compared to those with atrial pacing (Chapter 5). The confirmation of a significant impact of pacing modality on PWV can have implications on future studies looking at the acute heart rate dependency of arterial stiffness, meaning studies should be designed with inducing heart rate changes through cardiac pacing with a fixed pacing modality, or with the different pacing modalities accounted for in the analysis.

In light of recent studies that have shown that left ventricular ejection time (LVET), rather than heart rate, is related to PWV (Nürnberger et al., 2003; Salvi and Parati, 2013), the relationship between temporal changes with heart rate and arterial stiffness should be further explored. Whilst heart rate determines how fast or slow an artery is extended, the temporal changes with heart rate determine the duration of each extension. LVET can be easily determined from existing measurements of blood pressure and blood flow waveforms in the determination of PWV, and thus data can be re-examined and re-analysed to determine the presence of a significant relationship between LVET and PWV. Computational modelling can also be used to simulate varying LVET at the same heart rate to determine whether changes in measured PWV could be observed.

Finally, whilst the studies presented in this thesis centred around the measurement of PWV as a surrogate measure of arterial stiffness, mainly as carotid-femoral PWV is increasingly being

used as a clinical marker for cardiovascular disease, many other parameters of arterial stiffness exist (Section 2.2.2), and the work presented could be extended to investigate other stiffness parameters such as arterial compliance. A similar *ex vivo* study to that by Bergel (1960, 1961a) could be conducted, where arterial segments from rodents or humans undergo oscillatory pressure strain. Arterial compliance could be obtained as a parameter of local arterial stiffness using measurements of pressure oscillations and diameter changes, as opposed to the elastic modulus which is considerably more involving due to the requirement of measuring the internal radius of the vessel. The rodent work (Chapter 3) presented in this thesis could be extended to measure local compliance of the aorta through ultrasonic quantification of arterial diameters at different heart rates, coupled with invasive aortic pressure measurements. Similarly, the pacing study (Chapter 4) could be extended to include measurements of arterial diameter obtained in humans at various sites using ultrasound, such as the brachial and carotid arteries, coupled with blood pressure waveforms measured non-invasively with applanation tonometry at the brachial or carotid sites, respectively, allowing for local arterial compliance to be calculated.

Appendix A (pp. 1 8 184 of this thesis ha ee removed as t contain published material under copyright. Removed contents published as:
Isabella Tan, Mark Butlin, Ying Yi Liu, Keith Ng, & Alberto P. Avolio (2012), Heart Rate Dependence of Aortic Pulse Wave Velocity at Different Arterial Pressures in Rats. *Hypertension*, vol. 60, no. 2, pp. 528-533.
https://doi.org/10.1161/HYPERTENSIONAHA.112.194225

APPENDIX A

Heart rate dependence of aortic pulse wave velocity at different arterial pressures in rats (final peer-reviewed manuscript)

This is the non-final version of the article published in final form in:

Tan I, Butlin M, Liu YY, Ng K, Avolio AP. Heart rate dependence of aortic pulse wave velocity at different arterial pressures in rats. *Hypertension.* 2012;60(2):528-533.

Appendix B (pp. 186-222) of this thesis has been removed as it contains published material under copyright. Removed contents published as:
Isabella Tan, Bart Spronck, Hosen Kiat, Edward Barin, Koen D. Reesink, Tammo Delhaas, Alberto P. Avolio, & Mark Butlin (2016), Heart Rate Dependency of Large Artery Stiffness. *Hypertension*, vol. 68, no. 1, pp. 236-242. https://doi.org/10.1161/HYPERTENSIONAHA.116.07462

APPENDIX B Heart rate dependency of large artery stiffness (final peer-reviewed manuscript)

This is the non-final version of the article published in final form in:

Tan I, Spronck B, Kiat H, Barin E, Reesink KD, Delhaas T, Avolio AP, Butlin M. Heart Rate Dependency of Large Artery Stiffness. *Hypertension.* 2016;68(1):236-242.

APPENDIX C

Effects of pacing modality on non-invasive assessment of heart rate dependency of indices of large artery function (manuscript as published in final form)

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Effects of pacing modality on noninvasive assessment of heart rate dependency of indices of large artery function

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Tan I, Kiat H, Barin E, Butlin M, Avolio AP. Effects of pacing modality on noninvasive assessment of heart rate dependency of indices of large artery function. *J Appl Physiol* 121: 771–780, 2016. First published July 28, 2016; doi:10.1152/japplphysiol.00445.2016.—Studies investigating the relationship between heart rate (HR) and arterial stiffness or wave reflections have commonly induced HR changes through in situ cardiac pacing. Although pacing produces consistent HR changes, hemodynamics can be different with different pacing modalities. Whether the differences affect the HR relationship with arterial stiffness or wave reflections is unknown. In the present study, 48 subjects [mean age, 78 ± 10 (SD), 9 women] with in situ cardiac pacemakers were paced at 60, 70, 80, 90, and 100 beats per min under atrial, atrioventricular, or ventricular pacing. At each paced HR, brachial cuff-based pulse wave analysis was used to determine central hemodynamic parameters, including ejection duration (ED) and augmentation index (AIx). Wave separation analysis was used to determine wave reflection magnitude (RM) and reflection index (RI). Arterial stiffness was assessed by carotid-femoral pulse wave velocity (cfPWV). Pacing modality was found to have significant effects on the HR relationship with ED ($P = 0.01$), central aortic pulse pressure ($P = 0.01$), augmentation pressure ($P < 0.0001$), and magnitudes of both forward and reflected waves ($P = 0.05$ and $P = 0.003$, respectively), but not cfPWV ($P = 0.57$) or AIx ($P = 0.38$). However, at a fixed HR, significant differences in pulse pressure amplification ($P < 0.001$), AIx ($P < 0.0001$), RM ($P = 0.03$), and RI ($P = 0.03$) were observed with different pacing modalities. These results demonstrate that although the HR relationships with arterial stiffness and systolic loading as measured by cfPWV and AIx were unaffected by pacing modality, it should still be taken into account for studies in which mixed pacing modalities are present, in particular, for wave reflection studies.

arterial stiffness; heart rate; pulse wave velocity; wave reflection; pacing; pulse wave analysis

NEW & NOTEWORTHY

This was the first study to demonstrate significant differences in wave reflection indices with different cardiac pacing modalities. This has implications for future heart rate studies in wave reflections, and studies should either be designed to employ a single pacing modality for inducing heart rate changes, or to take pacing modality into account in the analysis.

ARTERIAL STIFFNESS AND ARTERIAL wave reflections have both been shown to be independent predictors and risk factors for all-cause and cardiovascular events and mortality (12, 26, 30, 44). Measures of arterial stiffness such as pulse wave velocity (PWV), and central systolic loading such as augmentation index (AIx), are now readily used in research, and there is an increased interest to include such indices in routine clinical procedures because of their prognostic value (32, 33). As such, large population studies have been conducted to establish reference values for these indices (20, 22, 51). Although blood pressure (BP) is associated with both PWV (6, 7, 23, 48) and AIx (64, 78), the effect of heart rate (HR), another hemodynamic parameter that can be highly variable between and within individuals, is less well established. Although AIx has been shown to decrease linearly with HR in both cross-sectional (22, 24, 36) and acute studies (59, 77, 79), a consensus has yet to be reached on the relationship between PWV and HR, particularly because concomitant changes in BP with HR have been observed in most acute studies (2, 29, 35).

Earlier investigations on the acute effects of HR on PWV or AIx commonly induced changes in HR by way of cardiac pacing (2, 3, 19, 25, 35, 59, 77, 79) to minimize confounding effects on the systemic circulation. When the study cohort consisted of subjects with in situ cardiac pacemakers, subjects could be paced from the right atrium (atrial pacing, Ap) (2, 19, 25, 77), right ventricle (ventricular pacing, Vp) (3, 25, 35, 59), or both the right atrium and the right ventricle (sequential atrioventricular pacing, ApVp) (3, 35, 77). Although hemodynamic consequences of pacing from different chambers in the heart have been well investigated (15, 17, 70, 76), whether or not these consequences affect the HR relationship with arterial stiffness, arterial waveform shape, or arterial wave reflections have not been studied in detail. Past studies have found temporal changes relating to HR, such as ejection duration (ED), had a stronger association with the resultant changes in PWV (54) and AIx (58) than HR itself. Furthermore, increased HR can lead to changes in left ventricular ejection, which can affect the forward-traveling pressure wave (73) and, in turn, lead to changes in AIx (63). Because pacing at different cardiac sites has been shown to influence ED (8, 14, 76) and left ventricular function (53), conclusions drawn from pacing studies regarding HR effects on arterial stiffness and wave reflections may need to take into account the pacing modalities that were undertaken. For example, Ap typically results in a greater ED than ApVp or Vp alone for the same HR, theoretically resulting in a later occurrence of the systolic peak, with greater pressure augmentation, depending on the timing of the peak in that wave, and a higher AIx. However, Ap is also associated with a greater cardiac output than ApVp or Vp alone for the same HR. For the same conditions in the vasculature, this would result in a higher pulse pressure and a decrease in AIx. This study aimed to determine whether different pacing modalities would affect the relationship between HR and indices of arterial stiffness, arterial waveform shape, and arterial wave reflections as determined from acute pacing studies.

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METHODS

Patients with implanted pacemakers or implantable cardioverter defibrillators with pacing function were recruited from the Cardiac Health Institute and Macquarie Heart clinics at Macquarie University. Exclusion criteria included unstable angina, prior myocardial infarction within the last 12 mo, and uncontrolled congestive heart failure. A total of 52 subjects entered the study, but 4 were excluded from analysis due to biventricular pacing ($n = 2$) or suboptimal electrocardiographic (ECG) recording ($n = 2$), with the latter resulting in the inability to identify their pacing modality at the time of measurement. Overall, 48 subjects [age, 40-93 (mean ± SD, 78 ± 10) yr, 9 women] were included in the analysis. The study protocol was approved by Macquarie University's Human Ethics Committee, and written consent to participate in the study was obtained from all subjects.

Pulse wave analysis. Brachial and central aortic BP, augmented pressure (AP) of the central aortic BP, AIx, time to first systolic inflection (T_i), and ED were determined by brachial cuff-based pulse wave analysis (SphygmoCor XCEL; AtCor, Sydney, Australia). Brachial BP was obtained via an oscillometric method with a brachial cuff positioned on the right arm, and central aortic waveform was derived from the brachial BP volume displacement waveform using a validated transfer function (9). AIx was defined as the ratio of AP to the central aortic pulse pressure (PP), expressed as a percentage. Pulse pressure amplification (PPA) was expressed as the ratio of brachial PP to central aortic PP. ED was defined as the duration between the end-diastolic time to the incisura, and T_i was defined as the time from end-diastole to the inflection point.

Arterial stiffness, waveform shape, and wave reflection indices. Arterial stiffness was assessed by means of carotid-femoral PWV (cfPWV). The carotid pulse waveform was obtained by tonometry on the skin above the right carotid artery with simultaneous acquisition of the femoral pulse waveform using a cuff on the right upper thigh (SphymoCor XCEL) (10, 21). The subtraction method for path length was used to calculate cfPWV, whereby the path length was calculated as the distance between the sternal notch and the carotid site subtracted from the distance between the sternal notch and top of the thigh cuff (10).

In addition to AIx, T_i, and PPA determined from pulse wave analysis, the magnitude of wave reflection was also investigated. Magnitudes of the forward (P_f) and backward (P_b) pressure waves, reflection magnitude (RM), and reflection index (RI) were determined with wave separation analysis using the triangulation method as described by Westerhof et al. (71) and equations as described by Murgo et al. (38). Briefly, aortic blood flow was approximated using a triangle, with the peak set at the first systolic shoulder (T_1) of the derived aortic pressure. The start and end of the triangle were set at the end-diastolic time and the time at incisura, respectively. Because calibration of the flow wave is not required (71), the peak of the triangle was set at unity. P_f and P_b waves were then calculated accordingly using the following equations:

$$P_f(t) = \left[P_m(t) + Z_c^* Q(t)\right]/2 \quad (1)$$

$$P_b(t) = \left[P_m(t) - Z_c^* Q(t)\right]/2, \quad (2)$$

where $P_m(t)$ is the averaged central aortic pressure waveform, $Q(t)$ is the constructed triangular blood flow, and Z_c is the characteristic impedance. Z_c was derived as the average of the fourth to seventh harmonics of the input impedance, which in turn were determined from the Fourier transforms of $P_m(t)$ and $Q(t)$. A smoothing function was applied to the inverse of the modulus of the $Q(t)$ Fourier transform to remove extreme peaks in the harmonics resulting from the sharp peak in the constructed flow wave. RM was defined as a ratio of the magnitudes (peak − trough) of the forward and reflected pressure waves, and allows for an estimation on the amount of wave reflection (71). RI is defined as the ratio of the magnitudes of the reflected and measured pressure waves. In this study, RM and RI were calculated with the following equations:

$$RM = \left|P_b\right| / \left|P_f\right|, \quad (3)$$

$$RI = \left|P_b\right| / \left(\left|P_f\right| + \left|P_b\right|\right). \quad (4)$$

Study protocol. Subjects were advised to continue with their current medications, but to refrain from caffeine and fatty meals 4 h before the study. Seated brachial BP was measured in duplicate (SphygmoCor XCEL) after 10 min of seated rest. After a further 10 min of supine rest, finger arterial pressure waveform was measured from the left middle finger (Finometer PRO; Finapres Medical Systems, Amsterdam, The Netherlands) to obtain beat-to-beat stroke volume (SV) and total peripheral resistance (TPR) measurements. Brachial and central aortic BP and cfPWV were then obtained (SphygmoCor XCEL). At the completion of baseline measurements, subjects were then paced in a randomized sequence of 60, 70, 80, 90, and 100 beats per min (bpm) using their prescribed pacemaker settings, with all measurements repeated 3 min after each pace rate change. ECG data were also acquired continuously for the duration of the study (PowerLab acquisition system, LabChart software; ADInstruments, Dunedin, New Zealand) for HR monitoring and identification of pacing modality. SV and TPR from the Finometer PRO device were also recorded via PowerLab and LabChart. The average duration for study protocol completion was 60 min.

Data analysis. Brachial and central aortic BP waveforms were averaged over 5 s, and cfPWV was averaged over 10 s. SV and TPR values were averaged across 10 cardiac cycles, and cardiac output (CO) was calculated as the product of SV and HR, where HR was determined from ECG data. The pacing modality at each pacing step was identified from ECG data. Observations with measured HR differing from the paced rate by more than 5 bpm were excluded from the analysis. Pacing rates of 60 and 70 bpm were not achievable in some subjects because they had a higher unpaced resting HR. Optimal finger arterial pressure waveform, and consequently SV, TPR, and CO measurements, could not be obtained in five subjects. For subjects who had pacemakers in DDD mode (dual chamber-sensed and dual chamber-paced), some were under Ap at the lower HRs but switched to ApVp at the higher HRs. Only data from a single pacing modality were included for analysis for these subjects.

Statistical analysis. A linear mixed model with maximum likelihood estimation was fitted to all measured parameters, with HR, pacing modality, and their interaction term modeled as fixed effects, and each subject's individual intercepts modeled as random effects (Eq. 5):

$$Y_{ij} = \beta_0 + \beta_1 \cdot \mathrm{HR} + \beta_2 \cdot \mathrm{PM} + \beta_3 \cdot \mathrm{HR} \cdot \mathrm{PM} + \varepsilon_{ij} + u_j \quad (5)$$

where HR is the paced HR at each pacing step (modeled as a continuous predictor), PM is the pacing modality (modeled as a categorical predictor), and HR·PM is the interaction term; ε_{ij} denotes the residual of variances, and u_j denotes the random effect due to individual subject variances (i.e., variance of the random intercepts). Treatment contrasts were used for pacing modality comparisons, with Ap being the reference group against which ApVp and Vp were compared, thus resulting in the following model (Eq. 6):

$$Y_{ij} = \beta_0 + \beta_1 \cdot \mathrm{HR} + \beta_2 \cdot \mathrm{ApVp} + \beta_3 \cdot \mathrm{Vp} + \beta_4 \cdot \mathrm{HR} \cdot \mathrm{ApVp} + \beta_5 \cdot \mathrm{HR} \cdot \mathrm{Vp} + \varepsilon_{ij} + u_j \quad (6)$$

where β_0 denotes the intercept of the HR trajectory for the Ap group; β_1 denotes the estimated effect of HR for the Ap group; β_2 and β_3 denote the difference in intercepts between Ap and ApVp, and Ap and Vp groups, respectively; β_4 and β_5 denote the differences in estimated HR effect between Ap and ApVp, and Ap and Vp groups, respectively. Thus, the estimated HR effect for ApVp can be calculated as $\beta_1 + \beta_4$, and the estimated HR effect for Vp can be calculated as $\beta_1 + \beta_5$. The correlation between repeated measures from the same subject is accounted for via the random effect. For parameters in which the HR·PM interaction was not significant, the model was refitted without the interaction term to determine the main effect of

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pacing modality, where β_1 denotes the overall average effect of HR, and β_2 and β_3 denote the differences in the measured parameter compared with Ap at a fixed HR with ApVp and Vp, respectively. One-way ANOVA was used to compare differences in baseline hemodynamics across pacing modalities, and post hoc Student's *t*-tests with Bonferroni correction were performed as appropriate. Categorical variables were compared using Pearson's χ^2 tests. Descriptive statistics are presented as means ± standard deviation (SD) unless otherwise stated, and model estimates are presented as means [95% confidence intervals (CI)]. A value of $P < 0.05$ was considered statistically significant. Data analysis was performed using the software R (49), and mixed modeling was performed using the R nlme package (47).

RESULTS

Subject clinical characteristics are outlined in Table 1. At baseline, all but four subjects were actively paced, and there were no significant hemodynamic differences among the three pacing modality groups except for AP and AIx (Table 2). Some subjects in the ApVp group were under Ap at baseline because their pacemakers were in DDD mode.

The estimated values for the effect of HR on measured hemodynamic parameters for each pacing modality group are shown in Table 3. Significant differences in the HR trajectories between pacing modality groups were observed for ED [$\chi^2(6) = 8.63$, $P = 0.01$], central aortic PP [$\chi^2(6) = 9.20$, $P = 0.01$], AP [$\chi^2(6) = 22.02$, $P < 0.0001$], $|P_f|$ [$\chi^2(6) = 6.05$, $P = 0.05$], $|P_b|$ [$\chi^2(6) = 11.99$, $P = 0.003$], and TPR [$\chi^2(6) = 7.67$, $P = 0.02$] (Fig. 1), but not for other hemodynamic measures (Table 3). Pacing modality was shown to have a main effect on PPA [$\chi^2(6) = 14.73$, $P < 0.001$], AIx [$\chi^2(6) = 19.31$, $P = 0.0001$], RM [$\chi^2(6) = 7.33$, $P = 0.03$], and RI [$\chi^2(6) = 7.10$, $P = 0.03$], with contrasts showing the Ap group having lower PPA, but higher AIx, RM, and RI than both ApVp and Vp groups at constant HR (Table 4). No significant differences in other hemodynamic parameters were observed (Table 4).

Table 1. *Clinical characteristics of subjects*

	Ap	ApVp	Vp	*P*
Number	14	21	13	
Men/Women	9/5	17/4	13/0	0.06
Age, yr	78 ± 6	77 ± 12	80 ± 8	0.70
Height, m	1.69 ± 0.1	1.72 ± 0.1	1.73 ± 0.8	0.32
Weight, kg	69 ± 12	79 ± 15	82 ± 13	0.05
Implant indications, *n*				
SSS	5	3	1	0.14
Bradycardia	8	2	6	0.01
Irregular HR	1	2	1	0.97
Heart block	0	7	3	0.06
Syncope	1	0	1	0.44
Atrial fibrillation	2	0	9	<0.001
Ventricular tachycardia	0	1	0	0.52
Cardiomyopathy	1	2	0	0.53
Other	1	3	1	0.74
Pacemaker mode, *n*				
AAI/DDD	3	3	0	0.23
DDD	11	18	1	<0.001
VVI	0	0	11	<0.001
VVD	0	0	1	0.25
Medications, *n*				
α blocker	1	2	0	0.53
β blocker	8	8	6	0.54
Calcium antagonists	3	6	1	0.35
Nitrates	0	2	3	0.14
ACE-inhibitors	2	6	4	0.54
AngII-blockers	3	7	5	0.61
Diuretic	1	4	6	0.05
Antiarrhythmics	6	4	4	0.31
Anticoagulants	7	5	10	0.01
Antiplatelets	2	4	3	0.84
Statins	7	13	8	0.75
Aspirin	2	7	0	0.05

AAI, atrial pacing and sensing; ACE, angiotensin-converting enzyme; AngII, angiotensin II; Ap, atrial pacing; ApVp, sequential atrioventricular pacing; DDD, dual chamber (atrial and ventricular) pacing and sensing; HR, heart rate; SSS, sick sinus syndrome; VDD, ventricular pacing and atrial sensing; Vp, ventricular pacing; VVI, ventricular pacing and sensing.

Table 2. *Baseline hemodynamic measurements*

	Ap	ApVp	Vp	*P*
HR, bpm	64 ± 6	63 ± 6	64 ± 10	0.70
Brachial SBP, mmHg	129 ± 15	126 ± 15	122 ± 15	0.51
Brachial DBP, mmHg	74 ± 11	71 ± 9	71 ± 3	0.55
Brachial PP, mmHg	55 ± 11	55 ± 13	52 ± 15	0.70
Aortic SBP, mmHg	119 ± 13	115 ± 13	111 ± 12	0.25
Aortic DBP, mmHg	74 ± 10	72 ± 9	71 ± 3	0.70
Aortic PP, mmHg	45 ± 7	43 ± 10	39 ± 12	0.32
MAP, mmHg	91 ± 11	87 ± 10	85 ± 5	0.30
AP, mmHg	16 ± 6	13 ± 4	9 ± 5*	0.004
AIx, %	36 ± 12	30 ± 6	24 ± 8*	0.004
cfPWV, m/s	9.0 ± 1.6	9.8 ± 1.5	10.0 ± 1.6	0.21

AIx, augmentation index; AP, augmentation pressure; bpm, beats per min; cfPWV, carotid-femoral pulse wave velocity; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure. *$P < 0.01$ compared with Ap.

DISCUSSION

To the best of our knowledge, this is the first study to demonstrate the relevance of pacing modality when analyzing data in acute HR studies that employ cardiac pacing for inducing HR changes, in particular for investigations of wave reflection characteristics.

Previous studies investigating the effects of acute changes in HR on arterial stiffness and wave reflections have mostly employed cardiac pacing as the means to induce HR changes (2, 3, 19, 25, 35, 59). Unlike pharmacological or exercise-induced heart changes that enact through systemic mechanisms such as the sympathetic and parasympathetic systems, HR pacing is likely to have fewer confounding effects on the systemic circulation. What is unknown is whether different pacing modalities can lead to different changes in arterial stiffness or wave reflections in response to acute HR changes. In our present study, it was shown that although the HR relationship with cfPWV and AIx were not significantly different between pacing modalities, the changes in the HR relationship with ED, magnitudes of forward and reflected waves, and thus central aortic PP and AP, were different. Furthermore, at constant HR, significant differences were observed between pacing modalities in PPA, AIx, RM, and RI.

With all three modes of pacing, cfPWV increased with increasing HR, with no significant differences in slope observed between the pacing modalities (Fig. 2*A*). Correspondingly, T_i also decreased with decreasing HR. At constant HR, cfPWV was on average 0.8 m/s lower with Ap than with ApVp and Vp (Table 4), although the differences did not reach statistical significance. The increase in cfPWV with increasing HR has been observed previously (2, 19, 25, 29, 35), mostly with a concomitant increase in BP (2, 29, 35). A study correcting for BP-related effects on cfPWV revealed an independent HR effect on cfPWV (61). When compared at baseline

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Table 3. *Estimated values for effect of HR per 10-bpm increase on measured hemodynamic parameters for each pacing modality group*

	Ap		ApVp	Vp
Parameter	Estimate	P^1	Estimate[2]	Estimate[2]
cfPWV, m/s	0.4 (0.3, 0.5)	<0.0001	0.3 (0.2, 0.4)	0.4 (0.3, 0.5)
T_i, ms	−2.1 (−2.8, −1.4)	<0.0001	−3.2 (−3.7, −2.6)	−2.8 (−3.5, −2.2)
AP, mmHg	−3.3 (−3.7, −2.8)	<0.0001	−2.3 (−2.6, −1.9)†	−1.7 (−2.1, −1.2)‡
AIx, %	−4.0 (−4.8, −3.2)	<0.0001	−3.6 (−4.3, −2.9)	−3.2 (−4.0, −2.4)
$\|P_f\|$, mmHg	−2.0 (−2.7, −1.3)	<0.0001	−2.1 (−2.6, −1.5)	−1.1 (−1.7, −0.5)
$\|P_b\|$, mmHg	−1.8 (−2.1, −1.4)	<0.0001	−1.4 (−1.7, −1.2)	−0.9 (−1.3, −0.6)‡
RM, $\times 10^{-3}$	−1.79 (−2.55, −1.04)	<0.0001	−1.33 (−1.94, −0.71)	−1.32 (−2.00, −0.65)
RI, $\times 10^{-3}$	−0.72 (−1.04, −0.40)	<0.0001	−0.57 (−0.83, −0.31)	−0.57 (−0.86, −0.28)
ED, ms	−17.8 (−20.1, −15.4)	<0.0001	−15.0 (−16.9, −13.1)	−13.0 (−15.1, −10.9)†
SV, ml	−6.9 (−8.4, −3.3)	<0.0001	−5.7 (−7.1, −4.3)	−5.1 (−6.5, −3.8)
CO, l/min	0.1 (0.0, 0.2)	0.05	0.3 (0.2, 0.4)	0.2 (0.1, 0.3)
TPR, CGS	9.9 (39.2, 59.0)	0.70	−83.0 (−125.4, −40.5)†	−45.1 (−86.7, −3.5)
bSBP, mmHg	1.0 (0.1, 2.0)	0.04	0.4 (−0.4, 1.1)	1.3 (0.4, 2.1)
bDBP, mmHg	4.1(3.3, 4.9)	<0.0001	3.56 (2.9, 4.2)	3.1 (2.4, 3.8)
bPP, mmHg	−3.1 (−4.1, −2.0)	<0.0001	−3.2 (−4.0, −2.3)	−1.9 (−2.8, −0.9)
cSBP, mmHg	0.1 (−0.8, 0.9)	0.89	−0.1 (−0.8, 0.6)	0.5 (−0.2, 1.3)
cDBP, mmHg	4.4 (3.6, 5.2)	<0.0001	3.8 (3.1, 4.5)	3.2 (2.4, 3.9)
cPP, mmHg	−4.3 (−5.1, −3.4)	<0.0001	−3.9 (−4.6, −3.3)	−2.7 (−3.4, −1.9)†
bPP:cPP	0.06 (0.05, 0.07)	<0.0001	0.06 (0.05, 0.07)	0.05 (0.04, 0.06)
MAP, mmHg	4.1 (3.3, 4.9)	<0.0001	3.6 (2.9, 4.2)	3.3 (2.6, 4.0)

AIx, augmentation index; AP, augmentation pressure; bDBP, brachial diastolic blood pressure; bPP, brachial aortic pulse pressure; bSBP, brachial systolic blood pressure; cDBP, carotid diastolic blood pressure; cfPWV, carotid-femoral pulse wave velocity; CO, cardiac output; cPP, central aortic pulse pressure, cSBP, carotid systolic blood pressure; ED, ejection duration; HR, heart rate; MAP, mean arterial pressure; $|P_f|$, magnitude of forward pressure wave; $|P_b|$, magnitude of backward reflected pressure wave; RI, reflection index; RM, reflection magnitude; SV, stroke volume; T_i, time to first systolic inflection; TPR, total peripheral resistance (CGS units being dyn·s·cm^{-5}); Significant differences in HR trajectories compared with Ap denoted by $*P < 0.05$ compared with Ap; $\dagger P < 0.01$ compared with Ap; $\ddagger P < 0.001$ compared with Ap. [1]Indicates statistical significance of β_1, the estimated HR effect for Ap. [2]Estimates calculated as $\beta_1 + \beta_4$ for ApVp, and $\beta_1 + \beta_5$ for Vp, from the coefficients of the fitted linear mixed model (see Eq. 6).

and at the same HR, however, those with Ap had lower cfPWV but higher brachial and central aortic pressures than those with ApVp and Vp (Table 4). These differences were not statistically different, but the trend was consistent with other studies that have shown that Vp led to lower arterial pressures than Ap (18) and ApVp (35, 62). Furthermore, although there was no difference in the average cfPWV at constant HR between ApVp and Vp, an earlier study demonstrated the apparent

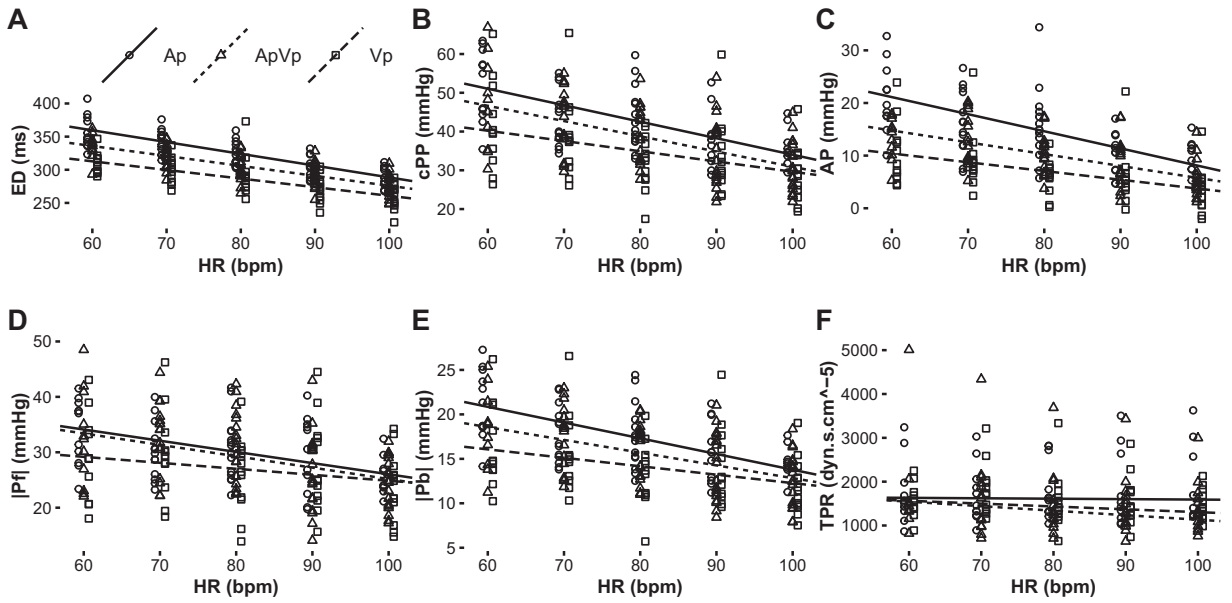

Fig. 1. Scatter plots for parameters that had significantly different heart rate (HR) trajectories with different pacing modalities. *A*: ejection duration (ED). *B*: central aortic pulse pressure (cPP). *C*: central aortic augmentation pressure (AP). *D*: magnitude of forward pressure wave ($|P_f|$). *E*: magnitude of backward reflected wave ($|P_b|$). *F*: total peripheral resistance (TPR). Values for the slopes can be found in Table 3.

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Table 4. *Estimated average differences in measured hemodynamic parameters between pacing modalities at a fixed heart rate*

	ApVp vs. Ap		Vp vs. Ap	
Parameters	Estimates[1]	*P*	Estimates[1]	*P*
cfPWV, m/s	0.8 (−0.4, 2.1)	0.19	0.8 (−0.6, 2.3)	0.24
T_i, ms	−0.7 (−4.2, 2.8)	0.69	−2.4 (−6.3, 1.6)	0.24
AIx, %	−7.8 (−13.5, −2.2)	0.01	−14.6 (−20.9, −8.4)	<0.0001
|Pf| , mmHg	−0.9 (−4.9, 3.1)	0.65	−3.0 (−7.5, 1.4)	0.18
RM	−0.04 (−0.08, −0.00)	0.03	−0.05 (−0.09, −0.01)	0.01
RI	−0.02 (−0.03, −0.00)	0.03	−0.02 (−0.04, −0.01)	0.01
SV, ml	8.9 (−5.6, 23.3)	0.23	−1.0 (−16.3, 14.4)	0.90
CO, l/min	0.7 (−0.4, 1.9)	0.20	−0.1 (−1.3, 1.1)	0.91
bSBP, mmHg	−3.8 (−14.9, 7.3)	0.50	−11.8 (−24.1, 0.6)	0.06
bDBP, mmHg	−0.7 (−7.1, 5.8)	0.84	−5.0 (−12.2, 2.2)	0.17
bPP, mmHg	−3.2 (−9.9, 3.6)	0.36	−6.7 (−14.3, 0.8)	0.08
cSBP, mmHg	−4.6 (−14.5, 5.3)	0.36	−13.0 (−24.0, −1.9)	0.02[2]
cDBP, mmHg	−0.7 (−7.2, 5.8)	0.83	−5.3 (−12.6, 1.9)	0.15
bPP:cPP	0.05 (0.01, 0.09)	0.01	0.09 (0.04, 0.13)	<0.001
MAP, mmHg	−2.8 (−10.6, 5.1)	0.49	−9.4 (−18.2, −0.7)	0.04[2]

AIx, augmentation index; bPP, brachial pulse pressure; cPP, central aortic pulse pressure; RI, reflection index. RM, reflection magnitude. Estimates denote the difference in measured parameters compared with Ap at a fixed HR. Negative values indicate a lower value than Ap. [1]Estimates were denoted by β_2 for ApVp vs. Ap, and by β_3 for Vp vs. Ap from the fitted linear mixed model without the HR·PM interaction term. [2]Although the contrasts were statistically significant, pacing modality did not improve the model with HR as the sole predictor [$\chi^2(6) = 5.41$, $P = 0.07$ for cSBP; $\chi^2(6) = 4.76$, $P = 0.09$ for MAP].

paradoxical increase in cfPWV with a decrease in BP with Vp compared with ApVp (4). As proposed by the investigators, a possible explanation to this observation could be the increased muscle sympathetic nerve activity (SNA) with Vp (62) in response to lower BP due to lower SV (4), resulting in lower arterial distensibility. Although TPR, which has been shown to strongly correlate with muscle SNA (11), was measured in this study, there was no significant association with pacing modality, thus it was not possible to ascertain whether SNA levels differed between pacing groups. Furthermore, although there were negligible differences in TPR between the pacing groups at 60 bpm, the differences increased as HR increased, with TPR decreasing with HR in the ApVp and Vp groups, but relatively unchanged in the Ap group.

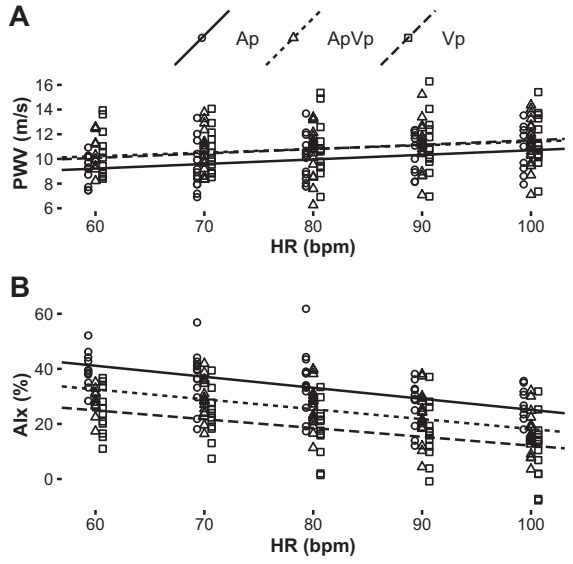

Fig. 2. Scatter plots for carotid-femoral pulse wave velocity (cfPWV) and central aortic augmentation index (AIx) with HR for different pacing modalities. *A*: cfPWV, *B*: AIx.

With increasing HR, diastolic filling time is shortened, leading to a reduction in SV (43, 69) and a decrease in ED (69), as was observed at all pacing modalities in the present study. Because of the large increases in HR induced in this study, CO increased despite the decrease in SV. The decline in ED with HR was steeper with Ap than with ApVp and Vp, with the difference being significant between Vp and Ap. As with previous studies, when compared at the same HR, subjects in the Ap group had longer ED than those with ApVp (76) and Vp (8, 14), with significant differences observed between Ap and Vp at all HRs, and between Ap and ApVp at lower HRs. Although ED has been shown to relate to SV (50, 68), and Vp has been shown to result in lower CO compared with Ap and ApVp due to disruption of ventricular activation sequence and atrioventricular synchrony (27), the differences in SV and CO between pacing modality groups did not reach statistical significance in the present study, whether at the same HR or with changes in HR. This may be due to variability in the measurements, because it was previously shown that the Finapres model flow method for determining CO was not entirely reliable without invasive calibration (52), thus comparison of absolute CO values determined from this method across individuals, and by implication SV, was not recommended.

The decrease in AP with HR in the present study followed a similar pattern as the decrease in ED at all three pacing modalities (Fig. 1*C*), with the steepest decrease in those with Ap, followed by those with ApVp and Vp. Significant differences in the HR trajectories were observed with ApVp and Vp compared with Ap. This was likely due to the reflected pressure wave augmenting the P_f wave during late systole to diastole as a result of reduced ED with HR (5). Similarly, central aortic PP also decreased due to an increase in central aortic diastolic pressure with no change in systolic pressure (Fig. 1*B*), leading to a decrease in AIx with HR at all pacing modalities (Fig. 2*B*). This is consistent with other HR studies

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that have shown a linear decrease in AIx with HR (59, 77, 79). The decrease in AIx with HR, although slightly higher in Ap, then followed by ApVp and Vp (Fig. 2*B*), was not significantly different among the pacing modalities. As such, the HR relationship with AIx would not change when paced HR changes are achieved with a single pacing modality.

Although pacing modality did not affect the relationship between HR and AIx when compared at the same HR, AIx was significantly higher in the Ap group than both ApVp and Vp, as was ED. Moreover, the differences in ED between pacing modalities, in particular at lower HRs (at 60 bpm, ED difference was 23 ms between Ap and ApVp, and 46 ms between Ap and Vp), were much larger compared with the decrease in ED with HR (13 ms/10 bpm with Vp to 18 ms/10 bpm with Ap). With similar heart period and time of arrival of the reflected wave, a reduced ED would result in the reflected wave augmenting the forward wave in late systole to early diastole to a much larger extent than a change in both HR and ED. Figure 3 shows the result of altered forward and backward wave interaction due to changes in ED with different pacing modalities at 60 and 100 bpm. Given the large differences in ED and AIx between pacing modalities at the same HR, particularly at lower HRs, studies concerning the association between HR and AIx should either design the study with HR changes induced by a single pacing modality, or account for the different pacing modalities in their analysis. Previous studies have demonstrated that ED had a higher correlation with AIx than HR (16, 54, 58, 67), and although fitting AIx with ED in place of HR did not improve the model in the present study as quantified by the Akaike Information Criterion (AIC = 1,330.17 with ED, AIC = 1,296.88 with HR), our results further support that the reduction in AIx with HR is related to the change in ED. This effect becomes more evident with large changes in ED in the presence of relatively small changes in HR. It should be noted that the average change in AIx with HR across the three pacing modalities observed in this study was −3.6%/10 bpm, slightly lower than the commonly used correction for AIx at 75 bpm (−4.8%/10 bpm), as implemented in the AtCor Medical SphygmoCor software. This correction was derived from two studies: one of subjects with in situ cardiac pacemakers (−3.9%/10 bpm) with both ApVp and Vp (77), and another study with subjects temporarily paced with Ap (−5.6%/10 bpm) (79). Although the difference in slopes did not reach statistical significance (79), possibly because of the small sample size of both studies (n = 22 and 20, respectively), this nonetheless demonstrated that individuals with permanent pacing may exhibit different changes in AIx with HR compared with those without, indicating that the relationship can be highly variable, and a uniform correction cannot be applied to all populations. As such, it has been suggested that a more appropriate method for correcting AIx for HR would be to include HR as an independent predictor or covariate (60). Furthermore, it has previously been shown that the correlation between AIx and HR was stronger in those with greater cfPWV (45), but the trend observed in the present study was that those with greater cfPWV, those with Vp, showed smaller changes in AIx with HR.

Reduced ED would result in an increase in central to peripheral PP amplification (31, 77) as shown in our results with increased HR and Vp. This is also consistent with the reduction in wave reflection because of a shift in the timing of

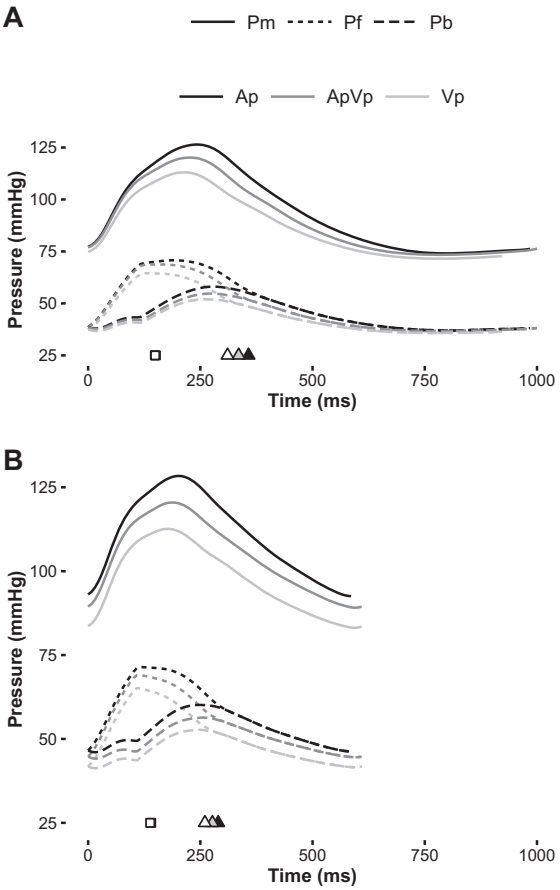

Fig. 3. Averaged aortic wave with calculated forward and backward waves for the three pacing modalities at 60 beats per min (bpm) (*A*) and 100 bpm (*B*). Because of the differences in ED, the backward waves augment the forward waves later in systole as ED decreased, resulting in a smaller AIx. Squares represent time to first systolic inflection (T_i), triangles represent ED [black, atrial pacing (Ap); white, ventricular pacing (Vp); gray, sequential atrioventricular pacing (ApVp)]. T_i remained unchanged with all three pacing modalities but ED was significantly shorter in Vp compared with Ap.

the reflected wave relative to the forward wave (5). However, our results showed that the reduction in wave reflection with the increase in HR is also observed in the magnitudes of the forward and reflected pressure waves. Both $|P_f|$ and $|P_b|$ decreased with HR at all pacing modalities (Fig. 1, *D* and *E*), and may also have contributed to the decrease in AIx with HR (63). Similar to AIx, both RM and RI decreased with increasing HR regardless of pacing modality. Magnitudes of the forward and reflected waves are not affected by confounding effects of timing (71), thus the decrease in RM and RI with HR may be a frequency-related effect, since RM is comparable to the frequency-dependent reflection coefficient (73). Furthermore, when compared at the same HR, despite there being no significant differences in $|P_f|$ and minimal significant differences in $|P_b|$, the magnitude ratios were significantly higher with Ap than with ApVp and Vp, meaning wave reflection was significantly reduced in the latter groups. These observations may be due in part to the different spread of cardiac frequency har-

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monics arising from the differences in ED, and in part to possible attenuation of the reflected waves resulting from decreased impedance mismatch (13), which in the present study, may have resulted from the trend of increased cfPWV with ApVp and Vp. In a recent study by Parragh et al. (46), subjects with reduced ejection fraction (EF) were shown to have significantly lower RM compared with subjects with normal EF. However, statistical significance was lost after correction for HR and ED, indicating that RM was likely influenced by both these parameters, as was observed in our study. Reduced wave reflection because of impedance matching can result in transmission of excessive pulsatile energy to the microvasculature, which can be especially damaging in low-impedance organs such as the brain and kidney (37).

Although it was not the purpose of this study to investigate the functional source of waveform features, throughout this article waveform parameters have been discussed in the context of reflection of waves from discontinuities in impedance, based on the conventional understanding of systemic hemodynamics. A theoretical proposal involving the empirical fitting and subtracting of a pressure component named the reservoir pressure, results in a model of the arterial system that is largely free from wave reflection (66). This theory has been applied to the understanding of the coupling of AIx and left ventricular dynamics, with AIx reducing with dobutamine induced stress, but not associated with wave reflection as evaluated using the reservoir pressure-based analysis (58). However, the various permutations of the proposed reservoir pressure, a theoretically zero dimensional phenomena, behaves as a wave itself, posing a conceptual anomaly in the theory (1, 39, 42, 72). The fitted reservoir pressure also has no correlation with intra-aortic volume (57), confounding the intrinsic assumption behind the theory. Given the current fundamental and practical problems that form the basis of the reservoir pressure theory, reviewed elsewhere more thoroughly (40, 41), the conventional understanding of AIx and T_i as parameters associated with wave propagation have been used in this article.

Limitations. The cohort in this study consisted mainly of elderly male subjects, thus age and gender differences in hemodynamic and arterial responses to acute HR changes could not be investigated. In addition, subjects were heterogeneous in their cardiac function and indication for pacemaker implantation, and the influence of potentially clinical pertinent factors such as left ventricular EF and diastolic dysfunction, were not stratified in the current study. Indeed, it has previously been shown that AIx is higher in women (34, 36, 56) and thus could contribute to the results observed in the present study in which Vp resulted in the lowest AIx, with no women in the Vp group. However, an analysis excluding women still showed a significant difference in AIx between Ap and both ApVp and Vp groups for average HR [Ap vs. ApVp, −8.2 (−15.0, −1.4), $P = 0.02$; Ap vs. Vp, −15.4 (−22.5, −8.4), $P = 0.0001$]. Furthermore, all subjects with Vp presented with atrial fibrillation (AF), which can significantly affect hemodynamics (55) and has been shown to be correlated with higher PWV (28). Thus, it cannot be discounted that AF may have been a contributing factor to the Vp group differences. As such, conclusions drawn from this study cannot be directly extrapolated to the general population nor beyond the studied HR range. Furthermore, responses to acute changes in HR by means of cardiac pacing may not be reflective of long-term changes caused by tachycardia.

In the present study, wave separation analysis for determination of wave reflection parameters were based on an estimate of the flow waveform using a triangular wave shape rather than with a measured flow wave. However, this method of estimation has been shown to correlate highly with parameters derived from direct measurements of pressure and flow (71).

Changes in hemodynamics and wave reflection parameters in response to HR changes for each pacing group may not be representative of the long-term effects of cardiac pacing with different pacemaker modes. In this study cohort, subjects in the Ap and ApVp groups had their pacemaker modes set to either AAI/DDD (AAI, atrial-sensed, atrial-paced pacemaker) or DDD, and proprietary algorithms were implemented in the pacemakers to reduce occurrence of Vp. Some subjects who had Ap at baseline switched to ApVp at higher HRs, thus changes observed in this study may be a combination of both long-term effects of pacing as well as acute effects dependent on the cardiac site(s) being paced. We did not account for atrioventricular delays for subjects with pacemakers set at DDD, which has been shown to affect hemodynamic responses to pacing, in particular BP (74, 75). Although long-term Vp in the right ventricular apex is known to result in increased incidence of myocardial perfusion and dysfunction (65), whether these adverse effects contributed to the increasing trend in arterial stiffness and reduced wave reflection as observed in the present study could not be determined. However, differences in various parameters already observed at baseline may indicate, at least in part, long-term effects of different modalities of cardiac pacing.

Finally, factors other than ED that could influence wave reflections were not investigated. Thus, although the changes in wave reflections observed in the present study were consistent with reduced ED, there may be other hemodynamic differences between pacing modalities that influenced the wave reflection parameters.

Conclusions. The present study demonstrates that pacing from different cardiac sites exhibited varying effects on central aortic hemodynamics, arterial stiffness, and wave reflection with acute changes in HR. Although the changes with HR in cfPWV and AIx, the common indices for large artery stiffness and central systolic loading, respectively, were unaffected by pacing modality, pacing modality significantly influenced wave reflection indices at a fixed HR level. The main driving factor behind differences in wave reflection parameters among pacing modality groups can be attributed to differences in ED. Thus, further studies that employ cardiac pacing as means to induce HR changes should either be designed to induce HR changes with a single pacing modality, or account for it in the analysis. In particular, for wave reflection studies in which large changes in ED are present, correction for wave reflection parameters may need to be made for ED rather than HR.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

I.T., M.B., and A.P.A. conception and design of research; I.T. and M.B. performed experiments; I.T. analyzed data; I.T., M.B., and A.P.A. interpreted results of experiments; I.T. prepared figures; I.T. drafted manuscript; I.T., H.K., E.B., M.B., and A.P.A. edited and revised manuscript; I.T., H.K., E.B., M.B., and A.P.A. approved final version of manuscript.

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Appendix D (pp. 236-244) of this thesis has been removed as it contains confidential material.

APPENDIX D Ethics approval for all research conducted in this thesis

This appendix contains the final ethics approval for the research presented in Chapters 3, 4, 5 and 7.

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