Aorto-Coronary Haemodynamics:

Studies in Cardiovascular Magnetic Resonance

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‘it’s all about the journey…’

TABLE OF CONTENTS

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Adam James Nelson

January 2019

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To Professor Stephen Worthley, thank you for your eternal optimism and continued belief in me. I deeply admire your vision and ambition and leave each of our meetings with renewed energy and drive. Although exceptionally busy, you always made time to talk, and more recently have been instrumental in helping to plot the next steps in my career. Thank you for your friendship and counsel.

Joining the Cardiovascular Research Centre as a medical student, I was spoilt by the continuous supply of PhD or post doc students who would work in the office in Level 6 of the old RAH. It was watching these individuals I aspired to be a clinician-scientist. In particular I would like to acknowledge the friendship, camaraderie and humour of Dr Rishi Puri as well as the support and mentorship of Dr James Richardson, Dr Ben Dundon, A/Prof Dennis Wong and Dr Sam Sidharta. I am particularly grateful to Dennis for his assistance with *Chapter 5*.

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4. Arterial stiffness predicts invasive coronary flow reserve. **Nelson AJ**.
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   (in submission)

ABBREVIATIONS

AA	Ascending aorta
ACE	Angiotensin converting enzyme
AGE	Advanced glycation end-products
AIx	Augmentation index
AP	Augmentation pressure
APV	Averaged peak velocity
aPWV	Aortic pulse wave velocity
ARB	Angiotensin II receptor blockers
baPWV	Brachial-ankle pulse wave velocity
bCBF	Basal coronary blood flow
bPP	Brachial pulse pressure
CBF	Coronary blood flow
CCB	Calcium channel blockers
cfPWV	Carotid-femoral pulse wave velocity
CFR	Coronary flow reserve
CFVR	Coronary flow velocity reserve
CKD	Chronic kidney disease
CMR	Cardiovascular magnetic resonance
CMR-DE	Cardiovascular magnetic resonance – delayed enhancement
CMR-PI	Cardiovascular magnetic resonance – perfusion imaging
cPP	Central pulse pressure
CSVD	Cerebral small vessel disease
CT	Computed tomography
CTO	Chronic total occlusion
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DDA	Distal descending aorta
DPP-4	Dipeptidyl peptidase-4
eGFR	Estimated glomerular filtration rate
ECM	Extracellular matrix

EDHF	Endothelial-derived hyperpolarising factor
FWA	Forward wave amplitude
GLP-1	Glucagon like peptide-1
GTN	Glyceryl trinitrate
GWAS	Genome-wide association studies
hCBF	Hyperaemic coronary blood flow
HDL	High density lipoprotein
HFHS	High fat high sucrose
HFpEF	Heart failure with preserved ejection fraction
IVUS	Intravascular ultrasound
LAD	Left anterior descending coronary artery
LCx	Left circumflex coronary artery
LDL	Low density lipoprotein
LOX	Lysyl oxidase
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVEDV	Left ventricular end diastolic volume
LVESV	Left ventricular end systolic volume
LVH	Left ventricular hypertrophy
MAP	Mean arterial blood pressure
MI	Myocardial infarct
MMP	Matrix metalloproteinase
MPR	Myocardial perfusion reserve
MPRI	Myocardial perfusion reserve index
NO	Nitric oxide
PC	Phase contrast (magnetic resonance imaging)
PCI	Percutaneous coronary intervention
PDA	Proximal descending aorta
P_{ex}	Excess pressure
PET	Positron emission tomography
PP	Pulse pressure

PPAR	Peroxisome proliferator-activated receptor
P_{res}	Reservoir pressure
PVR	Pulmonary vascular resistance
PWA	Pulse wave analysis
PWV	Pulse wave velocity
QCA	Quantitative coronary angiography
RCA	Right coronary artery
rCBF	Resting coronary blood flow
RPP	Rate pressure product
RWA	Reservoir wave analysis
SBP	Systolic blood pressure
SGLT-2	Sodium-glucose co-transporter 2
SSFP	Steady state free precession
TA	Thoracic aorta
TAC	Total arterial compliance
TG	Triglycerides
TNF	Tumour necrosis factor
TOE	Transoesophageal echocardiogram
VLA	Vertical long axis
VSMC	Vascular smooth muscle cell
WMLs	White matter lesions
Zc	Characteristic impedance

THESIS STRUCTURE

This thesis represents an evolution of ideas originating from our group's earliest exposure to vascular dynamic imaging almost 10 years ago. The final narrative has been an interrogation of the relationship between aortic stiffness and coronary blood flow through invasive and non-invasive means, in both health and disease.

As per the University of Adelaide guidelines, the format of this thesis is 'expanded publication'. This has allowed a more expansive description of methods and results than has been included in submitted manuscripts. Given the interrelationship of techniques across *Chapters*, a concerted attempt has been made to summarise and reference back to earlier *Chapters* where appropriate thereby avoiding large sections of repetition. Similarly, given the common content of concluding remarks across the publications, a deliberate effort was made to keep discussion focused on the key outcomes of each individual publication, and then to provide more encompassing comments as a separate Chapter across the body of work.

SYNOPSIS

Large artery stiffness has been shown to independently predict cardiovascular events and all-cause mortality in a broad range of cohorts. Despite its description almost half a century ago, the cardiovascular research community continues to grapple with a robust model for ventriculo-vascular interactions, let alone a sound basis for its predictive strength. A mechanistic understanding of the key sequelae arising from increased arterial stiffness is likely to provide instructive pathophysiological insight, but also pave the way for novel risk stratification and possible therapeutic targets. One powerful, putative effect of increased arterial stiffness is a reduction in coronary blood flow, however a reproducible, simultaneous and non-invasive methodology to evaluate both has been elusive. This thesis examines the relationship between myocardial perfusion and arterial stiffness.

In *Chapter 2*, invasive assessment of both coronary blood flow and coronary flow (velocity) reserve was undertaken with Doppler FloWires and then compared with cardiovascular magnetic resonance (CMR) derived assessment of arterial stiffness, aortic distensibility. In a cohort of subjects with a favourable traditional risk factor profile and no significant angiographic disease, aortic distensibility was linearly associated with measures of both resting and hyperaemic coronary blood flow as well as coronary flow velocity reserve. Moreover, aortic stiffness was associated with a reduced response to adenosine. The validation of this relationship was completed in non-invasive methodology by using CMR perfusion imaging (CMR-PI) to evaluate indices of myocardial blood flow and myocardial perfusion reserve in *Chapter 3*. In subjects with normal perfusion studies, aortic distensibility was again strongly correlated with resting and hyperaemic myocardial blood flow as well as myocardial perfusion reserve.

After evaluating 'normal' individuals in earlier studies, the later cohorts focused on disease states. In *Chapter 4,* the behaviour of this relationship in ischaemic and non-ischaemic myocardium was evaluated in a highly selected group with a single perfusion defect on CMR-PI confirmed on subsequent coronary angiography. In this group, arterial stiffness (once again, evaluated by CMR derived aortic distensibility) remained associated with resting and hyperaemic blood flow despite critical epicardial coronary disease.

In *Chapter 5,* subjects who had received primary percutaneous coronary intervention for acute ST elevation myocardial infarction (STEMI) were evaluated with CMR perfusion and delayed enhancement imaging on day three and again at three months. The myocardium was dichotomised to infarct and non-infarct territories with blood flow, perfusion reserve and indices of myocardial injury evaluated on a per segment basis. In this cohort, arterial stiffness was associated with reduced *early* and *late* hyperaemic response to adenosine in both infarct and non-infarct myocardium. On multiple linear regression aortic distensibility remained an independent predictor of improvement in myocardial perfusion reserve suggesting arterial stiffness may be a key feature for worse outcomes post STEMI.

This thesis not only confirms the putative relationship between large artery function and coronary blood flow but also establishes CMR-PI as a reproducible and robust modality in this space. Non-invasive evaluation of the aorto-coronary haemodynamic relationship may provide novel insight, not only as a potential clinical surrogate endpoint, but also a manner to evaluate therapeutic efficacy.

REVISIONS

I am grateful for the opportunity to revise aspects of the thesis following external assessment. Each of the points raised by both learned reviewers have been adopted, edited or included in this 'final' submission which has improved the quality of the work substantially.

In particular, clarity has been provided on the terminology around *increased* arterial stiffness, *functional* arterial stiffness and global prevalence sentiments. Additional references have been included with respect to the history of pulse pressure, adenosine effects on epicardial calibre and assessment of ascending aortic distensibility using transthoracic echocardiographic imaging.

Furthermore, the suggestions for tightening up the statistical description in each of the chapters has been heeded. Semantic and typographical errors have all been gratefully highlighted and amended.

I'd like to take the opportunity to thank both reviewers for their thorough and considered assessment of this thesis.

1. CHAPTER 1: Large arteries, arterial stiffness and coronary blood flow

Arterial stiffness: a review, and impact on coronary blood flow

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1.1 STATEMENT OF AUTHORSHIP

Manuscript details

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Principal Author Contributions

Candidate	Dr Adam J Nelson
Contribution to the Paper	Primary contributor to the conception and design of the work; Drafted the work; Provided final approval of the version to be published; Accountable for all aspects of the work.
Overall percentage	90%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

August 2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	A/Prof Matthew I Worthley
Contribution to the Paper	Helped drafting the work and revising it critically; Accountable for all aspects of the work

August 2018

Co-Author Contributions (continued)

Name of Co-Author	Prof Stephen G Worthley
Contribution to the Paper	Helped drafting the work and revising it critically; Accountable for all aspects of the work

August 2018

1.2 ABSTRACT

Large artery stiffness is an established mediator of cardiovascular disease and is now a recognised, independent predictor of both cardiovascular events and all-cause mortality. In this *Chapter*, the anatomy and physiology of large arterial function is discussed whilst summarising some of the key models and constructs that underpin these ideas. Common definitions and measures of arterial stiffness are outlined as are potential sequalae which may explain its mechanistic, integrative strength. The extent to which arterial stiffness may be modified by pharmacological and lifestyle therapy is also briefly reviewed. Finally, given the purported aorto-coronary relationship, measures and determinants of coronary blood flow are examined with a particular focus on both invasive and non-invasive measures covered in the body of this work.

1.3 THE LARGE ARTERIES

1.3.1 Physiology

Arteries have two main functions. The first is to transform pulsations generated by the high pressure of the left ventricle into near smooth, continuous flow at a capillary level, thereby performing a *cushion* function. The second is to deliver blood from the left ventricle to tissues and organ systems in an ordered manner by need, thereby performing the role of a *conduit*.

Elegant work in animals, largely canines, has demonstrated the efficiency of a young, healthy arterial system in both its cushion and conduit function. From a cushion perspective, arterial pulsations are effectively dampened in the larger arteries such that pulsatile flow is rarely seen in arterioles and capillaries of less than 200 micron diameter (Nicholls & O'Rourke 2005). Despite achieving this marked dampening of the pulse, there is minimal loss of energy with a fall of less than 5mmHg of mean pressure observed from the proximal aorta to a resistive arteriole (Christensen & Mulvany 2001). The net result is a progressive reduction of pulsatility through the arterial tree to the level of the micro-circulation of high flow renal and cerebral vascular beds minimising barotrauma that would result from exposure to pulsations at high systolic pressures.

Conduit function is dynamically mediated largely through shear stress and endothelial vasomotor control and characterized by low resistance to flow (Mulvany & Aalkjaer 1990). In contrast, the cushion function of large arteries is dependent on the structural, viscoelastic properties of the wall in addition to their diameter and length (Safar 1996).

1.3.2 Anatomy

Arteries throughout the human body consists of three walls; tunica adventitia, tunica media and tunica intima. The intima has a monolayer of endothelial cells which provides a lumen/wall interface and is a dynamic organ. The main components of the tunica media are elastin, collagen and smooth muscle. As mentioned, the large arteries

are setup for their cushion function by their structural composition which has a relatively high proportion of elastin compared to more distal, muscular arteries. As one moves distally, there is a progressive reduction in the elastin-collagen ratio– elastin representing 60% at the arch but only 20% at the abdominal aorta. This in part, forms the anatomical basis for the transition between the predominantly 'cushion' and reservoir function of the large arteries and the predominantly 'conduit' function of the more distal, muscular arteries.

1.3.3 Models

1.3.3.1 Windkessel

A variety of models have been proposed to understand the complexity of the cushion and conduit functions. The oldest and most primitive is the Windkessel model, conceived by Hales (Hales 1964) and developed by Frank (Frank 1899) named from the air filled dome seen on old fire engines that transformed pulsatile flow from a pump into a steady stream at the end of the fire hose. This 'lumped' model *(Figure 1.1)*, so named as it groups the circulation in terms of parallel resistance and capacitance components; the resistance corresponding to total peripheral vascular resistance (PVR, predominantly at the arteriolar level), and a total arterial compliance (TAC, mostly due to the large elastic arteries) (Westerhof & Stergiopulos 2000). This model suggests that as the left ventricle contracts, the elastic proximal arteries become distended and at the end of systole at aortic valve closure, blood is discharged to the periphery at a rate determined by PVR and TAC. Dichotomising resistance and compliance, although conceptually attractive, is limited as these functions are often interrelated across the entire vascular bed. Moreover, the arterial series of branching networks is oversimplified by the assumption that pressure change occurs instantaneously.

The addition of Fourier analysis to simultaneous pressure and flow signals allowed the calculation of input impedance – a measure of the resistance to flow, and a function of the proximal aorta subsequently termed characteristic impedance (Zc). The addition

of this 'third' element to the Windkessel model included components of wave theory as Zc is defined by wave speed times blood density divided by cross sectional area. The three-element Windkessel (Stergiopulos et al. 1995) remains the prevailing model in the literature and although a fourth element, inertance has been added by some (Burattini & Gnudi 1982; Stergiopulos et al. 1999), the three element remains conceptually attractive.

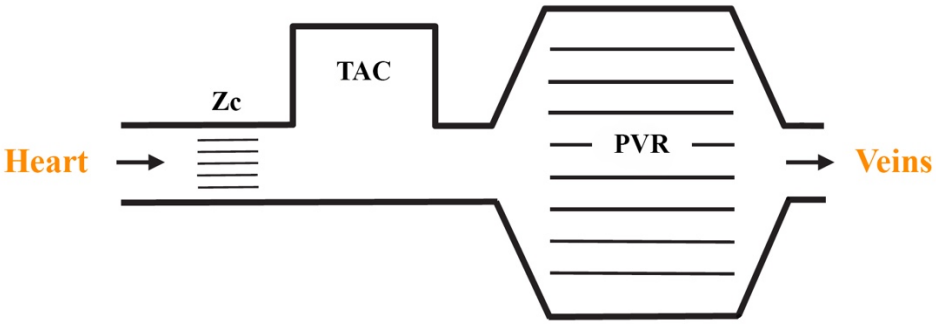

FIGURE 1.1 *Three-element Windkessel model. Zc – characteristic aortic impedance; TAC – total arterial compliance; PVR – peripheral vascular resistance*

1.3.3.2 Wave propagation

Wave propagation is a component of arterial haemodynamic theory that provided some of the basis for distributive models of arterial function. The simultaneous measurement of central and peripheral arterial waveforms in young healthy individuals reveals a lower central arterial systolic blood pressure compared to brachial systolic blood pressure whilst mean arterial and diastolic blood pressure tend to be consistent at both sites. This phenomenon was termed pulse pressure amplification and can be explained by wave reflection and propagative theory. In its most basic form, the ejection of blood into the proximal arterial tree, largely the proximal aorta, generates a pressure wave that propagates throughout the body. At points of structural or functional discontinuity,

specifically the transition between low resistance arteries to high resistance arterioles, reflection of this pressure wave occurs in a retrograde fashion towards the aortic valve *(Figure 1.2)*. This is perhaps not unexpected as the arterial system top to bottom is only 1-2m in length and yet the pulse wave travels between 4 and 15m/s between the proximal aorta and the peripheral arteries respectively (O'Rourke et al. 2014). As observed in a young vascular tree, this net reflected wave arrives following aortic valve closure. Furthermore, the arrival of this net reflected wave within the proximal aorta at diastole provides additional efficiency by augmenting coronary perfusion pressure – an event that largely occurs during diastole alone. Supportive ideas progressed by Taylor, O'Rourke and others with the use of frequency domain, or Fourier, analysis suggest the young cardiovascular system is optimally 'tuned' for this interaction – not only by dispersion of peripheral reflecting sites but also by the inverse relationship between heart rate and body length – variables that preserve this optimal ventriculo-vascular interaction and timing of the reflected wave.

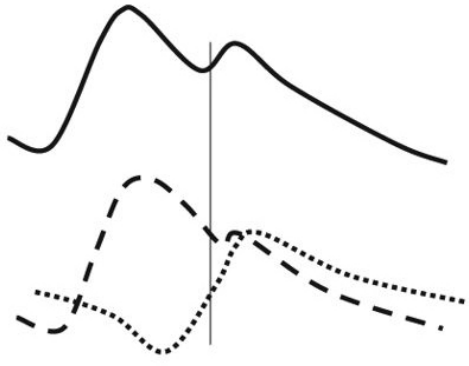

FIGURE 1.2 *Redrawn and adapted (Georgianos et al. 2018). Forward travelling wave in dashed line, reflected wave in dotted line and observed pulse wave in solid line.*

1.3.3.3 Reservoir wave analysis

Evolution of the 'single' reflected wave propagation theory came in the form of reservoir wave analysis. Reservoir wave analysis (RWA) was initially proposed in 2003 and is fundamentally based around Windkessel modelling and on the premise that measured pressure (P) is considered to be the sum of two independent components: a reservoir pressure (P_{res}) which relates to arterial compliance and is consistent throughout the large arterial system but relative to the wave properties of the arteries; and an excess pressure (P_{ex}) which is the difference between the total pressure waveform and the reservoir pressure waveform *(Figure 1.3)*.

A key assumption in RWA is that P_{res} is the net product of innumerable forward and backward waves rather than a net, 'single' reflected wave from peripheries. These derive from the forward travelling waves that are generated in the aortic root by the stroke volume from the left ventricle and undergo modification as they travel through the arterial circulation by reflection and re-reflection. Geographically disparate and of varying amplitude, these incident and reflected waves are therefore in constant interaction throughout the cardiac cycle and along the arterial tree.

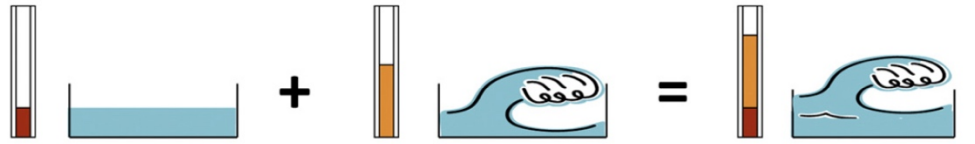

Figure 1.3 *Adapted from Tyberg (Tyberg et al. 2014). Observed pressure can be directly measured, P_{res} can be mathematically modelled and then P_{ex} calculated. Areas under the reservoir and excess pressure curves as well as their amplitudes*

This provides an explanation for a key shortcoming of the single wave reflection theory which had to provide the assumption that an equally large reflected wave returned in late diastole to 'cancel' the forward wave and thus, exponentially reduced pressure and flow (Tyberg et al. 2014). This lacks a physiological backing however as these waves are not visible during ectopic beats where they ought not have had time to decay. The reservoir wave pressure heuristic however, suggests the pressure observed in diastole is solely due to P_{res} which fits with the observation that P_{ex} during diastole approaches zero (Hughes, A et al. 2012).

The field of arterial mechanics remains vigorously debated in the literature with variations on multiple-element Windkessels and Fourier impedance modelling all providing alternate explanations of this complex physiology (Mynard & Smolich 2014).

1.4 ARTERIAL STIFFNESS

1.4.1 Background

Over the last 20 to 30 years of cardiovascular research, arterial stiffness has evolved from being an association to an independent predictor, and now to an established risk factor and mediator for a number of cardiovascular, renal and neurological diseases. Analogous in some ways to the haemoglobin A1c of diabetes, arterial stiffness appears to be a barometer for vascular health being the key interaction between inexorable ageing and vascular risk factors. Rates of increased arterial stiffness grows non-linearly and variably with age, demonstrating minimal change in the first 30 years of life (McEniery et al. 2005; Mitchell et al. 2010; Mitchell et al. 2004) but notably accelerates such that its prevalence approaches 70% by 70 years of age (Mitchell, Guo, Benjamin, et al. 2007). Given the high rates of increased stiffness in the elderly and an overall population that is ageing, together with greater life expectancy in developing nations, direct and indirect sequelae of arterial stiffness are likely to become increasingly burdensome on a global scale.

1.4.2 Definitions

Extrapolated from Hooke's Law in the setting of physics, stiffness is the relationship between a force applied to a material and its resultant deformation. Assessment therefore requires simultaneous measurement of both observed deformation and subjected force. In a cylindrical structure such as an artery, the force applied is three dimensional (longitudinal, radial and circumferential) and termed 'stress' with the resultant physical deformation termed 'strain'. This critical relationship between stress and strain defines an artery's stiffness with its inverse termed, 'elasticity'. Compliance or distensibility represent changes in volume related to changes in pressure, either absolute or normalised to initial volume, respectively. Applying Newton's second law of wave theory to a vessel wall, Moens and Korteveg (Korteveg 1878; Moens 1877) derived and later simplified by Bramwell and Hill (Bramwell & Hill 1922), that a

pressure wave's propagation speed in a non-compressible, non-viscous fluid is proportional to its stiffness. This observation spawned modern day interest in arterial haemodynamics and provided the foundation for the non-invasive 'gold standard' assessment of arterial stiffness today, carotid-femoral 'Pulse wave velocity'.

1.4.3 Measures of arterial stiffness

Arterial stiffness can be assessed using a variety of techniques. These broadly fall into general, systemic measures of compliance and wave reflection (e.g. pulse pressure, augmentation index), regional measures (e.g. pulse wave velocity) and local measures (e.g. distensibility and compliance) (Mackenzie et al. 2002).

1.4.3.1 Blood pressure

Flow and pressure are pulsatile and intrinsically linked to the interaction between the ventricle and the vasculature – so called, ventriculo-vascular coupling. The most clinically apparent aspect of this pulsatile interaction is blood pressure which has remained a cornerstone of every clinical cardiovascular evaluation for well over 100 years. Seminal work by Mahomed in the late 1880s not only described the pressure pulse with a sphygmogram he developed as a medical student, but also linked elevated blood pressure to the development of nephrosclerosis (O'Rourke 1992). It is now well established that non-invasive blood pressure obtained at the brachial artery is associated with cardiovascular events and mortality (WHO 1999). Although there has been varying interest in different aspects of the standard brachial blood pressure 'measurement' - initially systolic (Gubner 1962), then diastolic blood pressure (Kannel et al. 1971) - pulse pressure remains the most convenient, yet crude measure of pulsatile haemodynamics. Brachial pulse pressure (PP) increases with age, particularly in the 6th decade, and is more tightly linked to cardiovascular risk in this age-group than other metrics of the non-invasive blood pressure reading (Benetos et al. 1998; Benetos, Safar, et al. 1997; Franklin et al. 1999; Gasowski et al. 2002). As

described, there remains significant contention around the dominant process that contributes to increased pulse pressure with age. The prevailing view remains that a faster pulse wave generates earlier reflected waves, increasing systolic pressures and augmenting PP. A more recent paradigm, mainly driven by Framingham cohort observations, is that increased forward wave amplitude (FWA) is generated by aortic characteristic impedance – i.e. a mismatch between aortic root properties and peak aortic flow (Cooper et al. 2015; Torjesen et al. 2014).

Regardless of its exact mechanistic basis, guidelines currently suggest that a brachial PP of >60mmHg conveys increased risk for asymptomatic end organ damage in the elderly (Dart & Kingwell 2001; Mancia et al. 2013) and overall cardiovascular disease (Franklin et al. 1999; Mitchell et al. 1997).

Beyond pulse pressure, central aortic blood pressure is widely acknowledged as being haemodynamically and physiologically more relevant as it is the site of systolic left ventricular afterload and the origin of coronary diastolic perfusion. Invasive assessment, however is impractical for widespread use and thus attempts at correcting a peripheral waveform are attractive and have been used as endpoints in clinical trials (see below).

1.4.3.2 Pulse waveform analysis

As described, the arterial waveform is the net outcome of the interaction between the forward pressure waveform created by the ventricular stroke volume and reflected waves *(Figure 1.4)*. Augmentation of central blood pressure by the return of these waves from the peripheral circulation can be quantified by pulse wave analysis (PWA) and the calculation of an augmentation index (AIx). Initially described in the 1980s (Karamanoglu et al. 1993; Kelly, R et al. 1989), measurement involves discerning a shoulder on the upstroke of the pulse wave which corresponds to the forward wave (P1). The merging of the forward wave with the incoming reflected wave then generates an inflection point. A second systolic peak (P2) is observed as the maximum

effect of the reflected wave on the central pressure contour. The degree of 'augmentation' can be calculated mathematically by the equation P2 – P1 and is termed Augmentation Pressure (AP) (Davies, JI & Struthers 2003). Augmentation pressure, when expressed as a ratio to pulse pressure, (AP/PP) is called Augmentation Index (AIx) (Mackenzie et al. 2002).

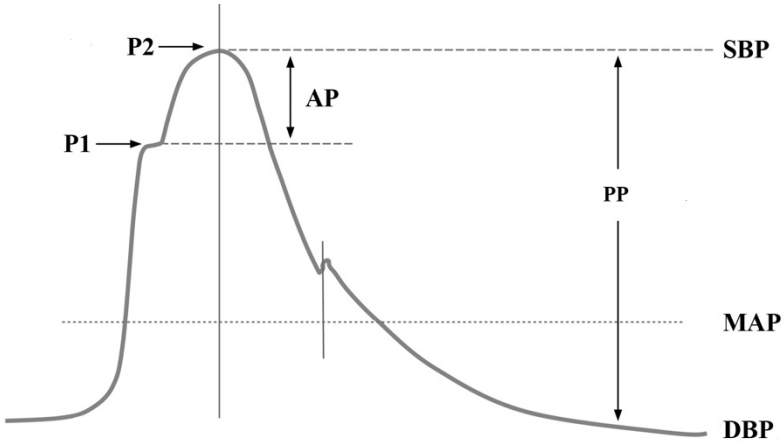

FIGURE 1.4 *Arterial pressure waveform allowing calculation of augmentation index (AP/PP). AP – augmentation pressure; PP – pulse pressure; SBP - systolic blood pressure; DBP – diastolic blood pressure.*

Augmentation Index depends on the timing of the reflected waves, and thus pulse wave velocity and arterial stiffness in addition to the amount of wave reflection which is largely governed by resistance of peripheral arteries. A large body of evidence demonstrates AIx increases with age (Nicholls & O'Rourke 2005) and is elevated in patients with diabetes (Wilkinson et al. 2000), dyslipidaemia (Wilkinson et al. 2002) and hypertension (Nicholls & O'Rourke 2005). In the large community-based Multiethnic Study of Atherosclerosis (MESA), PWA was performed in 5960 participants and followed for a median of 7.6 years (Chirinos et al. 2012). After adjustment for traditional risk factors, AIx was independently predictive of cardiovascular events (HR for 10% increase 1.08; 95%CI 1.01 – 1.14, p = 0.016) however, reflection magnitude (the ratio of reflected to forward wave amplitude) was a stronger predictor (HR for 10% increase 1.34; 95%CI 1.08 – 1.67, p = 0.009).

The central aortic waveform is inaccessible in routine clinical practice and thus there has been considerable interest in acquiring a peripheral waveform and reconstructing its constituents to not only estimate aortic pressures but also generate a central aortic waveform. This is most often performed at the radial artery as it is easily accessible and compressible, requires minimal expertise and is reproducible (Wilkinson et al. 1998). A number of transfer functions have been described (Chen, CH et al. 1997; Hope et al. 2003; Pauca et al. 2001) and demonstrate robust validity against aortic pressures (Hope et al. 2008) using different modelling and mathematical techniques (Fetics et al. 1999). Nonetheless, a significant degree of contention in the literature exists about how generalizable the radial waveform is to the central waveform (O'Rourke 2007) not only because the properties of the upper limb vasculature are not constant in all ages (Millasseau et al. 2002) or can be variably affected by vascular disease (McLeod et al. 2004), but also because they are exposed to a single, fixed mathematical equation. Whether the transfer function successfully reproduces the central waveform or not, radial PWA clearly has predictive capacity (Hope et al. 2008) and may instead simply be an integrated measure of arterial stiffness, in and of itself.

1.4.3.3 Pulse wave velocity

As Bramwell and Hill applied Moens and Korteveg's equations, the speed of propagation of the pulse wave (pressure or flow) in an artery is directly related to the stiffness of the vessel. Simply put, this can be assessed by dividing the distance a pulse wave travels by its transit time *(Figure 1.5)*. Pulse wave velocity (PWV) is generally accepted as the most simple, non-invasive and robust method for determining arterial stiffness. Although theoretically assessable in any blood vessel, carotid-femoral PWV earned the label of the 'gold standard' modality from the 2006 consensus statement (Laurent et al. 2006) and has remained so in subsequent statements (Townsend et al. 2015).

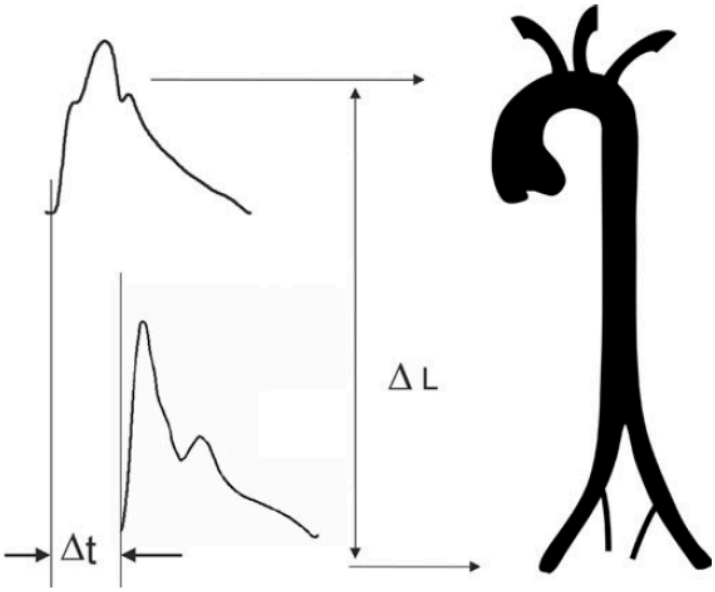

FIGURE 1.5 *Adapted from Laurent (Laurent et al. 2006) demonstrating concept behind PWV calculation. Carotid waveform at top, femoral waveform at bottom. Estimation of length, or distance, is a point of contention mentioned in text.*

From a cardiac frame of reference, the aorta and its most proximal vessels are exposed to the largest amount of pulsatile energy and as described, provide a dominant contribution to the arterial cushion function. Thus, assessment of the pulse wave velocity in these vessels would likely provide the most information on elastic or cushion arterial function, and thus earlier demonstrable changes from increasing arterial stiffness. Given the carotid to femoral distance approximates the aortic 'path', carotid-femoral PWV is occasionally (albeit erroneously) referred to as aPWV, and although they are highly correlated (Podolec et al. 2007), aPWV has traditionally been measured invasively (Lim et al. 2004) whereas cfPWV is non-invasive. Brachial-ankle PWV, described mainly in Asian populations until recently, likely represents a composite of both elastic and muscular artery interaction but is strongly correlated to cfPWV and has similar prognostic capacity (Ninomiya et al. 2013; Ohkuma et al. 2017; Sheng et al. 2014; Turin et al. 2010). Femoral-ankle or femoral-tibial however, appears of less prognostic value as it assesses muscular rather than elastic vessel function (Meyer et al. 2016).

Measurement of cfPWV involves recording of pulse waves at the common carotid and the femoral artery using pressure sensors (mechanotransducers or applanation tonometers) or Doppler ultrasound. The transit time can be measured directly using simultaneous recordings at both carotid and femoral sites, or alternatively can be derived from the ECG if waveforms are acquired sequentially. Exactly what point constitutes the 'foot' of the pulse wave varies between algorithms and devices, although the most widely used is the intersecting tangent algorithm. The other points of contention are the calculation of travel distance (which is usually assimilated to the surface distance between the two recording sites) and how readily carotid-femoral PWV approximates 'true' aortic PWV. Carotid-femoral PWV involves the detection of two waves – one heading in a cephalad (carotid) and the other in a caudal direction (femoral) whereas true 'aortic' PWV ought simply be antegrade flow. This complicates the exact determination of not only transit time but also distance. Cardiac magnetic resonance imaging which utilises phase-contrast (PC) sequences are able to generate flow-time curves at multiple sites and thus can measure directly within the aortic course. With multi-planar and cross-sectional imaging afforded by CMR, an exact measure of transit distance can be made. Phase contrast imaging remains at risk of noise and sampling error which hinders an accurate determination of the 'foot' or 'peak' of the pulse wave which is further compounded by the lack of commercially available software or standardised guidance (Wentland et al. 2014). Correlation studies of cfPWV with aPWV assessed by both CMR (Huybrechts et al. 2011) and invasive (Weber et al. 2011) methods have demonstrated a consistent overestimation by around 2m/s and thus a correction is made to the carotid-femoral distance. This is achieved either by subtracting 20% off the surface distance (Van Bortel et al. 2012) or by subtracting the suprasternal notch-carotid distance from the suprasternal notch-femoral distance (Weber et al. 2009). Inherent limitations to either correction persist when there is abdominal obesity or large volumes of breast tissue (Van Bortel et al. 2002). A variation on the concept of PWV involves evaluating the 'QKD' interval

which is the time between the onset of the QRS on the ECG, and the last Korotkoff sound at the brachial artery. Although initially described in the 1990s (Gosse et al. 1994), a large longitudinal study has confirmed its independent prognostic capacity for CV events (Gosse et al. 2013).

The growth of cfPWV as the 'gold standard' has not only resulted in the development of reference values (Arterial Stiffness Reference 2010) but also appears in the current (due for updating in 2018) ESC guidelines for hypertension prognostication (Mancia et al. 2013) where a cfPWV of >10m/s carries a high risk of both subclinical end organ damage and cardiovascular events.

1.4.3.4 Distensibility

Compared to more general, indirect measures such as cfPWV, arterial distensibility is a direct, local measure of a vessel's stress/strain relationship. Distensibility evaluates the normalised change in arterial diameter (or cross-sectional area) in relation to distending pressure and has been widely used in a variety of vascular territories including the carotid (Barenbrock et al. 2002; Dijk et al. 2005; Paini et al. 2006), brachial (Urbina et al. 2002), radial (Giannattasio et al. 1999), aorta (Groenink et al. 1998; Metafratzi et al. 2002) and recently, the pulmonary arteries (Ray et al. 2018).

1.4.3.4.1 Ultrasound/echocardiography

Ultrasound has historically been the modality of choice in view of its ability to measure superficial arteries with excellent temporal resolution. The spatial resolution however, can limit the detection of small luminal diameter changes which, in addition to operator dependence, can limit reproducibility particularly with serial evaluations. Although the ascending aorta can be visualised with transthoracic windows (Sabia et al. 2018), the majority of the thoracic aorta is only visible with transoesophageal echocardiography (TOE). Although extensively published in recent years by Nemes et al. (Nemes 2008; Nemes et al. 2012; Nemes, Forster & Csanady 2008; Nemes, Forster,

Geleijnse, et al. 2008; Nemes et al. 2009; Nemes, Geleijnse, et al. 2008), the use of TOE for measurement of aortic dynamic function dates back to the late 1980s (Isnard et al. 1989) and aortic distensibility back to the early 1990s (Lang et al. 1994). The largest study utilising TOE measures of aortic compliance, aortic stiffness and stiffness index involved a review of 500 patients' studies who underwent a clinically indicated procedure and were analysed retrospectively. After a median follow up of just over 5 years in a high-risk population (44% mortality), tertiles of all indices were predictive using Log-Rank Kaplan-Meier survival analysis. Once corrected for traditional risk factors however, the independent strength of each measure was attenuated such that only stiffness index remained significant (Go OD Echocardiography 2014). Although feasible, its uptake beyond small cross-sectional studies (Nemes et al. 2007) or retrospective analysis has been limited as it remains relatively invasive, operator dependent and requires a physician to perform the assessment.

1.4.3.4.2 Cardiac magnetic resonance

First described in the late 1980s (Mohiaddin et al. 1989), cardiac magnetic resonance offers the ability to evaluate vascular dynamic function within almost any vascular bed and across any plane. CMR is not limited by body habitus (aside from the morbidly obese who cannot fit in the tunnel) or vascular calcification, nor does it suffer from operator dependence.

Although tonometry cfPWV is able to provide an overall 'value' for aortic stiffness, it makes assumptions about distance travelled and neglects the ascending aorta. While some of this can be overcome using phase contrast, flow-based CMR sequences, the value for any assessed segment remains a vessel's 'average' and as our group has demonstrated (Nelson et al. 2009), as have others (Kim, EK et al. 2013), there is regional heterogeneity. It is therefore of significant interest to evaluate not only an 'average' stiffness for the aorta but also regional changes as it may be a more sensitive

marker of disease (Hundley et al. 2001). Similar findings were reported by Redheuil et al. (Redheuil et al. 2010) in an asymptomatic population aged 20-50 years (a subgroup of their larger analysis) where proximal aortic distensibility fell sharply before 50 years of age even after multivariate analysis included cfPWV, carotid distensibility and AIx. This highlights its potential utility at detecting early, subclinical aortic dysfunction.

Cardiovascular magnetic resonance derived arterial distensibility can be theoretically performed at any vascular bed although it has been used most for the measurement of aortic distensibility. In brief (*see Chapter 2*), cross sectional cine images are acquired, and the inner lumen traced throughout the cardiac cycle. Conventionally this is done at three levels providing measurements at the 'ascending aorta (AA)', 'proximal descending aorta (PDA)' and 'distal descending aorta (DDA)'. Maximum and minimum values are obtained, and a relative area change calculated indexed to baseline vessel size. This is then indexed to the distending pulse pressure to provide a value for distensibility in its final units, $10^{-3}mmHg^{-1}$. Whilst some have advocated for a transfer function from the radial artery waveform to deduce central PP (Redheuil et al. 2010), thereby reducing the potential impact of pressure amplification and subsequent under-estimation of distensibility (particularly in the young), most have simply used brachial readings and accepted this limitation (Rerkpattanapipat et al. 2002). Both methods have been validated against 'gold standard' measures of arterial stiffness, in particular tonometry-derived cfPWV (all $R^2 > 0.7$, $p<0.001$) (Lehmann et al. 1993; Nelson et al. 2009). CMR aortic distensibility is highly reproducible with a coefficient of variation between 1 and 2% for intra- and inter-observer variability, respectively (Nelson et al. 2009). In addition, distensibility-specific sequences and analysis take less than 5 minutes and although unlikely to be a sole indication for a clinical CMR request given its cost and accessibility, could represent a key component

of a comprehensive cardiovascular examination or an addition to an otherwise clinically indicated scan.

The largest study to date involving aortic distensibility was performed by Maroules et al. (Maroules et al. 2014) in the Dallas Heart study. They evaluated 2122 individuals without established CVD and performed aortic arch PWV, aortic distensibility and total arterial compliance (TAC – calculated as LV stroke volume/pulse pressure (Kawaguchi et al. 2003)) and were followed for a mean duration of 7.8 years. Total arterial compliance was modestly associated with the composite event endpoint per standard deviation (HR 1.07, p = 0.03) however both TAC (HR 1.11, p = 0.011) and distensibility (HR 1.45, p = 0.0005) strongly associated with non-fatal cardiovascular events where arch PWV did not. Of note there was no significant improvement in the c statistic when adding either TAC or distensibility CMR indices into the Framingham Risk score. This relatively modest incremental benefit observed may well be explained by the low risk of the population and small numbers of events.

1.4.4 Epidemiology

Almost 100 years after the introduction of the sphygmomanometer in clinical practice (O'Brien 1996), appreciation of arterial stiffness as a more specific measure of vascular health has grown markedly in the last 20 years. Evidence of this is in the number of PubMed listings by year of publication *(Figure 1.6)*. In the early years seminal and founding work in the field was largely mechanistic and challenging to synthesize at a population or even individual patient level. This was due to myriad methods of assessment, variously studied vascular beds and a paucity of reference or 'normal' values to apply to clinical decision making. In 2006, the European Society of Cardiology published a consensus document providing a precis on arterial stiffness measurement and more importantly, recognised cfPWV as the 'gold standard' method for clinical evaluation (Laurent et al. 2006). Used largely in cross sectional or smaller

longitudinal studies until that time, this statement called for reference values which were subsequently produced by a similar group in 2010 from nearly 17,000 patients, of which 1,500 were healthy 'normal' controls (Arterial Stiffness Reference 2010). The consensus guidelines reported a 'reference' PWV by stratum of decade and blood pressure status with subsequent modification of the 'at risk' cut-off (Van Bortel et al. 2012). The American Heart Association then published their own consensus statement in 2015 and provided a Class IIa recommendation to measure arterial stiffness in clinical practice or those with hypertension on the grounds of Level A evidence support (Townsend et al. 2015).

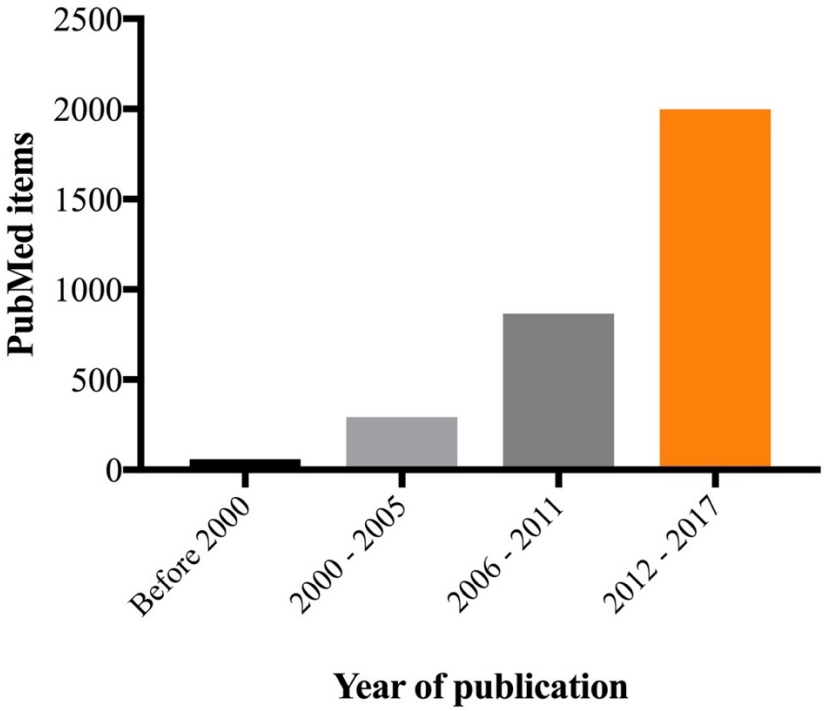

FIGURE 1.6 *Column graph representing items indexed on PubMed using 'arterial stiffness' search.*

Measures of arterial stiffness have been shown to strengthen traditional risk assessment and be independent predictors for cardiovascular events, cardiovascular death and all-cause mortality. Almost exclusively with cfPWV, given its relative simplicity and accessibility, this has been shown across a number of cohorts including 'healthy' volunteers (Mattace-Raso et al. 2006; Mitchell et al. 2010; Willum-Hansen

et al. 2006), the elderly (Meaume et al. 2001; Sutton-Tyrrell et al. 2005), those with established coronary disease (Kaneko et al. 2013), hypertension (Boutouyrie et al. 2002; Laurent et al. 2001; Terai et al. 2008), diabetes (Cardoso et al. 2013; Levisianou et al. 2013; Roos et al. 2013) and renal disease (Blacher et al. 2002; Pannier et al. 2005; Shoji et al. 2001; Zoungas et al. 2007).

The most comprehensive systematic review of the predictive capacity of cfPWV was reported by Ben-Shlomo et al. (Ben-Shlomo et al. 2014) in which they performed an individual patient analysis of 17 cohorts totalling 17,635 participants. For inclusion in the analysis, studies had to have a) directly measured carotid-femoral PWV, b) followed subjects for a minimum of 1 year and c) collected outcome data on all-cause mortality, coronary outcomes (MI, revascularization) and stroke events. After adjusting for cholesterol, smoking status, diabetes and hypertension, using pooled adjusted hazard ratios, a 1 SD increase in $\log_e$ transformed cfPWV was associated with increased risk of all-cause mortality (HR 1.17; 95%CI 1.11-1.22), CV mortality (HR 1.28; 95%CI 1.15-1.43), CHD events (HR 1.23; 95%CI 1.11-1.35) and CVD events (HR 1.30; 95%CI 1.18 – 1.48). The predictive capacity appeared stronger in younger individuals although saliently was not modified by hypertension, smoking, gender, diabetes or kidney disease. The addition of cfPWV to standard risk prediction models like Framingham, whilst only modestly improving the c statistic, did assist with the separation of younger individuals of intermediate risk. These findings were similar to an earlier meta-analysis which included just over 15,000 patients for a mean follow up of just over 7 years (Vlachopoulos et al. 2010).

1.4.5 Underlying mechanisms of arterial stiffness

Arterial stiffness is associated with modifications to the properties of load bearing structural components of the arterial wall which, in turn, alters the stress and strain relationship. The mechanisms that underpin these changes are the result of a complex interaction between mechanical (haemodynamic forces) and humoral (hormones such as angiotensin II, salt, glucose) factors which impact on the material properties of the arterial wall. Dichotomising these into either a) material properties, or 'passive' mechanisms, or b) cellular or molecular signalling, or 'active' mechanisms provides a framework for description.

1.4.5.1 Passive

1.4.5.1.1 'Functional' stiffness

As described (*1.3.2 Anatomy*), the anatomical makeup of large arteries generates intrinsic functional arterial stiffness (Shadwick 1999; Wagenseil & Mecham 2009); as wall tension and distending pressure increases, the load-bearing burden of the arterial wall shifts from predominantly elastin recruitment to collagen which is materially stiffer and less compliant. An artery's stiffness, therefore, is fundamentally and non-linearly pressure dependent (Cox 1975). Applying this observation, an increased distending pressure will generate a 'functionally' stiffer vessel which in turn will increase PWV and pulse pressure thereby providing the substrate for a positive feedback loop. Teasing out the direction of causality is inherently challenging with these observations.

1.4.5.1.2 Mechanical fatigue

As described in (*1.3.2 Anatomy*), elastin has rubber-like characteristics being highly resilient, deformable and of comparatively low stiffness; properties which facilitate its capacity to store strain energy. The pulsatile nature of blood flow subjects the arteries to continuous and repetitive strain throughout life with the aorta, for example,

passively dilating up to 15% of its size with each heartbeat (Nicholls & O'Rourke 2005). Applying the modern engineering principles of rubber fatigue, if distended 15% per cycle it would take approximately 1 billion beats to fracture a component of elastin. This corresponds to 30 years at a rate of 70 beats/min (O'Rourke & Hashimoto 2007). Radiocarbon data shows elastin to have the greatest longevity of any protein in the human body with a half-life of between 40-174 years (mean 74 years) (Shapiro, SD et al. 1991) supporting a theory that elastin may undergo a qualitative rather than quantitative change when exposed to mechanical fatigue. Cross-sectional studies of a range of species of varying body size, heart rate and life span show an association between the degree of elastin fragmentation, disorganisation and fracture, and the total number of cardiac cycles (Avolio, A et al. 1998). Supporting this underlying mechanism comes from an elastin haplodeficient mouse where a functional loss of elastin results in the development of precocious arterial stiffness followed by hypertension (Le et al. 2011).

1.4.5.2 Active

1.4.5.2.1 Endothelial cell function

Once conceived as an inert monolayer forming the interface between the arterial wall and circulating blood, it is now recognised that the innermost component of the intima is indeed highly functional and dynamic. This has been elegantly described at the small vessel level (Furchgott & Zawadzki 1980), although its role in large artery behaviour is less certain. More recently, increased pulsatility has been shown to alter the phenotypic behaviour of both endothelial and vascular smooth muscle cells via complex mechano-transduction pathways (Lehoux et al. 2006; Resnick & Gimbrone 1995). In bovine endothelial cell culture, increased amplitude and frequency of mechanical forces were associated with increased expression of endothelin-1 and reduced expression of endothelial nitric oxide synthase (Ziegler et al. 1998); changes

which themselves would generate arterial stiffness and through increased pulse pressure, perpetuate a positive feedback loop.

1.4.5.2.2 Vascular smooth muscle cell function

A large body of evidence supports the role of vascular smooth muscle cell (VSMC) activity in small vessels regulating vascular tone and peripheral resistance, however, their role in large artery stiffness appears phenotypically, and potentially adaptively, different (Campbell & Campbell 2012; Cox 1979). The predominant change in vascular smooth muscle cell behaviour is a trans-differentiation leading to osteogenesis and calcium deposition largely in the media of the arterial wall (Johnson, RC et al. 2006). The exact precipitants of this change remain elusive, however a variety of mediators including calpain-1, a regulator of MMP activity, (Jiang et al. 2012) and transglutimase 2, a cell programmer (Johnson, KA et al. 2008) have been implicated. In addition to VSMC direct trans-differentiation, the degradation of elastin appears to promote the signalling pathway for vascular calcification and ultimately the process of elastocalcinosis, a finding seen even in presumed 'healthy' individuals (Atkinson 2008; McEniery et al. 2009). As with other mechanisms of arterial stiffness, the directional relationship between calcification and arterial stiffness is complex. Evidence of a bidirectional or positive feedback process comes from a recent study by Guo et al. (Guo et al. 2017) who evaluated the aorto-iliac arteries in just over 600 middle aged men over a period of six years. In this study, the annual increase in calcification was strongly associated with an increase in baPWV; moreover, in those without initial calcification, baseline elevated baPWV predicted the development of calcification.

1.4.5.2.3 Extracellular matrix

As described, the arterial wall comprises extracellular matrix (ECM) proteins which include collagen, elastin, proteoglycans and glycoproteins; the collagen and elastin

providing most of the structural integrity and elasticity, respectively. The ECM is in a constant state of tension between the inter and intramolecular cross-linking of collagen and elastin which is catalysed by lysyl oxidase (LOX) and their turnover by matrix metalloproteinases (MMPs) into advanced glycation end-products (AGEs). Formation of AGE in the ECM leads to increased arterial stiffness, a feature commonly observed with increasing age (Bailey 2001). Conditions that either down-regulate LOX such as obesity (Chen, JY et al. 2013) or up-regulate MMP expression (Galis & Khatri 2002) or activity via haemodynamic stressors (Galis & Khatri 2002), inflammation (Visse & Nagase 2003) or reactive oxygen species (Rajagopalan et al. 1996), therefore, have been shown to increase arterial stiffness. Promotion of fibrosis within the ECM, potentially driven by the pro-inflammatory cardiotropin-1, has also been implicated in increased arterial stiffness (Lopez-Andres et al. 2013).

1.4.5.2.4 Neuroendocrine

Remodelling of the arterial wall in arterial stiffness, particularly in aging, appears to be associated with a pro-inflammatory milieu (Lakatta & Levy 2003) and for the most part, mediated by increased levels of angiotensin II. The multiple effects of increased angiotensin II include the stimulation of collagen production (Benetos, Levy, et al. 1997), VSMC expression, endothelin receptor signalling and reactive oxygen species in addition to a reduction in NO-dependent signalling (Wang, M et al. 2010). These effects have been closely associated with ageing implicating a variety of mediators such as transforming growth factor beta 1, monocyte chemoattractant protein-1 and matrix metalloproteinases (Lakatta et al. 2009; Wang, M & Lakatta 2002; Wang, M et al. 2007).

1.4.5.2.5 Genetics

1.4.5.2.5.1 Genome-wide association

Closely linked with arterial stiffness, interest in the genetics of hypertension has revealed up to 50% (h2 = 0.5) of individuals may have a hereditary basis to their elevated blood pressure (Padmanabhan et al. 2015). Notwithstanding this, contributions from epigenetics and behavioural influence are likely to modify single, isolated gene effect size and thus the prevailing aetiology of hypertension is felt to be multifactorial and probably polygenic (Reich & Lander 2001). Widened brachial pulse pressure, a feature of isolated systolic hypertension and a crude measure of arterial stiffness, was shown in large genome-wide association studies (GWAS) to have modest heritability in Nigerian (Adeyemo et al. 2002) and Mexican-American (Atwood et al. 2001) individuals with similar findings demonstrated in the Framingham cohort (h2 = 0.5) (DeStefano et al. 2004). Genetic correlation studies have been performed with more specific measures of arterial stiffness parameters although these have been either small cross-sectional studies or limited to twin cohorts (Snieder et al. 2000; Tarnoki et al. 2012). Collectively the heritability estimates have reached as high as 0.48 (Mitchell et al. 2005) although the individual genetic associations have been weak despite a number of potential, biologically plausible candidate genes including those coding for Angiotensin II receptors (Benetos et al. 1995), fibrillin-1 (Medley et al. 2002), endothelin receptors (Lajemi et al. 2001), matrix metalloproteinases (Medley et al. 2003), and nitric oxide synthase (Mitchell, Guo, Kathiresan, et al. 2007). Given a large number of these studies were underpowered, a large GWAS meta-analysis compiled data from over 26,000 individuals from Europe (Mitchell et al. 2012). A genome-wide association was found between cfPWV and a region on chromosome 14, a location coding for human cardiac and vascular cell lines suggesting putative relevance. When applied to the Framingham cohort, the presence of the allele was associated with increased risk of coronary artery disease HR 1.05 (95%CI 1.02 – 1.08, p = 0.0013) and heart failure, HR 1.10 (95%CI

1.03 – 1.16, p = 0.004). In GWAS terms, this is a highly significant association but at an individual level, these hazard ratios represent only a modest modifier of absolute risk. Nonetheless, it remains of significant interest that this gene, and others, may not only reveal instructive pathophysiological insights for arterial stiffness but perhaps provide a background to an individual's variable development of arterial dysfunction when exposed to traditional vascular risk factors (Logan et al. 2015).

1.4.5.2.5.2 Single genes

From an animal model perspective, evidence of genetic causation in arterial stiffness has come in the form of the Klotho gene; an antiaging gene which when mutated results in a number of aging phenotypes and early death (Kuro-o et al. 1997). Overexpression has been shown to reverse ageing, prevent hypertension and extend lifespan in mice (Gao et al. 2016; Kurosu et al. 2005; Xiao et al. 2004). Recent mechanistic studies by Chen and others with Klotho haplodeficient mice exhibited higher aortic PWV compared to wild type animals (Chen, K et al. 2015). Moreover, the development of faster PWV preceded the development of hypertension suggesting stiffness was causal of hypertension, rather than a sequel of it. At a histological level, haplodeficient mice had reduced elastin and increased collagen in the tunica media of the aorta; a finding that was not observed in the carotid or femoral arteries of these animals.

1.4.6 Causes of arterial stiffness

A large body of evidence has linked arterial stiffness to the presence of traditional risk factors including increasing age (O'Rourke & Hashimoto 2007), hypertension (Benetos et al. 1993), diabetes (Wilkinson et al. 2000), dyslipidaemia (Wilkinson et al. 2002), smoking (Mahmud & Feely 2004), obesity (Chau et al. 2018), renal disease (Blacher et al. 2001; Blacher et al. 2002; London et al. 2001; Mourad et al. 2001) and sedentary lifestyle (Tanaka et al. 1998). Whilst these associations do not prove

causation, a number of these relationships are particularly strong and postulated mechanisms deserve noting.

1.4.6.1 Ageing

Early studies in arterial stiffness suggested a linear relationship with increasing age (Avolio, AP et al. 1983; Smulyan et al. 2001; Vaitkevicius et al. 1993). Over the last 10 years, however, it has become clear that the relationship is more likely curvilinear and markedly increases after the fifth decade (Greenwald 2007; McEniery et al. 2005). A point of ongoing contention remains whether arterial stiffening is an inexorable feature of aging (McEniery et al. 2007) or whether the changes observed are a reflection of long term, cumulative comorbidity exposure thereby supporting the notion of a 'vascular age' versus 'chronological age' (Lakatta & Levy 2003; Lee, HY & Oh 2010).

Regardless, the major alterations observed with increasing age characterised by the imbalance between collagen and elastin in the ECM. With the largest relative abundance of elastin, our group (Nelson et al. 2009), and others (Rogers et al. 2001) have shown this process appears to occur preferentially in the proximal aorta. Although the traditional view is of cyclical material stress as described earlier (O'Rourke & Hashimoto 2007), there is additional evidence of increased matrix metalloproteinase (MMP) expression and subsequent elastin degradation in animal studies of aged rodents (Li et al. 1999; Wang, M et al. 2005). Not only is there a reduction in the elastin quality and quantity, there is a commensurate increase in collagen synthesis and deposition which steadily increases after the sixth decade (Cattell et al. 1996; Wang, M et al. 2007; Yu & Blumenthal 1963).

Largely animal based work has demonstrated that age-associated remodelling has a pro-inflammatory profile characterized by increased VSMC expression and increased secretion of angiotensin II which contributes to both material and structural change (Lakatta et al. 2009). The role of amyloid in other age-associated conditions such as

dementia, renal dysfunction and more recently diabetes (Westermark 2005) is well established, however has more recently also been linked to the arterial wall. An elegant autopsy study from the 1970s proposed this theory with aortic amyloid present in 51% of patients in their 40s but 95% of those in their eight decade of life – saliently this was geographically distant from atheroma (Iwata et al. 1978). Components of the inflammatory milieu implicated in the 'aging' vascular phenotype have also been observed in animal models of induced obesity, diabetes and hypertension. The corollary that these vascular risk factors may be inducing a form of 'early' or precocious ageing – extending the notion of a 'vascular age' (Cunha et al. 2017; Najjar et al. 2005).

1.4.6.2 Hypertension

A large body of evidence supports a strong association between hypertension and aortic stiffness (Beltran et al. 2001). The traditional view has been that hypertension stimulates aortic remodelling and subsequently stiffening, as an adaptive process to increase the wall-to-lumen ratio (La Place's law) in response to chronic elevation of distending pressure (Boutouyrie et al. 1999; Gibbons & Dzau 1994). In vivo aortic banding confirms that the aorta materially stiffens in response to increased pressure (Fridez et al. 2002) and ex vivo mechanosensitive cell culture studies produce matrix when exposed to increased stress (Humphrey et al. 2014). However, the potential for bidirectionality of this association, and even subclinical arterial stiffness causing hypertension, has been the focus of both population studies and more recently, animal-based enquiry. Seminal work from Weisbrod et al. demonstrated in a mouse model of diet-induced obesity (high fat high sucrose, HFHS) that arterial stiffness (PWV, measured invasively and non-invasively) develops rapidly and precedes the onset of hypertension and overt diastolic dysfunction (Weisbrod et al. 2013). The most compelling human work comes from Kaess et al. (Kaess et al. 2012) who evaluated the non-hypertensive offspring cohort from Framingham. In this group they showed

that higher baseline PWV was a risk factor for progression of hypertension but also predicted incident hypertension during a seven-year period of follow up. When evaluating predictors of follow up PWV from baseline PWV, no parameter of BP (systolic, diastolic, mean) was significant enough to enter the model suggesting it was causal of BP rather than a complication of it. Similar findings were earlier reported by Dernellis et al. (Dernellis & Panaretou 2005) in other smaller, association studies although of varying ages (Liao et al. 1999; Takase et al. 2011). Whether there is a predominant direction of causality or whether there are phenotypes of hypertension which are generated by arterial stiffness (isolated systolic hypertension, or resistant hypertension) or possibly a degree of positive feedback between the two, remains a focus of ongoing research (Humphrey et al. 2016; Laurent & Boutouyrie 2015).

1.4.6.3 Glucose and insulin

Arterial stiffness is consistently observed across all age groups with diabetes and metabolic syndrome: a core feature being insulin resistance (Salomaa et al. 1995; Sutton-Tyrrell et al. 2001). Not only do the earliest changes in both arterial stiffness and endothelial dysfunction appear in obese children with the metabolic syndrome (Tounian et al. 2001), there is evidence of a 'metabolic derangement' dose effect relationship on indices of arterial stiffness (Scuteri et al. 2004). Mechanistically, hyperinsulinaemia and hyperglycaemia induce chronic over-activity of the renin-angiotensin-aldosterone system (RAAS) which drives expression of angiotensin type I receptors in vascular tissue (Nickenig et al. 1998) and subsequently wall hypertrophy and fibrosis (Jesmin et al. 2003; Rizzoni et al. 2001). Chronic fasting glycaemia appears to promote non-enzymatic glycation of proteins with covalent cross-linking of collagen, advanced glycation end-products (AGEs) which result in expansion of the media and alteration of the arterial wall mechanics (Brownlee et al. 1988).

1.4.7 Implications of arterial stiffness

A growing body of evidence now suggests that the high flow, low resistance organs such as the brain and kidney are most at risk of subclinical complications from large artery stiffness. Inefficient damping of the arterial pressure waveform in the large arteries allows elevated pulsatile flow and pulse pressure to extend to arterioles *(Figure 1.7)*. Not only does this potentially drive vascular remodelling and endothelial dysfunction at a smaller artery/arteriolar level but subjects these vascular beds to barotrauma. Description of these changes has established a mechanistic basis for the predictive strength of arterial stiffness in these cohorts and has re-shaped it from being an 'association', to an established 'risk factor'.

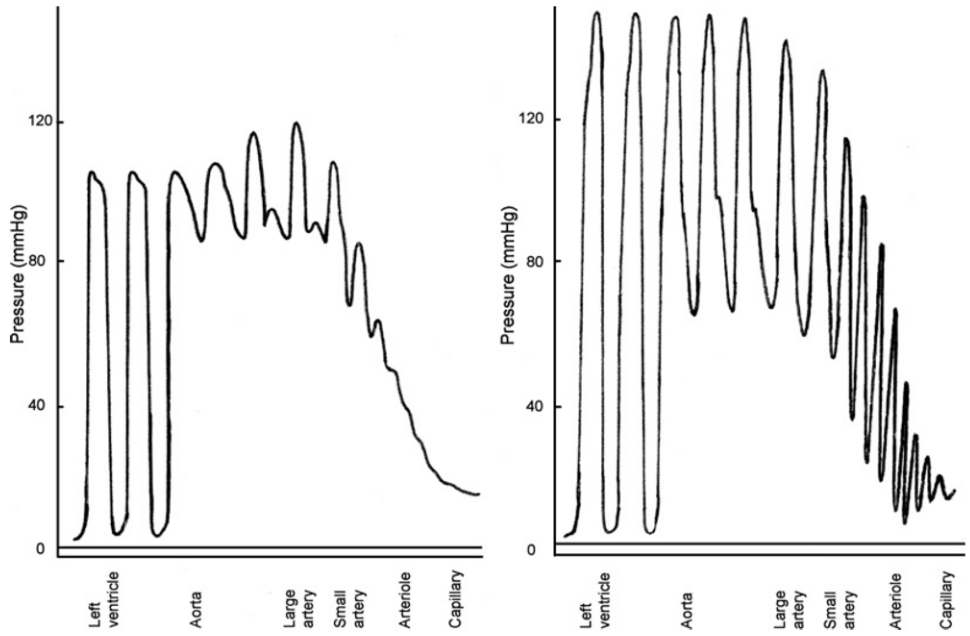

FIGURE 1.7 *Adapted from O'Rourke (O'Rourke & Hashimoto 2007) demonstrating inadequate dampening of the arterial pulse pressure and persistence of pulsatility into small arterioles in those with stiff arteries (right) compared to normal (left).*

1.4.7.1 Neurological sequelae

The large arteries (i.e. middle cerebral artery calibre) provide over 50% of the cerebrovascular resistance (Faraci & Sigmund 1999), however to maintain continuous and diastolic flow, even some of the most distal perfusing vessels are exposed to a degree of pulsatility. The relatively short vascular distance to achieve this distribution

of pressure (Fukasawa 1969) places the microcirculation at risk of barotrauma (Blanco et al. 2017; Snyder et al. 2015) and is demonstrably less efficacious with age and the presence of traditional vascular risk factors (Toth et al. 2017). Mechanistically, the persistence of a pulsatile arterial wave into the small vessels of the brain from increased arterial stiffness is believed to directly cause cerebral microvascular damage or cerebral small vessel disease (CSVD) (O'Rourke & Safar 2005; Roher et al. 2011; Tzourio et al. 2014). Furthermore, the increased pulsatile load may induce a remodelling response in the smaller calibre vessels which, although protective initially against the intravascular pressure, may potentiate microvascular ischaemia through reduced vasoreactivity (O'Rourke & Safar 2005).

Confirmed in a recent meta-analysis (van Sloten et al. 2015), smaller cross-sectional studies involving a variety of measures of arterial stiffness have been strongly associated with MRI manifestations of CSVD including white matter lesions (WMLs), lacunar infarcts and cerebral microbleeds CMB (Hatanaka et al. 2011; Henskens et al. 2008; Kim, DH et al. 2008; Kuo, HK et al. 2010; Matsumoto et al. 2007). These radiological findings are believed to represent the basis for stroke syndromes and a loss of cognitive function.

In large community studies of individuals without stroke or dementia, higher cfPWV has been associated with lower memory scores and subcortical white matter hyperintensities in septuagenarians (Cooper et al. 2016; Hughes, TM et al. 2018; Mitchell et al. 2011; Tsao et al. 2016) and in younger individuals (Poels et al. 2012). Longitudinally in the Framingham cohort, cfPWV predicted the ten-year incidence of both mild cognitive impairment and dementia in the non-diabetic elderly (Pase, Beiser, et al. 2016). Evidence of earlier changes comes from studies in middle-aged adults aged 45 to 65 where cfPWV was associated with slower processing speed, executive function and white matter changes (Pase, Himali, et al. 2016). Furthermore, the change

in cfPWV over four years predicted a decline in executive function and memory scores after adjustment for measured BP and the presence of hypertension (Hajjar et al. 2016).

1.4.7.2 Renal sequelae

Autoregulation of blood flow across the kidney is achieved through myogenic tone in the afferent arteriole, largely under the control of complex tubule-glomerular feedback mechanisms (Loutzenhiser et al. 2002). Through the presence of the efferent arteriolar resistance, the mean and pulsatile pressures in the glomerulus are maintained at approximately 60% of the medium to large arterial values (Mitchell 2004). This relatively low resistance to high pressure preserves glomerular filtration but comes at the cost of exposing the glomerular capillary bed to the potentially damaging barotrauma of increased peak and pulsatile pressures (Bidani & Griffin 2004). This is evidenced by a tight association between both increased SBP (Gosse & Safar 2005) and widened PP (Fesler et al. 2007) with an increased rate of decline in renal function. Consistent with these findings, arterial stiffness, which is associated with both of these coarse haemodynamic consequences, has also been more tightly linked to an increased rate of GFR decline across the spectrum of renal disease (Ford et al. 2010; Taal et al. 2007; Tanaka et al. 1998; Townsend et al. 2018). Given the pathophysiological links, it is consistent that arterial stiffness, aside from minor exceptions (Briet et al. 2011; Chandra et al. 2014), has independent prognostic capacity with respect to progression of renal disease but also cardiovascular and non-cardiovascular mortality in this cohort of patients (Baumann et al. 2014; Ford et al. 2010; Guerin et al. 2001; Karras et al. 2012; Madero et al. 2013; Sedaghat et al. 2016).

1.4.7.3 Cardiac sequelae

Aortic stiffness, either through increased aortic impedance, reduced aortic reservoir function or through return of reflected waves, increases the systolic load on the left ventricle. Additional load on the left ventricle (both early and late systolic) through

Laplace's law is offset by concentric hypertrophy, which although initially compensatory (Hori et al. 1985; Kohno et al. 1996), ultimately reduces efficiency and increases myocardial oxygen demand (Kelly, RP et al. 1992). Through increased filling pressures, arterial stiffness and concentric hypertrophy are both associated with the development of diastolic dysfunction (Russo et al. 2012) and can result in heart failure with preserved ejection fraction, HFpEF (Abhayaratna et al. 2008; Chae et al. 1999; Dart & Kingwell 2001). Increased filling pressures within the left ventricle are translated to the left atrium which is associated with fibrosis and an increased risk of atrial fibrillation (Mitchell, Vasan, et al. 2007).

Increased arterial stiffness, particularly when accompanied with age, broadens pulse pressure and reduces mean diastolic pressure relative to mean systolic. As covered later, given the coronary vessels are extrinsically compressed during systole, flow almost exclusively occurs during diastole. With a reduction in mean diastolic pressure observed in stiffer arteries, coronary perfusion may be compromised by a variety of putative mechanisms (Kass et al. 1996).

1.4.8 Stiffness as a therapeutic target

The strength of data linking increased arterial stiffness with a number of cardiovascular and non-cardiovascular outcomes makes it an attractive therapeutic target. Moreover, its appearance before clinically manifest cardiovascular disease raises the potential for not only an early risk stratification but also a biomarker for temporal modification.

1.4.8.1 Pharmacological

1.4.8.1.1 Antihypertensives

Traditional approaches at reducing arterial stiffness have targeted a reduction in blood pressure and as described above, a reduction in operating MAP will reduce 'functional' stiffness and confound measurement of PWV. Positive control arms which reduce operating BP to the same degree as the studied agent are therefore required to prove effects on stiffness independent of passive mechanisms.

1.4.8.1.1.1 ACE inhibitors

Angiotensin converting enzyme (ACE) inhibitors have the strongest evidence which is biologically plausible from their anti-fibrotic and anti-inflammatory activity at the vessel wall, mediated mainly through angiotensin II activity. Confirming earlier reviews (Ong et al. 2011), and positive results from shorter term individual studies (Mahmud & Feely 2002), the largest meta-analysis (Shahin et al. 2012) of ACE inhibitors and PWV included just under 500 individuals and demonstrated an improvement in central haemodynamic indices (including AIx) when compared to other anti-hypertensive agents although their comparative effect on PWV was modest overall. Robust smaller studies, however, suggest these effects are amplified by higher dose and longer treatment (Ong et al. 2011) and occur in a variety of vascular territories (Tropeano et al. 2006).

The largest study to assess the impact of ACE inhibitors on measures of arterial stiffness and wave reflection indices and subsequent cardiovascular events was the Conduit Artery Function Evaluation (CAFÉ) study (Williams et al. 2006) which was an arm of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) (Sever et al. 2001). In the CAFÉ study the perindopril/amlodipine arm experienced a significant improvement in wave reflection indices including aortic systolic pressure and aortic PP when compared to the atenolol/thiazide comparator arm. These differences occurred despite similar brachial BP measurements between the groups raising a potential mechanism for the differential CV outcomes in other BP lowering trials (Dahlof et al. 2002; Heart Outcomes Prevention Evaluation Study et al. 2000).

1.4.8.1.1.2 Angiotensin II receptor blockers

Angiotensin II receptor blockers (ARBs) appear to improve aortic stiffness independent of BP, superior to calcium channel blockers (CCBs) (Boutouyrie et al. 2010) and to an extent that is comparable to ACE inhibitors in both short and long term studies (Julius et al. 2004; Laurent et al. 2014; Mahmud & Feely 2000; Peng et al. 2015). Smaller trials have suggested the addition of an ARB to an ACE may provide incremental reductions in aortic stiffness (Frimodt-Moller et al. 2012) although this did not translate to net clinical benefit in a large outcome study secondary to adverse drug reactions (Yusuf et al. 2008).

1.4.8.1.1.3 Other agents

Calcium channel blockers have been used in the comparator arms of most ACE/ARB studies and their impact on measures of aortic or arterial stiffness independent of BP is uncertain and probably small (Boutouyrie et al. 2011). The effect of beta blockers on arterial stiffness is less clear (Salvi et al. 2013) and may differ between agents. A reduction in heart rate alone has been shown to alter the visco-elastic properties, however a slower heart rate can prolong ejection time and result in increased wave

reflection and pressure amplification (Mahmud & Feely 2008). Moreover, the non-selective atenolol has a vasoconstrictive effect at the peripheries potentially driving further wave reflection and providing a mechanistic basis for the superior performance of the vasodilating and NO donating agents in this class (Dhakam et al. 2008; McEniery et al. 2004). Although mechanistically of potential benefit given their role in the RAAS pathway, aldosterone antagonists (eplerenone and spironolactone) have shown only modest impact on arterial stiffness indices (Edwards et al. 2009) with their BP-independent effects probably no greater than CCBs (Janic et al. 2014). Nitrates, although not formally anti-hypertensives, are vasoactive but largely have their effects at the muscular and microvascular levels. One relatively large study demonstrated an improvement in augmentation index but no significant impact on cfPWV (Stokes et al. 2003).

1.4.8.1.2 Lipid lowering therapy

Data on the ability for statins to modify arterial stiffness is conflicting due to the variety of cohorts studied, intensity of therapy and time frame assessed. The largest meta-analysis of the impact of statin therapy on PWV included six trials totalling 303 participants (Upala et al. 2017). Statin use overall was associated with a statistically significant reduction in cfPWV despite low to moderate intensity regimens. On balance, this was consistent with earlier reviews of smaller trials, some of which yielded conflicting results by including measures other than cfPWV (Rizos et al. 2010). It is likely that patients with an underlying pro-inflammatory phenotype of stiffness will derive the most anti-stiffening benefit from statins. Of note, other measures of stiffness were also improved by statin therapy in subsequent trials evaluating fluvastatin (Lunder et al. 2011) and atorvastatin (Sadat et al. 2013) where improvement in carotid stiffness was observed, possibly mediated by reductions in oxidative stress (Wang, J et al. 2012) or inflammation (Wallace et al. 2010).

1.4.8.1.3 Diabetic drugs

Improvement in glycaemic control, potentially via reduction in AGE formation (Aronson 2003), has demonstrable effects on arterial stiffness (Ikonomidis et al. 2016) although it is of interest whether drug-specific, disease modifying pathways can enhance this effect. Treatment with the peroxisome proliferator-activated receptor gamma – (PPAR-γ) glitazone class has been shown to reduce arterial stiffness in patients with diabetes, and more recently, chronic inflammatory disease (Marder et al. 2013). Small studies have shown some potential benefit of metformin on indices of arterial stiffness although this has been limited to cohort studies in selected populations (Agarwal et al. 2010). Recent positive cardiovascular outcomes trials of the newer anti-diabetic agents, independent of their glycaemic control, have generated interest in deciphering potential mechanisms of benefit. Improvements in a variety of indices measuring arterial stiffness have been demonstrated in small studies involving liraglutide (glucagon like peptide-1) (Lambadiari et al. 2018), empagliflozin (sodium-glucose co-transporter 2 (SGLT-2) inhibitor) (Cherney et al. 2014), tofogliflozin (SGLT-2) (Bekki et al. 2018) and anagliptin (dipeptidyl peptidase-4 (DPP-4) inhibitor) (Duvnjak & Blaslov 2016; Tahara et al. 2016). The mechanisms behind these benefits and whether this will extend to non-diabetic cohorts remains of great interest to the field.

1.4.8.1.4 AGE breakers

Following the non-enzymatic glycation of vascular proteins, particularly collagen, AGEs form and facilitate collagen cross-linking. As covered earlier, AGEs are a key underlying mechanism behind the development of arterial stiffness, particularly in individuals with diabetes and hypertension (Susic 2007). Animal studies (Asif et al. 2000; Wolffenbuttel et al. 1998) of the successful AGE breaker, algebrium, were confirmed in a small human study of hypertensive individuals with diabetes (Kass et al. 2001); however, a follow up study in non-diabetics was disappointing (Steppan et

al. 2012). Other agents directed at either blocking AGE formation (Corman et al. 1998), or their activity at the receptor level (Geronikaki et al. 2007) remain in development.

1.4.8.1.5 Anti-inflammatory drugs

Inflammation remains a central component of arterial stiffness associated with conditions such as rheumatoid arthritis and inflammatory bowel disease and although associated with the aging and hypertensive phenotype (Mahmud & Feely 2005), it is unclear how much of this is modifiable in 'traditional' arterial stiffness. Antibodies against tumour necrosis factor alpha (TNF-alpha) have shown efficacy in patients with a demonstrable, diagnosed chronic inflammatory disease (Maki-Petaja et al. 2012; Tam et al. 2014; Zanoli et al. 2014) although no such data exist for 'standard' cohorts. In the atherosclerotic field, recent interest has been buoyed by the results of the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) which showed a significant reduction in cardiovascular events by reducing inflammation (Ridker et al. 2017). Whether such potent and non-selective agents will have a net clinical benefit targeting what may be a comparatively small component of arterial stiffness pathophysiology remains unclear. Moreover whether inflammation can be so broadly targeted as being 'negative' is also uncertain with an observational study of non-steroidal anti-inflammatory drugs (NSAIDs) in patients screened for abdominal aortic aneurysms demonstrating a signal towards increased, rather than decreased, arterial stiffness (Claridge et al. 2005).

1.4.8.2 Non-pharmacological

1.4.8.2.1 Weight loss

The largest meta-analysis evaluating the effect of weight loss looked at 20 studies (three were randomised) including 1259 participants with PWV as the measured outcome. A modest weight loss (mean 8% from initial weight) achieved through diet

and lifestyle measures resulted in a significant reduction in PWV (Petersen et al. 2015) although this was significantly confounded by improvements in blood pressure. Similar results from a meta-analysis of caloric restriction were reported on indices of aortic distensibility and pulse wave analysis (Petersen et al. 2016), again also associated with a favourable improvement in blood pressure.

1.4.8.2.2 Exercise

Increased physical activity is associated with a plethora of cardiometabolic benefits which contribute to the observed cardiovascular mortality benefit when compared to sedentary individuals (Lavie et al. 2015; Nocon et al. 2008). Repeated bouts of cyclical, laminar stress on the vascular wall with increased cardiac output may assist with improvements in endothelial function (Joyner & Green 2009) but also advantageous vessel remodelling (Dinenno et al. 2001; O'Donovan et al. 2014). One of the largest cross-sectional studies involving over 5000 subjects demonstrated that the age driven increases in PWV in the control arm could be mitigated by moderate to vigorous activity, although not with light activity; an effect that was seen up to 70 years of age (Ahmadi-Abhari et al. 2017). The benefits of exercise on aortic stiffness, however, are less proven in subjects with established hypertension (Kraft et al. 2007; Pierce 2017), or those with obesity (Montero et al. 2014). The largest meta-analysis of RCTs published to date evaluated 1627 participants and concluded aerobic exercise was associated with improvements in both cfPWV and peripheral PWV, with a degree of dose dependence observed with higher intensity and in those with higher baseline stiffness (Ashor et al. 2014).

1.5 CORONARY BLOOD FLOW

1.5.1 Coronary anatomy

1.5.1.1 Epicardial vessels

The two main coronary arteries arise from coronary ostia located within the sinuses of Valsalva of the aorta. The left posterior sinus gives rise to the left main coronary artery which has daughter vessels, the left anterior descending (LAD) and the left circumflex (LCx) arteries. The LAD runs along the interventricular groove from base to apex and through its multiple daughter diagonal and septal branches, supplies the anterior and anterolateral surface of the LV as well as the anterior septum. The LCx tracks along the atrioventricular groove and through its daughter marginal vessels, supplies the left atrium and the remainder of the lateral wall.

Arising from the anterior coronary sinus, the right coronary artery (RCA) tracks along the atrioventricular groove and also gives rise to marginal branches. Blood from this system supplies the right atrium and right ventricular free wall.

The posterior descending artery (PDA) runs along the posterior atrioventricular groove and in 90% of the population arises from the RCA; in the remaining 10% it arises from the LCx where the system is said to be ‘left dominant’.

1.5.1.2 Micro and macro-circulation

Cardiac blood supply is provided from outside to in, with epicardial vessel calibre diminishing as the vessels divide and penetrate the myocardium. As the internal diameter reaches 1-3mm, tributaries arise that run perpendicular to the parent vessel. Further tributaries then descend (~ 400-1500μm) from the outer epicardium to the outer endocardium where a network of smaller arteries and arterioles (100-400μm) transition functionally from being ‘macrocirculation’ to feed the coronary ‘microcirculation’ with diameters of <100μm. At this level, the density of myocardial capillaries exceeds 3000 capillaries/mm^2 – far denser than is seen in skeletal muscle where densities of 600 capillaries/mm^2 are observed.

1.5.1.3 Architecture

As with the large arteries described above, the coronary vessels each have three layers of varying proportions depending on the calibre, and hence function, of the vessel – tunica intima, tunica media and tunica adventitia. Although anatomically a simple monolayer of cells, the endothelium is physiologically active and is present throughout the macro and microcirculation. This monolayer is thromboresistant, anti-inflammatory and semipermeable. Vasomotor tone is tightly regulated by paracrine pathways originating from the endothelium and mediated via secondary messengers to nearby smooth muscle cells within the intima and media: the outcome is a dynamic state of relaxation or contraction. By way of comparison, the media of the coronaries is collagen rich where the media of the aorta is elastin rich – this distinction providing one of the key anatomical differences between a resistance and conduit vessel respectively.

The adventitia is a more nebulous layer with connective tissue formed by collagen fibrils, fibroblasts and nerve endings providing a mechanism for neurogenic control of vasomotor tone. Coronary vessels of >1mm internal diameter receive blood supply extrinsically from a dense network of small vessels called vaso vasorum which course throughout the adventitia.

1.5.2 Determinants of coronary blood flow

Coronary flow can be broadly summarised in electrical terms according to Ohm's law, whereby flow is directly proportional to the pressure gradient across the vascular bed (arterial to venous) and inversely proportional to the overall resistance of the vascular tree. The mechanisms behind these broad determinants are myriad and yet after many years of research, many remain elusive or poorly understood. Given the critical nature of coronary blood flow to organ function, it is unsurprising there are a number of interrelated and complex mechanisms which contribute to its control. Indeed, the parallel activity and redundancy probably allows for a degree of compensation during

disease whereby other pathways will take up activity from a dysfunctional component. Nonetheless, a detailed description of all of these mechanisms is outside the scope of this body of work but some key determinants of coronary blood flow deserve review.

1.5.2.1 Structural contributors

1.5.2.1.1 Extravascular compressive resistance

Coronary blood flow is dependent on perfusion pressure and resistance to flow within the vascular tree. The effective perfusion pressure is the gradient between the input aortic pressure and the pressure to outflow in the venous system at the level of the right atrium, however there is additional resistance from extravascular forces exerted by the contracting myocardium. Seminal work from Gregg et al. (Gregg & Green 1940) demonstrated the phasic nature of blood flow with predominant flow occurring in diastole and minimal, if not reverse flow, in systole. An extension of this theory related the increased wall tension from the left ventricle extrinsically compressing intramyocardial vessels thereby permitting arterial inflow in diastole but impeding it during systole (Downey & Kirk 1975). The explanation for increased observed venous flow in systole, when one would expect these vessels also to be compressed, came from Spaan who described an intramyocardial 'pump' (Spaan 1985). In this model the microcirculatory vessels are compressed during systole and produce a capacitive discharge at both 'ends' of the vessel. Directed upstream this impedes coronary inflow, and yet simultaneously directed downstream, this accelerates flow into the venous system thereby forming a 'pump'. An extension of this theory reveals a form of compliance within the system such that an effective 'sucking' or 'charging' effect occurs during diastole of a healthy microcirculation (Hoffman & Spaan 1990).

1.5.2.1.2 Fixed microvascular resistance

The effects of systolic contraction appear to be more pronounced on the subendocardial compared to the subepicardial vessels (LeGrice et al. 1995) which

ought to confer a greater vulnerability to ischaemia. This effect, however, appears to be overcome, or at least compensated for, by a lower intrinsic microvascular resistance (Chilian 1991) observed within the subendocardium, largely driven by an increased density of arterioles and capillaries. This resultant vascular gradient favours transmural flow (Goto et al. 1991) to the subendocardium during maximal vasodilatation to the point that in a non-beating, maximally vasodilated heart there is comparatively more perfusion at the level of the subendocardium than subepicardium (Hoffman & Spaan 1990). Moreover, there is evidence that this transmural pressure gradient may indeed be augmented by microvascular recoil and 'suction' (Davies, JE et al. 2006), at which time there is a commensurate observed reduction in venous outflow.

Although in health it appears the effects of extravascular compression are mitigated by intrinsic microvascular characteristics, it remains of interest whether this is overcome in states of increased effective back pressure (e.g. rise in the pressure at which flow = 0, e.g. diastolic dysfunction, LVH) or reduction in antegrade perfusion pressure (Duncker & Bache 2008; Duncker et al. 1993) such as significant epicardial stenosis or increased pulse pressure.

1.5.2.2 Dynamic microcirculation

1.5.2.2.1 Autoregulation

Autoregulation is characterised by a vascular bed's ability to maintain constant blood flow across a breadth of perfusion pressures. The coronary, much like the cerebral and the renal vasculature, has an impressive autoregulatory capacity which is controlled mainly through changes in the calibre of the microcirculation, and subsequently, resistance.

The prevailing theory is that control of the microcirculation is via three main factors although the dominance of each at any one time is dynamic and informed by location: 1) shear stress, 2) wall stress and 3) metabolism. Small resistance arteries, 100-400μm in diameter, appear to regulate their diameter mainly in response to wall shear stress

(flow) and vascular wall stress (myogenic) whereas arterioles (<100μm) appear to be more sensitive to changes in local metabolism.

1.5.2.2.2 Shear stress and the endothelium

Small arteries and arterioles respond to changes in local shear stress by increasing their calibre; a phenomenon called flow-mediated dilatation. This process appears to be predominantly driven by release of nitric oxide, possibly via mechanosensors such as integrins, and relies on an intact, functional endothelium (Kuo, L et al. 1995). Also associated with this phenomenon is endothelial-derived hyperpolarising factor (EDHF) whose identity (or identities) and behaviour remains controversial but is thought to be hydrogen peroxide or C-type natriuretic peptide or potentially even potassium (Beyer & Gutterman 2012). The relative contribution of each compound appears to vary on vessel size (and possibly animal model studied) as well as potentially disease states where the role of EDHF could be increased when NO pathways are dysfunctional (Miura et al. 2001). Nitric oxide dependent changes appear to occur primarily upstream (~100-300μm) as NO synthase inhibition diminishes resting epicardial diameter and abolishes shear-mediated dilatation in the 'larger' arterioles (~150μm). It is unclear whether smaller vessels (<100μm) have the NO 'machinery' to facilitate this response (Sellke et al. 1990), or instead whether any potential effects are overwhelmed by other local autoregulatory pathways (Jones et al. 1996). Regardless, the physiological relevance of this process to baseline coronary flow has not been demonstrated in a large number of studies (Altman et al. 1994; Bernstein et al. 1996; Davis et al. 1998). Similarly, its role in hyperaemic flow during exercise (Quyyumi et al. 1995) and other forms of metabolic vasodilatation has been questioned (Duncker & Bache 2008).

Other contributors to endothelial derived vasodilation include prostacyclin (probably in part through NO dependent pathways) in addition to vasoconstrictors such as endothelin (Hillier et al. 2001) and thromboxane A2 (Eidt et al. 1989). These all appear

to be implicated (Sorop et al. 2008) in disease states such as spasm and following ischaemia, although their independent role in governing 'physiological' blood flow is unclear.

1.5.2.2.3 Wall stress

The myogenic response is the ability of vascular smooth muscle to respond to changes in vascular transmural pressure and thus, changes in wall stress. Present throughout vascular tissue, this phenomenon was first described by Bayliss (Bayliss 1902) and extrapolated as a mechanism for not only protection from injury but also maintenance of flow; increased intravascular pressure results in vasoconstriction and a reduction in flow, reduced intravascular pressure leads to vasodilatation and preservation of flow. The cellular mechanism is poorly understood, however current theories invoke the role of stretch activated L-type Ca channels to instigate smooth muscle responses.

1.5.2.2.4 Metabolic

Given the highly metabolic nature of cardiac tissue, coronary blood flow must be carefully controlled in order to provide oxygen and nutrition to cardiomyocytes which in turn permits maintenance of cardiac output and systemic tissue perfusion (Duncker & Bache 2008); periods of increased demand must be met with a commensurate increase in supply. This is incrementally more important in the myocardium where as much as 80% of the arterially delivered O_2 is removed from the blood under resting conditions, leaving little more efficiency to be derived by mechanisms of increased extraction observed in skeletal and other vascular beds. Seminal work from Fick (Shapiro, E 1972) confirmed the corollary, now well established, that myocardial oxygen demand and coronary blood flow must be near-linearly related.

Although there is acceptance of the role of locally derived vasodilators in the microcirculation, the feedback mechanism from various metabolites remains incompletely understood and the subject of ongoing study (Deussen et al. 2012).

Various potential metabolic stimuli and pathways felt to increase coronary blood flow include hypoxia (Gremels & Starling 1926) – either directly (Gauthier-Rein et al. 1997) or through a secondary messenger such as adenosine (Deussen et al. 1988) or prostaglandins (Busse et al. 1984), hypercapnia – either directly (Case et al. 1978) or through acidaemia (Wexels et al. 1985), reactive oxygen species (Saitoh et al. 2006) as well as ATP-sensitive potassium channels (Deussen et al. 2006; Farouque et al. 2002).

1.5.2.2.5 Large artery mechanics

As described above, a large body of evidence, albeit partially understood, has shown the key role of the microcirculation in controlling resistance and flow. Perhaps underappreciated in this relationship however, is the potential impact that arterial (and in particular, aortic) stiffness may have on coronary blood flow. As described earlier *(1.4.7.3 Cardiac sequelae)*, increased aortic stiffness has a number of key sequelae which could impact upon coronary blood flow: 1) early systolic augmentation increases LV wall stress driving hypertrophy and myocardial oxygen demand, potentially reducing maximal absolute coronary flow; 2) increased characteristic aortic impedance (and thereby reduced aortic 'reservoir) or via wave reflection phenomena, allows systolic run off and a reduction in diastolic aortic 'capacitance' which in turn can reduce coronary perfusion pressure; and 3) via direct damage to microcirculation through enhanced pulsatility driving capillary and arteriolar rarefaction and dysregulation of vasomotor tone.

Until the late 1990s, the basis of this physiology had been tested only in canines whereby the aorta was either artificially stiffened (with bandages) or replaced with a rigid tube. In Watanabe's (Watanabe et al. 1992) and Ohtsuka's (Ohtsuka et al. 1994) experiments of aortic bandaging from the same group, a reduction in aortic compliance was observed which in turn drove a widening in pulse pressure and an increase in left

ventricular cardiac work. The increase in myocardial oxygen demand overall increased coronary blood flow, however during periods of increased contractility or heart rate, the endocardial/epicardial blood flow ratio was reduced, suggesting a shift in coronary blood away from the subendocardium. The findings from Saeki's group were contrasting as although ejecting blood into a rigid tube caused a dramatic increase in PP and a demonstrable reduction in DBP, coronary blood flow overall was increased without a shift in blood flow away from the subendocardium (Saeki et al. 1995). The model in the latter experiment has been criticised for its abrupt and artificial changes to the arterial haemodynamics which are unlikely to accurately represent the chronic, progressive and compensatory features of large artery stiffening that occurs in human disease.

Beyond animal work, there have been a handful of human studies. Fukuda et al. evaluated 192 patients following clinically indicated angiography and compared epicardial atherosclerotic burden and invasive coronary flow velocity reserve (CFVR) with brachial-ankle pulse wave velocity (baPWV) as their measure of arterial stiffness (Fukuda et al. 2006). CFVR was obtained in non-diseased arteries of subjects classified as 'normal', 'single vessel disease' or 'double vessel disease' (79 in total). Regression analysis revealed a moderate relationship between baPWV and CFVR, R -0.45, $p < 0.0001$. In multiple linear regression, baPWV was independently predictive of CFVR across the studied cohort (β -0.44 , $p < 0.01$). Of note, atherosclerotic burden correlated with measures of arterial stiffness but did not correlate with CFVR. Moreover, this study did not report on the relative association with resting or hyperaemic blood flow.

Leung et al. compared invasive coronary blood flow with both aortic PWV and carotid-femoral PWV in 18 subjects with angiographically severe coronary disease pre and post percutaneous coronary intervention (PCI) (Leung et al. 2006). Aortic PWV

was strongly associated with resting and hyperaemic CBF ($R^2 > 0.5$ both pre and post, $p < 0.01$) although the relationship with resting CBF was stronger pre- (R^2 0.452, $p < 0.01$) than it was post-PCI (R^2 0.261, $p = 0.043$). Additionally, after dividing their cohort into 'stiff' and 'non-stiff', hyperaemic blood flow was reduced in the former group compared to the latter suggesting improvement from PCI in the context of a stiff aorta may be attenuated. Of particular interest was that cfPWV did not correlate with any measure of coronary blood flow, nor did it correlate with aPWV. Also, it is unclear whether these aorto-coronary relationships can be extended out to non-stented, native vessels.

In the non-invasive arena, there have been two key studies. Saito et al studied 102 hypertensive subjects and obtained baPWV, augmentation index (calculated from a carotid tracing) as well as resting and hyperaemic coronary flow velocity measurements by echocardiogram (Saito et al. 2008). Coronary flow velocity reserve was associated with baPWV (R -0.46, $p < 0.001$) and AIx (R -0.41, $p < 0.001$) although on multivariate analysis only AIx remained significant (R -0.32, $p < 0.001$). There were a number of key limitations. None of these subjects had angiography and thus epicardial disease couldn't be excluded. Although baPWV has independent prognostic capacity, it measures the interface of elastic and muscular arteries and thus, may not be a sensitive parameter to evaluate more specific changes in elastic, cushion arterial function where stiffness has been documented to occur preferentially in a proximal distribution (Nelson et al. 2009).

Extending observations into a clinical cohort, Kingwell et al. sought to determine whether large artery stiffness impacted upon ischaemic threshold in patients with coronary artery disease (Kingwell et al. 2002). Ninety-six patients with angiographically proven coronary artery disease (mean, maximum stenosis, 85%; mean vessels stenosed > 50%, 1.6) underwent carotid applanation tonometry where

augmentation index, distensibility index, cfPWV and systemic arterial compliance were obtained. Each subject then underwent treadmill exercise test and time to ST segment depression was analysed. All indices of arterial stiffness listed above correlated in univariate analysis with time to ischaemia (R -0.25 to 0.27, all $p < 0.05$). On multivariate analysis, distensibility index, AIx and systemic arterial compliance continued to be independently predictive however cfPWV was not independent of age. An extension of this finding is that restriction of hyperaemic flow through a stiff aorta in the context of a mild to moderate epicardial stenosis could simulate a severe stenosis in the context of a 'non-stiff' aorta.

1.5.3 Invasive methods of evaluating coronary blood flow

1.5.3.1 Coronary flow reserve

The notion of 'reserve' within the coronary circulation was proposed in seminal work from Gould (Gould & Lipscomb 1974). These studies asserted that resting coronary blood flow was effectively maintained until the stenosis became critical (approximately 85% of cross-sectional area). It was at this point that additional demand would exceed supply, as the microvasculature was fully 'compensated' or had exceeded its 'reserve'. Coronary flow reserve (CFR), as it was first described, is expressed as the quotient of maximal flow to resting flow and encompasses the entire vessel's capacity (epicardial to microcirculation) to increase blood flow. With preserved microvascular function in the absence of epicardial stenosis, the maximum or hyperaemic flow is approximately five-fold that of resting flow, providing a CFR of 4-5. In the clinical context of a chest pain evaluation, inducing maximal hyperaemia of the distal coronary microcirculation can reveal a flow limiting epicardial stenosis. While a stenosis may need to exceed 85% narrowing of a coronary vessel before it reduces resting blood flow, in the context of hyperaemia, even a 50% stenosis can reduce hyperaemic blood flow (Gould et al. 1990).

Given CFR is a ratio, one of its strengths (and inherent weaknesses), is that vessel diameter and flow stream are not required for its derivation although the assumption is made that these do not change between resting and hyperaemic assessment. Given the ease of obtaining flow measurements, the CFR is generally obtained with the use of an intracoronary Doppler velocity guidewire (or Doppler FloWire) which provides a measure of flow 'velocity'. By obtaining a flow 'velocity' at rest and another at hyperaemia, the quotient can be performed which provides an index, coronary flow velocity reserve (CFVR). Values of 2 and 2.5 have been used as a cut-off point for an inadequate hyperaemic response although 3 has been shown in young healthy controls to be 'normal' (Chauhan et al. 1993; Perchet et al. 1995). There exists, therefore a reasonably large 'grey zone' between 2 and 3 which has been a weakness of the modality, particularly when trying to assess discrete epicardial lesions. In the absence of flow-limiting epicardial disease, the CFVR therefore is a robust measure of microcirculatory function. Wider application of CFVR has been limited due to its invasive nature and in the context of assessing epicardial disease, its sensitivity to haemodynamic changes such as HR and BP.

1.5.3.2 Coronary blood flow

Coronary blood flow can be assessed with either Doppler velocity or via thermodilution methods however both require engaging a coronary artery and placing a guidewire. As described above, the coaxial positioning of the Doppler FloWire will provide a value for flow 'velocity'. The derivation of flow requires an assessment of vessel size which can be obtained through intravascular ultrasound (IVUS) (Puri et al. 2012) or more commonly through quantitative coronary angiography (QCA). Using QCA, coronary blood flow can then be derived with the formula π x [Coronary Diameter]^2 x Average Peak Velocity x 0.125 (Deanfield et al. 2005). Limitations include the sensitivity of the Doppler wire to movement and turbulence, in addition to

the potential error multiplication by deducing cross sectional area from a 2D diameter method.

1.5.4 Non-invasive methods of evaluating myocardial blood flow

1.5.4.1 Myocardial perfusion reserve

The physiological basis for perfusion imaging rests on the concept of coronary flow reserve covered above. Although intrinsically related, where blood flow is volume per unit time, perfusion is indirectly measured as blood flow through mass (or volume) per unit time. Flow through mass can be measured via high fidelity nuclear cardiology by labelling blood and observing the signal intensity changes as it passes through the myocardium. Alternatively, a blood-borne detectable tracer can be introduced and its passage through the myocardium observed. Techniques have been described using both echocardiography (Wei et al. 1998) and computed tomography (George et al. 2007) however spatial resolution and ionising radiation, respectively, are key limitations. Magnetic resonance imaging using gadolinium is now increasingly available, has sufficient spatial resolution to detect regional defects, and is safe.

As gadolinium passes through blood, there is shortening of the T1 signal making the agent appear ‘white’. During its passage through the microcirculation of perfused myocardium, the myocardial signal becomes ‘brighter’, however in an area of reduced or absent perfusion, the T1-shortening is reduced, and the tissue appears hypo-intense or ‘darker’. The change in myocardial ‘brightness’ or signal over time during the ‘first pass’ of gadolinium is a measure of myocardial blood flow and thus, perfusion. In vessels with a significant epicardial stenosis, adenosine has reduced capacity to augment flow as the downstream microvasculature is already maximally dilated in a compensatory manner to reduce microcirculatory resistance. In this context, the administration of a contrast agent will be preferentially distributed to regions of relative hyperaemia due to coronary ‘steal’ phenomenon. This will leave areas of delayed contrast wash-in which appear dark and are termed ‘perfusion defects’.

By evaluating the change in signal over time, one can generate signal-intensity time curves for both myocardium and blood pool at rest and with hyperaemia. The maximal upslope of this curve has been shown to be sensitive to changes in blood flow. Evaluating the quotient of the maximal upslope at hyperaemia and with the maximal upslope at rest, provides an index of myocardial perfusion – termed myocardial perfusion reserve (MPR) or myocardial perfusion reserve index (MPRI). This value is analogous to the CFVR, covered above. Studies comparing CMR-derived MPR with microsphere-derived MPR have demonstrated a strong relationship in normal swine coronaries ($R = 0.88$, $p<0.01$). Similarly comparing MPR with intracoronary Doppler FloWire CFVR in normal human coronaries there was a robust agreement between the modalities ($R = 0.80$, $p<0.01$) (Wilke et al. 1997). In ischaemic segments, MPR demonstrated a strong correlation with both positron emission tomography (PET) perfusion reserve ($R = 0.76$, $p<0.01$) (Schwitter et al. 2001) and Doppler CFVR ($R = 0.87$, $p<0.01$) (Kurita et al. 2009).

1.5.4.1.1 Myocardial blood flow quantification

Quantitative myocardial blood flow can be derived from signal intensity curves of the myocardium and of the blood pool, as described above, and can be obtained with CT, PET or CMR. A variety of models can be used to estimate the behaviour of contrast in the circulatory system with each having strengths and weaknesses. The ‘linear shift invariant’ models are simpler (and thus attractive for potential clinical use) and assume the circulatory response is linear and does not vary over time – i.e. if two contrast boluses are administered, the uptake is the linear sum had they been given separately; and if a second bolus is given after a period of time, its uptake is shifted by the same amount of time. By making these assumptions, the system can be described by a transfer function, i.e. the myocardial time intensity curve is equal to the arterial input (LV blood pool time intensity curve) merged with the transfer function by deconvolution. Where convolution is a mathematical technique that folds two curves

together, deconvolution is the process of 'unfolding' the curves to approximate a shape that defines its behaviour. Complex mathematical derivation can show that the myocardial blood flow can be approximated to the maximal value of Fermi function which, itself, is governed by delay, amplitude, shift and decay *(Figure 1.8)*. This technique has been validated extensively in animals (Christian et al. 2009; Jerosch-Herold et al. 2002; Jerosch-Herold et al. 2000) with robust correlation against microspheres (R >0.9) (Schmitt et al. 2005) and studied widely in humans (Arnold et al. 2011; Cheng et al. 2008; Heydari et al. 2015; Selvanayagam et al. 2005).

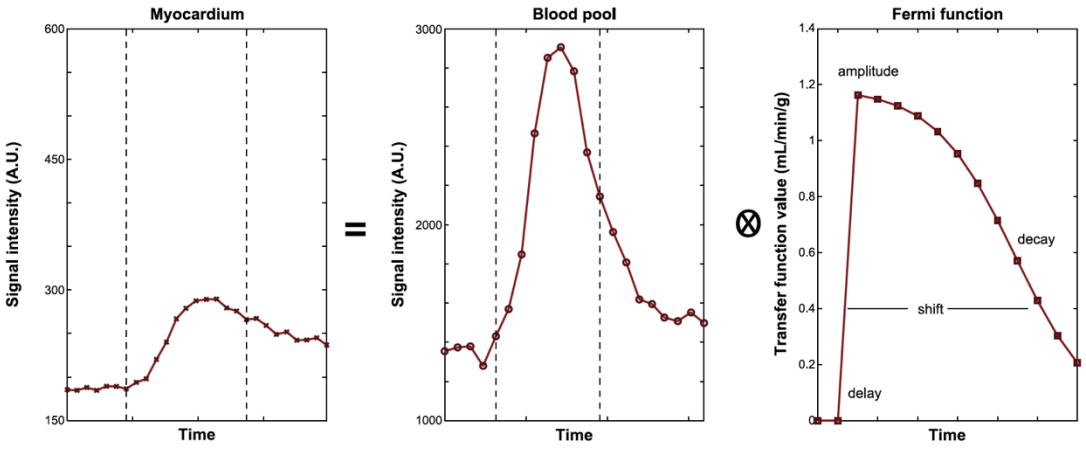

FIGURE 1.8. *Adapted from (Lee, DC & Johnson 2009). Representative image of myocardial time intensity curve (left), blood pool time intensity curve (middle) convolved with Fermi transfer function (right). The maximum amplitude of the transfer function equals absolute myocardial blood flow (MBF).*

1.5.4.2 Adenosine activity

Adenosine is a nonselective ligand for four receptors: A_1, A_{2A}, A_{2B}, A_3 (Olanrewaju et al. 2000). It appears to be released during periods of hypoxaemia and ischaemia although its role in 'physiology' or health is unclear as described earlier. The exact biological action of adenosine remains elusive. The main effect is vasodilation of the microcirculation which reduces resistance and increases coronary blood flow, up to fivefold in healthy individuals. The mechanism is widely felt to be largely endothelial-

independent and triggered through membrane bound 2A and 2B receptors (Morrison et al. 2002) located on smooth muscle cells (Shryock & Belardinelli 1997) and mediated by cyclic AMP pathways (Hussain & Mustafa 1993).

Some degree of endothelial-dependence is postulated, however, as animal studies of denuded coronary arteries have an attenuated hyperaemic response to adenosine, particularly in canines (Nees et al. 1985). Whether this is as a result of the loss of 'secondary' flow mediated dilatation owing to an increase in blood flow is also unclear. Cell culture studies have demonstrated endothelial-based adenosine receptors assist with mediating NO release (Olanrewaju & Mustafa 2000), although the functional studies supporting or elucidating the role of this pathway in vasodilation are sparse (Abebe et al. 1995).

Adenosine has a very short half-life making it an ideal agent for hyperaemic stress imaging. Whilst it can be administered intravenously, dosing for intracoronary routes is well established and reliably generates hyperaemia (De Bruyne et al. 2003; Farouque et al. 2002). There is minimal effect on heart rate or blood pressure (Wilson et al. 1990). There is no direct action on the epicardial vessel (Sudhir et al. 1993) although some have observed the indirect effects of flow-mediated dilatation (Lupi et al. 1997).

1.6 AIM OF THESIS

The aim of this thesis is to investigate the relationship between aortic stiffness and coronary blood flow through invasive and non-invasive means in both health and disease. The specific aims are as follows:

- To evaluate the relationship between invasive, gold-standard measures of both resting and hyperaemic blood flow with cardiovascular magnetic resonance derived aortic distensibility in subjects free from epicardial coronary disease
- To validate the relationship between resting and hyperaemic coronary blood flow in a completely non-invasive methodology in subjects with normal myocardial perfusion studies
- To validate a novel measure of aortic distensibility which can be obtained from standard, clinically indicated CMR studies
- To evaluate the relationship between resting and hyperaemic coronary blood flow with aortic distensibility in subjects with confirmed perfusion defects, allowing a comparison of this relationship in both ischaemic and non-ischaemic territories
- To evaluate the relationship between resting and hyperaemic coronary blood flow with aortic distensibility in subjects who have sustained an acute myocardial infarct allowing a comparison between recently infarcted myocardium and remote, non-infarct myocardium
- To evaluate whether increased aortic stiffness is predictive of myocardial blood flow at three months post myocardial infarction.

2. CHAPTER 2: Invasive proof of concept

Cardiac magnetic resonance imaging derived aortic distensibility is associated with coronary blood flow

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2.1 STATEMENT OF AUTHORSHIP

Manuscript details

Title of paper	*Cardiac magnetic resonance imaging derived aortic distensibility is associated with coronary blood flow*
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Principal Author Contributions

Candidate	Dr Adam J Nelson
Contribution to the Paper	Primary contributor to the conception and design of the work; Drafted the work; Provided final approval of the version to be published; Accountable for all aspects of the work.
Overall percentage	90%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

August 2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	A/Prof Matthew I Worthley
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Contribution to the Paper	Helped drafting the work and revising it critically; Provided final approval of the version to be published; Accountable for all aspects of the work

August 2018

2.2 ABSTRACT

Background: A large body of data supports the independent prognostic capacity of arterial stiffness for not only cardiovascular events, but all-cause mortality. An unfavourable aorto-coronary relationship is felt to be a key mechanistic factor driving these events, however human studies that confirm this association are lacking. This study sought to evaluate the relationship between coronary blood flow (Doppler FloWire) and cardiac magnetic resonance (CMR) derived aortic distensibility.

Methods: Following clinically indicated coronary angiography, a 0.014-inch Doppler FloWire and infusion catheter were advanced into the study vessel. Average peak velocity (APV) was acquired at baseline and following intracoronary adenosine administration to derive coronary flow velocity reserve (CFVR = peak APV/baseline APV), and coronary blood flow (CBF) (π x [Coronary Diameter]^2 x Average Peak Velocity x 0.125). Directly after angiography, patients underwent CMR for evaluation of aortic distensibility at the ascending aorta (AA), proximal descending aorta (PDA) and distal descending aorta (DDA) as previously described.

Results: Fifteen subjects (53 ±13 years) with only minor epicardial disease (<30%) were enrolled. Baseline CBF for the cohort was 62.6±16mL/min and hyperaemic CBF was 192.7 ± 66.2mL/min respectively. Coronary flow velocity reserve for the cohort was 3.15 ± 0.48. Aortic distensibility was 3.89 ± 1.72·10^{-3}mmHg^{-1} at the AA, 4.08 ± 1.80·10^{-3}mmHg^{-1} at the PDA and 4.42 ± 1.67·10^{-3}mmHg^{-1} at the DDA. All levels of distensibility correlated with rCBF (R^2 0.350 – 0.373, $p < 0.05$), hCBF (R^2 0.453 – 0.464, $p < 0.01$), and CFVR (R^2 0.442 – 0.511, $p < 0.01$).

Conclusion: Extending prior observations in animal models and other invasive cohorts, there was a strong correlation between CMR-derived aortic distensibility and CFVR. There was a modest correlation with rCBF although hCBF was more strongly related to aortic distensibility. This confirms the purported theory that arterial stiffness, at least in part, governs hyperaemic although to a lesser extent, resting blood flow.

2.3 INTRODUCTION

A large body of evidence now supports arterial stiffness as an independent prognostic marker for, not only cardiovascular events (Vlachopoulos et al. 2010), but all-cause mortality (Ben-Shlomo et al. 2014). This predictive capacity has been demonstrated in a variety of longitudinal cohorts including 'healthy' community population studies (Mattace-Raso et al. 2006; Mitchell et al. 2010; Willum-Hansen et al. 2006) in addition to those with diabetes (Levisianou et al. 2013; Roos et al. 2013), hypertension (Boutouyrie et al. 2002; Laurent et al. 2001), chronic kidney disease (Blacher et al. 2002; Shoji et al. 2001; Zoungas et al. 2007) and established coronary artery disease (Kaneko et al. 2013). On the strength of this research, consensus statements from both the European (Laurent et al. 2006) and American (Townsend et al. 2015) cardiology societies have espoused the role of arterial stiffness measurements not only as key research tools and endpoints in cardiovascular trials, but also for prognostication in the clinic.

Arterial stiffness remains a nebulous umbrella term which incorporates the structural and functional changes that describes the large arteries' response to intravascular pressure. Mechanistically, contemporary arterial haemodynamic theories suggest a stiffer arterial system will result in less cushioning of the arterial pulse and a faster forward pulse wave. A faster pulse wave will arrive at points of functional discontinuity quicker, such as arterial bifurcations, and generate reflected waves which return at end-systole rather than diastole. This will increase afterload and myocardial oxygen demand but in the context of reduced diastolic pressure, potentially reduce coronary blood flow.

Evidence of this physiology is sparse and comes almost exclusively from animal work with conflicting results – some associating arterial stiffness with reduced coronary blood flow (Watanabe et al. 1992), others with increased coronary blood flow (Saeki

et al. 1995). Using applanation tonometry derived pulse wave velocity and intracoronary Doppler FloWire measurements, Leung et al. (Leung et al. 2006) were the first to demonstrate in humans that a stiffer aorta governs not only hyperaemic coronary blood flow (hCBF) but, to a lesser extent, resting coronary blood flow (rCBF) as well.

Cardiovascular magnetic resonance has evolved in recent years to offer a comprehensive evaluation of not only cardiac but also novel indices of vascular dynamic structure and function (Redheuil 2014). The aim of this study was to evaluate the aorto-coronary relationship using the gold standard measure of coronary blood flow, intracoronary Doppler FloWire against CMR-derived aortic distensibility in patients with no more than minor angiographic coronary disease.

2.4 METHODS

2.4.1 Subjects

2.4.1.1 Inclusion criteria

The study was approved by the Human Research Ethics Committee and the Investigational Drugs Subcommittee at the Royal Adelaide Hospital.

Patients referred to the Royal Adelaide Hospital Cardiovascular Investigation Unit for a clinically indicated diagnostic coronary angiogram were eligible for participation. Informed consent was obtained prior to the commencement of the procedure and following an angiographically 'normal' angiogram (no more than luminal irregularities), subjects were enrolled into the study. While this involved consent prior to the knowledge that they meet the angiographic inclusion criteria, it negated the need to take them off the table for the consenting process and allowed the administration of procedural sedation (intravenous fentanyl).

2.4.1.2 Exclusion criteria

Exclusion criteria included age > 75 years, use of vasoactive medications < 48 hours of angiography (calcium channel blockers, nitrates, beta blockers), established coronary disease (angina, history of myocardial infarction, prior revascularisation), atrial fibrillation/flutter, aortopathy (history of intervention, dissection or aneurysm), magnetic resonance chamber incompatibility (pacemakers, aneurysm clips, claustrophobia), impaired LV function (LVEF < 45%), severe valvular heart disease, renal impairment (estimated glomerular filtration rate < 30 mL/min/1.73m^2) and significant bleeding risk (requirement for heparin administration).

2.4.1.3 Demographics

Prescribed medications and risk factors were recorded. Risk factors were defined as cigarette smoking (previously or currently smoking tobacco), hypercholesterolemia (use of cholesterol-lowering therapy or total cholesterol level > 5.2mmol/L), hypertension (blood pressure > 140/90mm Hg or on medical antihypertensive therapy or subject to lifestyle intervention), obesity (BMI > 30kg/m^2), diabetes, and family history of cardiovascular disease (index event in a first-degree relative, male ≤ 55 years and female ≤ 60).

Fasting blood was obtained at the time of coronary angiography and sent to a central laboratory for total cholesterol, low-density lipoprotein, high-density lipoprotein and triglyceride levels, high sensitivity C reactive protein (hsCRP, Beckman Immage Immunochemistry System, Fullerton, California), fasting glucose, complete blood examination as well as renal and liver function.

2.4.2 Invasive coronary protocol

2.4.2.1 Intracoronary setup

After diagnostic angiography, measures of myocardial blood flow and coronary flow reserve were performed according to standardised practice (Anderson et al. 1995) *(Figure 2.1).* The study vessel in all cases was the proximal segment of the left anterior descending (LAD) coronary artery. A 6Fr XB3.5 left guiding catheter was engaged in the ostium of the left main coronary artery. Therapeutic heparin anticoagulation was administered at 80IU/kg. A 2.7Fr coaxial infusion microcatheter (Progreat, Terumo, Tokyo, Japan) was placed within the study vessel at the origin of the LAD. A 0.014-inch Coronary Doppler FloWire (ComboWire XT, Volcano Corporation, San Diego, California) was then advanced through the microcatheter into the LAD. The FloWire was positioned in the mid-section of the study vessel and manipulated until a stable Doppler flow signal was obtained.

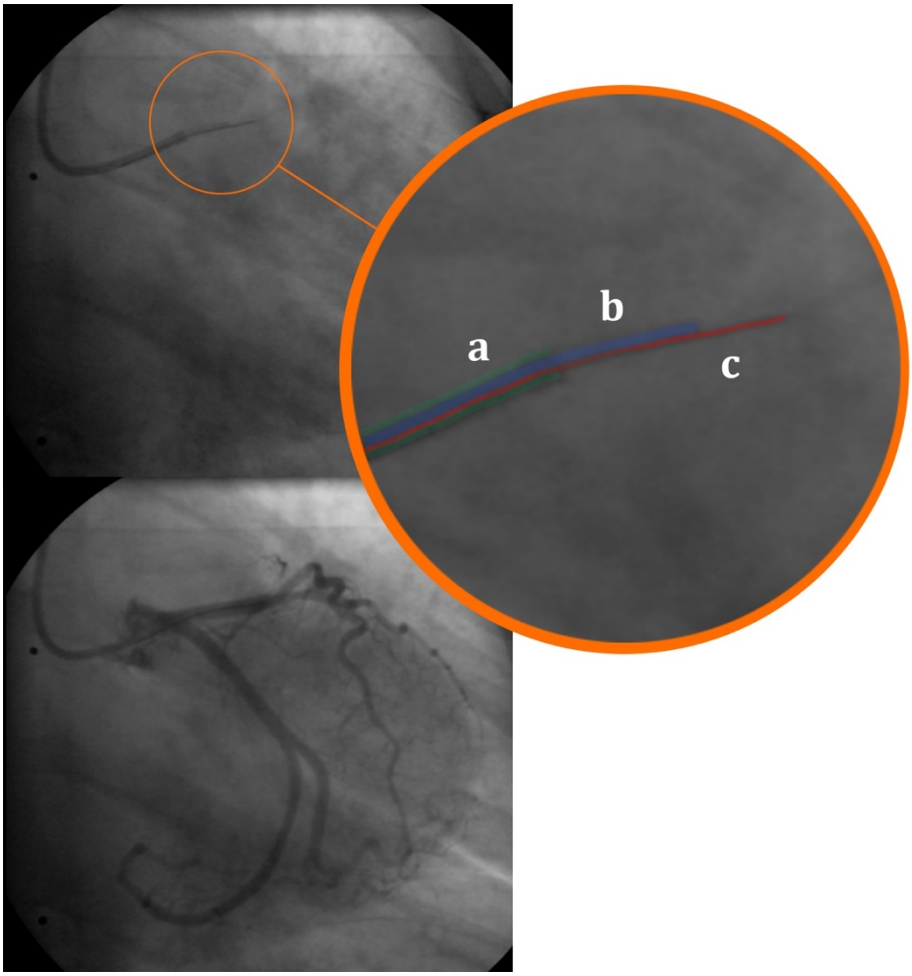

FIGURE 2.1: *Invasive coronary protocol setup. XB3.5 6Fr guiding catheter (a), housing a Progreat Infusion microcatheter (b) and a Doppler FloWire (c).*

2.4.2.2 Haemodynamics

Invasive central haemodynamics were acquired prior to the commencement of the intracoronary protocol. This included central systolic, diastolic and mean arterial pressure. This was then analysed offline to calculate central pulse pressure (cPP).

2.4.2.3 Coronary blood flow

The assessment of coronary blood flow (CBF) was undertaken as per previously validated studies (Anderson et al. 2005; Doucette et al. 1992). An infusion of intracoronary 5% dextrose was run for 5 minutes. At the completion of the infusion, baseline Averaged Peak Velocity (APV) was obtained. This was defined as the steady segment of APV over ten cardiac cycles. A coronary angiogram was then performed using 9mL of non-ionic contrast (Omnipaque, GE Healthcare, Waukesha, USA) injected through a Medrad infusion pump (IMaxeon, New South Wales) at 5mL/s. Angiographic images were then stored offline.

Continuous instantaneous peak velocity and APV indices were continuously acquired at a sampling rate of 200Hz using the ComboMap console (Volcano Corporation) and extracted for offline analysis. Haemodynamic data (systemic blood pressure and heart rate) and ECG rhythm strip were recorded continuously throughout the study for off-line analysis.

2.4.2.4 Coronary flow reserve

Following the acquisition of the angiogram for coronary blood flow (CBF) quantification, a further infusion of 5% dextrose was run for five minutes. Intracoronary nitro-glycerine (200μg) was administered to minimize epicardial artery vasomotion.

After obtaining an optimal and stable velocity signal, a bolus of 48microg of intracoronary adenosine was administered (Duffy et al. 1999; Farouque et al. 2002).

Continuous instantaneous peak velocity and APV indices, haemodynamic data (systemic blood pressure and heart rate) and ECG rhythm strip were recorded continuously throughout the study for off-line analysis. Baseline APV was defined as the steady segment of APV over ten cardiac cycles immediately before the adenosine-induced rise in APV. Peak APV was the highest APV after the adenosine bolus.

2.4.2.5 Offline analysis

2.4.2.5.1 Coronary blood flow

An independent and trained observer carried out the offline angiographic analysis blinded to the CMR analysis. As per previously published methods (Goodhart & Anderson 1998; Zeiher et al. 1991), an automated edge-detection program was used to obtain the diameter of the study vessel (CMS software, Medis Corp, Leiden). A region of interest approximately 5mm in length and within 10mm from the end of the infusion catheter was selected for quantitative coronary angiography (QCA) of the baseline angiogram *(Figure 2.2)*.

Coronary blood flow was then calculated from the formula (Deanfield et al. 2005):

$$\pi \times [\textit{Coronary Diameter}]^2 \times \textit{Average Peak Velocity} \times 0.125$$

where diameter is measured in mm and Average Peak Velocity (APV) is measured in cm/s. As basal CBF is closely related to the rate-pressure product (RPP) (Czernin et al. 1993), an index of myocardial oxygen consumption, values for basal flow in each subject were corrected for the respective rate-pressure product. This was performed by multiplying basal flow by the mean RPP of the cohort, divided by the RPP in the individual patient (Uren, Melin, et al. 1994).

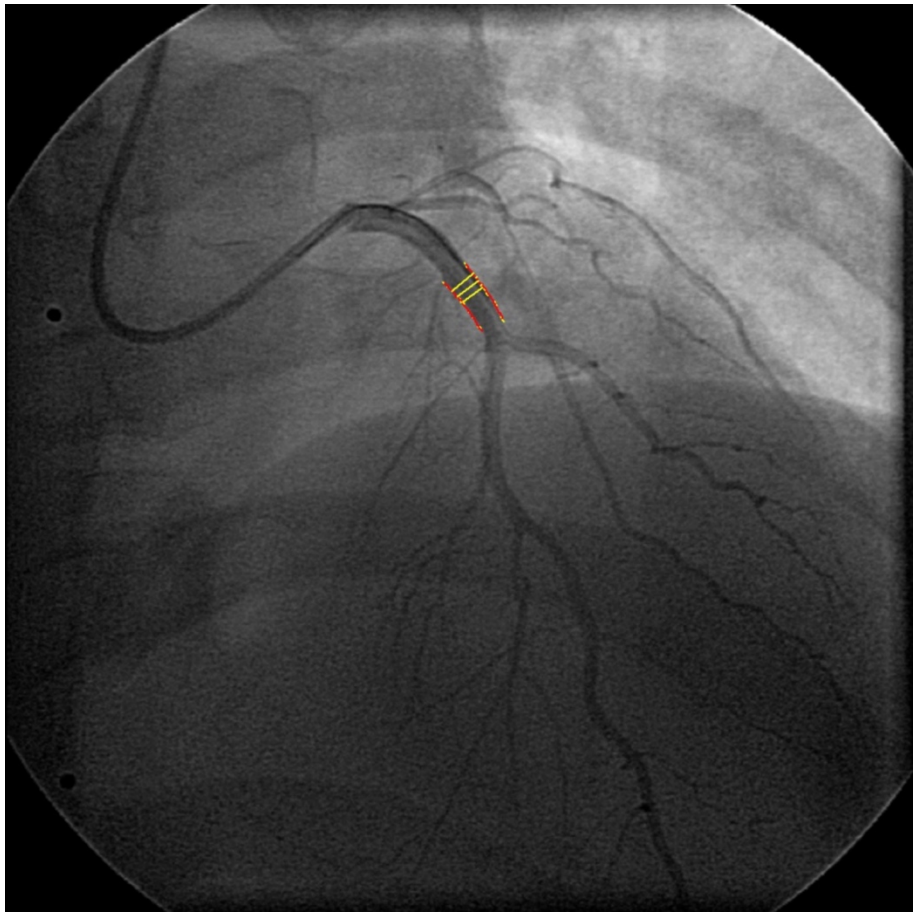

FIGURE 2.2: *A region of interest 5mm in length (distance between proximal and distal yellow bars) and within 10mm from the end of the infusion catheter. Semi-automated edge-detection software allowed calculation of vessel diameter.*

2.4.2.5.2 Coronary flow velocity reserve

Coronary flow velocity reserve (CFVR) was determined offline as a ratio of peak APV / resting APV *(Figure 2.3)*. A normal response is considered to have occurred if the CFVR is greater than 2.5 following adenosine (Hasdai & Lerman 1999).

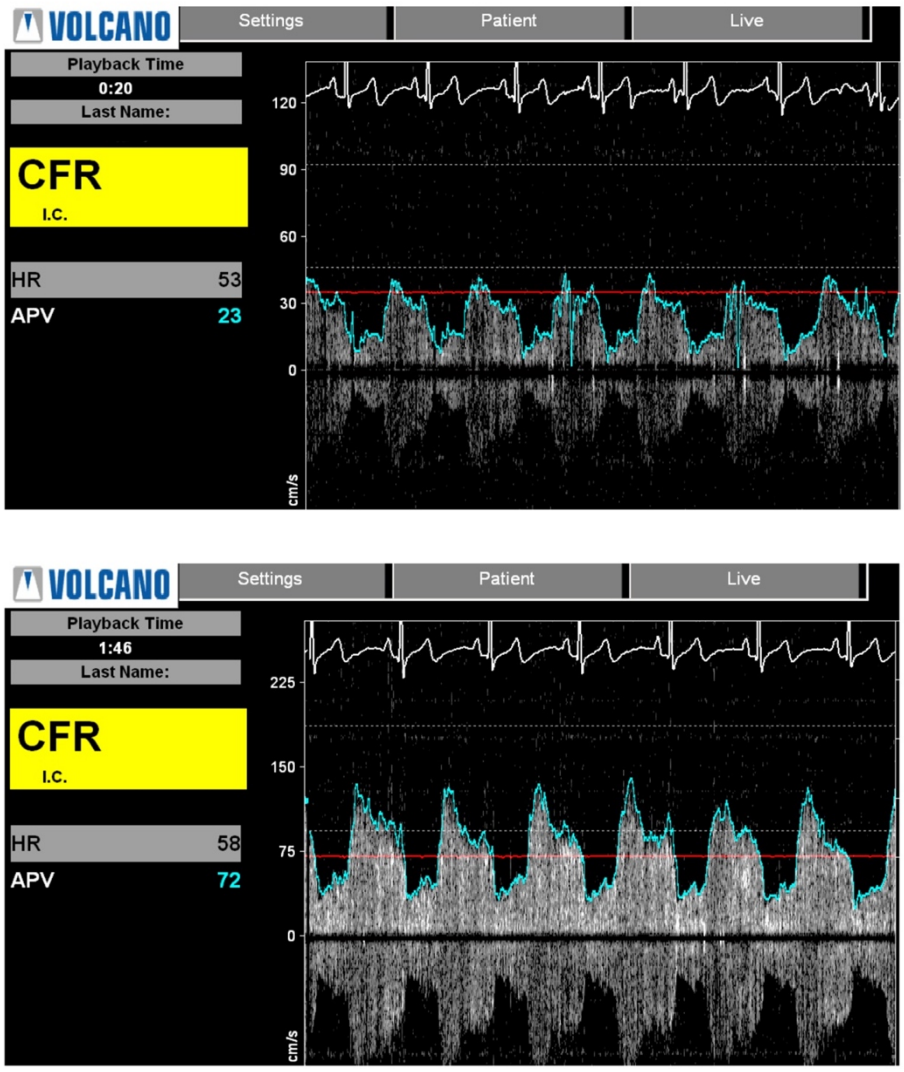

FIGURE 2.3. *Representative images of Volcano console Doppler FloWire tracing, (top) resting APV and (bottom) peak APV following adenosine administration. In this example, CFVR = 3.13.*

2.4.3 Non-invasive cardiac magnetic resonance protocol

All cardiovascular magnetic resonance (CMR) studies were performed utilising the Siemens Avanto 1.5 Tesla magnetic resonance imaging scanner (Siemens Medical Imaging, Erlangen, Germany) located within the Cardiovascular Investigation Unit of the Royal Adelaide Hospital. All CMR studies were performed with subjects in a supine position with a phased-array surface coil positioned over the thorax.

2.4.3.1 Cardiac structure and function

Initial long axes reference views were used to plan seven to ten parallel short-axis slices from the mitral annulus to and inclusive of the left ventricular apex *(Figure 1.4)*. Images were acquired during expiratory breath hold with retrospectively ECG-gated steady state free precession (SSFP) sequences. Acquisition time was determined to include 90% of the R-R interval, image matrix 256 x 150, field of view (FOV) 380mm, repetition time (TR) 52ms, echo time (TE) 1.7ms, flip angle 70°. Slice thickness was 6 mm with inter-slice gaps of 4mm.

Offline analysis was performed to evaluate LV size and function utilizing proprietary software (Argus software, Siemens Medical Imaging, Erlangen, Germany). Endocardial contours were manually traced at end-diastole (start of R-wave) and at end-systole (visually smallest cavity). As per local protocol and established guidance (Hudsmith et al. 2005), non-contiguous papillary muscles were included within the blood pool. The most basal LV slice was determined as the slice where at least 50% of the cavity was surrounded by ventricular myocardium. The most apical slice was determined by the slice within visible blood pool. End-systolic and end-diastolic volumes were obtained and thus left ventricular ejection fraction could be calculated.

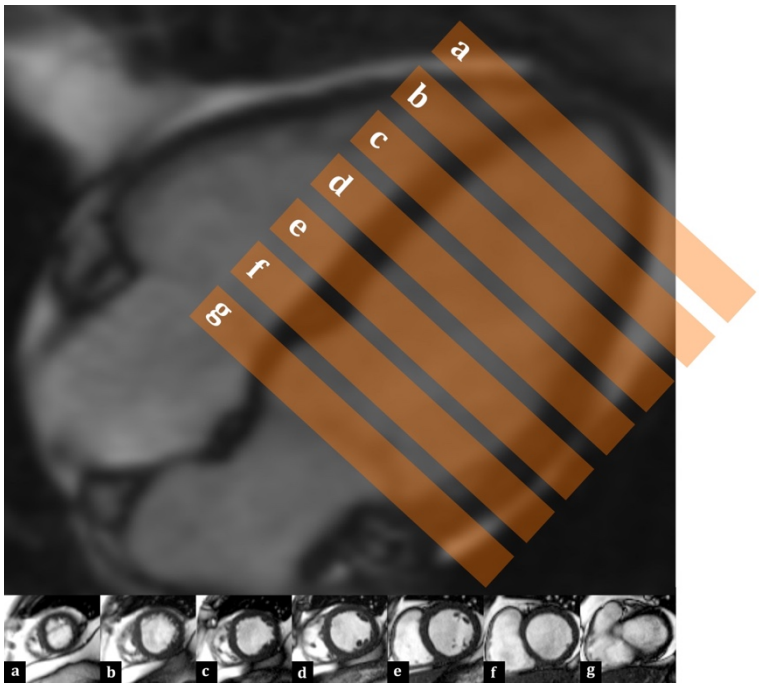

FIGURE 2.4. *Representative image of short axis images 'stack' acquired from mitral annulus (g) through to apex (a).*

2.4.3.2 Aortic distensibility

Following the cardiac protocol, aortic imaging was performed using the same phased-array surface coil placed on the thorax in addition to a spine-coil-array positioned within the patient bed. Images were acquired during expiratory breath hold with retrospectively ECG-gated SSFP sequences. Acquisition time was determined to include 90% of the R-R interval, image matrix 256 x 150, FOV 320mm, TR 40ms, TE 1.4ms, Flip Angle 40°. Slice thickness was 6 mm. Sagittal oblique aortic imaging was initially acquired from the level of the aortic sinuses to the renal arteries. Using the right pulmonary artery as a reference level, a perpendicular cross-sectional cine image was acquired of the ascending aorta (AA) and proximal descending aorta (PDA). A further SSFP cine image was acquired of the distal descending aorta (DDA) approximately 5cm beyond the level of the diaphragm *(Figure 2.5)*.

Brachial artery blood pressure was taken simultaneously using a CMR compatible automated non-invasive sphygmomanometer. Three results during both the cardiac and vascular protocols were obtained, and the measurements averaged. Outliers were discarded and repeat readings taken as required.

Aortic distensibility (AD) was evaluated offline by manually tracing aortic cross-sectional images at the level of the ascending aorta, proximal descending aorta and distal descending aorta. Maximal and minimal lumen areas were calculated and distensibility derived by a previously published formula (Groenink et al. 1998):

$$\frac{\textit{aortic area at end systole } (mm^2) - \textit{aortic area at end diastole } (mm^2)}{\textit{brachial pulse pressure } (mmHg) \times \textit{aortic area at end diastole } (mm^2)}$$

The pulse pressure was calculated from the difference between the systolic and diastolic blood pressures and measured in mmHg. The aortic areas were measured in mm^2 and after multiplying by 1000, aortic distensibility is reported in its final unit of $mmHg^{-1}$.

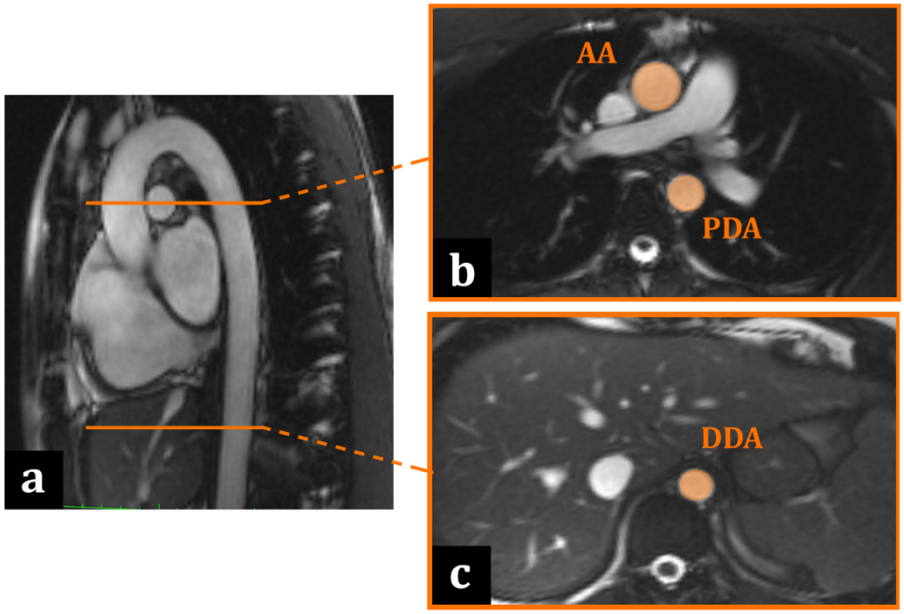

FIGURE 2.5 *Representative image of sagittal or 'candy cane' view of aorta (a), from which cross-sectional planes can be acquired at the level of the pulmonary artery to generate 'Ascending Aorta' [AA] and Proximal Descending Aorta [PDA] (b), and 5cm below the diaphragm to generate 'Distal Descending Aorta [DDA] (c).*

2.4.4 Statistical Analysis

Baseline characteristics are represented as mean ± standard deviation unless stated otherwise. Comparison of mean values between groups were by paired t-test or two-way ANOVA.

The relationship between continuous variables was determined by simple linear regression. Adjustment was performed by partial correlation. Analysis of covariance was applied in the presence of both continuous and categorical variables.

Statistical significance was defined as a two-sided $p<0.05$. Statistical analyses were performed using SPSS statistical software (v24, IBM SPSS, New York, USA) and graphs were drawn using GraphPad Prism 7 (GraphPad Software, La Jolla California USA).

Reproducibility

The reproducibility of aortic distensibility measures has previously been published by our group (Nelson et al. 2009) in a similar cohort of individuals. In that study, coefficients of variation (CV) were 1 and 2% for intra and inter-observer variability respectively. Reproducibility of intracoronary flow measurements was not assessed as this technique is well established, our group has had extensive experience in this space (Anderson et al. 2005; Worthley et al. 2009; Worthley et al. 2007), and to avoid the inherent risk of protracted instrumentation of the coronary vessels following 'near normal' clinical findings. Contemporary studies have reported a biological variation in measures of Doppler FloWire CBF to yield a CV <3.5% (Leung et al. 2006).

2.5 RESULTS

2.5.1 Sample size

Consent was obtained in 51 consecutive patients. Of those, 32 patients had evidence of >30% luminal stenosis and thus were not enrolled in the study. Eighteen subjects successfully completed the invasive coronary protocol. In one subject, the guiding catheter was not able to safely engage the left main and thus the invasive protocol was abandoned. Three subjects were unable to complete the non-invasive CMR protocol and thus 15 individuals had both invasive and non-invasive datasets for comparison *(Figure 2.6)*.

2.5.2 Cohort

The clinical characteristics of the cohort are listed *(Table 2.1)*. The majority of patients were male (n = 9, 60%) and the mean age was 53 ± 13 years. Consistent with the attempt to identify a population less likely to have angiographic coronary disease, the majority of patients had fewer than three traditional risk factors for coronary disease (73%), the most prevalent being hypertension (53%).

The most common class of medication was angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) (67%) although the majority were on < 3 cardiovascular medications (87%).

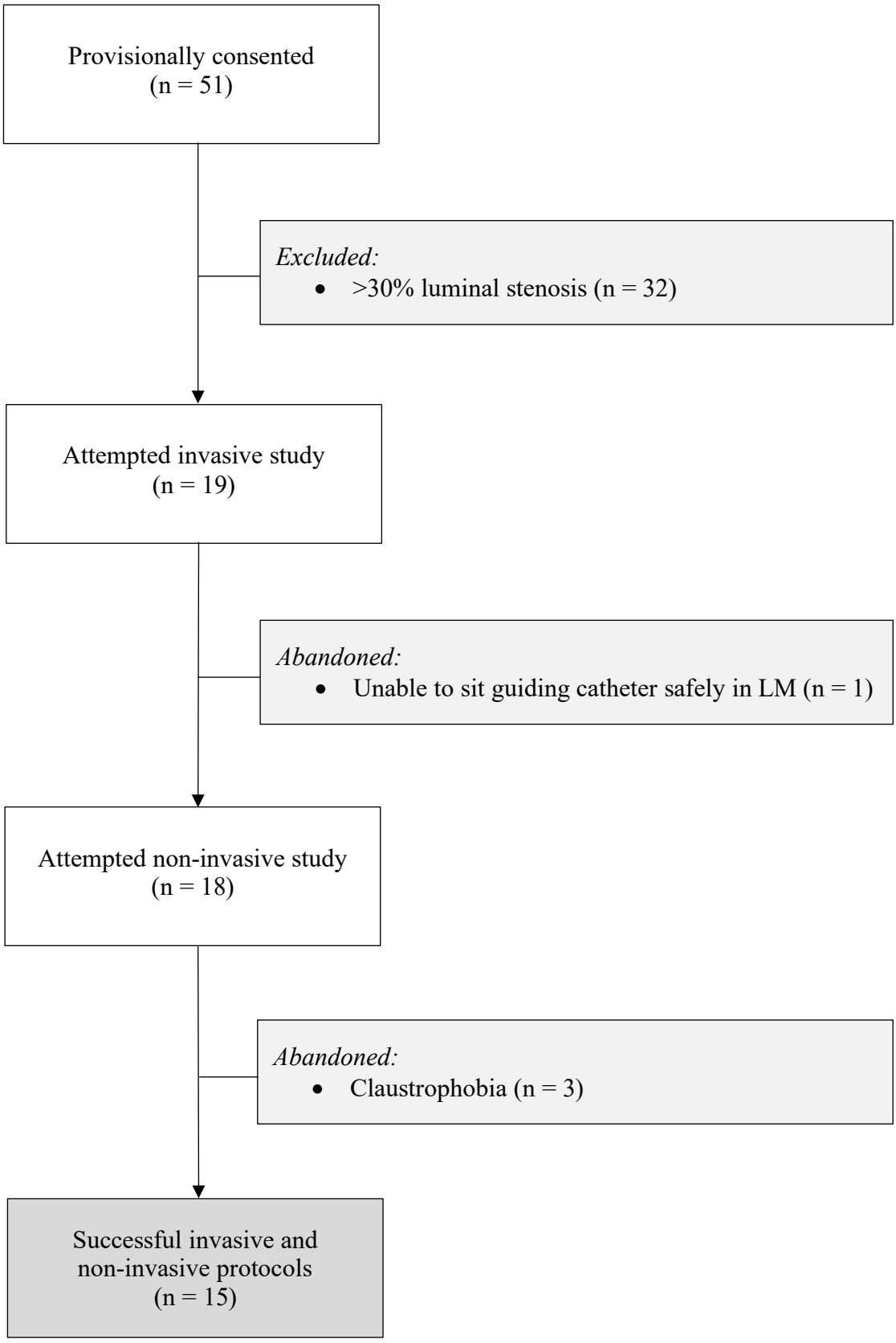

FIGURE 2.6. *Cohort build*

Clinical characteristic	Cohort
Mean age ± SD [years]	53 ± 13
Male sex – no. (%)	9 (60)
Diagnostic angiogram indication – no. (%)	
Chest pain for investigation	11 (73)
Dyspnoea for investigation	2 (13)
Positive stress test	2 (13)
Mean BMI ± SD [kg/m^2]	29.1 ± 4.1
Risk factors – no. (%)	
Family history	3 (20)
Cigarette smoking	2 (13)
Hypertension	8 (53)
Dyslipidaemia	7 (46)
Diabetes	4 (26)
≤ 3 traditional risk factors	11 (73)
Medications – no. (%)	
Aspirin	6 (40)
ACE/ARB	10 (67)
Statin	7 (47)
Diuretic	2 (13)
≤ 3 medications	14 (87)

TABLE 2.1 *Clinical characteristics*

Laboratory tests *(Table 2.2)* revealed normal kidney and liver function in addition to gender-appropriate haematological indices without viscosity. Lipid profile overall was only minimally outside the reference ranges – mean LDL 2.3 ± 0.6mmol/L, mean HDL 1.1 ± 0.2mmol/L and TG 1.1 ± 0.8mmol/L. There were no new diagnoses of diabetes with a mean cohort fasting glucose of 4.1 ± 0.6mmol/L. Mean c-reactive protein for the cohort was normal 2.2 ± 1.4mmol/L.

Laboratory characteristics	**Cohort**
Lipid profile ± SD [mmol/L]	
Total cholesterol	4.2 ± 0.6
Triglycerides	1.2 ± 0.7
LDL-C	2.6 ± 0.5
HDL-C	1.1 ± 0.4
GGT ± SD [mmol/L]	61 ± 14.1
Hb ± SD [mg/dL]	131 ± 14
Fasting glucose ± SD [mmol/L]	4.7 ± 0.7
C-reactive protein [mg/L]	2.7 ± 1.8
eGFR [mL/min/1.73m^2]	71 ± 11.2

TABLE 2.2 *Laboratory characteristics. Abbreviations: low-density lipoprotein (LDL), high-density lipoprotein (HDL), gamma-glutamyl transferase (GGT), haemoglobin (Hb), estimated glomerular filtration rate (eGFR)*

2.5.3 Invasive studies

Aside from the inability to safely (for research purposes) engage the left main coronary ostium in one patient, the invasive protocol was uneventful and well tolerated. One patient experienced four seconds of AV block following adenosine although this was asymptomatic and without appreciable change in invasive blood pressure. The LAD was studied in all patients given the ease of instrumentation.

A summary of the invasive indices is listed *(Table 2.3)*. The mean LAD diameter was 3.33 ± 0.6mm. Baseline APV was 14.7 ± 5.7cm/s producing a resting CBF of 62.2 ± 16.0mL/min. Peak APV was 46.8 ± 20.3cm/s producing a hyperaemic CBF of 192.7 ± 66.2mL/min. An intact microvascular response was observed in each subject with all CFVR > 2.5.

The mean CFVR for the cohort was 3.15 ± 0.48 (Range, 2.5 – 4.9). Individual APV response curves to adenosine bolus are displayed *(Figure 2.7)*.

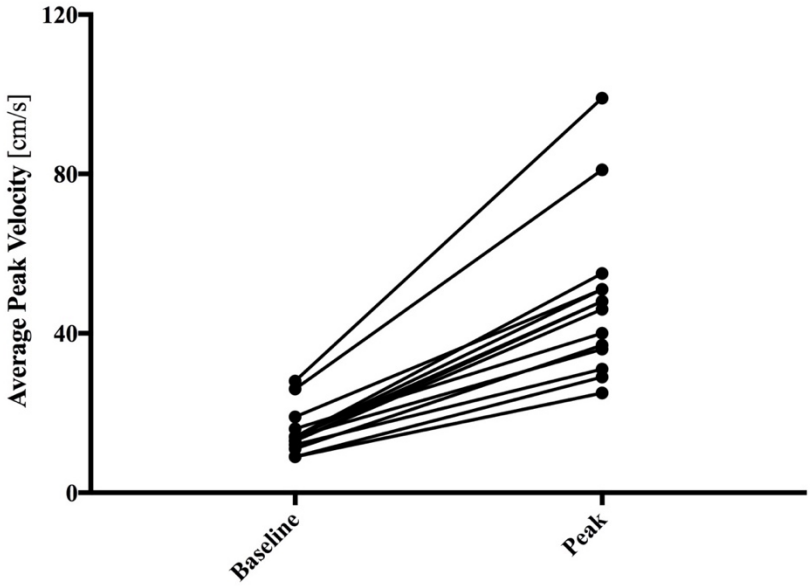

FIGURE 2.7 *Average peak velocity response curves to adenosine bolus.*

Variables	Mean ± SD
Baseline haemodynamics	
bSBP [mmHg]	136 ± 12
bDBP [mmHg]	83 ± 9
bPP [mmHg]	54 ± 8
HR [bpm]	67 ± 11
Rate pressure-product	8599 ± 2016
Invasive indices	
QCA LAD diameter [mm]	3.33 ± 0.6
Resting CBF [mL/min]	62.2 ± 16.0
Hyperaemic CBF [mL/min]	192.7 ± 66.2
CFVR	3.15 ± 0.48
cPP [mmHg]	61 ± 11
Cardiac structure and function	
LVEDV [mL]	113.7 ± 10.8
LVESV [mL]	34.6 ± 7.1
LVEF [%]	66.7 ± 4.9
LV mass [g]	106.4 ± 16.6
Aortic distensibility [$10^{-3}mmHg^{-1}$]	
AA	3.89 ± 1.72
PDA	4.08 ± 1.80
DDA	4.42 ± 1.67

TABLE 2.3 *Investigative variables. Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), brachial pulse pressure (bPP), heart rate (HR), quantitative coronary angiography (QCA), left anterior descending (LAD), coronary blood flow (CBF), coronary flow velocity reserve (CFVR), central pulse pressure (cPP), left ventricular end diastolic and systolic volume (LVEDV, LVESV), left ventricular ejection fraction (LVEF).*

2.5.4 Non-invasive studies

Aside from the three patients with claustrophobia, the CMR protocol was well tolerated and uneventful. The approximate time spent in the chamber was 20 minutes.

2.5.4.1 Cardiac structure and function

All subjects had normal left ventricular cavity size (LVEDV 113.7 ± 10.8mL. Reference ranges: males 115-198mL; females 88-168mL) and systolic function (LVEF 66.7 ± 4.7%. Normal range: 58-76%) (Maceira et al. 2006).

Although hypertension was the most prevalent risk factor and our cohort had a mean brachial SBP of 135 ± 12mmHg, there was no evidence of left ventricular hypertrophy (LV mass 106.4 ± 16.6g. Reference ranges: males 108-184g; females 72-144g).

2.5.4.2 Aortic distensibility

Measures of AD were relatively consistent across all three measured levels: AA (3.89 ± $1.72 \cdot 10^{-3}$mmHg^{-1}), PDA (4.08 ± $1.8 \cdot 10^{-3}$mmHg^{-1}) and DDA (4.42 ± $1.67 \cdot 10^{-3}$mmHg^{-1}). There was a numerical reduction in AD moving proximally along the aorta, a phenomenon associated with ageing our group has reported on previously (Nelson et al. 2009) although this did not reach statistical significance ($p = 0.127$).

2.5.5 Comparison of invasive and non-invasive aortic stiffness measures

Central pulse pressure correlated strongly with all levels of aortic distensibility: vs. AA ($R^2 = 0.525$, $p = 0.002$), PDA ($R^2 = 0.560$, $p = 0.001$) and DDA ($R^2 = 0.509$, $p = 0.0028$). Central and brachial pulse pressure had strong agreement (R^2 0.425 $p = 0.0084$).

2.5.5.1 Resting coronary blood flow

All measures of aortic distensibility were associated with rCBF: AA, R^2 0.373 (p = 0.016); PDA, R^2 0.361 (p = 0.018); and DDA, R^2 = 0.350 (p = 0.020). After adjusting for age and gender, the relationships remained statistically significant at all levels ($p<0.05$).

Univariate analysis revealed an association between rCBF and cSBP (R -0.578, p = 0.024) although minimal association with cPP (R -0.359, p = 0.189) or cDBP (R -0.233, p = 0.404). There was no relationship with measures of brachial blood pressure. Resting CBF did not correlate with indices of cholesterol (TG, LDL, HDL), inflammation (CRP), obesity (BMI) or renal function.

2.5.5.2 Hyperaemic coronary blood flow

A modest inverse relationship was observed between cPP and hCBF, R^2 0.345 (p = 0.021), however after adjustment for age and gender, this was no longer statistically significant (p = 0.223). There was a strong correlation between hCBF and all levels of aortic distensibility: AA, R^2 0.453 (p = 0.006); PDA, R^2 0.455 (p = 0.005); and DDA, R^2 0.464 (p = 0.005). These relationships remained highly statistically significant after adjustment for age and gender ($p<0.05$).

Univariate analysis revealed an association between hCBF and cSBP (R -0.667, p = 0.007) as well as hCBF and cPP (R -0.727, p = 0.002) although minimal association with cDBP (R -0.233, p = 0.404).

Hyperaemic CBF did not correlate with indices of cholesterol (TG, LDL, HDL), inflammation (CRP), obesity (BMI) or renal function.

2.5.5.3 Coronary flow velocity reserve and aortic stiffness

A strong association was observed between cPP and CFVR, R^2 0.532 (p = 0.002). Similarly, robust relationships were observed between CFVR and the non-invasive measures of aortic stiffness at all levels of the aorta: AA, R^2 0.511 (p = 0.003); PDA, R^2 0.451 (p = 0.006); and DDA, R^2 0.442 (p = 0.007). All of these relationships remained highly statistically significant after adjustment for age and gender ($p < 0.01$). There was no relationship between measures of either central or brachial blood pressures and CFVR.

Coronary flow velocity reserve did not correlate with indices of cholesterol (TG, LDL, HDL), inflammation (CRP), obesity (BMI) or renal function.

	rCBF	**hCBF**	**CFVR**
Unadjusted			
AA	**0.611 (0.016)**	**0.673 (0.006)**	**0.715 (0.003)**
PDA	**0.600 (0.018)**	**0.675 (0.006)**	**0.672 (0.006)**
DDA	**0.591 (0.020)**	**0.682 (0.005)**	**0.665 (0.007)**
cPP	-0.438 (0.103)	**-0.587 (0.021)**	**-0.729 (0.002)**
Adjusted			
AA	**0.565 (0.044)**	**0.655 (0.011)**	**0.695 (0.006)**
PDA	**0.545 (0.046)**	**0.634 (0.015)**	**0.623 (0.017)**
DDA	**0.569 (0.042)**	**0.670 (0.009)**	**0.670 (0.009)**
cPP	-0.154 (0.616)	-0.348 (0.223)	**-0.599 (0.024)**

TABLE 2.4 *Correlation between coronary blood flow and indices of arterial stiffness. R value with p value in brackets. Statistically significant association denoted by bolding of text. rCBF adjusted for age, gender and rate pressure product. hCBF and CFVR adjusted for age and gender.*

2.5.5.4 Effect of arterial stiffness on degree of hyperaemic response

Figure 2.8 demonstrates the relationship between rCBF and hCBF against measures of aortic distensibility. The resting and hyperaemic slopes converge when plotted against all levels of aortic distensibility. There was a statistically significant difference between the slopes of the respective resting and hyperaemic CBF regression lines; AA, $p = 0.017$; PDA, $p = 0.018$; and DDA, $p = 0.015$, suggesting a stiffer aorta is associated with a reduced hyperaemic response to adenosine. These relationships persisted after adjusting both CBF curves for age and gender as well as RPP for rCBF.

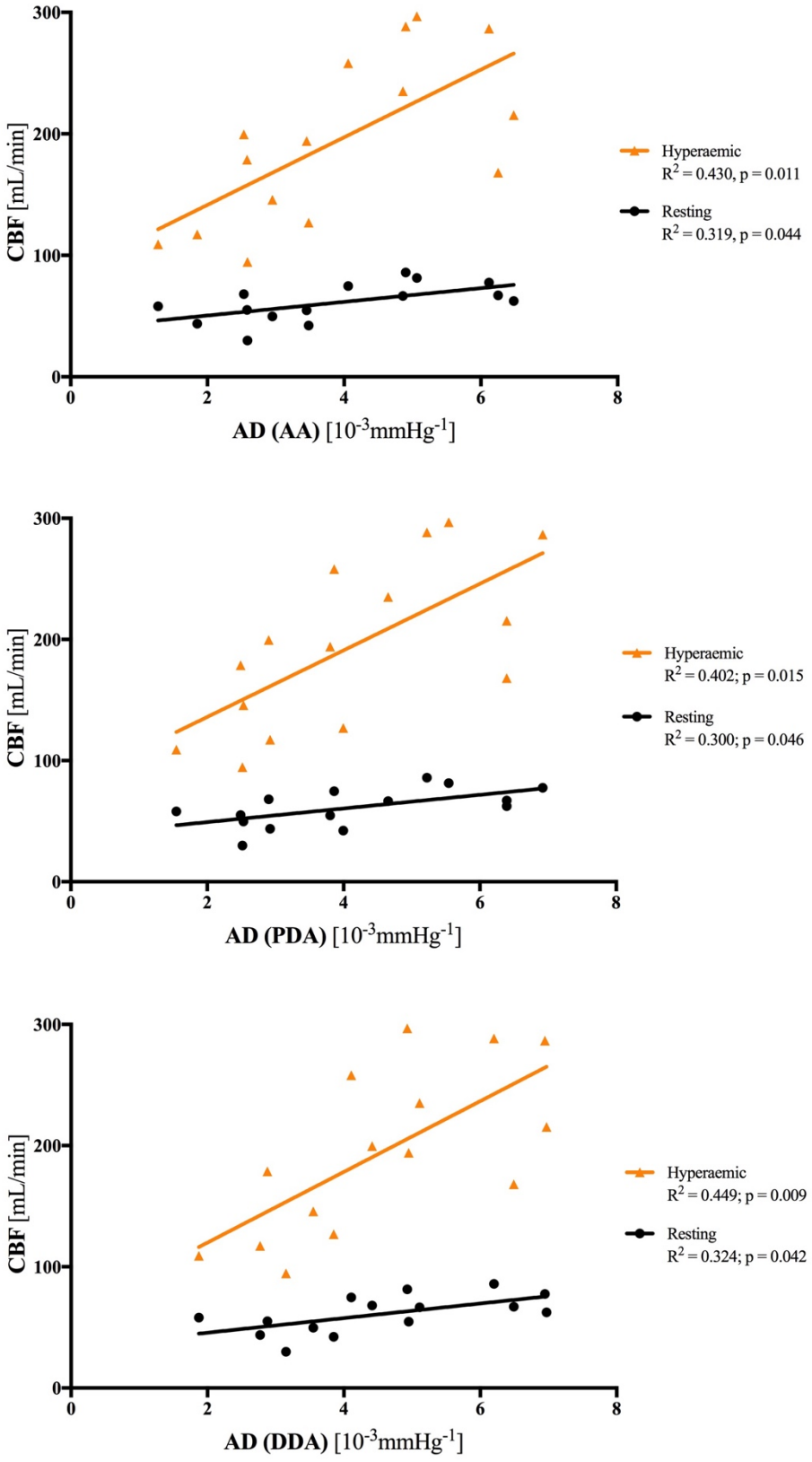

FIGURE 2.8 *Resting and hyperaemic CBF versus aortic distensibility at the AA (top), PDA (middle) and DDA (bottom). Differences between the slopes of rCBF and hCBF lines were all <0.05.*

2.6 DISCUSSION

To our knowledge, this is the first study to evaluate the relationship between invasive indices of coronary blood flow and CMR derived measures of arterial stiffness.

Aortic stiffness and coronary blood flow.

This study demonstrates that hyperaemic and to a lesser extent, resting coronary blood flow are associated with measures of aortic stiffness in patients with no more than minor coronary artery disease. As evidenced by the linear relationships, a stiffer aorta is associated with reduced resting and hyperaemic blood flow.

Differential impact on rCBF

All three levels of measured aortic distensibility correlated with both resting and hyperaemic blood flow. The association, however, was more robust with hCBF than it was with rCBF. A similar finding was observed with cPP, a coarser marker of stiffness, where its association no longer maintained statistical significance following adjustment for other known determinants of rCBF. With modest correlation coefficients and borderline statistical significance, rCBF therefore appears to be predominantly governed by factors other than large artery stiffness such as myogenic autoregulation. Although difficult to tease out with CFVR given its 'whole of vessel' functional assessment, and particularly with this cohort all having a CVFR > 2.5, it remains of interest whether aortic stiffness would play a more 'dominant' role in governing rCBF when either the microvascular reserve is fully 'compensated' or if the microvasculature is dysfunction. Extending this out to epicardial disease, Leung et al. demonstrated a tighter relationship between aPWV and rCBF in the presence of a flow limiting stenosis (R^2 = 0.453) compared to post PCI when the lesion had been effectively treated (R^2 = 0.261). Whether this represents exhaustion of the microcirculatory reserve to accommodate supply/demand, or concomitant

microcirculatory dysfunction, or features of both, large artery stiffness may have a greater impact on rCBF in states of 'disease' than in 'health'.

Hyperaemic response to adenosine

The convergence of the hCBF and rCBF curves when plotted against aortic distensibility support earlier work from Leung et al. (Leung et al. 2006) that a stiffer aorta conveys a reduced hyperaemic response to adenosine. Leung et al. demonstrated this relationship in both pre and post PCI coronary vessels, however our study has extended this observation to subjects with near normal coronary arteries.

Although our contention is that a stiff aorta is less capable of accommodating an increase in flow, it is plausible that there are other contributors to this relationship. Increased aortic stiffness has been associated with endothelial function and thus endothelial-mediated adenosine effects may be reduced by synchronous microvascular dysfunction.

An extension of this relationship has broad clinical implications. In our subjects with the least distensible aortas, their CFVRs approached 2.5 - the threshold considered significant for ischaemia. In an individual with a chest pain syndrome and only minor obstructive coronary disease, a very stiff aorta may be enough to exhaust perfusion reserve and could provide a causal link to 'microvascular angina'. As proposed by Kingwell et al. (Kingwell et al. 2002) , extending this to individuals with epicardial disease, a mild to moderate stenosis may behave like a severe stenosis in the setting of a stiff aorta.

Blood pressure and coronary blood flow

Univariate correlation revealed significant associations between cSBP and both resting and hyperaemic blood flow. Given CBF predominantly occurs in diastole, an association between SBP and both rCBF and hCBF invokes an indirect explanation. Systemic hypertension is associated with changes in the microvasculature such as

rarefaction (reduced number) and peri-arteriolar fibrosis (Feihl et al. 2008). It may therefore be that these chronic changes associated with elevated SBP may impact on both rCBF and hCBF and thus be a surrogate for microvascular function and intrinsic resistance. Supporting this is evidence that CFVR is reduced in patients with hypertension compared to normotensive controls (Rimoldi et al. 2014). Our data suggests this process may only partially associate with arterial stiffness, as there was minimal correlation between SBP and aortic distensibility in this cohort. An assessment of endothelial function, also known to be reduced in hypertension, may provide some insight as a potential mediator of the SBP and CBF relationship (Bolad & Delafontaine 2005).

Furthermore, there was no association between cDBP and indices of coronary blood flow suggesting there may be a greater contribution from diastolic resistance on coronary flow than from central diastolic driving pressure per se. Simultaneous assessment of coronary blood flow and pressure would have allowed a better understanding of this interaction.

The lack of association between any peripheral measure of blood pressure and coronary flow indices again highlights the key role central pressure assessment has not only in elucidating putative mechanisms, but why its predictive strength may be far superior to brachial measures (McEniery et al. 2014).

Measures of arterial stiffness

Aortic stiffness increased in a linear manner moving from the abdomen towards the aortic valve and, although not statistically significant, is a well described phenomenon with ageing (Hundley et al. 2001; Nelson et al. 2009; Rogers et al. 2001). Conceivably the proximal aorta may be incrementally more important to the aorto-coronary relationship than the abdominal aorta given its predisposition to stiffening, in addition to its geographical proximity to the coronary ostia; however, in our cohort all levels correlated with similar strength to the observed measures of CBF and MPR. This may

be reflective of the relatively similar values for distensibility that were obtained at all three sites or possibly that we were underpowered to detect these differences. Alternatively, it could be that the aorto-coronary relationship is reflective of 'net' large artery function and thus all measures will confer agreement.

Limitations

As with other studies in this field, the invasive and involved nature of the protocol means the sample size is small. Despite this limitation, our correlations were statistically robust. Although the CMR component of the study was performed within two hours of the invasive protocol, the assessment of distensibility and coronary blood flow was not simultaneous and although unlikely, potentially affected by different loading and haemodynamic conditions. An inherent limitation of aortic distensibility assessment is the reliance on bPP rather than central distending pressure. Although we had access to this value by virtue of the invasive arm of the protocol, our work in distensibility to date has utilised bPP, and as with other larger studies in this area, provides a reasonable approximation in most individuals.

To complete the translation from invasive to non-invasive validation, it would have been ideal to have performed a comparison between coronary blood flow with Doppler FloWire and myocardial blood flow with CMR perfusion imaging (CMR-PI). This would have required patients to have consented to gadolinium administration after having undergone coronary angiography within a matter of hours. This was felt to be unethical in view of these patients already having received a diagnosis of non-obstructed coronary arteries. Other studies in this space, however, have shown robust correlation indices of myocardial perfusion in both ischaemic and non-ischaemic myocardium against invasive Doppler FloWire (Kurita et al. 2009), invasive QCA (Mordini et al. 2014) and PET (Morton et al. 2012). A further potential limitation in the study relates to the certainty of obtaining maximal hyperaemia. More recent

evidence suggests some patients do not obtain maximal hyperaemia at this dose and that doses as high as 200μg are recommended by some (Adjedj et al. 2015).

Conclusion

To our knowledge, this is the first study to establish the purported relationship between arterial stiffness and coronary blood flow using CMR derived aortic distensibility and invasive Doppler coronary FloWire in essentially 'normal' coronary arteries.

3. CHAPTER 3: Non-invasive proof of concept

Cardiovascular magnetic resonance imaging can non-invasively evaluate aorto-coronary haemodynamics

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3.1 STATEMENT OF AUTHORSHIP

Manuscript details

Title of paper	*Cardiovascular magnetic resonance imaging can non-invasively evaluate aorto-coronary haemodynamics*
Publication Status	Submitted for publication

Principal Author Contributions

Candidate	Dr Adam J Nelson
Contribution to the Paper	Primary contributor to the conception and design of the work; Drafted the work; Provided final approval of the version to be published; Accountable for all aspects of the work.
Overall percentage	90%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

August 2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	A/Prof Matthew I Worthley
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August 2018

3.2 ABSTRACT

Background: Invasive evidence supports an association between large artery function and indices of myocardial blood flow. Further evaluation of this relationship has been limited by the invasive nature of current techniques, requirement for multi-modality or sequential studies, or by inaccurate, inadequate imaging resolution. This study sought to validate the use of cardiovascular magnetic resonance (CMR) to evaluate both aortic distensibility and myocardial blood flow as a method to demonstrate the aorto-coronary relationship in a completely non-invasive, simultaneous manner.

Methods: Patients referred for a clinically indicated CMR perfusion imaging (CMR-PI) study were invited to participate. Aortic distensibility was evaluated and after excluding studies with perfusion defects or scar, semi-quantitative and quantitative perfusion analysis was performed using proprietary software. Signal-intensity time curves were generated from short-axis slices allowing myocardial perfusion reserve (MPR) as well as both absolute resting and hyperaemic blood flow (rMBF, hMBF) to be obtained.

Results: Fifty-three subjects (61.2 ± 10.4 years), the majority being male (62%), were included in the study. Aortic distensibility was mildly reduced across the cohort (average $3.43 \pm 1.58 \cdot 10^{-3} mmHg^{-1}$), however all had a normal response to adenosine (MPR 2.9 ± 0.65). Aortic distensibility was associated with rMBF (R^2 0.231 – 0.281, $p < 0.01$), hMBF (R^2 0.282 – 0.409, $p < 0.01$) and MPR (R^2 0.421 – 0.517, $p < 0.01$). Furthermore, CMR-PI was repeatable with robust intra-class coefficients: MPR (0.96); rMBF (0.83); and hMBF (0.91).

Conclusion: This is the first study to utilise CMR derived indices of both myocardial blood flow and aortic distensibility to evaluate the aorto-coronary relationship. Aortic distensibility correlated strongly with hMBF and MPR although only modestly with rMBF. This repeatable and non-invasive technique may facilitate a broader evaluation of this physiology in different subgroups, and potentially in a serial manner over time.

3.3 INTRODUCTION

Aortic stiffness has emerged as an independent predictor of cardiovascular outcomes in a broad cohort of patients (Ben-Shlomo et al. 2014). Moreover, it provides incremental prognostic capacity above standard risk stratification algorithms (Vlachopoulos et al. 2010) and now appears in European guidelines (Van Bortel et al. 2012). Although associated with neurological (van Sloten et al. 2015) and renal (Ford et al. 2010; Townsend et al. 2018) sequelae due to increased transmission of pulsatility at a microvascular level (O'Rourke & Safar 2005), it is the putative impact on the heart that is likely to drive its cardiovascular predictive capacity.

Mechanistically a stiffer aorta results in a faster pulse wave which results in an earlier return of reflected waves at end-systole rather than diastole. The net effect is an increase in left ventricular cardiac work, an increase in myocardial oxygen demand and with time, compensatory hypertrophy. The earlier return of the reflected wave fails to augment diastolic pressure leading to an increase in pulse pressure but importantly, a reduction in coronary perfusion pressure.

Seminal work in canines provided evidence of this relationship (Ohtsuka et al. 1994; Watanabe et al. 1992), however more recently, Leung et al. (Leung et al. 2006) confirmed the association between resting and hyperaemic blood with large artery stiffness. Although compelling, we recently have extended their observations using cardiac magnetic resonance (CMR) derived aortic distensibility in individuals with no significant epicardial coronary disease *(Chapter 2)*. A similar relationship was found whereby a stiffer artery confers not only reduced rCBF but also a blunted response to adenosine. As with Leung et al. this study still required an invasive arm to evaluate measures of coronary flow which limits its use to small scale, cross-sectional cohorts. With the advent of improved 1st pass perfusion techniques in CMR it has now become possible to quantitate absolute myocardial blood flow (MBF) and myocardial

perfusion reserve (MPR) using adenosine as the hyperaemic stimulus and gadolinium as the contrast agent. A number of studies have shown these values to approximate those obtained in animal models with microspheres (Christian et al. 2009; Jerosch-Herold et al. 2002) and more recently, in larger human studies (Arnold et al. 2011; Cheng et al. 2008; Heydari et al. 2015; Selvanayagam et al. 2005)

The aim of this study was to perform simultaneous measurements of aortic stiffness and both MBF and MPR to demonstrate this relationship in a completely non-invasive manner.

3.4 METHODS

3.4.1 Subjects

3.4.1.1 Inclusion criteria

The study was approved by the Human Research Ethics Committee at the Royal Adelaide Hospital. Patients referred for a CMR perfusion imaging (CMR-PI) scan at the Royal Adelaide Hospital to evaluate for evidence of ischaemic heart disease, either chest pain or dyspnoea, were invited to participate. Subjects were enrolled consecutively between January 2012 until the September 2013. Patients had abstained from caffeine and xanthine-containing products for at least 24 hours prior to scanning as per local protocol.

3.4.1.2 Exclusion criteria

Exclusion criteria included age >75 years, use of vasoactive medications <48 hours of angiography (calcium channel blockers, nitrates, beta blockers), established coronary disease (angina, history of myocardial infarction, prior revascularisation), atrial fibrillation/flutter, aortopathy (history of intervention, dissection or aneurysm), magnetic resonance chamber incompatibility (pacemakers, aneurysm clips, claustrophobia), impaired LV function (LVEF < 45%), severe valvular heart disease, renal impairment (estimated glomerular filtration rate < 30mL/min/1.73m^2) or atrioventricular nodal disease (Grade II or higher) were excluded.

3.4.1.3 Demographics

Prescribed medications and risk factors were recorded. Risk factors were defined as cigarette smoking (previously or currently, smoking tobacco), hypercholesterolemia (use of cholesterol-lowering therapy or total cholesterol level > 5.2mmol/L), hypertension (blood pressure > 140/90mm Hg or on medical antihypertensive therapy or subject to lifestyle intervention), obesity (BMI > 30kg/m^2), diabetes (plasma haemoglobin A1c > 6.5%, or on glycaemic medications), and family history of

cardiovascular disease (index event in a first-degree relative, male ≤ 55 years and female ≤ 60).

3.4.2 Cardiac magnetic resonance protocol

All cardiovascular magnetic resonance (CMR) studies were performed utilizing the Siemens Avanto 1.5 Tesla magnetic resonance imaging scanner (Siemens Medical Imaging, Erlangen, Germany) located within the Cardiovascular Investigation Unit of the Royal Adelaide Hospital. All CMR studies were performed with subjects in a supine position with a phased-array surface coil positioned over the thorax.

3.4.2.1 Cardiac structure and function

Methods for evaluating left ventricular structure and function are covered in *Chapter 2*. In brief, short-axis slices from the mitral annulus to and inclusive of the left ventricular apex were acquired during expiratory breath hold.

Offline analysis was performed to evaluate LV size and function utilizing proprietary software (Argus software, Siemens Medical Imaging, Erlangen, Germany). Endocardial contours were manually traced at end-diastole (start of R-wave) and at end-systole (visually smallest cavity). End-systolic and end-diastolic volumes were obtained and thus left ventricular ejection fraction could be calculated.

3.4.2.2 Aortic distensibility

Following the cardiac protocol, aortic imaging was performed using the same phased-array surface coil placed on the thorax in addition to a spine-coil-array positioned within the patient bed. Images were acquired during expiratory breath hold with retrospectively ECG-gated SSFP sequences. Acquisition time was determined to include 90% of the R-R interval, image matrix 256 x 150, FOV 320mm, TR 40ms, TE 1.4ms, Flip Angle 40°. Slice thickness was 6 mm.

Sagittal oblique aortic imaging was initially acquired from the level of the aortic sinuses to the renal arteries. Using the right pulmonary artery as a reference level, a perpendicular cross-sectional cine image was acquired of the ascending aorta (AA) and proximal descending aorta (PDA) as well as a further SSFP cine image of the distal descending aorta (DDA) approximately 5cm beyond the level of the diaphragm.

In addition, with plans to use sequences acquired already as part of a clinically indicated scan, aortic measurements were also performed on the vertical long axis (VLA) image. This sequence is obtained from an axial scout image by defining a sagittal oblique plane from the LV apex through the mitral valve. The resultant image is the VLA and includes a 'two chamber' picture of the myocardium with a lateral view of the LA and LV *(Figure 3.1)*. From a thoracic cavity perspective, this transects the aortic arch around the level of the great vessels and approximates a level of the thoracic aorta (TA) between the conventional distensibility fiduciary points of AA and PDA. Given this sequence is planned on myocardial structures – i.e. visibility of the four intracardiac chambers – there is the potential for this acquisition to be slightly oblique with respect to the aorta. This would generate a cross section of the aorta in the shape of an ellipse rather than a circle. Although vessels that appear ellipsoid at low pressure often distend to a circle under high pressure (Melbin & Noordergraaf 1971) the assumption in the distensibility formula is that of 'circular' expansion. Major and minor axes were therefore drawn and if the ellipticity index was <0.9 (minor/major), the patient was excluded from analysis.

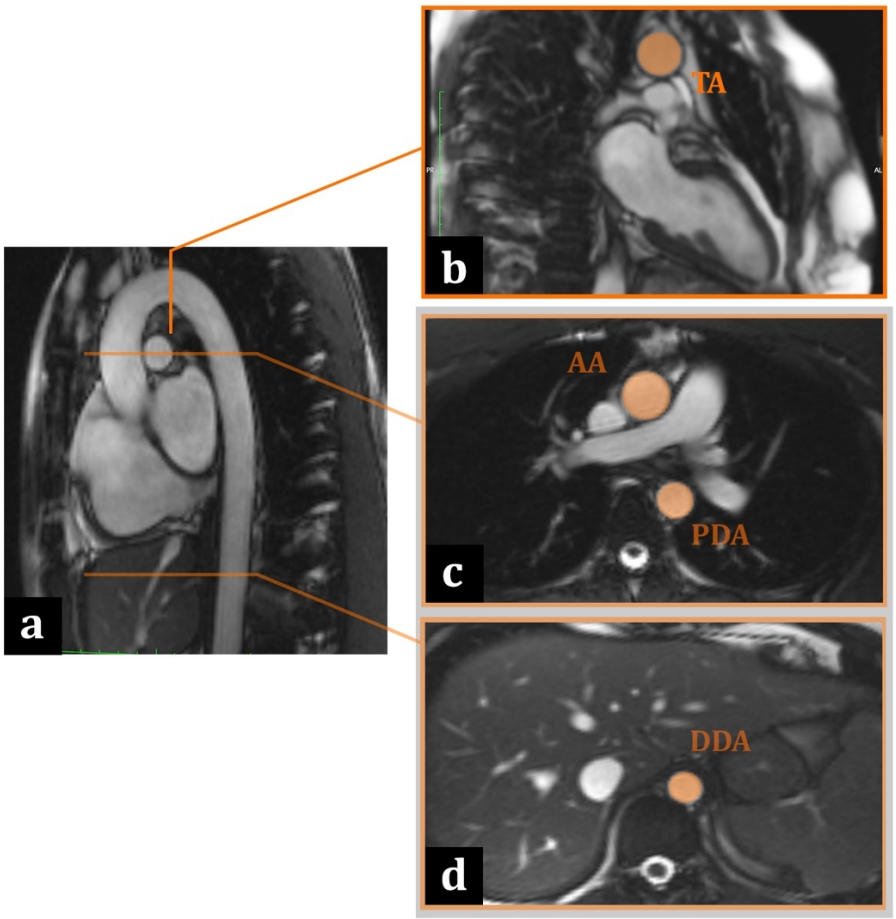

FIGURE 3.1 *Expanded from Figure 2.5. Representative sagittal or 'candy cane' view of aorta which shows the point at which the vertical long axis plane (b) transects the aortic arch allowing calculation of 'Thoracic Aorta' (TA) distensibility. Images (c) and (d) reflect the 'traditional' cross sectional planes and are acquired at the level of the pulmonary artery to generate 'Ascending Aorta' [AA] and Proximal Descending Aorta [PDA] (c), and 5cm below the diaphragm to generate 'Distal Descending Aorta [DDA] (d).*

Brachial artery blood pressure was taken simultaneously using a CMR compatible automated non-invasive sphygmomanometer. Three results during both the cardiac and vascular protocols were obtained, and the measurements averaged. Outliers were discarded and repeat readings taken as required.

Aortic distensibility was evaluated offline by manually tracing aortic cross-sectional images at the level of the ascending aorta, proximal descending aorta, distal descending aorta and thoracic aorta. Maximal and minimal lumen areas were calculated and distensibility derived by a previously published formula (Groenink et al. 1998):

$$\frac{\textit{aortic area at end systole } (mm^2) - \textit{aortic area at end diastole } (mm^2)}{\textit{brachial pulse pressure (mmHg) x aortic area at end diastole } (mm^2)}$$

The pulse pressure was calculated from the difference between the systolic and diastolic blood pressures and measured in mmHg. The aortic areas were measured in mm^2 and after multiplying by 1000, aortic distensibility is reported in its final unit of $10^{-3}mmHg^{-1}$.

3.4.2.3 CMR Perfusion imaging

3.4.2.3.1 Adenosine infusion protocol

As per local protocol, a physician with a cardiac defibrillator was present for all stress perfusion imaging. Advanced resuscitation medications were also available including aminophylline for adenosine receptor antagonism, glyceryl trinitrate (GTN) for ongoing chest pain and atropine for symptomatic AV block. A 20G cannula was inserted into each antecubital fossa – one for adenosine and one for contrast. Adenosine (AdenoScan, Sanofi-Aventis, Surrey, UK) was infused at 140μg/kg/min using a precise syringe pump (Graesby 3500, Minneapolis, Minnesota, USA) to a target duration of 3 minutes. The infusion was ceased if the patient developed symptomatic AV block, severe hypotension (<90mmHg) or bronchospasm.

3.4.2.3.2 Image acquisition

During the last minute of the adenosine infusion, the gadolinium-based contrast agent, Magnevist (dimeglumine gadopentetate, Bayer, Leverkusen, Germany) was administered in the contralateral arm. This was at a dose of 0.2mmol/kg body weight and a rate of 7ml/s followed by a 30 mL saline flush at the same rate (Kramer et al. 2008; Lyne et al. 2007).

Perfusion imaging was performed with a T1-weighted fast low-angle singe shot gradient echo (GRE) sequence and was acquired every cardiac cycle during first pass. Perfusion pulse sequence parameters were as follows: echo time 1.04ms; repetition time 165ms; saturation recovery time 100ms; voxel size 2.7 x 2.2 x 10mm; flip angle 12°. Parallel acquisition method using generalised auto-calibrating partially parallel acquisition (GRAPPA) was utilised (Griswold et al. 2002) which has been shown to increase signal to noise ratio and improve image quality. Perfusion images were obtained at three short axis planes - basal, mid and apical levels - of the left ventricle (Cerqueira et al. 2002).

The protocol was subsequently repeated 20 minutes later without adenosine for 'rest' images. Throughout the CMR protocol heart rate and ECG were monitored continuously. A CMR compatible cuff allowed blood pressure to be acquired at 5-minutely intervals.

3.4.2.3.3 Visual analysis

Visual analysis of each perfusion study was undertaken off-line and a consensus reached between two experienced observers (KST, AJN). Rest and stress perfusion and the late gadolinium enhancement images of three short axis sections (base, mid and apex) were viewed side by side. If the signal intensity on stress perfusion appeared lower in an area of myocardium for at least three dynamic images compared with remote myocardium, it was considered to be ischaemic (Ingkanisorn et al. 2006). If the same signal intensity abnormality was seen in the rest and stress perfusion images and there was no evidence of scar on late contrast enhanced images, the defect was considered an artefact. Similarly, if the perfusion defect lasted for less than 3 dynamic images it was also considered artefactual (Ingkanisorn et al. 2006).

For this proof of concept project, studies demonstrating any evidence of matched (infarcted, non-viable myocardium) or reversible defect (ischaemia) on their perfusion maps were subsequently excluded.

3.4.2.3.4 Quantitative analysis

Studies without evidence of ischaemia were then subject to quantitative analysis. The endocardial and epicardial contours of three short axis sections (base, mid and apex) were traced (QMASS, v7 .2, Medical Imaging Solutions, Leiden, Netherlands) and corrected manually for displacements such as breathing *(Figure 3.2)*. Short axis sections were not further segmented into coronary territories as these were 'normal' studies and thus only global, transmural measures of signal intensity were required.

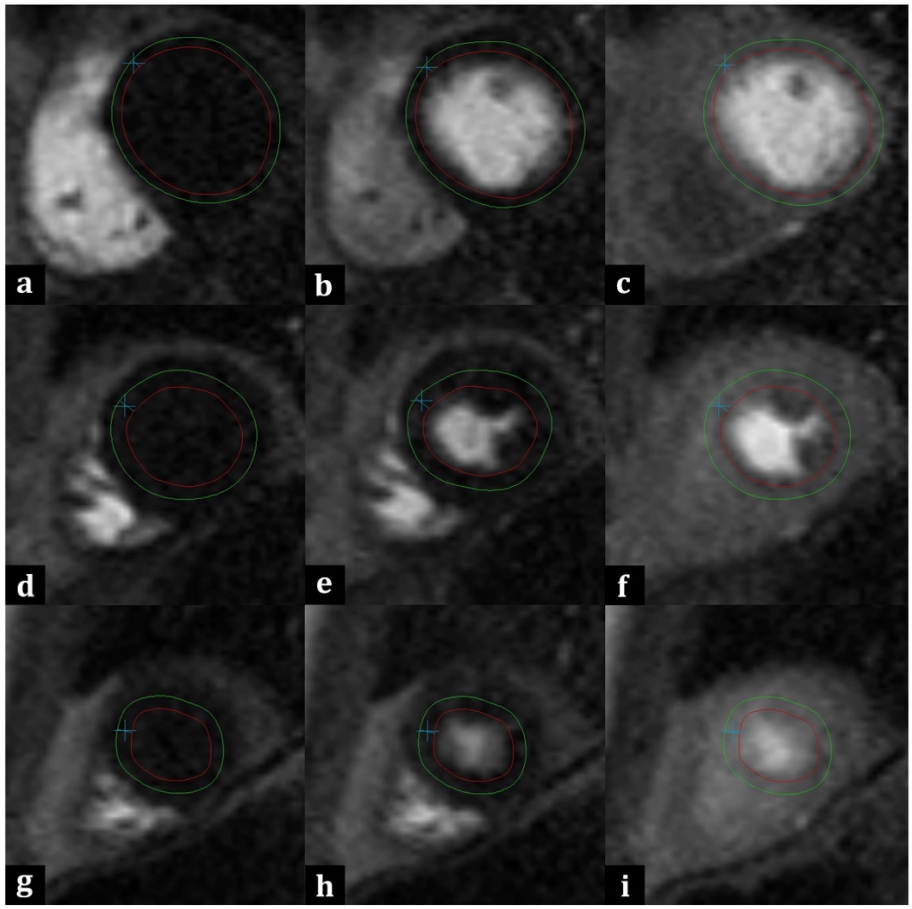

FIGURE 3.2 *Representative perfusion images demonstrating passage of gadolinium contrast over time at basal (a) – (c), mid (d) – (e) and apical (g) – (i) levels. Endocardial borders are depicted in red, epicardial borders in green (weighted towards endocardium).*

Within each section, signal intensity was measured by defining regions of interest that excluded the inner 10% and outer 30% of the myocardium to get stronger weighting of the subendocardium (where perfusion changes are more likely to occur) and reduce influence from the LV as previously described (Nagel et al. 2003).

The subendocardial signal intensity-time curves were generated for all segments by obtaining signal intensity on consecutive images before and during arrival of contrast material. The signal intensity-time curve for the left ventricle was generated on the basal section as a measure of input function *(Figure 3.3)*.

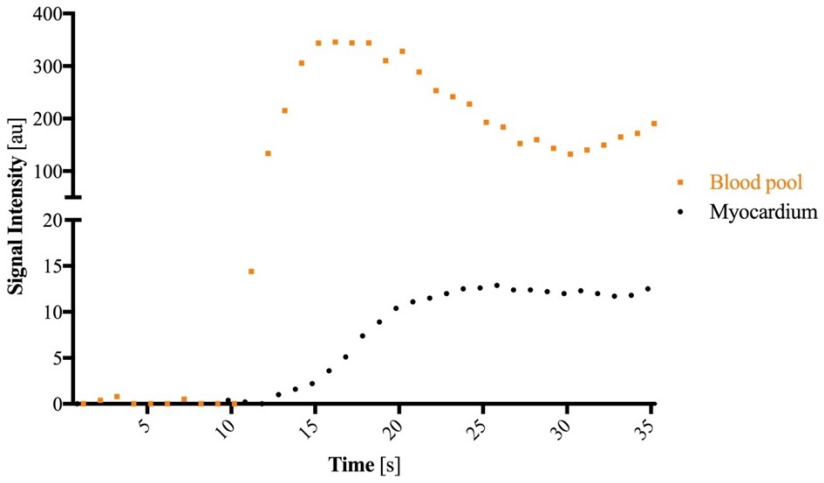

FIGURE 3.3 *Signal-intensity of contrast passage (arbitrary units) through blood pool (orange) and myocardium (black) over time.*

Myocardial perfusion reserve

The maximal initial upslope of the signal intensity-time curve was determined by using a sliding window with a four- point linear fit for the myocardium and a three-point fit for the left ventricle as previously described (Al-Saadi et al. 2000). For each segment, the myocardial perfusion reserve (MPR) was calculated by the quotient of maximal upslope at hyperaemia over maximal upslope rest *(Figure 3.4)*. This was performed for each of the three slices and then averaged for a 'global' MPR.

Myocardial blood flow

Deconvolution of the myocardial signal intensity time (SIT) curve and the blood pool SIT curve (as the arterial input function) was performed and fitted to a Fermi function. The maximum point on the resultant curve (as described in *Chapter 1*), closely approximates the absolute myocardial blood flow and has been extensively described (Cheng et al. 2008; Jerosch-Herold et al. 2002; Jerosch-Herold et al. 2000; Selvanayagam et al. 2005). Signal intensity time curves were generated in each short axis slice at rest and at hyperaemia allowing calculation of global values of resting (rMBF) and hyperaemic MBF (hMBF).

As basal CBF is closely related to the rate-pressure product (RPP) (Czernin et al. 1993), an index of myocardial oxygen consumption, values for basal flow in each subject were corrected for the respective RPP. This was performed by multiplying rMBF by the mean RPP of the cohort, divided by the RPP in the individual patient as previously described (Uren, Melin, et al. 1994).

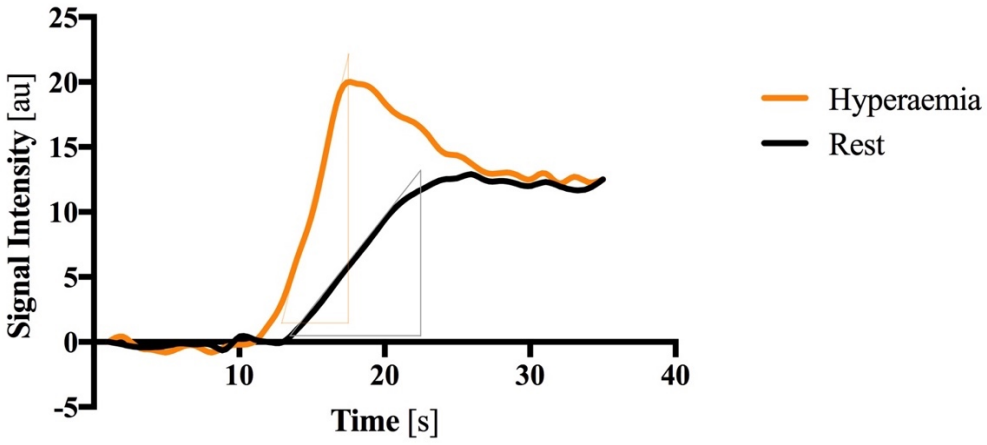

FIGURE 3.4 *Signal intensity-time curve comparing enhancement during hyperaemia (orange) vs. rest (black). Slopes of maximal intensity drawn on hyperaemic (light orange) and rest (grey) curves.*

3.4.3 Repeatability

Interobserver variability was performed on 20 de-identified scans and subject to Bland-Altman analysis (Bland & Altman 1986). Resting and hyperaemic MBF as well as MPR were all assessed. Aortic distensibility reproducibility has been previously published by our group (Nelson et al. 2009).

3.4.4 Statistical Analysis

Baseline characteristics are represented as mean ± standard deviation where normally distributed, or median and IQR for parameters with non-Gaussian distribution.

Regional variation in measures of aortic distensibility was assessed using 1-way ANOVA. The comparison of CBF and MPR against CMR measures of aortic distensibility was performed by regression analysis.

Reproducibility of CMR-PI measures was performed with Bland-Altman analysis.

Statistical significance was defined as a two-sided $p < 0.05$. Statistical analyses were performed using SPSS statistical software (v22, SPSS Inc. Chicago, Il) and graphs were drawn using GraphPad Prism 7 (GraphPad Software Inc, San Diego).

3.5 RESULTS

3.5.1 Sample size

Consent was obtained in 84 consecutive patients. Of those, seven patients were unable to complete their clinically indicated CMR-PI with six patients suffering claustrophobia and one patient sustaining intolerable chest heaviness after only 30 seconds of adenosine infusion. Of the 77 patients who completed the scan, three had poor image quality limiting even visual assessment of perfusion defects, five had reversible perfusion defects consistent with ischaemia, four had matched perfusion defects consistent with prior infarct and three were found to have presumed new left ventricular dysfunction.

The remaining 62 patients were subjected to both quantitative perfusion and aortic distensibility analysis. Of these, seven had suboptimal image quality owing largely to poor breath holding and two had an ellipsoid thoracic aorta on the VLA image plane. This left 53 subjects with complete data *(Figure 3.5)*.

3.5.2 Cohort

The clinical characteristics of the cohort are listed *(Table 3.1)*. The majority of patients were male (n = 33, 62%) with a mean age of 61 ± 10 years. As expected, the majority of patients were referred through with chest pain for investigation (87%) with a smaller number reporting dyspnoea as their main indication (13%). The most prevalent risk factors were hypertension (73%) and dyslipidaemia (68%) with just over a third being diabetic (36%). Consistent with these individuals being referred for an imaging-based functional test, over half had 3 or more traditional risk factors for coronary disease (51%). The most commonly prescribed medication was aspirin (64%) followed by either an ACE or an ARB (59%) with just less than half on statins (45%). Just less than a third were on a diuretic (25%), calcium channel blocker (30%) or beta blocker (20%).

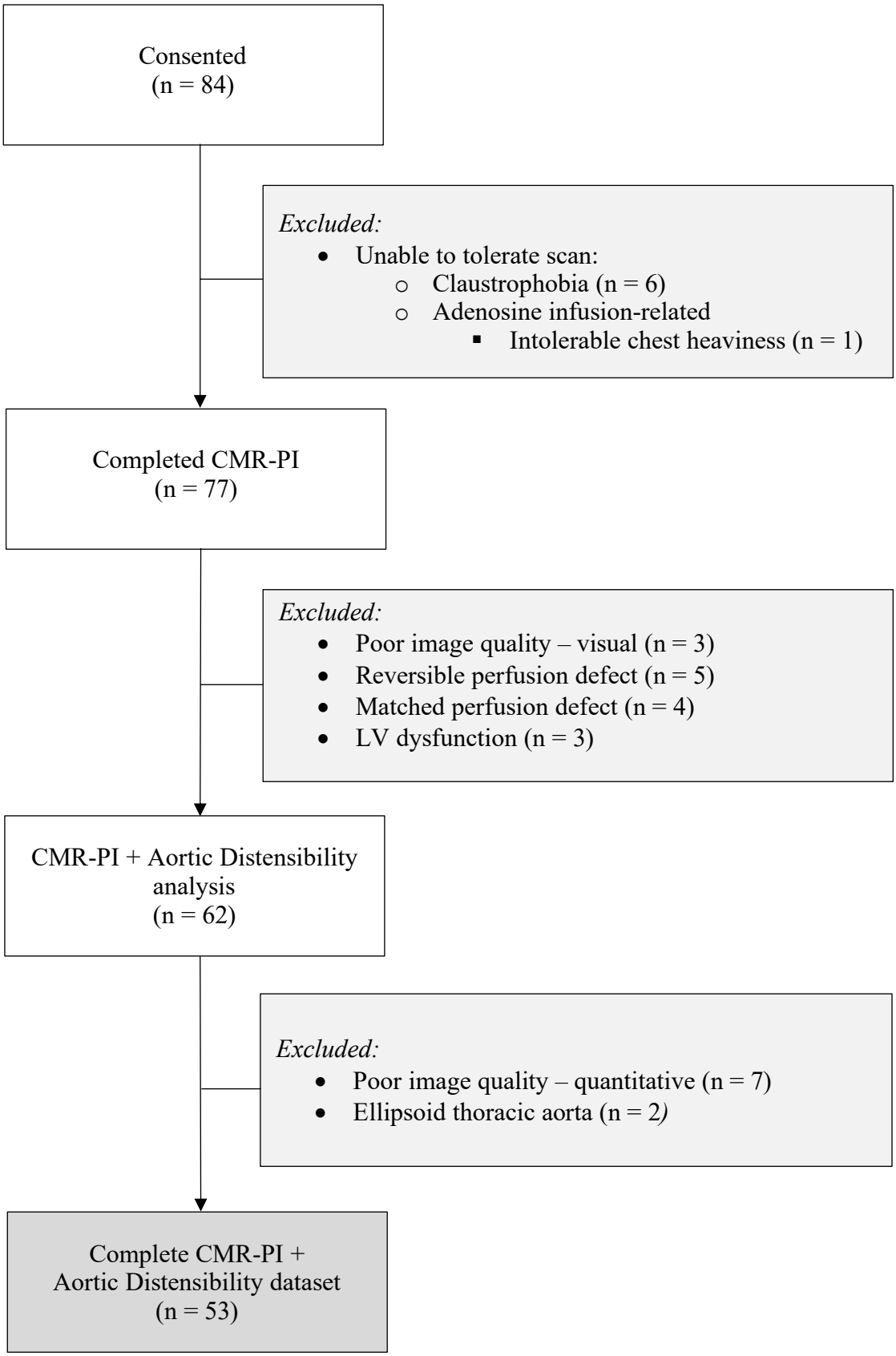

FIGURE 3.5 *Cohort build*

Clinical characteristic	Cohort
Mean age ± SD [years]	61.2 ± 10.4
Male sex – no. (%)	33 (62)
Stress test indication – no. (%)	
Chest pain for investigation	46 (87)
Dyspnoea for investigation	7 (13)
Mean BMI ± SD [kg/m2]	29.5 ± 3.2
Risk factors – no. (%)	
Family history	14 (26)
Cigarette smoking	18 (34)
Hypertension	39 (73)
Dyslipidaemia	36 (68)
Diabetes	19 (36)
≥ 3 risk factors	27 (51)
Medications – no. (%)	
Aspirin	34 (64)
ACEi/ARB	31 (59)
Statin	24 (45)
Diuretic	13 (25)
Calcium channel blocker	16 (30)
Beta blocker	13 (25)

TABLE 3.1 *Clinical characteristics. Abbreviations: BMI – body mass index; ACEi – angiotensin converting enzyme inhibitor; ARB – angiotensin receptor II blocker*

Laboratory investigations *(Table 3.2)* revealed normal kidney, liver function and red blood cell indices. Lipid profile overall was consistent with a primary prevention cohort: mean LDL 2.6 ± 0.5mmol/L; mean HDL 1.1 ± 0.4mmol/L; and TG 1.2 ± 0.7mmol/L. Despite one third being classed as diabetic, the fasting glucose for the cohort was 4.7 ± 0.7mmol/L. Mean c-reactive protein for the cohort was 2.7 ± 1.8mmol/L.

Laboratory characteristics	**Cohort**
Lipid profile ± SD [mmol/L]	
Total cholesterol	4.2 ± 0.6
Triglycerides	1.2 ± 0.7
LDL	2.6 ± 0.5
HDL	1.1 ± 0.4
GGT ± SD [mmol/L]	61 ± 14.1
Hb ± SD [mg/dL]	131 ± 14
Fasting glucose ± SD [mmol/L]	4.7 ± 0.7
C-reactive protein [mg/L]	2.7 ± 1.8
eGFR [mL/min/1.73m^2]	71 ± 11.2

TABLE 3.2 *Laboratory characteristics. Abbreviations: LDL – low-density lipoprotein; HDL – high density lipoprotein; GGT – gamma-glutamyl transferase; Hb – haemoglobin; eGFR – estimated glomerular filtration rate*

3.5.3 Cardiac Structure and Function

All subjects had normal left ventricular cavity size (LVEDV 121.4 ± 13.4mL. Reference ranges: males 115-198mL; females 88-168mL) and systolic function (LVEF 62.7 ± 5.3%. Normal range: 58-76%) (Maceira et al. 2006).

Left ventricular mass was within normal limits (LV mass 114.8 ± 19.5g. Reference ranges: males 108-184g; females 72-144g).

3.5.4 Aortic distensibility

3.5.4.1 Overall measures

Aortic distensibility for our cohort was similar to our cohort in *Chapter 2* with a mild increase in stiffness compared to 'healthy' controls (Nelson et al. 2009). As previously described, there was a linear reduction in aortic stiffness moving from the abdominal aorta proximally to the aortic valve. Of the 'traditional' measures of aortic distensibility, the lowest distensibility was observed at the level of the AA (3.11 ± $1.60 \cdot 10^{-3}$mmHg^{-1}) followed by the PDA (3.41 ± $1.57 \cdot 10^{-3}$mmHg^{-1}), and the DDA (3.76 ± $1.84 \cdot 10^{-3}$mmHg^{-1}).

The average distensibility (AA, PDA and DDA) was 3.43 ± $1.58 \cdot 10^{-3}$mmHg^{-1}. The mean of our new measure at the level of the TA was 3.23 ± $1.58 \cdot 10^{-3}$mmHg^{-1} which was numerically and anatomically between the AA and PDA.

Although there were numerical differences between all measures of distensibility *(Figure 3.6)*, this did not reach statistical significance ($p = 0.71$).

3.5.4.2 Agreement with TA distensibility

There was strong agreement between TA and all other measures of distensibility on regression analysis: AA (R^2 0.849, $p<0.0001$); PDA (R^2 0.821, $p<0.0001$); DDA (R^2 0.747, $p<0.0001$); and average distensibility (R^2 0.938, $p<0.0001$) .

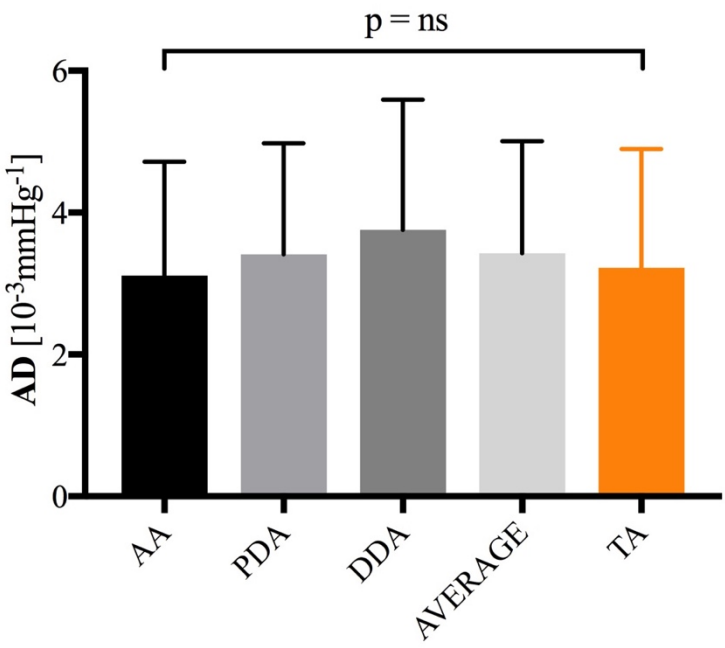

FIGURE 3.6 *Column graph of aortic distensibility measures at the level of AA (ascending aorta), PDA (proximal descending aorta), DDA (distal descending aorta) and TA (thoracic aorta). Average of the 'traditional' measures - AA, PDA and DDA - also depicted.*

3.5.5 CMR Perfusion imaging

As described in the cohort build *(Figure 3.5)*, just over 10% of the total population consented could not have either visual or quantitative perfusion analysis owing to suboptimal image quality. The vast majority of patients reported at least one symptom consistent with a hyperaemic response (n = 51, 96%), e.g. flushing, headache, dyspnoea, chest tightness.

There was a significant difference between rMBF (0.92 ± 0.17mL/min/g) compared with hMBF (2.95 ± 1.03mL/min/g) in response to adenosine infusion *(Figure 3.7)*.

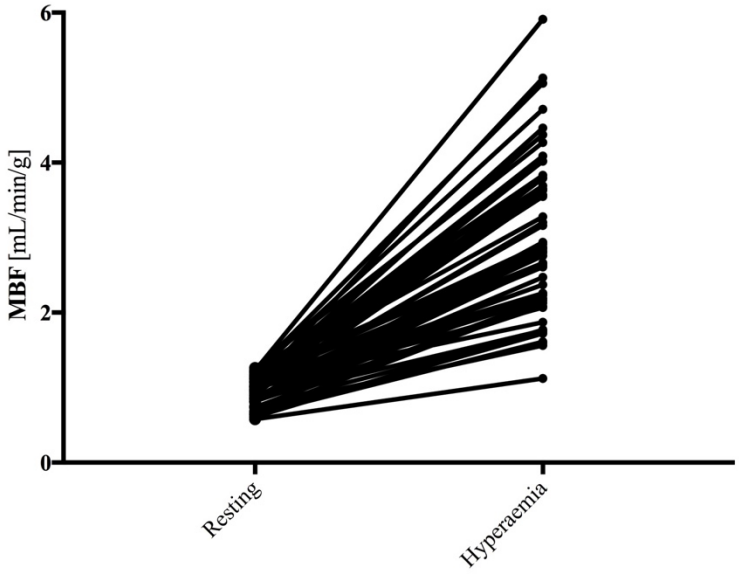

FIGURE 3.7 *Ladder plot of subject-level MBF response to adenosine infusion.*

Variables	Mean ± SD
Baseline haemodynamics	
SBP [mmHg]	146 ± 21
DBP [mmHg]	88 ± 12
PP [mmHg]	58 ± 13
HR [bpm]	61 ± 14
Cardiac structure and function	
LVEDV [mL]	121.4 ± 13.4
LVESV [mL]	39.1 ± 9.2
LVEF [%]	62.7 ± 5.3
LV mass [g]	114.8 ± 19.5
Aortic distensibility [10^{-3}mmHg^{-1}]	
AA	3.11 ± 1.60
PDA	3.41 ± 1.57
DDA	3.76 ± 1.84
Average (AA + PDA + DDA)	3.43 ± 1.58
TA	3.23 ± 1.64
Stress perfusion imaging	
Resting RPP	8960 ± 2620
Stress RPP	11810 ± 2130
rMBF [mL/min/g]	0.92 ± 0.17
hMBF [mL/min/g]	2.95 ± 1.03
MPR	2.91 ± 0.65

TABLE 3.3 *CMR-PI variables. Abbreviations: SBP – systolic blood pressure; DBP – diastolic blood pressure; PP – pulse pressure; HR – heart rate; LVEDV – left ventricular end-diastolic volume; LVESV – left ventricular end-systolic volume; LVEF – left ventricular ejection fraction; AA – ascending aorta; PDA – proximal descending aorta; DDA – distal descending aorta; TA – thoracic aorta; RPP – rate-pressure product; rMBF – resting myocardial blood flow; hMBF – hyperaemic myocardial blood flow; MPR – myocardial perfusion reserve.*

3.5.6 Association with indices of myocardial perfusion

3.5.6.1 Resting myocardial blood flow

All 'traditional' measures of aortic distensibility were modestly associated with rMBF *(Figure 3.8)*: AA, R^2 0.231 ($p = 0.0003$); PDA, R^2 0.280 ($p < 0.0001$); and DDA, $R^2 = 0.276$ ($p < 0.0001$). After adjusting for age and gender, the relationships remained statistically significant at all levels ($p < 0.01$).

Thoracic aortic distensibility was also modestly related to rMBF, R^2 0.281 ($p < 0.0001$) and after adjustment for age and gender, remained significant ($p < 0.01$).

Resting CBF did not correlate with indices of cholesterol (TG, LDL, HDL), inflammation (CRP), obesity (BMI) or renal function.

3.5.6.2 Hyperaemic myocardial blood flow

Of the 'traditional' measures of aortic distensibility, both AA and DDA were strongly associated with hCBF *(Figure 3.8)*; AA, R^2 0.391 ($p < 0.0001$); and DDA, R^2 0.410 ($p < 0.0001$). PDA remained strongly associated although this was more modest than the others, $R^2 = 0.282$ ($p < 0.0001$). After adjusting for age and gender the relationships remained statistically significant at all levels ($p<0.01$).

Thoracic aortic distensibility was strongly related to hCBF R^2 0.409 ($p < 0.0001$) and after adjustment for age and gender, remained significant ($p < 0.01$).

Hyperaemic CBF did not correlate with indices of cholesterol (TG, LDL, HDL), inflammation (CRP), obesity (BMI) or renal function. A modest, inverse correlation was observed between pulse pressure and hMBF, $r = -0.328$ ($p = 0.016$).

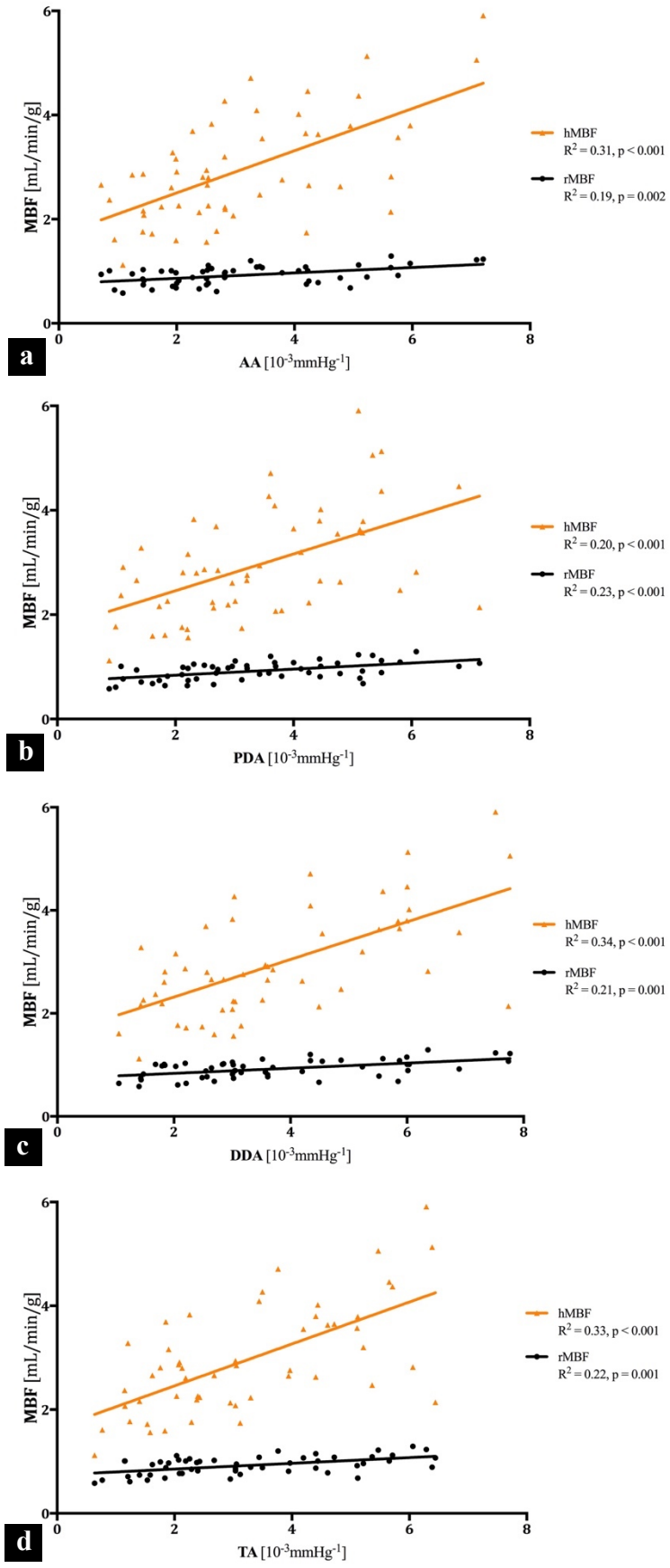

FIGURE 3.8 *Resting and hyperaemic CBF versus aortic distensibility at (a) AA, (b) PDA, (c) DDA and (d) TA. Differences between the slopes of rCBF and hCBF lines were all $p < 0.001$. Adjusted R^2 values (rCBF - age, gender (normalised RPP); hCBF - age, gender).*

3.5.6.3 Myocardial perfusion reserve

All levels of aortic distensibility were strongly associated with MPR *(Figure 3.9)*: AA, R^2 0.487 ($p < 0.001$); PDA, R^2 0.421 ($p < 0.001$); DDA, R^2 0.517 ($p < 0.001$); and TA R^2 0.497 ($p < 0.001$). All of these relationships remained highly statistically significant after adjustment for age and gender ($p<0.001$) *(Table 3.4)*.

Myocardial perfusion reserve did not correlate with indices of cholesterol (TG, LDL, HDL), inflammation (CRP), obesity (BMI) or renal function.

Modest, inverse correlations were observed between MPRI and both SBP ($r = -0.345$, $p = 0.011$) and PP ($r = -0.425$, $p = 0.001$).

	rMBF	**hMBF**	**MPR**
Unadjusted			
AA	0.481 (<0.001)	0.625 (<0.001)	0.681 (<0.001)
PDA	0.529 (<0.001)	0.531 (<0.001)	0.649 (<0.001)
DDA	0.525 (<0.001)	0.641 (<0.001)	0.719 (<0.001)
TA	0.526 (<0.001)	0.639 (<0.001)	0.751 (<0.001)
Adjusted			
AA	0.433 (0.002)	0.561 (<0.001)	0.622 (<0.001)
PDA	0.479 (<0.001)	0.452 (0.001)	0.586 (<0.001)
DDA	0.458 (0.001)	0.582 (<0.001)	0.675 (<0.001)
TA	0.465 (0.001)	0.578 (<0.001)	0.710 (<0.001)

TABLE 3.4 *Correlation coefficients between coronary blood flow and indices of arterial stiffness (r value with p value in brackets). rCBF adjusted for age and gender (normalised to RPP). hCBF and MPR adjusted for age and gender.*

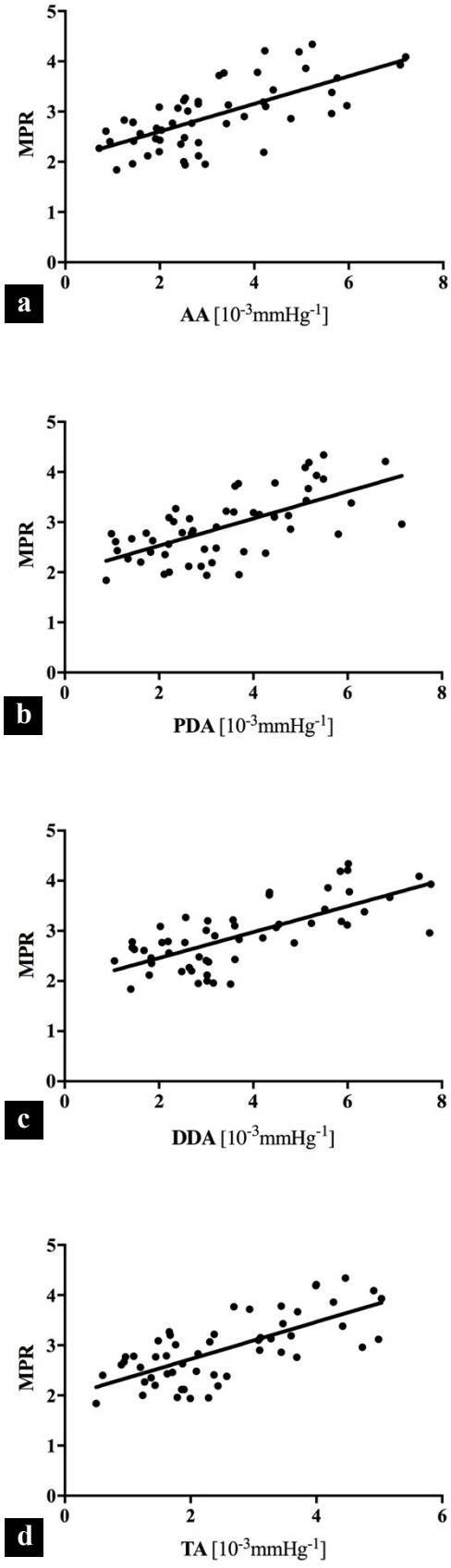

FIGURE 3.9. *Myocardial perfusion reserve versus aortic distensibility at (a) AA, (b) PDA, (c) DDA and)d) TA. All p<0.001 adjusted for age and gender.*

3.5.6.4 Effect of arterial stiffness on degree of hyperaemic response

As described earlier *(see Chapter 2)*, the aorto-coronary relationship between aortic distensibility, rMBF and hMBF is demonstrated in *Figure 3.8*. Across all levels of measured aortic distensibility, these slopes converge as arterial stiffness increases, i.e. a stiffer aorta confers a reduced hyperaemic response to adenosine. Statistically this is confirmed by a significant difference in the slopes of each line across all four levels when both were corrected for age and gender, $p<0.0001$.

3.5.7 Repeatability

Twenty consecutive studies were subjected to post-processing interobserver repeatability analysis. This was performed by another experienced observer (SD) blinded to the initial result and the outcome from distensibility analysis.

Intra-class coefficients were 0.96 for MPR, 0.83 for rMBF and 0.91 for hMBF.

Bland-Altman analysis demonstrated consistent levels of agreement across the spectrum of measures: MPR bias -0.02 ± 0.16 (95%CI -0.32 – 0.29); rMBF bias -0.04 ± 0.12 (95%CI -0.28 – 0.20); and hMBF bias -0.04 ± 0.22 (95%CI -0.46 – 0.39).

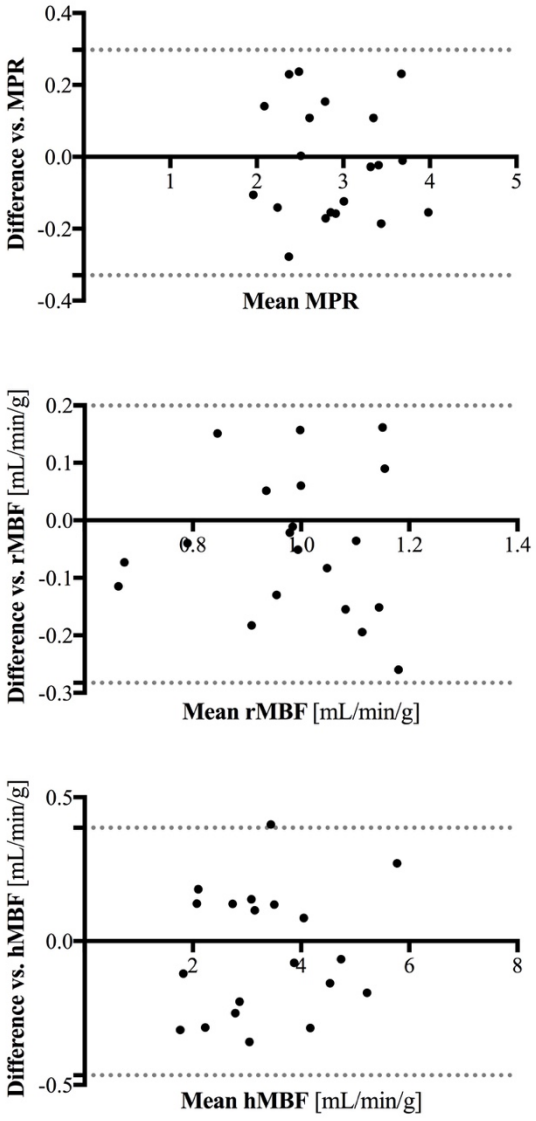

FIGURE 3.10 *Bland-Altman analysis of inter-observer agreement for MPR (top), rMBF (middle) and hMBF (bottom).*

3.6 DISCUSSION

To the best of our knowledge this study confirms for the first time the relationship between myocardial blood flow and aortic distensibility using CMR-derived indices.

Myocardial perfusion and arterial stiffness

Confirming our earlier invasive proof of concept, myocardial blood flow was again correlated with aortic distensibility at all measured levels. The regression coefficients were more robust for hMBF than they were for rMBF suggesting large artery stiffness has only a minor role in governing resting blood flow but may be a significant determinant of hyperaemic flow. Similar results were reported by Nemes et al. who have utilised transoesophageal echocardiography to evaluate both diastolic coronary flow velocities and aortic distensibility simultaneously (Nemes et al. 2007). In a study of 55 subjects, there was a moderate association with hyperaemic coronary flow (r -0.41, $p < 0.01$), a modest relationship with CFVR (r -0.21, $p < 0.01$) and a trend with resting coronary flow velocity (r -0.16, $p = 0.23$).

In our study, the strongest observed relationship was between aortic distensibility and MPR. This measure, analogous to CFVR, indexes to baseline myocardial blood flow and may provide a more physiological relevant measure of aortic reservoir function, as a measure of capacity to increase flow from baseline.

Feasibility and repeatability.

Of the subjects who were able to tolerate the CMR-PI scan, quantitative analysis was technically possible on the vast majority of subjects (85%). Although time consuming at >2 hours per patient for MBF, the analysis yielded comparable values to those observed in other similar studies (Cheng et al. 2008; Papanastasiou et al. 2016). Bland-Altman analysis revealed minimal interobserver bias and standard deviations of repeated observations were within clinically relevant boundaries of <0.2mL/min/g for rMBF, and <0.3mL/min/g for hMBF. Bland-Altman analysis of MPR demonstrated

high levels of repeatability with a low standard deviation and the tightest confidence intervals of all three indices. This is likely to result from the simplicity of the formula which is a quotient of slopes as opposed to a signal-intensity time curve which undergoes mathematical constraint and is sensitive to noise (Lee, DC & Johnson 2009).

Hyperaemic response to adenosine

As expected from the exclusion criterion, all individuals in the study had a normal response to adenosine with MPR values >1.5. As observed in the invasive proof of concept, the convergence of blood flow curves at the lower values of distensibility support the notion that a stiff aorta has less capacity to increase flow in response to a hyperaemic stimulus. Synchronous microvascular dysfunction remains a potential alternate, or at least contributing explanation for this observed phenomenon.

Subjects with the stiffest aortas had perfusion reserve values which approached the threshold for an ischaemic diagnosis (1.1 to 1.5 in the literature (Barmeyer et al. 2007; Nagel et al. 2003)). Given clinically indicated CMR-PI scans are subject to only visual inspection for a perfusion defect, these changes would not be detected unless quantitative analysis is performed. In this setting, a reduction in MPR may be evidence of global hypoperfusion. A recent study utilising CMR-PI demonstrated a reduced global MPR in high coronary risk individuals with non-obstructive epicardial disease compared to normal controls (Zorach et al. 2018). The conclusion was that of underlying microvascular disease although a key contributor to this phenomenon could also be reduced aortic reservoir capacity. Hypothetically, a completely rigid aorta (with a theoretical distensibility of 0) would near approximate a hypo-perfused MPR threshold of 1.5.

Validation of 'thoracic aorta' distensibility

We have described for the first time, the utility of a 'thoracic aorta' distensibility which can be evaluated in patients undergoing routine cardiac structure and function examinations. Predictably, the values obtained were closely related to the anatomical 'neighbours' on the aorta, AA and PDA. The standard 'VLA' sequence is acquired as part of initial planning and transected the aortic arch at a perpendicular plane in >90% of our subjects making this suitable for distensibility analysis. Of note, our initial pilot work had suggested up to 25% of patients would have oblique transection and thus be excluded. The younger mean age of this cohort may have resulted in less 'unwinding' of the arch and thus a greater proportion having a perpendicular plane through the aorta. Although the dedicated sequences can be acquired quickly if desired, analysis of the VLA sequence would facilitate evaluation of distensibility on patients who have had clinically indicated structure and function scans and have had a brachial blood pressure measured shortly before or after.

Limitations

Although post-processing repeatability was performed, test-retest agreement was not. This would have been preferable however would have involved a re-exposure to gadolinium in individuals who have been given a 'normal' test result. This was felt to be unethical. Other studies in this space have shown intra-class coefficients of between 0.80 and 0.89 with repeatability performed approximately 3 months apart (Goykhman et al. 2012). In a test-retest scenario, co-efficients of variation were 5% (R = 0.94) for non-ischaemic and 9% (R = 0.93) for ischaemic segments suggesting overall excellent reproducibility (Chih et al. 2010).

The post-processing time required for quantification of myocardial blood flow is significant. Approximately 20 minutes is required to trace the endo- and epicardial tracings per slice and thus with three slices, 60 minutes per time point and a total of 2 hours for rest and stress imaging. The mathematical manipulation is a further 20

minutes per study and thus this is not a technique which is ready for clinical application. The development of semi-automated techniques, however, will generate significant reductions in processing time, and will facilitate its expansion into the clinical arena.

As described above, although the relationship between distensibility and measures of perfusion was linear, it is impossible to exclude not only diffuse coronary disease as these individuals did not proceed to angiography, nor underlying endothelial dysfunction and microvascular disease as a cause of reduced hyperaemic flow.

There is some uncertainty in the literature as to whether 140microg/kg/min is an adequate dose of adenosine to induce maximal hyperaemia (Karamitsos et al. 2010). Local protocols dictate that adenosine is administered through a large bore cannula via an antecubital vein and symptoms of hyperaemia documented. In this cohort, the vast majority of patients reported at least one symptom of hyperaemia with a significant change in mean rate-pressure product. Although it is possible some subjects experienced a submaximal hyperaemic response, the independent predictors for this are those with LV dysfunction and age >65, the former being an exclusion for this study and less than a quarter of our study being > 65 years of age.

Conclusion

This is the first study to utilise CMR derived indices of both myocardial blood flow and aortic distensibility to evaluate the aorto-coronary relationship. In this cohort, aortic distensibility correlated strongly with both hyperaemic MBF and MPR although only modestly with resting MBF.

4. CHAPTER 4: Arterial stiffness and ischaemic myocardium

Impact of arterial stiffness on myocardial blood flow: ischaemic and non-ischaemic myocardium

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4.1 STATEMENT OF AUTHORSHIP

Manuscript details

Title of paper	*Impact of arterial stiffness on myocardial blood flow: ischaemic and non-ischaemic myocardium*
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Principal Author Contributions

Candidate	Dr Adam J Nelson
Contribution to the Paper	Primary contributor to the conception and design of the work; Drafted the work; Provided final approval of the version to be published; Accountable for all aspects of the work.
Overall percentage	90%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

August 2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	A/Prof Matthew I Worthley
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August 2018

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Contribution to the Paper	Helped drafting the work and revising it critically; Provided final approval of the version to be published; Accountable for all aspects of the work

August 2018

4.2 ABSTRACT

Background: The links between large artery function and myocardial blood flow have been previously established either with invasive techniques, or in 'normal' cohorts. We sought to evaluate the aorto-coronary relationship using cardiovascular magnetic resonance perfusion imaging (CMR-PI) and aortic distensibility in subjects with demonstrable perfusion defects and corresponding significant lesions at angiography.

Methods: The CMR-PI database at the Royal Adelaide Hospital was used to identify subjects with a single perfusion defect and corresponding angiography. Aortic distensibility was evaluated from standard planning sequences as previously described. Myocardium was divided into equi-angular segments and adjudicated as 'ischaemic' or 'non-ischaemic' based on angiography findings. Resting and hyperaemic blood flow (rMBF, hMBF) as well as myocardial perfusion reserve (MPR) were obtained from the signal-intensity time curves.

Results: Of the 3,729 CMR-PI studies performed, 229 had a single reversible perfusion defect ratified at angiography, with a complete set of perfusion and distensibility data available for 86 subjects. Mean age was 67.1 ± 9.8 years. The majority of lesions were in the LAD (49%) with a total of 507 'ischaemic' and 1041 'non-ischaemic' segments analysed. Aortic distensibility correlated in ischaemic and non-ischaemic myocardium, respectively; rMBF (R^2 0.154 and 0.107, $p < 0.01$); hMBF (R^2 0.292 and 0.258, $p < 0.01$) and MPR (R^2 0.483 and 0.504, $p < 0.001$).

Conclusion: The similar aorto-coronary regression coefficients suggests large arterial stiffness continues to govern myocardial blood flow despite critical epicardial disease. The linear reduction in myocardial blood flow observed with increasing aortic stiffness not only provides a mechanism for amplifying the functional significance of a 'moderate' stenosis but has the potential to extend the ischaemic zone in watershed territories.

4.3 INTRODUCTION

Arterial stiffness is now an established, independent predictor of adverse cardiovascular outcomes (Ben-Shlomo et al. 2014). This has been shown in a number of cohorts including otherwise 'healthy' individuals (Mattace-Raso et al. 2006; Mitchell et al. 2010; Willum-Hansen et al. 2006), hypertensives (Boutouyrie et al. 2002; Laurent et al. 2001; Terai et al. 2008) and in those with both established chronic kidney (Blacher et al. 2002; Pannier et al. 2005) and coronary artery disease (Kaneko et al. 2013). The basis for the strength of this predictive capacity, however, remains mechanistically unclear.

Although its neurological and renal sequelae are well described (O'Rourke & Safar 2005), given its deleterious effects on both sides of the myocardial supply-and-demand equation, there may be a central, coronary component to its predictive strength. With a stiffer aorta, the pulse wave travels faster and returns earlier in the cardiac cycle. It's arrival in systole increases afterload on the left ventricle driving compensatory left ventricular hypertrophy and increases myocardial oxygen demand. The earlier pulse wave arrival negates diastolic 'augmentation' thereby reducing the trans-coronary pressure gradient at the level of the aorta, putatively reducing flow to the myocardium.

Recent studies (Leung et al. 2006), including our own (unpublished, *Chapters 2 and 3*), have shown a stiffer aorta is associated with reduced resting coronary blood flow but critically results in a blunted response to hyperaemia, even in relatively 'normal' coronary arteries. In the context of epicardial disease, the inability to increase flow as observed in aortic stiffness, may render a fixed stenosis functionally more significant. Supportive evidence comes from Kingwell et al. (Kingwell et al. 2002) who demonstrated a number of measures of large artery stiffness to be independent predictors of ischaemic threshold on treadmill testing.

We aimed to evaluate whether the relationship between arterial stiffness and coronary blood flow continued to hold in both ischaemic and non-ischaemic myocardium as assessed with cardiac magnetic resonance perfusion imaging (CMR-PI) in patients with established significant epicardial coronary artery disease.

4.4 METHODS

4.4.1 Subjects

4.4.1.1 CMR database

The study was approved by the Human Research Ethics Committee at the Royal Adelaide Hospital. Since its establishment in 2008, The Royal Adelaide Hospital is home to one of the largest CMR departments in Australia and performs close to 1500 studies per year. For a number of years, it was the sole provider of adenosine and dobutamine stress CMR studies in South Australia, Northern Territory and large parts of western New South Wales and Victoria making it a quaternary referral centre for CMR. The CMR department maintains a prospectively collected database of indications, baseline clinical characteristics and results of all scans undertaken for both research and clinical purposes.

4.4.1.2 Inclusion criteria

The Royal Adelaide Hospital CMR database was queried for stress imaging scans from 2008 until 2016. This was subsequently narrowed to CMR-PI and then to all those reported as having a ‘reversible perfusion defect’.

As part of local protocol, all patients abstained from caffeine and xanthine-containing products for at least 24 hours prior to scanning.

4.4.1.3 Exclusion criteria

In order to obtain a cohort of patients whose myocardium could be dichotomised into 'ischaemic' or 'non-ischaemic' for comparison, other contributors to abnormal myocardial blood flow were excluded. From a patient characteristic perspective, those with a documented history of established coronary disease (angina, history of myocardial infarction, prior revascularisation) or severe valvular disease were excluded. Patients with evidence of late gadolinium enhancement (e.g. scar), left ventricular systolic dysfunction (LVEF < 50%) or multiple perfusion defects – matched or reversible – were excluded.

In order to confirm the veracity of the reversible perfusion defect, those who a) did not have angiography at the Royal Adelaide Hospital, b) were medically managed upfront or c) had evidence of < 50% visual angiographic stenosis at angiography (false positive) were excluded. Given the complexity of hibernating myocardial blood flow in chronically occluded territories (Cheng et al. 2008; Selvanayagam et al. 2005), chronic total occlusions (evidence of collateralisation) were also excluded.

4.4.1.4 Demographics

Prescribed medications and risk factors were collected at time of CMR. Risk factors were defined as cigarette smoking (previously or currently smoking tobacco), hypercholesterolemia (use of cholesterol-lowering therapy or total cholesterol level > 5.2 mmol/L), hypertension (blood pressure > 140/90mmHg or on medical antihypertensive therapy or subject to lifestyle intervention), obesity (BMI > 30kg/m^2), diabetes (plasma haemoglobin A1c > 6.5%, or on glycaemic medications), and family history of cardiovascular disease (index event in a first-degree relative, male ≤ 55 years and female ≤ 60 years).

4.4.2 Cardiac magnetic resonance protocol

All cardiovascular magnetic resonance (CMR) studies were performed utilising the Siemens Avanto 1.5 Tesla magnetic resonance imaging scanner (Siemens Medical Imaging, Erlangen, Germany) located within the Cardiovascular Investigation Unit of the Royal Adelaide Hospital. All CMR studies were performed with subjects in a supine position with a phased-array surface coil positioned over the thorax.

4.4.2.1 Cardiac structure and function

Methods for evaluating left ventricular structure and function are covered in *Chapter 2*. In brief, short-axis slices from the mitral annulus to and inclusive of the left ventricular apex were acquired during expiratory breath hold.

Offline analysis was performed to evaluate LV size and function utilizing proprietary software (Argus software, Siemens Medical Imaging, Erlangen, Germany). Endocardial contours were manually traced at end-diastole (start of R-wave) and at end-systole (visually smallest cavity). End-systolic and end-diastolic volumes were obtained and thus left ventricular ejection fraction could be calculated.

4.4.2.2 Aortic distensibility

Methods for evaluating aortic distensibility from already acquired cardiac structure and function studies is covered in detail in *Chapter 3*. In brief, the vertical long axis (VLA) planning image transects the aorta at the level of the arch between the previously described levels of the ‘Ascending Aorta’ and ‘Proximal Descending Aorta’.

Aortic distensibility was evaluated offline by manually tracing aortic cross-sectional images at the level of the 'thoracic aorta' (TA). Major and minor axes were drawn on each VLA image and if the ellipticity index was <0.9 (minor/major), the patient was excluded from the study. Maximal and minimal lumen areas were calculated and distensibility derived by a previously published formula (Groenink et al. 1998):

$$\frac{\textit{aortic area at end systole } (mm^2) - \textit{aortic area at end diastole } (mm^2)}{\textit{brachial pulse pressure } (mmHg) \times \textit{aortic area at end diastole } (mm^2)}$$

The baseline heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) taken at commencement of CMR imaging was used for distensibility calculation. The pulse pressure was calculated from the difference between the systolic and diastolic blood pressures and measured in mmHg. The aortic areas were measured in mm^2 and after multiplying by 1000, aortic distensibility is reported in its final unit of $10^{-3}mmHg^{-1}$.

4.4.2.3 CMR perfusion imaging

4.4.2.3.1 Adenosine infusion protocol

As per local protocol, a physician with a cardiac defibrillator was present for all stress perfusion imaging. All patients received adenosine (AdenoScan, Sanofi-Aventis, Surrey, UK) at a dose of 140μg/kg/min using a precise syringe pump (Graesby 3500, Minneapolis, Minnesota, USA) via a cannula in the antecubital vein.

4.4.2.3.2 Image acquisition

CMR perfusion imaging acquisition has been covered in detail in *Chapter 3*. In brief, during the last minute of the adenosine infusion, the gadolinium-based contrast agent, Magnevist, (dimeglumine gadopentetate, Bayer, Leverkusen, Germany) was administered. Perfusion imaging was performed with a T1-weighted fast low-angle single shot gradient echo (GRE) sequence and was acquired every cardiac cycle during first pass. Perfusion images were obtained at three short axis planes - basal, mid and apical levels - of the left ventricle (Cerqueira et al. 2002).

Haemodynamic data was acquired at five-minutely intervals throughout the adenosine protocol and recorded.

4.4.2.3.3 Visual analysis

Confirmation of the clinically documented perfusion defect was performed by an experienced observer (KST, AJN) blinded to the original report. Rest and stress perfusion and the late gadolinium enhancement images of three short axis sections (base, mid and apex) were viewed side by side. If the signal intensity on stress perfusion appeared lower in an area of myocardium for at least three dynamic images compared with remote myocardium, it was confirmed to be ischaemic (Ingkanisorn et al. 2006). If the same signal intensity abnormality was seen in the rest and stress perfusion images and there was no evidence of scar on late contrast enhanced images, the defect was considered an artefact and the patient excluded. Similarly, if the perfusion defect lasted for less than three dynamic images, it was also considered artefactual and the subject was excluded (Ingkanisorn et al. 2006).

4.4.2.3.4 Semi-quantitative analysis

Following visual confirmation, semi-quantitative analysis was undertaken. The endocardial and epicardial contours of three short axis sections (base, mid and apex) were traced (QMASS, v7 .2, Medical Imaging Solutions, Leiden, Netherlands) and

corrected manually for displacements (e.g. breathing). As per *Figure 4.1*, each short axis slice was divided into six equiangular segments in a clockwise manner beginning at the anteroseptal insertion of the right ventricle (Barmeyer et al. 2007). Segments were then assigned to coronary territories according to the 18-segment model of the American Society of Echocardiography (Lang et al. 2015). Segments 1, 2 and 6 were assigned to the left anterior descending (LAD); 2,3 and 4 to the left circumflex (LCx) and segments 4 and 5 to the right coronary artery (RCA). Within each section, signal intensity was measured by defining regions of interest that excluded the inner 10% and outer 30% of the myocardium to get stronger weighting of the subendocardium *(Figure 4.2)* and reduce influence from the LV as previously described (Nagel et al. 2003).

The subendocardial signal intensity-time curves were generated for all segments by obtaining signal intensity on consecutive images before and during arrival of contrast material. The signal intensity-time curve for the left ventricle was generated on the basal section as a measure of input function *(Figure 4.3)*. The maximal initial upslope of every signal intensity-time curve was determined by using a sliding window with a four- point linear fit for the myocardium and a three-point linear fit for the left ventricle as previously described (Al-Saadi et al. 2000).

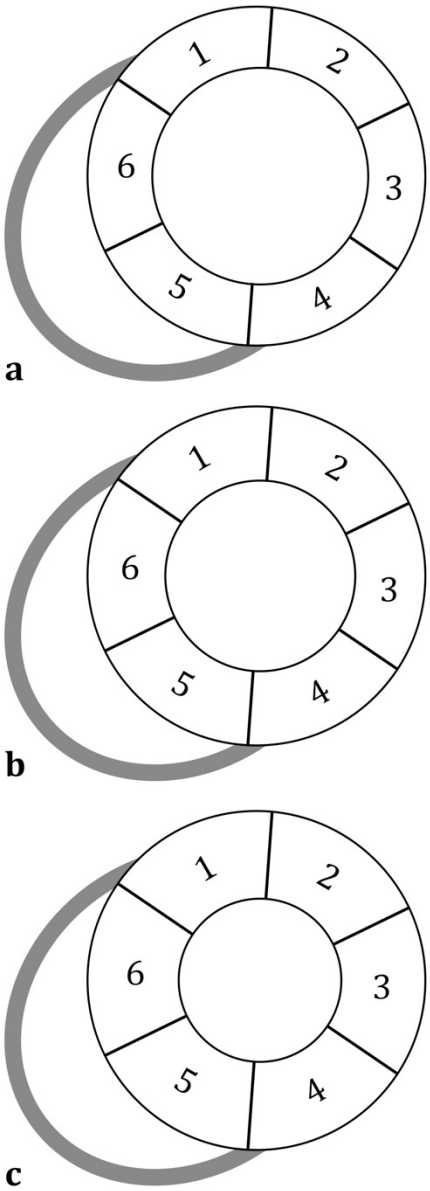

FIGURE 4.1. *Representative figure of 6 equi-angular segments at basal (a), mid (b) and apical (c) levels. Segments 1 (anterior), 2 (anterolateral) and 6 (anteroseptal) were assigned to the LAD; 2 (anterolateral), 3 (lateral) and 4 (inferior) to the LCx and segments 4 (inferior) and 5 (inferoseptal) to the RCA*

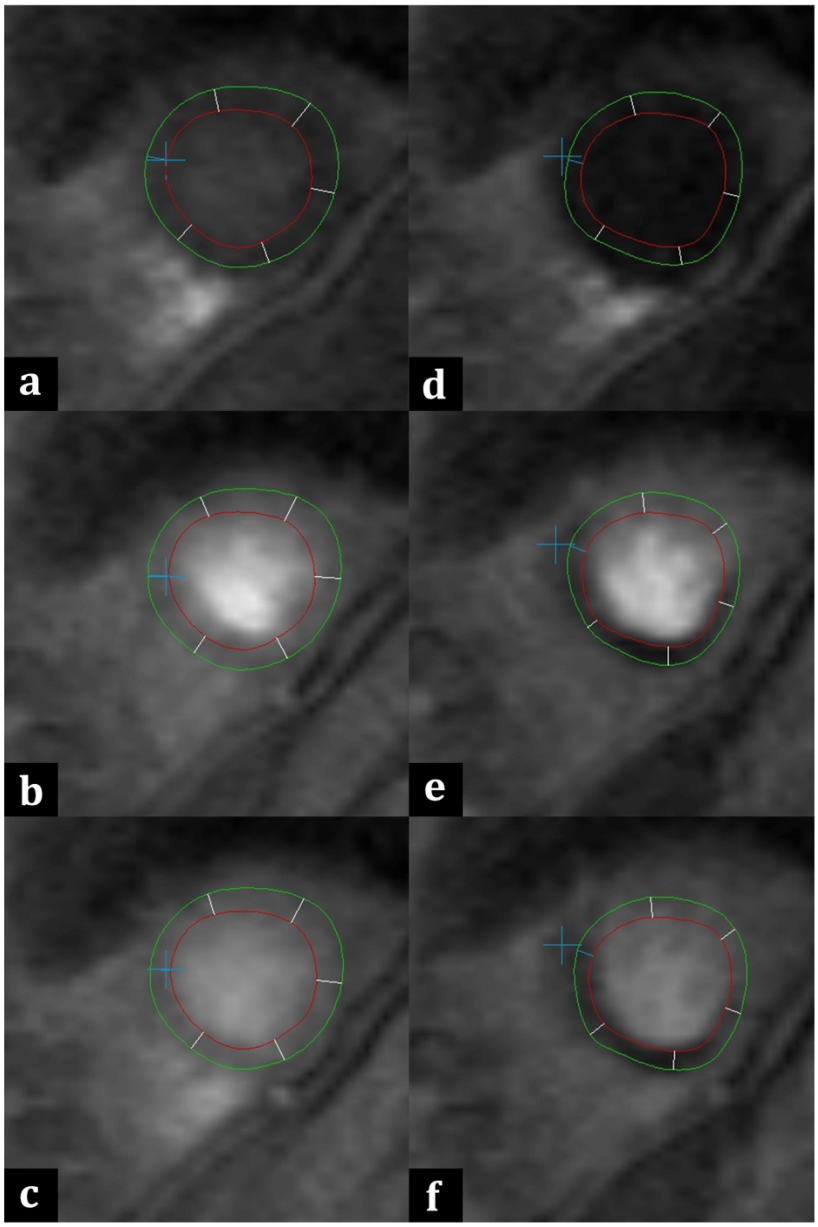

FIGURE 4.2 *Representative figure of basal level perfusion sequences acquired at rest (a-c) and hyperaemic (d-f) phases. Earlier images (a,d) compared with later images (c,f) demonstrating contrast enhancement from blood pool to myocardium progressively. Perfusion defect noted at peak hyperaemia in (e) and (f) compared to equivalent rest images (b) and (c).*

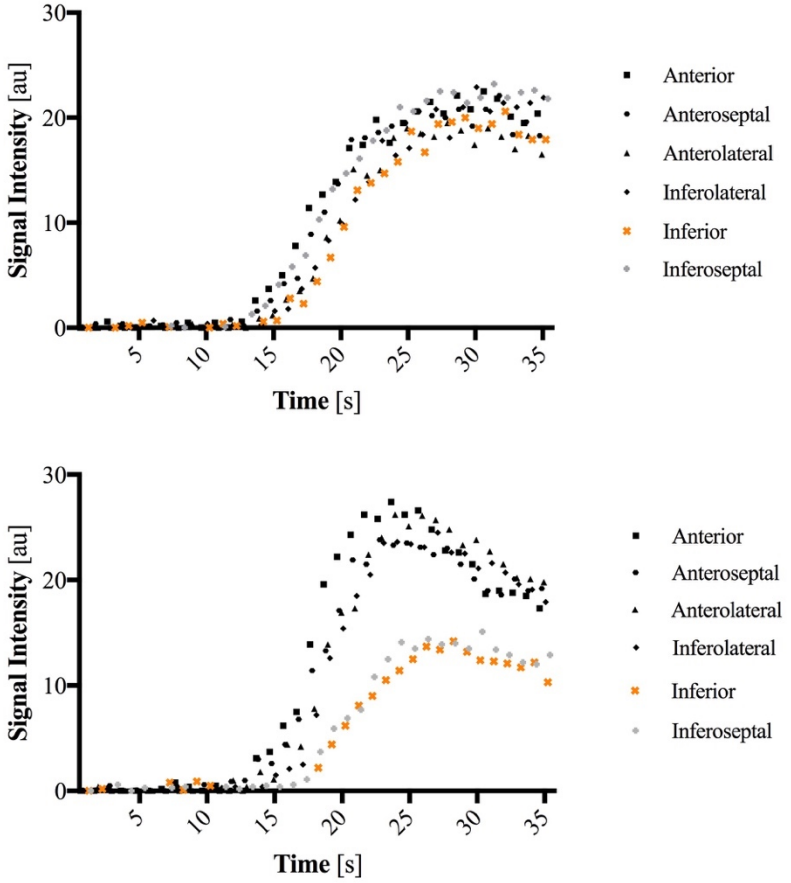

FIGURE 4.3 *Signal intensity-time curve by region. Segments 4 (inferior) and 5 (inferoseptal) demonstrate reduced velocity and intensity of contrast enhancement*

4.4.2.3.5 Quantitative analysis

Myocardial Blood Flow (MBF) was determined in each equiangular section and then averaged per coronary territory in mL/min/g. This was performed by deconvolution of the signal intensity time curves constrained to a Fermi function using an arterial input from the left ventricular blood pool signal, specifically accounting for any delay in the arrival of the tracer (Jerosch-Herold et al. 2004; Jerosch-Herold et al. 2002; Jerosch-Herold et al. 2000; Selvanayagam et al. 2005). This was performed on both rest and stress images to generate resting MBF (rMBF) and hyperaemic MBF (hMBF). As basal CBF is closely related to the rate-pressure product (RPP) (Czernin et al. 1993), an index of myocardial oxygen consumption, values for basal flow in each subject were corrected for the respective RPP. This was performed by multiplying rMBF by the mean RPP of the cohort, divided by the RPP in the individual patient (Uren, Melin, et al. 1994).

For each of the 18 segments, myocardial perfusion reserve (MPR) was calculated by dividing the maximal upslope value at stress over rest. Although multiple cut-offs exist in the literature, an MPR of 1.5 achieves a >90% sensitivity for detecting ischaemia (Al-Saadi et al. 2000).

4.4.3 Quantitative coronary angiography

Angiography films for each patient were obtained. Analysis of all angiography films were de-identified and analysed without knowledge of the CMR results.

Quantitative coronary angiography (QCA) was performed on end-diastolic frames using orthogonal views. Where orthogonal views obtained at time of clinically driven angiography were inadequate to accurately perform QCA, patient was excluded. As per previously published methods (Goodhart & Anderson 1998; Zeiher et al. 1991), reference diameter, lesion length and minimal luminal diameter were measured using proprietary software (CMS software, Medis Corp, Leiden). The lesion diameter was then generated in a semi-automated manner at the interpolation line between the normal segments proximal and distal to the stenosis (de Feyter et al. 1991; Garrone et al. 2009) *(Figure 4.4)*.

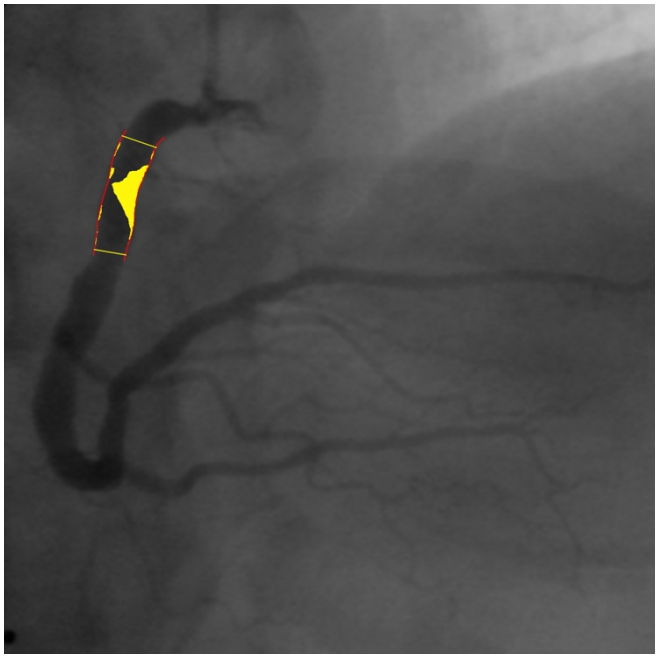

FIGURE 4.4 *Representative image of a coronary angiogram with QCA analysis.*

4.4.4 Linkage of CMR and angiography findings

Based on QCA evidence of the presence and location of a lesion, each of the 18 myocardial segments were dichotomized as either 'ischaemic' or 'non-ischaemic'. i.e. if mid LAD was stenosed, mid and apical segments 1, 2 and 6 were determined to be 'ischaemic' (i.e. a total of 6 ischaemic and 12 non-ischaemic segments). 'Ischaemic' and 'non-ischaemic' averages for rMBF, hMBF and MPR were calculated on a per patient level.

4.4.5 Statistical Analysis

Baseline characteristics are represented as mean ± standard deviation where normally distributed, or median and interquartile range (IQR) for parameters with non-Gaussian distribution.

The relationship between continuous variables was determined by simple linear regression, and adjustment for age and gender was by partial correlation. Analysis of covariance was used when both continuous and categorical variables were present.

Statistical significance was defined as a two-sided $p<0.05$. Statistical analyses were performed using SPSS statistical software (v24, IBM SPSS, New York, USA) and graphs were drawn using GraphPad Prism 7 (GraphPad Software, La Jolla California USA).

4.5 RESULTS

4.5.1 Sample

For a detailed cohort build, see *Figure 4.5*. Of the 3,729 CMR-PI studies evaluated, 344 had reversible perfusion defects. From there, 105 were excluded on the basis of multiple perfusion defects, LV dysfunction or evidence of late gadolinium enhancement. A further ten were of insufficient image quality to undertake quantitative analysis. After linking with medical history, a further 62 subjects were unable to be paired with angiography data. After angiography, nine patients were excluded with evidence of a chronic total occlusion, 15 had false positive scans and 28 had false negative CMR-PI scans. After successful CMR-PI analysis, a further 19 were unable to have distensibility analysis due to either poor image quality or being off plane/eccentric. The final sample consisted of 86 patients with CMR-PI, distensibility and QCA analysis.

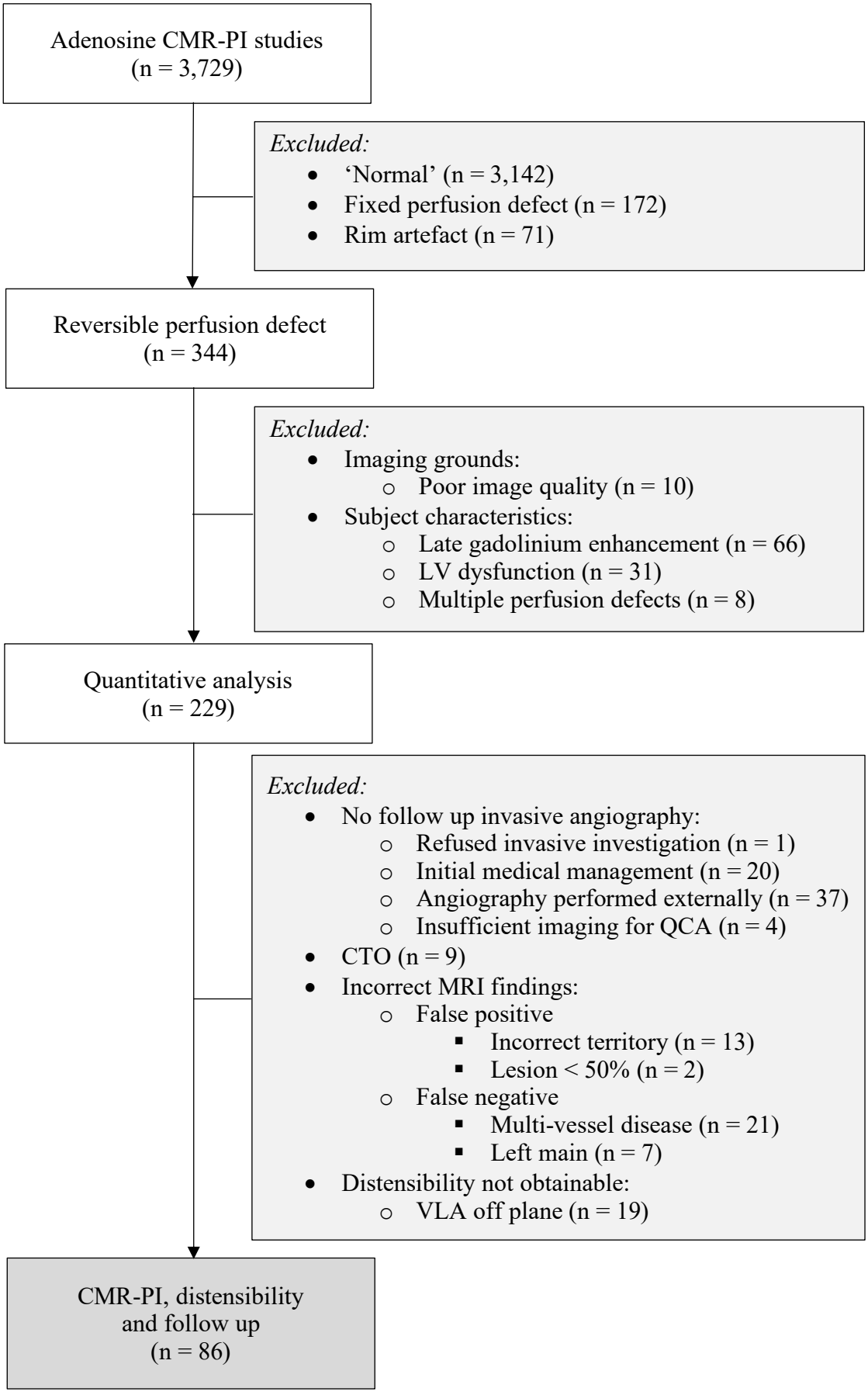

FIGURE 4.5 *Cohort build*

4.5.2 Cohort

The clinical characteristics of the cohort are listed *(Table 4.1)*. The majority of patients were male (n = 54, 63%) with a mean age of 67 ± 9.8 years. As expected, the majority of patients were referred through with chest pain for investigation (91%) with only a few reporting 'dyspnoea' as their presenting complaint (9%). The most prevalent risk factors were hypertension (84%) and dyslipidaemia (79%) with nearly half of patients diagnosed with diabetes (47%). The overall population was relatively high risk with close to two thirds having three or more traditional risk factors (64%). The most commonly prescribed medication was an ACE or an ARB (72%) followed by aspirin (69%). Just over half were on a statin (56%) and although nearly 50% were diagnosed with diabetes, less than half were treated with either insulin or tablets (21% total cohort).

Clinical characteristic	Cohort
Mean age ± SD [years]	67 ± 9.8
Male sex – no. (%)	54 (63)
Stress test indication – no. (%)	
Chest pain for investigation	79 (91)
Dyspnoea for investigation	7(8)
Mean BMI ± SD [kg/m^2]	27.7 ± 6.6
Risk factors – no. (%)	
Family history	26 (30)
Cigarette smoking	39 (45)
Hypertension	72 (84)
Dyslipidaemia	68 (79)
Diabetes	40 (47)
≥ 3 risk factors	55 (64)
Medications – no. (%)	
Aspirin	59 (69)
ACE/ARB	61 (72)
Statin	48 (56)
Diuretic	21 (24)
Calcium channel blocker	36 (41)
Beta blocker	38 (44)
Insulin	4 (5)
OHG	14 (16)

TABLE 4.1 *Clinical characteristics. Abbreviations: BMI – body mass index; ACEi – angiotensin converting enzyme inhibitor; ARB – angiotensin receptor II blocker; OHG – oral hypoglycaemic (medication)*

Laboratory investigations *(Table 4.2)* revealed normal kidney function and haemoglobin. Lipid profile overall was reasonable for a cohort without established CVD and with less than half treated with a statin: mean LDL 2.5 ± 1.1mmol/L, mean HDL 1.2 ± 0.4mmol/L and TG 1.8 ± 1.1mmol/L.

Laboratory characteristics	**Cohort**
Lipid profile ± SD [mmol/L]	
Total cholesterol	5.3 ± 1.5
Triglycerides	1.8 ± 1.1
LDL-C	2.5 ± 1.1
HDL-C	1.2 ± 0.4
Hb ± SD [mg/dL]	135 ± 14
eGFR [mL/min/1.73m^2]	78 ± 10

TABLE 4.2 *Laboratory characteristics. Abbreviations: LDL – low-density lipoprotein; HDL – high density lipoprotein; Hb – haemoglobin; eGFR – estimated glomerular filtration rate*

4.5.3 Cardiac Structure and Function

All subjects had normal left ventricular cavity size (LVEDV 122.9 ± 32.6mL. Reference ranges: males 115-198mL; females 88-168mL) and systolic function (LVEF 69.9 ± 7.1%. Normal range: 58-76%) (Maceira et al. 2006).

Left ventricular mass was within normal limits (LV mass 104.1 ± 23.5g. Reference ranges: males 108-184g; females 72-144g).

4.5.4 Aortic distensibility

Aortic distensibility was reduced consistent with increased arterial stiffness in this cohort. Although there are no established 'reference' ranges for our TA level, the mean of this cohort, ($2.34 \pm 0.82 \cdot 10^{-3} mmHg^{-1}$) is reduced compared to the 'normal' CMR-PI cohort of *Chapter 3* ($3.23 \pm 1.64 \cdot 10^{-3} mmHg^{-1}$). This is consistent with the overall increased burden of traditional vascular risk factors and hitherto unknown, presence of CAD in this cohort.

4.5.5 Coronary artery evaluation

Coronary angiography metrics are listed *(Table 4.3)*. The majority of patients had a right-dominant circulation (94%). All lesions were severe with a mean stenosis of 81 ± 6.9% by QCA. All lesions were treated, the majority with percutaneous coronary intervention (98%) with two patients undergoing coronary artery bypass grafting due to lesion complexity (2%).

Coronary characteristics	**Value**
Right dominant circulation - no. (%)	81 (94)
Ischaemic vessel – no. (%)	
LAD	42 (49)
LCx	15 (17)
RCA	29 (34)
Quantitative coronary angiography - Mean ± SD	
Minimal lumen diameter [mm]	0.75 ± 0.5
Reference diameter [mm]	3.23 ± 0.7
Stenosis [%]	81 ± 14.7
Mean stenosis length [mm]	17.8 ± 6.1
Treatment of lesion – no. (%)	
Stent	83 (97)
POBA	1 (1)
CABG	2 (2)

TABLE 4.3 *Coronary characteristics. Abbreviations: LAD – left anterior descending; LCx – left circumflex; RCA – right coronary artery; POBA – plain old balloon angioplasty; CABG – coronary artery bypass grafting.*

4.5.6 CMR perfusion imaging

Only 3% of the population with single perfusion defects had inadequate image quality to perform quantitative perfusion analysis. This is less than expected in view of the highly selected nature of the cohort from which rim artefacts and non-diagnostic scans had already been excluded.

The majority of patients reported at least one symptom of hyperaemia (n = 81, 94%), e.g. flushing, headache, dyspnoea, chest tightness.

After linking location of ischaemic lesion with subtended segments, there were 507 'ischaemic' segments' and 1041 'non-ischaemic' segments.

4.5.6.1 Myocardial blood flow

Resting myocardial blood flow was significantly lower in the ischaemic compared to non-ischaemic segments (0.80 ± 0.14 vs. 1.06 ± 0.19mL/min/g, $p < 0.0001$). Hyperaemic myocardial blood flow was significantly lower in the ischaemic compared to the non-ischaemic segments (1.05 ± 0.27 vs. 3.34 ± 0.81mL/min/g, $p < 0.0001$).

4.5.6.2 Myocardial perfusion reserve

As expected from the visual perfusion defect, MPR was significantly lower in the ischaemic compared to the non-ischaemic segments (1.20 ± 0.20 vs. 2.99 ± 0.52, $p < 0.0001$).

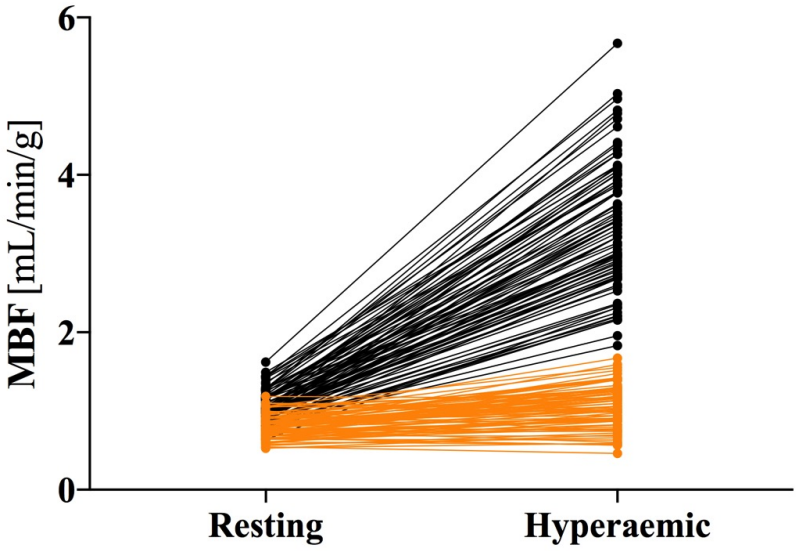

FIGURE 4.6 *Ladder plot of resting and hyperaemic MBF. Ischaemic segments in orange, non-ischaemic segments in black.*

CMR Variables	Mean ± SD
Baseline haemodynamics	
SBP [mmHg]	151 ± 19
DBP [mmHg]	83 ± 13
PP [mmHg]	67 ± 16
HR [bpm]	73 ± 12
Cardiac structure and function	
LVEDV [mL]	122.9 ± 32.6
LVESV [mL]	38.7 ± 17.8
LVEF [%]	69.9 ± 7.1
LV mass [g]	104.1 ± 23.5
Aortic distensibility [10^{-3}mmHg^{-1}]	2.34 ± 0.82
Stress perfusion imaging	
Resting RPP	9004 ± 2355
Stress RPP	12630 ± 3025
Segments analysed	
Ischaemic	507
Non-ischaemic	1041

TABLE 4.3 *CMR variables. Abbreviations: SBP – systolic blood pressure; DBP – diastolic blood pressure; PP – pulse pressure; HR – heart rate; LVEDV – left ventricular end-diastolic volume; LVESV – left ventricular end-systolic volume; LVEF – left ventricular ejection fraction; AA – ascending aorta; PDA – proximal descending aorta; DDA – distal descending aorta; TA – thoracic aorta; RPP – rate-pressure product.*

4.5.7 Associations with indices of myocardial perfusion

4.5.7.1 Resting myocardial blood flow

There was an overall modest correlation between rMBF and aortic distensibility. Comparing ischaemic and non-ischaemic myocardium, a stronger relationship was observed between rMBF and aortic distensibility in ischaemic segments (R^2 0.207, p <0.0001) compared with non-ischaemic segments (R^2 0.113, p = 0.0015).

After adjusting for age and gender, relationships remained statistically significant, although the strength of the association weakened *(Table 4.4)*.

Resting MBF did not correlate with indices of cholesterol (TG, LDL, HDL), measures of obesity (BMI) or renal function (GFR).

4.5.7.2 Hyperaemic myocardial blood flow

There was a strong correlation observed between hMBF and aortic distensibility. Comparing ischaemic and non-ischaemic myocardium, a stronger relationship was observed between hMBF and aortic distensibility in ischaemic segments (R^2 0.367, p <0.0001) compared with non-ischaemic segments (R^2 0.319, p = 0.0015). After adjusting for age and gender, these relationships remained statistically significant with minimal weakening of the association *(Table 4.4)*.

Neither ischaemic or non-ischaemic hMBF correlated with indices of cholesterol (TG, LDL, HDL), measures of obesity (BMI) or renal function (GFR).

Weak correlations were observed between ischaemic hMBF and both SBP (r -0.234 0.032) and PP (r -0.266, p = 0.013) although not DBP (r -0.027 p = 0.8).

Similarly, weak correlations were observed between non-ischaemic hMBF and both SBP (r -0.283, p = 0.009) and DBP (r -0.231, p = 0.035) but not PP (r -0.178, p = 0.1)

4.5.7.3 Myocardial perfusion reserve

A strong correlation was observed between MPR and aortic distensibility. Relatively similar agreement was observed between aortic distensibility and MPR in both ischaemic (R^2 0.483, $p < 0.0001$) and non-ischaemic (R^2 0.504, $p < 0.0001$) myocardium.

Corrected for age and gender, these relationships held with minimal change in the strength of the association *(Table 4.4)*.

Neither ischaemic or non-ischaemic MPR correlated with indices of cholesterol (TG, LDL, HDL), measures of obesity (BMI) or renal function (GFR).

Weak correlation was observed between MPR and both SBP (r -0.228, p = 0.037) and PP (r -0.296, p = 0.006) although not with DBP (r 0.014, p = 0.9)

	rMBF		**hMBF**		**MPR**	
	I	**NI**	**I**	**NI**	**I**	**NI**
Unadjusted						
Distensibility	0.455 <0.0001	0.373 0.002	0.578 <0.0001	0.565 <0.0001	0.706 <0.0001	0.710 <0.0001
Adjusted						
Distensibility	0.393 0.002	0.327 0.004	0.540 <0.0001	0.508 <0.0001	0.690 <0.0001	0.697 <0.0001

TABLE 4.4 *Correlation between myocardial blood flow and MPR with aortic distensibility (r value with p value below). rCBF, hCBF and MPR adjusted for age and gender. Abbreviations: I – ischaemic; NI – non-ischaemic.*

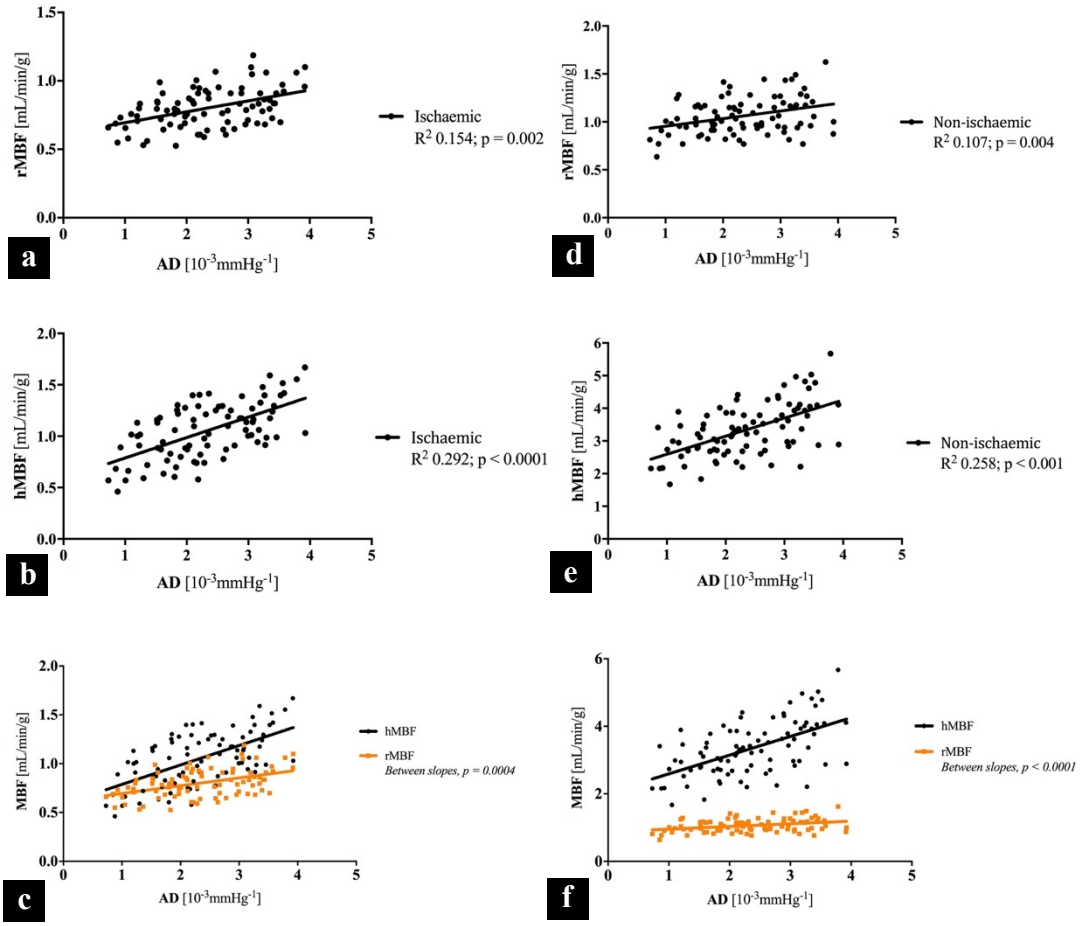

FIGURE 4.7 *Relationship between both resting and hyperaemic MBF in ischaemic (a – c) and non-ischaemic (d - f) myocardium. Significant differences in slopes between hMBF and rMBF in ischaemic (c) and non-ischaemic (f) myocardium.*

4.5.7.4 Diabetic subgroup

Given the propensity for abnormal microvascular function in diabetic patients, a pre-specified analysis was performed in the 40 subjects diagnosed with diabetes. With the adjustments for age and gender, similar associations were observed between aortic distensibility and CMR-PI indices. In ischaemic segments, the relationships were as follows; rMBF (R^2 0.381, $p = 0.012$); hMBF (R^2 0.450, $p = 0.004$) and MPR (R^2 0.513, $p = 0.001$). In non-ischaemic segments, the relationships were as follows; rMBF (R^2 0.338, $p = 0.021$); hMBF (R^2 0.573, $p < 0.001$) and MPR (R^2 0.674, $p < 0.001$).

4.5.7.5 Impact of stenosis severity

Although dichotomised into 'ischaemic' and 'non-ischaemic' on the grounds of an angiographically significant lesion and subtended perfusion territory, stenosis severity is a continuous variable. In univariate analysis however, stenosis percentage did not correlate with rMBF (r -0.256, $p = 0.31$), hMBF (r -0.123, $p = 0.36$) or MPR (r -0.184, $p = 0.47$). Moreover, when adjusted for in regression analysis between indices of perfusion and distensibility, percentage stenosis did not significantly impact on any of the relationships.

4.6 DISCUSSION

This is the first study to compare the interaction between aortic stiffness and myocardial perfusion in 'ischaemic' and 'non-ischaemic' myocardium using magnetic resonance imaging.

Hyperaemic response to adenosine

A less distensible aorta conferred a reduced hyperaemic response to adenosine in both 'ischaemic' and 'non-ischaemic' territories. This is evidenced by convergence of the flow curves at the lower values of distensibility. Of particular interest in the ischaemic cohort is that the slopes of resting and hyperaemic flow intersect at a distensibility value above zero. This would imply the potential for values of hyperaemic flow that are at, or potentially below, resting flow in the least distensible aortas. A reduction in hyperaemic flow is described in coronary 'steal' phenomenon (Akinboboye et al. 2001) whereby adjacent non-ischaemic territories draw blood away from ischemic myocardium. This phenomenon is driven by a reduction in microvascular resistance generated by dilation of intact, non-ischaemic microvasculature, particularly in the context of collateral vessels (angiographically visible or not). Extending this observation, the near complete loss of aortic reservoir capacity in individuals with the least distensible aortas may render ischaemic territories at greater vulnerability through complete reliance on autoregulatory control of flow. Although maximal hyperaemia with adenosine is felt to be supraphysiological, this provides an additional theoretical basis for a reduction in ischaemic threshold proposed by Kingwell et al. (Kingwell et al. 2002). A key alternate explanation is that a less distensible aorta may be a marker for more diffuse coronary disease which could generate a graded reduction in flow over the length of the coronary circulation.

Arterial stiffness vs. ischaemic and non-ischaemic perfusion

The regression between rMBF and hMBF with aortic distensibility was similar throughout the myocardium although numerically more constant and robust in the 'ischaemic' territories. A similar trend was described by Leung et al. and suggests arterial stiffness may be a more consistent determinant of blood flow in the context of significant epicardial disease where other contributors to MBF are exhausted, or fully compensated. Percentage stenosis in our cohort did not correlate with rMBF, nor did it affect the relationship with stiffness after adjustment in the regression analysis. It is possible that given the majority of this cohort had 'severe' stenoses, further curvilinear reduction in flow would have been difficult to detect. Moreover, such small incremental change is probably at the limits of measurement for both QCA (a 2D approximation for a 3D process) and CMR-PI.

A less distensible aorta was associated with reduced overall absolute myocardial blood flow as evidenced by the ongoing, linear aorto-coronary relationship in both 'ischaemic' and 'non-ischaemic' myocardium. This is consistent with the findings of *Chapter 3* in subjects with normal myocardial perfusion. Applied clinically, a reduction in overall myocardial blood flow has the potential to extend ischaemia in watershed territories and may provide a mechanistic basis to the association between aortic stiffness and the development of collateral formation (Baykan et al. 2015; de Marchi 2014).

Although more broadly spread, the slope of the aorto-coronary regression in the 'non-ischaemic' territory was steeper suggesting a greater reduction in MBF for each stepwise reduction in distensibility. This flattening of the 'ischaemic' curve may reflect a threshold of the aorto-coronary relationship's contribution to blood flow in the context of an already maximally dilated microcirculation from a critical epicardial

stenosis. A broader cohort of subjects with moderate and severe epicardial disease with greater hyperaemic potential may allow further graded, delineation of this relationship.

Performance of quantitative assessment

Quantitative analysis of myocardial blood flow was possible in the vast majority of studies with <5% excluded on imaging grounds. This is significantly better performance than could be expected in an unselected population as equivocal findings and rim artefacts were already excluded upstream and are a common cause of false positive results. Similarly, those with claustrophobia or intolerance to the CMR chamber would not have completed their study and thus wouldn't have been recorded in the database.

Implications

An intervention to reduce arterial stiffness may facilitate an increase in hyperaemic myocardial blood flow. Traditionally this has not been a target for anti-anginal agents although conceivably a rightward shift on this curve could increase the anginal threshold in patients with either diffuse or non-revascularisable coronary disease. Evaluating the performance of agents not only on arterial stiffness indices but its relationship with myocardial blood flow may allow greater insights into which are more likely to have specific anti-ischaemic benefit. The ability to use CMR-PI as a 'one stop shop' to evaluate both parameters simultaneously would make this an attractive modality for clinical trials, particularly if able to semi-automate the quantitative analysis. The protocol in this study required gadolinium which, although generally safe, is not completely without risk. Risk of gadolinium deposition (mainly cutaneous and cerebral) is increased in populations with chronic and acute kidney disease although has been described in those with preserved renal function in the context of repeated administration (Layne et al. 2018). With over 10 million contrast enhanced MRI scans performed globally, mainly for musculoskeletal and neurological indications, this will need surveillance and may ultimately limit widespread and

repeated examinations. Of growing interest, therefore, is the ability to perform blood flow measurements without contrast using a technique called 'arterial spin labelling' (Wacker et al. 2003; Zhang et al. 2005). This technique has been extensively employed in cerebral blood flow studies (Detre et al. 2009), and ultimately may provide similar information and obviates the concerns surrounding gadolinium (Zun et al. 2011).

Limitations

Compared to our earlier work, and that of Leung et al., the strength of the relationship between stiffness and MBF, even in the 'non-ischaemic' or normal territories, was less robust. Although carefully traced and planimetered, the equiangular nature of myocardial segmentation results in some territories containing both ischaemic and non-ischaemic myocardium. This may have affected the strength of the observed signal. Similarly, the acquisition of three slices through the LV, although standard for CMR-PI, is only a representative estimate of the total myocardium. Superior results for quantitative assessment have recently been seen with contemporary 3D techniques although at present these remain labour intensive (Motwani et al. 2014).

This cohort was highly selected to include patients with demonstrable perfusion defects and a lesion confirmed at angiography. Using the presence of a visual perfusion defect as the entrance point of this study will have excluded a number of patients with hypoperfusion evident only on quantitative analysis. The presence of a visual perfusion defect in our study seemed to select a cohort with severe angiographic stenosis (mean 81%). Two patients from 227 were reported to have a visual perfusion defect and were subsequently excluded as there was no obvious corresponding stenosis. Post hoc exploratory analysis in these two patients however, revealed normal quantitative myocardial perfusion measures suggesting an error of visual analysis only. To extend the hypothesis that more diffuse or non-severe lesions may subtend ischaemic myocardium with stiffer aortas would require analysis of visually 'normal'

CMR-PI scans and a subsequent evaluation for coronary artery disease, likely CT coronary angiography, in those with abnormal scans. With semi-automated techniques, this analysis will be possible in the future.

Conclusion

This is the first study to demonstrate the relationship between aortic stiffness and both ischaemic and non-ischaemic myocardial perfusion indices using cardiovascular magnetic resonance. Aortic stiffness appears to have a more significant contribution to resting and hyperaemic blood flow in ischaemic compared to non-ischaemic myocardium. In this highly selected cohort, percentage stenosis was of a severe to critical nature in most patients and was not associated with rMBF or hMBF.

5. CHAPTER 5: Arterial stiffness and infarct myocardium

Increased arterial stiffness mitigates improvement in myocardial perfusion reserve post ST elevation myocardial infarction

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5.1 STATEMENT OF AUTHORSHIP

Manuscript details

Title of paper	*Increased arterial stiffness mitigates improvement in myocardial perfusion reserve post ST elevation myocardial infarction*
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Principal Author Contributions

Candidate	Dr Adam J Nelson
Contribution to the Paper	Primary contributor to the conception and design of the work; Drafted the work; Provided final approval of the version to be published; Accountable for all aspects of the work.
Overall percentage	90%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

August 2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	A/Prof Matthew I Worthley
Contribution to the Paper	Provided a contribution to the design of the work; Helped drafting the work and revising it critically; Provided final approval of the version to be published; Accountable for all aspects of the work

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August 2018

5.2 ABSTRACT

Background: Large artery stiffness has been associated with both a reduction in myocardial perfusion and an increase in myocardial oxygen demand. The impact of increased arterial stiffness on blood flow within 'infarct' and 'non-infarct' myocardium following ST elevation myocardial infarction (STEMI) is unknown.

Methods: Subjects underwent cardiovascular magnetic resonance on day three and day ninety post primary percutaneous coronary intervention for STEMI. Left ventricular myocardium was dichotomised into 'infarct' or 'non-infarct'. Aortic distensibility (AD), resting and hyperemic blood flow (rMBF, hMBF) and myocardial perfusion reserve (MPR) were obtained from first pass perfusion imaging. Myocardial injury was evaluated by infarct size (IS) and microvascular obstruction (MVO) using delayed enhancement sequences, in addition to creatine kinase levels.

Results: Twenty-seven subjects (mean age 59.7 ± 10 years, male 89%) were recruited. LAD infarcts were most common (41%) with cohort peak creatine kinase 2282 ± 1520units/L. Smoking was prevalent (59%) although only 22% had > 2 traditional risk factors. Baseline left ventricular ejection fraction was 44.7 ± 9.9%. MVO was present in 25 subjects (mean area 534 ± 493mm2) with mean cohort IS 26.9 ± 10.8%. Mean cohort AD was 3.59 ± 1.52·10-3mmHg-1. In both 'infarct' and 'non-infarct' myocardium at early imaging, AD was associated with hMBF (R 0.547 and 0.557 respectively, $p < 0.01$) and MPR (R 0.626 and 0.578 respectively, $p < 0.01$). These relationships remained similar at three months. There was no relationship between AD and rMBF at either time point. On multivariate analysis (IS, MVO, creatine kinase), AD independently predicted late MPR in the 'infarct' territory, and was associated with change in MPR from baseline (R 0.44, $p = 0.02$).

Conclusion: A stiff aorta was associated with reduced early and late hyperemic response to adenosine in both the 'infarct' and 'non-infarct' myocardium. On multivariate analysis, AD was independently associated with change in MPR from baseline.

5.3 INTRODUCTION

An evolving body of evidence now supports increased arterial stiffness as a key risk factor for cardiovascular events. The mechanisms behind this association appear to be driven by both increased cardiac work and resultant left ventricular hypertrophy, but potentially also through a reduction in coronary blood flow.

Initially described in animal models of aortic banding, an increase in arterial stiffness resulted in a widening of pulse pressure, an augmentation of systolic pressure and a reduction in coronary perfusion pressure, particularly at the subendocardial level (Ohtsuka et al. 1994; Watanabe et al. 1992). In humans, Leung et al. (Leung et al. 2006) subsequently demonstrated that although resting coronary blood flow was at least partially governed by large arterial mechanics, the hyperaemic response to adenosine both pre and post successful percutaneous coronary intervention (PCI) was significantly reduced in a stiff aorta. Others have shown a relationship between pulse wave velocity, a surrogate of arterial stiffness, and both echo (Saito et al. 2008) and invasive measures of coronary flow velocity reserve (Fukuda et al. 2006).

Our group has recently demonstrated the utility of cardiac magnetic resonance imaging to evaluate the relationship between aortic stiffness and myocardial perfusion. This technique is non-invasive, radiation-free and can provide near simultaneous assessment of both parameters. *Chapter 3* (normal subjects) and *Chapter 4* (subjects with established symptomatic stable coronary artery disease) confirmed a strong relationship between arterial stiffness and both resting and hyperaemic myocardial blood flow. Moreover, the relationship appeared to strengthen in ischaemic territories suggesting arterial stiffness may have a more dominant governance of blood flow under these conditions.

As described in *Chapter 1*, myocardial blood flow is tightly controlled by autoregulatory microvascular function. Post myocardial infarction, the microvasculature is often compromised, either through distal embolisation or oedema generated prior to or during PCI. Patients with reduced hyperaemic blood flow following revascularisation have worse functional and clinical outcomes (Ibanez et al. 2015; Wakatsuki et al. 2000). Recent animal model and human studies have shown reduced coronary flow velocity reserve in both infarct and non-infarct territories and have ascribed this to microvascular dysfunction. Other invasive studies post infarct have shown increased microcirculatory resistance (Ahn et al. 2016) in infarct territories consistent with microvascular dysfunction.

We hypothesised that the aorto-coronary relationship is likely to be overwhelmed by both local and systemic contributors to blood flow early but may have returned by three months. We hypothesised that a stiff aorta may mitigate improvement in hyperaemic flow following successful revascularisation as described in the stable setting by Leung et al. We sought to evaluate the aorto-coronary relationship in patients who have recently sustained a ST segment elevation myocardial infarction (STEMI). We evaluated these patients at baseline (within three days) and again at three month follow up to see how this relationship evolved and its interaction with indices of myocardial injury as assessed with late gadolinium enhancement.

5.4 METHODS

5.4.1 Subjects

5.4.1.1 Selection

The study was approved by the Human Research Ethics Committee at the Royal Adelaide Hospital. As part of a larger study (Wong et al. 2012), subjects with acute STEMI who underwent primary angioplasty between April 2009 and July 2011 were prospectively recruited. As part of the larger trial, participants underwent CMR perfusion (CMR-PI) and delayed enhancement (CMR-DE) imaging on day three and again at three months. A retrospective analysis was performed on individuals who participated at both time points.

5.4.1.2 Inclusion criteria

STEMI was defined as chest pain for at least 30 minutes and an ECG demonstrating ST-segment elevation of >0.1mV in two or more contiguous leads. CMR imaging was acquired at both day three and again at three months post STEMI. Vasoactive medications were not withheld in view of clinical indication. Patients had abstained from caffeine and xanthine-containing products for at least 24 hours prior to scanning as per local protocol.

5.4.1.3 Exclusion criteria

Exclusion criteria included atrial fibrillation/flutter, aortopathy (history of intervention, dissection or aneurysm), CMR chamber incompatibility (pacemakers, aneurysm clips, claustrophobia), pre-existing LV systolic dysfunction (LVEF < 45%), severe valvular heart disease, severe airways disease (chronic obstructive pulmonary disease or asthma on inhalers), renal impairment (estimated glomerular filtration rate < 30 mL/min/1.73m^2) or atrioventricular nodal disease (Grade II or higher) were excluded.

Patients were excluded from the retrospective analysis if there was a prior history of established ischaemic heart disease, evidence of reversible ischaemia in a non-culprit territory or if aortic distensibility measurements could not be obtained.

5.4.1.4 Demographics

Discharge medications from index hospitalisation and risk factors were recorded. Risk factors were defined as cigarette smoking (previously or currently smoking tobacco), hypercholesterolemia (use of cholesterol-lowering therapy or total cholesterol level > 5.2 mmol/L), hypertension (blood pressure > 140/90mmHg or on medical antihypertensive therapy or subject to lifestyle intervention), obesity (BMI > 30kg/m^2), diabetes (plasma haemoglobin A1c > 6.5%, or on glycaemic medications), and family history of cardiovascular disease (index event in a first-degree relative, male ≤ 55 years and female ≤ 60 years).

5.4.1.5 STEMI characteristics

As part of the larger study (Wong et al. 2012) evaluating novel predictors of functional outcome post STEMI, clinically relevant metrics around the STEMI hospitalisation were recorded. These included peak creatine kinase (CK), door-to-balloon time and pain-to-balloon time.

5.4.2 Cardiac magnetic resonance protocol

All CMR studies were performed utilizing the Siemens Avanto 1.5 Tesla magnetic resonance imaging scanner (Siemens Medical Imaging, Erlangen, Germany) located within the Cardiovascular Investigation Unit of the Royal Adelaide Hospital. All CMR studies were performed with subjects in a supine position with a phased-array surface coil positioned over the thorax.

5.4.2.1 Cardiac structure and function

Methods for evaluating left ventricular structure and function are covered in *Chapter 2*. In brief, short-axis slices from the mitral annulus to and inclusive of the left ventricular apex were acquired during expiratory breath hold.

Offline analysis was performed to evaluate LV size and function utilizing proprietary software (Argus software, Siemens Medical Imaging, Erlangen, Germany). Endocardial contours were manually traced at end-diastole (start of R-wave) and at end-systole (visually smallest cavity). End-systolic and end-diastolic volumes were obtained and thus left ventricular ejection fraction could be calculated.

5.4.2.2 Aortic distensibility

Methods for evaluating aortic distensibility from already acquired cardiac structure and function studies is covered in detail in *Chapter 3*. In brief, the vertical long axis (VLA) planning image transects the aorta at the level of the arch between the previously described levels of the 'Ascending Aorta' and 'Proximal Descending Aorta'.

Aortic distensibility was evaluated offline by manually tracing aortic cross-sectional images at the level of the 'thoracic aorta' (TA). Major and minor axes were drawn on each VLA image and if the ellipticity index was <0.9 (minor/major), the patient was excluded from the study. Maximal and minimal lumen areas were calculated and distensibility derived by a previously published formula (Groenink et al. 1998):

$$\frac{\textit{aortic area at end systole } (mm^2) - \textit{aortic area at end diastole } (mm^2)}{\textit{brachial pulse pressure } (mmHg) \times \textit{aortic area at end diastole } (mm^2)}$$

The baseline heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) taken at commencement of CMR imaging was used for distensibility calculation. The pulse pressure was calculated from the difference between the systolic and diastolic blood pressures and measured in mmHg. The aortic areas were measured in mm^2 and after multiplying by 1000, aortic distensibility is reported in its final unit of $10^{-3}mmHg^{-1}$.

5.4.2.3 CMR Perfusion Imaging (CMR-PI)

5.4.2.3.1 Adenosine infusion protocol

As per local protocol, a physician with a cardiac defibrillator was present for all stress perfusion imaging. All patients received adenosine (AdenoScan, Sanofi-Aventis, Surrey, UK) at a dose of 140μg/kg/min using a precise syringe pump (Graesby 3500, Minneapolis, Minnesota, USA) via a cannula in the antecubital vein.

5.4.2.3.2 Image acquisition

CMR perfusion imaging acquisition has been covered in detail in *Chapter 3*. In brief, during the last minute of the adenosine infusion, the gadolinium-based contrast agent, Magnevist (dimeglumine gadopentetate, Bayer, Leverkusen, Germany), was administered. Perfusion imaging was performed with a T1-weighted fast low-angle single shot gradient echo (GRE) sequence and was acquired every cardiac cycle during first pass. Perfusion images were obtained at three short axis planes - basal, mid and apical levels - of the left ventricle (Cerqueira et al. 2002).

Haemodynamic data was acquired at five-minutely intervals throughout the adenosine protocol and recorded.

5.4.2.3.3 Myocardial blood flow and perfusion reserve

The endocardial and epicardial contours of three short axis sections (base, mid and apex) were traced (QMASS, v7 .2, Medical Imaging Solutions, Leiden, Netherlands) and corrected manually for displacements (e.g. breathing). Each short axis slice was divided into six equiangular segments in a clockwise manner beginning at the anteroseptal insertion of the right ventricle (Barmeyer et al. 2007).

Segments were then assigned to coronary territories according to the 18-segment model of the American Society of Echocardiography (Lang et al. 2015). Segments 1, 2 and 6 were assigned to the left anterior descending (LAD); 2,3 and 4 to the left circumflex (LCx) and segments 4 and 5 to the right coronary artery (RCA). Within each section, signal intensity was measured by defining regions of interest that excluded the inner 10% and outer 30% of the myocardium to get stronger weighting of the subendocardium and reduce influence from the LV as previously described (Nagel et al. 2003) *(Figures 4.1 and 4.2)*.

The subendocardial signal intensity-time curves were generated for all segments by obtaining signal intensity on consecutive images before and during arrival of contrast material. The signal intensity-time curve for the LV was generated on the basal section as a measure of input function *(Figure 4.3)*. The maximal initial upslope of every signal intensity-time curve was determined by using a sliding window with a four-point linear fit for the myocardium and a three-point linear fit for the LV as previously described (Al-Saadi et al. 2000).

Myocardial Blood Flow (MBF) was determined in each equiangular section and then averaged per coronary territory in mL/min/g. This was performed by deconvolution of the signal intensity time curves (Selvanayagam et al. 2005) constrained to a Fermi function using an arterial input from the left ventricular blood pool signal, specifically accounting for any delay in the arrival of the tracer (Jerosch-Herold et al. 2004;

Jerosch-Herold et al. 2002; Jerosch-Herold et al. 2000). This was performed on both rest and stress images to generate resting MBF (rMBF) and hyperaemic MBF (hMBF). As resting MBF is closely related to the rate-pressure product (RPP) (Czernin et al. 1993), an index of myocardial oxygen consumption, values for basal flow in each subject were corrected for the respective RPP. This was performed by multiplying rMBF by the mean RPP of the cohort, divided by the RPP in the individual patient (Uren, Melin, et al. 1994).

For each of the 18 segments, myocardial perfusion reserve (MPR) was calculated by dividing the maximal upslope value at stress over rest.

Segments were denoted as 'infarct' or 'non-infarct' based on angiography findings and corresponding, subtended CMR segments.

5.4.2.3.4 Myocardial injury assessment

As part of our hypothesis, microvascular obstruction and larger infarct size may impact on coronary blood flow, and thus, aorto-coronary relationships. Although the larger study evaluated a number of procedural, invasive and non-invasive indices of myocardial injury (Wong et al. 2012), we evaluated only late gadolinium enhancement images for infarct size and microvascular obstruction (MVO). Although there are a number of sequences and techniques, our group and operators have experience in this metric.

Delayed enhancement (CMR-DE) imaging was obtained by acquiring an inversion-recovery segmented gradient echo T1 weighted sequence 15 minutes after the second contrast dose. The parameters were as follows: echo time (TE) 3.32ms; flip angle = 25degrees; field of view (FOV) 38cm; matrix size 176 x 256.

Infarct size

Validated initially in the chronic phase of MI, delayed hyperenhancement has been shown to correlate well with fibrosis and be a marker of irreversible injury (Kim, RJ et al. 2000). In the acute phase, different pulse sequence parameters, timing of imaging post contrast administration and varied planimetry methods have traditionally resulted in overestimation of infarct size compared to necropsy studies (Saeed et al. 1999). Moreover, myocardial oedema may contribute to hyperenhancement in the absence of overt cell necrosis (Judd et al. 1995; Lima et al. 1995). In this study infarct size was determined in a semi-automated manner using the 'full width at half the maximum (FWHM) criterion' which has been validated against necropsy data ($R = 0.94$, $p<0.0001$) (Amado et al. 2004). In brief, this is performed by identifying a region of hyperenhancement and selecting a 'seed' or bright point. Using proprietary QMASS software (Medis, Leiden, Netherlands), this seed point is extended to all pixels with signal intensity of greater than 50% in all short axis slices. The maximum signal intensity inside this region is then determined, and the final infarct size is defined as the area with a signal intensity 50% above the maximum of the initial region. Infarct size was calculated at both time points as well as a 'change' in infarct size.

Microvascular obstruction

Microvascular obstruction was defined on LGE as regions of subendocardial hypoenhancement within a hyperenhanced region (Hombach et al. 2005). These were manually delineated on the short axis images using QMASS and summed together to give an 'area' of MVO. This was performed on 'early' imaging only.

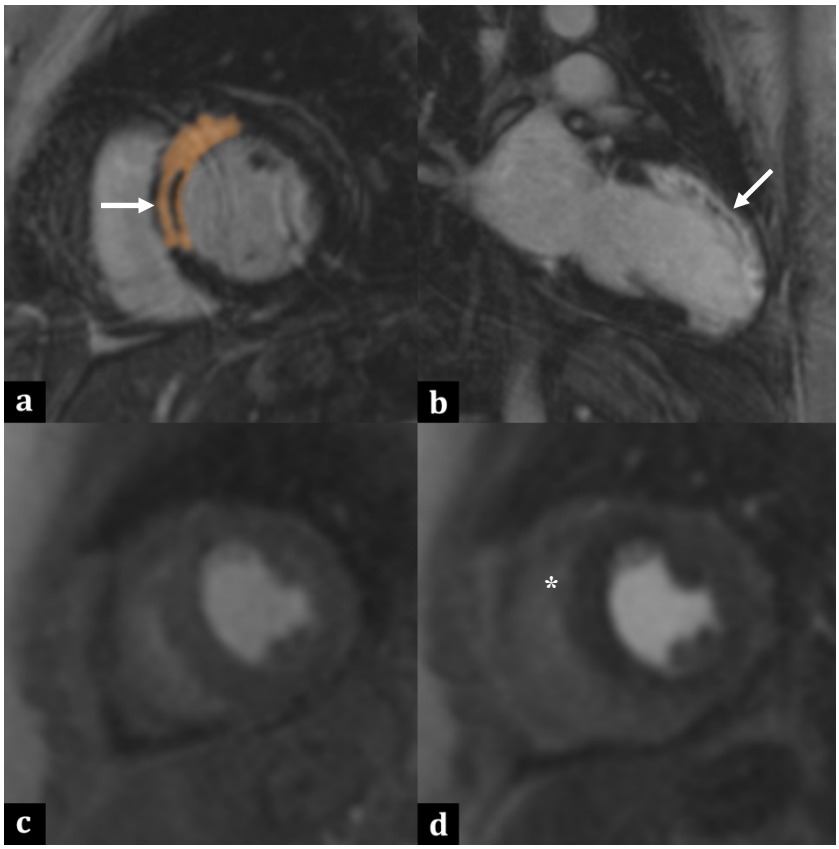

FIGURE 5.1. *Late gadolinium enhancement short axis (a) and vertical long axis (b) images demonstrating near transmural hyperenhancement in LAD territory – representative image analysis in (a) enhanced with orange; image (b) included for comparison in the VLA plane. Hypoenhanced 'lakes' of microvascular obstruction shown in both (a) and (b) with arrows. Myocardial perfusion imaging at rest (c) and stress (d) showing hypoenhancement in infarct-affected zones (labelled with *).*

5.4.3 Statistical Analysis

Baseline characteristics are represented as mean ± standard deviation where normally distributed, or median and IQR for parameters with non-Gaussian distribution.

The relationship between continuous variables was determined by simple linear regression, and adjustment for additional variables was by partial correlation. Analysis of covariance was used when both continuous and categorical variables were present. Comparisons of mean values between groups were by paired t-test or by ANOVA. Multiple linear regression was performed to determine predictors of *late* infarct territory MPR. Given the sample size, four variables with $p<0.2$ were included in regression model. Statistical significance was defined as a two-sided $p<0.05$. Statistical analyses were performed using SPSS statistical software (v24, IBM SPSS, New York, USA) and graphs were drawn using GraphPad Prism 7 (GraphPad Software, La Jolla California USA).

5.5 RESULTS

5.5.1 Sample

For a detailed cohort build, see *Figure 5.2*. Of the 61 patients recruited for the larger study, 11 did not participate in the CMR sub-study due to claustrophobia or limiting image quality. Of the 50 patients who underwent 'early' CMR (day 3 ± 1.5 days), seven had evidence of reversible ischaemia in a non-culprit territory, six withdrew consent and three had a relative contraindication to gadolinium with an eGFR ≤ 30ml/min/1.73m^2. Four patients had oblique imaging for aortic distensibility and a further two had inadequate quality 'late' CMR studies. This left a total of 27 individuals with both 'early' and 'late' CMR-PI, CMR-DE and distensibility data to evaluate.

5.5.2 Cohort

The clinical characteristics of the cohort are listed *(Table 5.1)*. The majority of patients were male (n = 24, 89%) with a mean age of 59.7 ± 10 years.

Overall the population had a paucity of vascular risk factors with less than one quarter diagnosed with three or more traditional risk factors. The most prevalent risk factors were smoking (59%) followed by hypertension (44%) and dyslipidaemia (37%).

Consistent with STEMI guidelines, all patients were treated with aspirin and a $P2Y_{12}$ inhibitor and the vast majority on statins and either an angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB).

With regards to infarct characteristics, there were equal numbers of LAD and RCA events (n = 11) with the remainder occurring in the main body of the LCx. Infarct sizes were modest by CK measure and likely secondary to a combination of short pain-to-balloon times and open arteries at the end of the case (TIMI flow 2.8 ± 0.4).

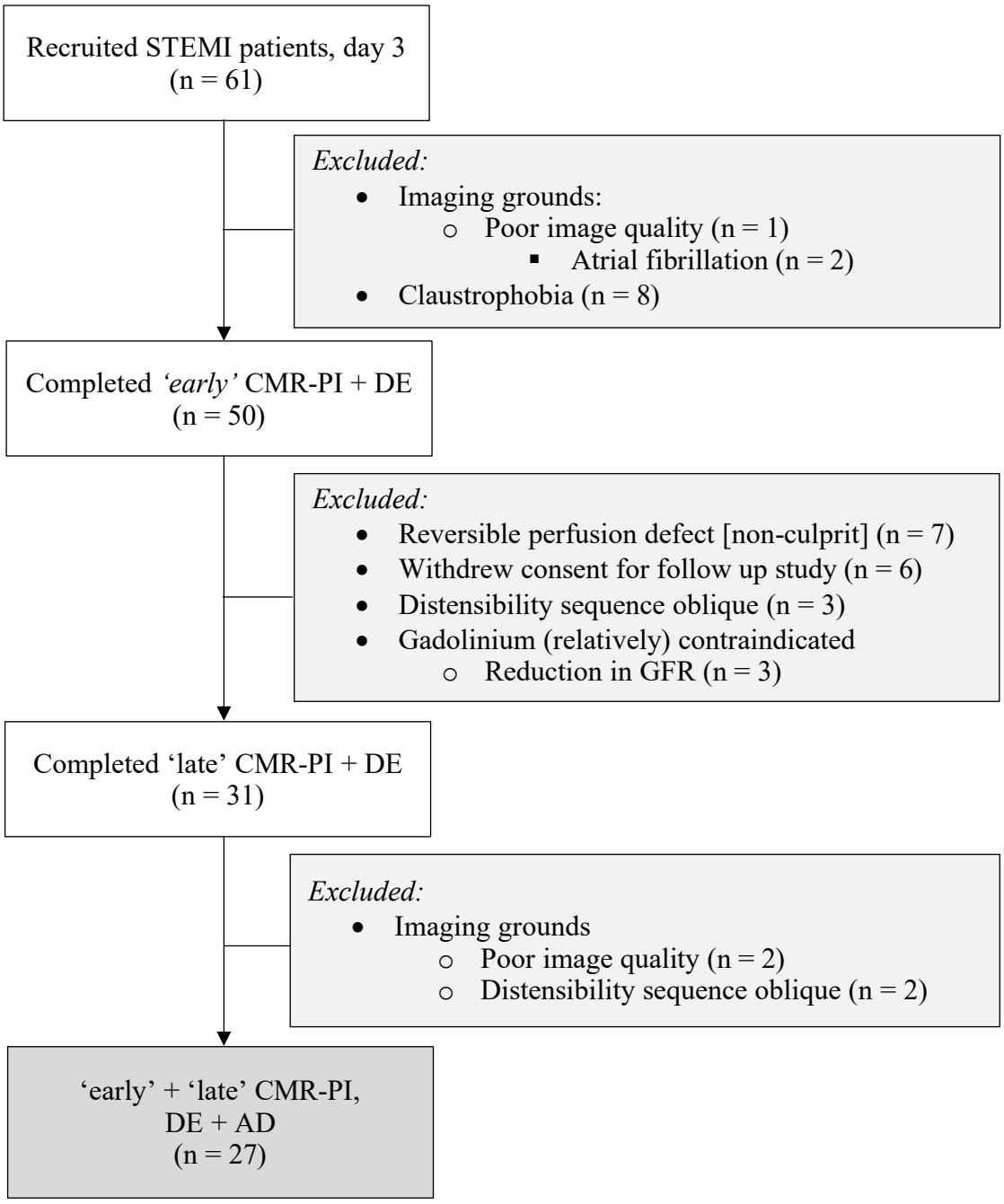

FIGURE 5.2 *Cohort build*

Clinical characteristic	Cohort
Mean age ± SD [years]	59.7 ± 9.6
Male sex – no. (%)	24 (89)
Mean BMI ± SD [kg/m^2]	28.4 ± 8.2
Risk factors – no. (%)	
Family history	4 (15)
Cigarette smoking	16 (59)
Hypertension	12 (44)
Dyslipidaemia	10 (37)
Diabetes	4 (15)
≥ 3 risk factors	6 (22)
Discharge medications – no. (%)	
Aspirin	27 (100)
$P2Y_{12}$ inhibitor	27 (100)
ACE/ARB	25 (93)
Statin	25 (93)
Beta blocker	19 (70)
Coronary anatomy	
Right dominant circulation - no. (%)	25 (93)
Infarct related vessel – no. (%)	
LAD	11 (41)
LCx	5 (19)
RCA	11 (41)
Infarct characteristics	
CK peak – Mean ± SD (units/L)	2282 ±1520
Time - Median ± SD (mins)	
Door-to-balloon	82 ± 56
Pain-to-balloon	228 ± 173
TIMI flow pre - mean ± SD	0.9 ± 0.8
TIMI flow post - mean ± SD	2.8 ± 0.4

TABLE 5.1 *Clinical and infarct characteristics. Abbreviations: $P2Y_{12}$ inhibitor (clopidogrel or ticagrelor); LAD – left anterior descending artery; LCx – left circumflex artery; RCA – right coronary artery; CK – creatine kinase.*

Laboratory investigations *(Table 5.2)* revealed normal kidney function and haemoglobin. Baseline lipid profile overall revealed a mildly elevated LDL and mildly reduced HDL.

Laboratory characteristics	**Cohort**
Lipid profile ± SD [mmol/L]	
Total cholesterol	5.3 ± 1.8
Triglycerides	1.8 ± 1.2
LDL-C	3.2 ± 1.5
HDL-C	0.9 ± 0.5
Hb ± SD [mg/dL]	136 ± 11
eGFR [mL/min/1.73m^2]	76 ± 13

TABLE 5.2 *Laboratory characteristics. Abbreviations: LDL – low-density lipoprotein; HDL – high density lipoprotein; Hb – haemoglobin; eGFR – estimated glomerular filtration rate*

5.5.3 Cardiac Structure and Function

Subjects at 'early' CMR had mild systolic dysfunction (LVEF 44.7 ± 9.9%) which by 'late' imaging at three months had improved to be low-normal (LVEF 50.9 ± 9.8%). This was driven by a numerical improvement in end-systolic volume (84.4 ± 21.4 vs. 72.9 ± 27.8mL). Left ventricular mass was within normal limits with no significant interval change between time points (LV mass 151.7 ± 34.9g. Reference ranges: Males 108-184g; Females 72-144g).

5.5.4 Aortic distensibility

5.5.4.1 Overall measures

Aortic distensibility was overall preserved in this cohort and was comparable to our 'normal' cohort in *Chapter 3* (3.59 ± 1.52 vs. 3.23 ± 1.64·10^{-3}mmHg^{-1}). This is consistent with the low burden of traditional vascular risk factors and a younger cohort age consistent with other contemporary STEMI studies (Berwanger et al. 2018). There was a small numerical reduction in distensibility between time points which did not reach statistical significance.

5.5.5 CMR Perfusion imaging

CMR perfusion imaging was well tolerated with majority of patients reporting at least one symptom of hyperaemia (e.g. flushing, headache, dyspnoea, chest tightness). There was no limiting hypotension or AV block. After correlating angiographic lesion location and subtended myocardium, there were 168 'infarct' segments' and 318 'non-infarct' segments.

5.5.5.1 Myocardial blood flow

Early rMBF was numerically less in the 'infarct' compared to 'non-infarct' segments although this did not reach statistical significance (0.88 ± 0.21 vs. 0.95 ± 0.19mL/min/g, p = 0.210). In response to adenosine infusion at the *early* time point, hMBF was significantly lower in the 'infarct' compared to the 'non-infarct' segments (1.15 ± 0.32 vs. 1.51 ± 0.34mL/min/g, p < 0.001).

By *late* imaging, the difference in rMBF in 'infarct' and 'non-infarct' segments had widened with both a reduction and increase in respective territories (0.80 ± 0.17 vs. 1.11 ± 0.22mL/min/g, p <0.0001). Although hMBF had improved in both territories, *late* hMBF remained significantly higher in 'non-infarct' compared to 'infarct' segments (1.38 ± 0.32 vs. 2.74 ± 0.56mL/min/g, p <0.0001)

5.5.5.2 Myocardial perfusion reserve

At the *early* time point, MPR was significantly lower in the 'infarct' compared to the 'non-infarct' segments (1.21 ± 0.29 vs. 1.68 ± 0.40, $p < 0.001$). By *late* imaging, although MPR had improved in the 'infarct' territory, the 'non-infarct' territory remained significantly higher (1.58 ± 0.35 vs. 2.58 ± 0.57, $p < 0.001$).

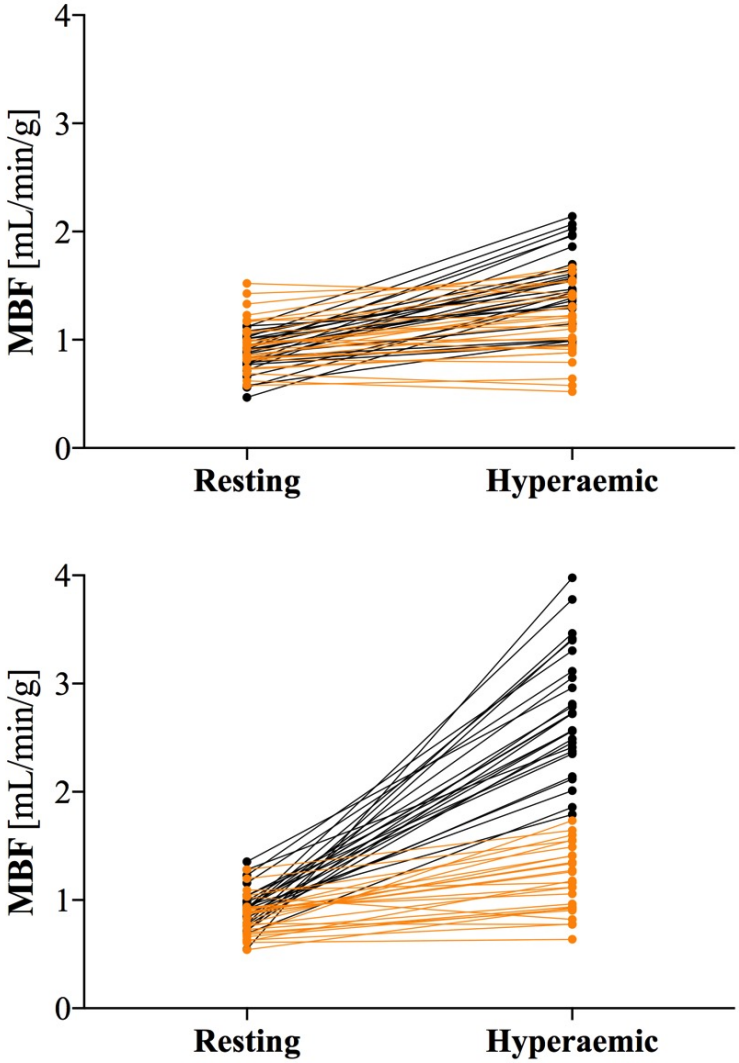

FIGURE 5.3 *Ladder plots of resting and hyperaemic MBF at early (top) and late (bottom) time points. Infarct segments in orange, non-infarct segments in black.*

5.5.6 Myocardial injury

The majority of patients had evidence of microvascular obstruction (n = 25, 93%) with a mean area of 534 ± 493mm^2. Infarct size at baseline was 26.9 ± 10.8%, although by three months this had improved to 20.6 ± 9.3% ($p < 0.001$) at repeat imaging.

	Early *(3 days)*	**Late** *(90 days)*	**P value**
Resting haemodynamics			
SBP [mmHg]	137 ± 27	135 ± 19	0.846
DBP [mmHg]	82 ± 17	81 ± 13	0.939
PP [mmHg]	54 ± 13	53 ± 16	0.944
HR [bpm]	68 ± 10	71 ± 9	0.415
Cardiac structure and function			
LVEDV [mL]	150.9 ± 27.1	153.6 ± 42.8	0.211
LVESV [mL]	84.4 ± 21.4	72.9 ± 27.8	0.061
LVEF [%]	44.7 ± 9.9	50.9 ± 9.8	**0.036**
LV mass [g]	151.7 ± 34.9	143.4 ± 29.5	0.218
Aortic distensibility [10^{-3}mmHg^{-1}]	3.59 ± 1.52	3.47 ± 1.74	0.973
Infarct size – [%] mean ± SD	26.9 ± 10.8	20.6 ± 9.3	**<0.001**
MVO area – [mm^2] ± SD	534 ± 493	-	
Stress perfusion imaging			
Resting RPP	9457 ± 2929	9814 ± 1910	0.598
Stress RPP	12060 ± 3305	11681 ± 2913	0.201
Infarct territory			
rMBF [mL/min/g]	0.88 ± 0.21	0.80 ± 0.17	**0.007**
hMBF [mL/min/g]	1.15 ± 0.32	1.38 ± 0.31	**0.001**
MPR	1.21 ± 0.29	1.58 ± 0.35	**<0.001**
Non-infarct territory			
rMBF [mL/min/g]	0.95 ± 0.19	1.11 ± 0.22	**<0.001**
hMBF [mL/min/g]	1.51 ± 0.34	2.74 ± 0.56	**<0.001**
MPR	1.68 ± 0.40	2.58 ± 0.57	**<0.001**

TABLE 5.3 *CMR perfusion imaging and delayed enhancement outcomes. Abbreviations: SBP – systolic blood pressure; DBP – diastolic blood pressure; PP – pulse pressure; HR – heart rate; LVEDV – left ventricular end diastolic volume; left ventricular end systolic volume; MVO – microvascular obstruction;*

5.5.7 Arterial stiffness and myocardial perfusion

5.5.7.1 Myocardial blood flow

There was no relationship between rMBF and distensibility in either 'infarct' or 'non-infarct' territories at either *early* or *late* time points *(Figure 5.4, grey dashed lines)*.

At the *early* time point, hMBF (after correction for age and gender) was related to aortic distensibility in both 'infarct' (R^2 0.299, p = 0.004) and 'non-infarct' territories (R^2 0.310, p = 0.003) with equal strength *(Figure 5.4, top panels)*. At the *late* time point however, hMBF was more strongly related to aortic distensibility in the 'non-infarct' territories (R^2 0.456, p < 0.001) than the 'infarct' territories (R^2 0.338, p = 0.001), although both were statistically significant *(Figure 5.4, bottom panels)*.

5.5.7.2 Myocardial perfusion reserve

After correcting for age and gender, MPR was linearly correlated with measures of aortic distensibility at both *early* and *late* time points in both 'infarct' and 'non-infarct' territories *(Figure 5.5)*: *early* 'infarct' R^2 0.470 (p = 0.001); *late* 'infarct' R^2 0.530 (p < 0.001), *early* 'non-infarct' R^2 0.334 (p = 0.002); *late* 'non-infarct' R^2 0.345 (p = 0.002).

	rMBF		**hMBF**		**MPR**	
	I	**NI**	**I**	**NI**	**I**	**NI**
'Early'						
Distensibility	0.219 (0.385)	0.203 (0.420)	**0.547 (0.004)**	**0.557 (0.003)**	**0.626 (0.001)**	**0.578 (0.002)**
'Late'						
Distensibility	0.282 (0.172)	0.267 (0.198)	**0.581 (0.001)**	**0.675 (<0.001)**	**0.728 (<0.001)**	**0.587 (0.002)**

TABLE 5.4 *Correlations at early and late time points between myocardial blood flow and MPR with aortic distensibility. I = infarct, NI = non-infarct (r value with p value below in parentheses). Bold denotes a significant relationship. rCBF adjusted for age and gender (already corrected for RPP). hCBF and MPR adjusted for age and gender.*

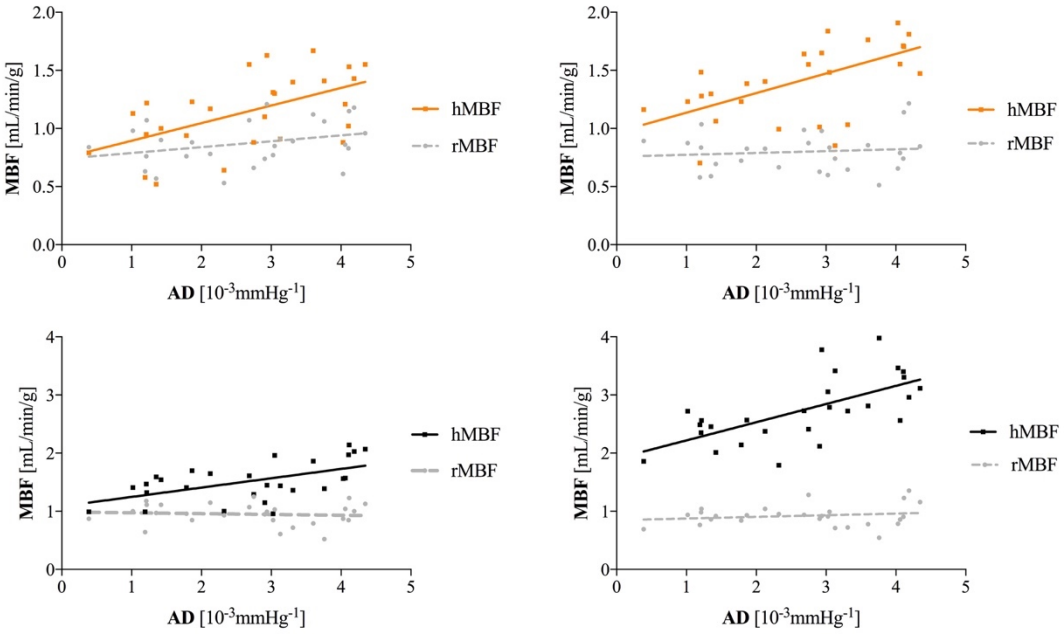

FIGURE 5.4 *Relationships between indices of myocardial perfusion and aortic distensibility. 'Infarct' territories shown on left, 'non-infarct' territories on right. Early time point, above, late time point, below.*
Dashed lines represent regression analysis with non-significant p value.

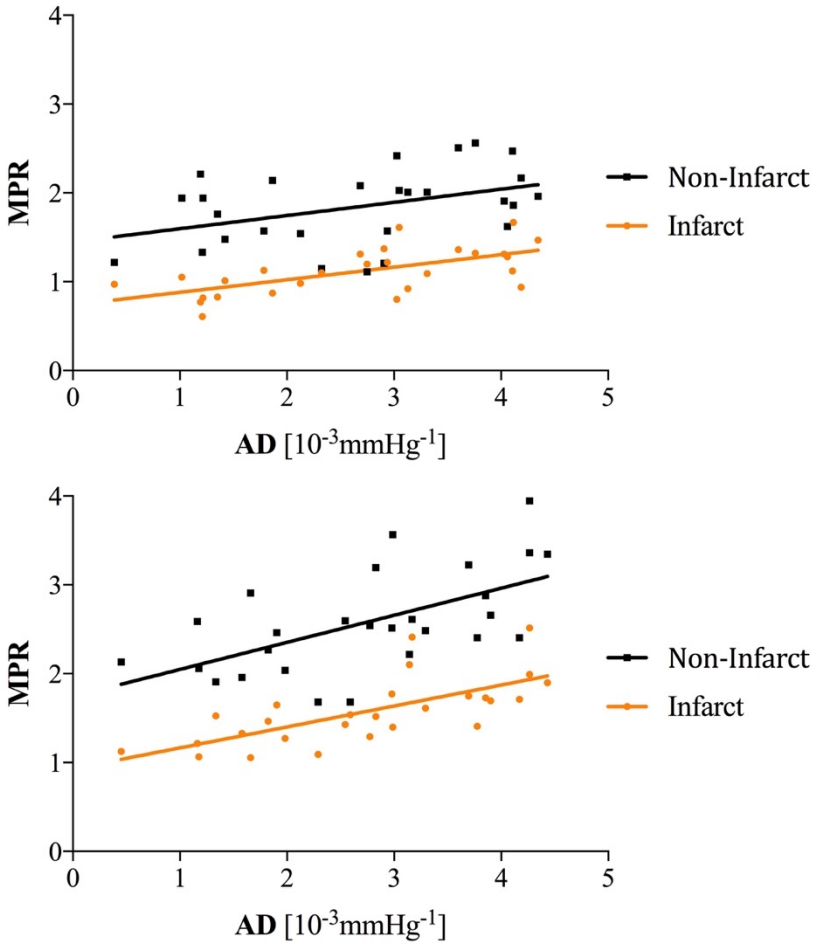

FIGURE 5.5 *Relationship between MPR and aortic distensibility in 'infarct' (orange) and 'non-infarct' (black) territories at early (top) and late (bottom) time points.*

5.5.7.3 Stiff versus compliant

In a pre-defined exploratory analysis, to further evaluate the impact of arterial stiffness on these measures, the cohort was divided into 'stiff' and 'compliant' at the mean value of aortic distensibility *(Figure 5.6)*. At the *early* time point in 'infarct' territories, a stiff aorta was associated a blunted response to adenosine evidenced by reduced hMBF (0.97 ± 0.29 vs. 1.31 ± 0.26mL/min/g, p = 0.004) and MPR (1.06 ± 0.21 vs. 1.36 ± 0.28, p = 0.004). Although there was a numerical difference between rMBF in stiff and compliant aortas, this did not reach statistical significance (0.83 ± 0.16 vs. 0.94 ± 0.18mL/min/g, p = 0.124). By the *late* time point, the was no relationship with rMBF, however the association with hMBF remained (1.21 ± 0.25 vs. 1.52 ± 0.36mL/min/g, p = 0.026). Although the MPR had improved in 'infarct' territories, a stiff aorta remained linked to reduced MPR (1.31 ± 0.20 vs. 1.82 ± 0.36 p <0.001).

In 'non-infarct' territories, a stiff aorta was associated with reduced hMBF (1.38 ± 0.25 vs. 1.64 ± 0.37mL/min/g, p = 0.049) and MPR (1.43 ± 0.27 vs. 1.92 ± 0.39, p = 0.001) although did not affect rMBF (1.01 ± 0.16 vs. 0.90 ± 0.20mL/min/g, p = 0.133). In the 'non-infarct' territories however, a stiffer aorta was more strongly associated with hMBF (2.34 ± 0.31 vs. 3.11 ± 0.50mL/min/g, p < 0.001) and MPR (2.22 ± 0.38 vs. 2.92 ± 0.52, p = 0.001) than it had been *early* post MI.

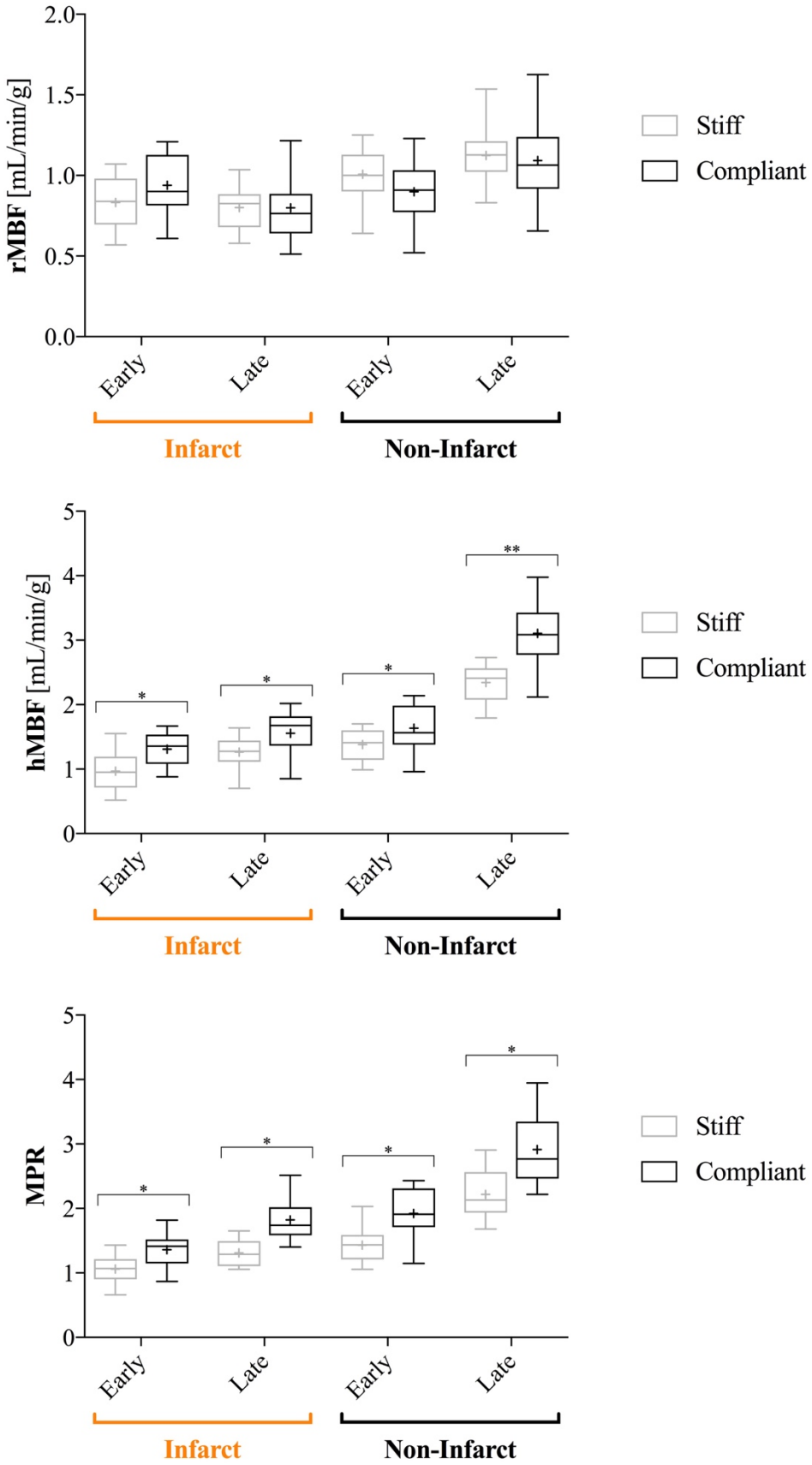

FIGURE 5.6 *Effect of a stiff vs. compliant aorta in both infarct and non-infarct territories, on rMBF (top), hMBF (middle) and MPR (bottom). *denotes significance <0.05, ** denotes significance <0.001*

5.5.8 Other predictors of myocardial perfusion

We sought to evaluate markers of myocardial injury which may impact on MBF and alter the aorto-coronary relationship.

In the 24 individuals with MVO, at the *early* time point in 'infarct' territories, MVO area correlated with MPR (R -0.489, p = 0.021), and although demonstrating a trend, was not significantly associated with either rMBF (R -0.381, p = 0.181) or hMBF (R -0.348, p = 0.11). At the *late* time point, baseline MVO area again was associated with MPR (R -0.481, p = 0.023), although the trend with hMBF weakened (R -0.273, p = 0.21) and was not present with rMBF (R -0.110, p = 0.6).

Early (R 0.422, p = 0.049) and *late* infarct size (R 0.456, p = 0.033) correlated with MVO area, however there was no association with rMBF, hMBF or MPR.

Peak CK also correlated with MVO area (R 0.494 p = 0.019), although not infarct size. With respect to myocardial perfusion, peak CK correlated with *early* MPR (R -0.446, p = 0.020) although not *late* MPR or other measures of blood flow.

In other univariate analyses, aortic distensibility was correlated with MVO area (R-0.394, p = 0.044) but not with CK or either time point of infarct size.

There was no significant difference between indices of myocardial injury or perfusion at any time point when comparing LAD vs. non-LAD infarcts.

5.5.9 Predictors of follow up MPR in infarct territory

To understand the dominant predictor of *late* MPR in the 'infarct' territory, these variables were evaluated with multiple linear regression. On multiple linear regression, aortic distensibility remained an independent predictor of 'infarct' territory MPR at three months *(Table 5.5)*.

	Univariate		**Multivariate**	
	β	p value	β	p value
MVO area	-0.481	0.023	-	0.352
Infarct size (early)	-0.218	0.091	-	0.393
Peak CK	-0.150	0.128	-	0.257
Distensibility	0.728	<0.001	**0.562**	**0.003**

TABLE 5.5 *Multiple linear regression for the prediction of MPR in infarct territory at 90 days.*

To assess whether MVO area or distensibility were predictive of MPR improvement, both were evaluated against Δ MPR. Regression analysis revealed no significant relationship between Δ MPR and MVO area (R -0.184, p = 0.314), however there was a modest association with aortic distensibility (R 0.444, p = 0.020) *(Figure 5.7)*.

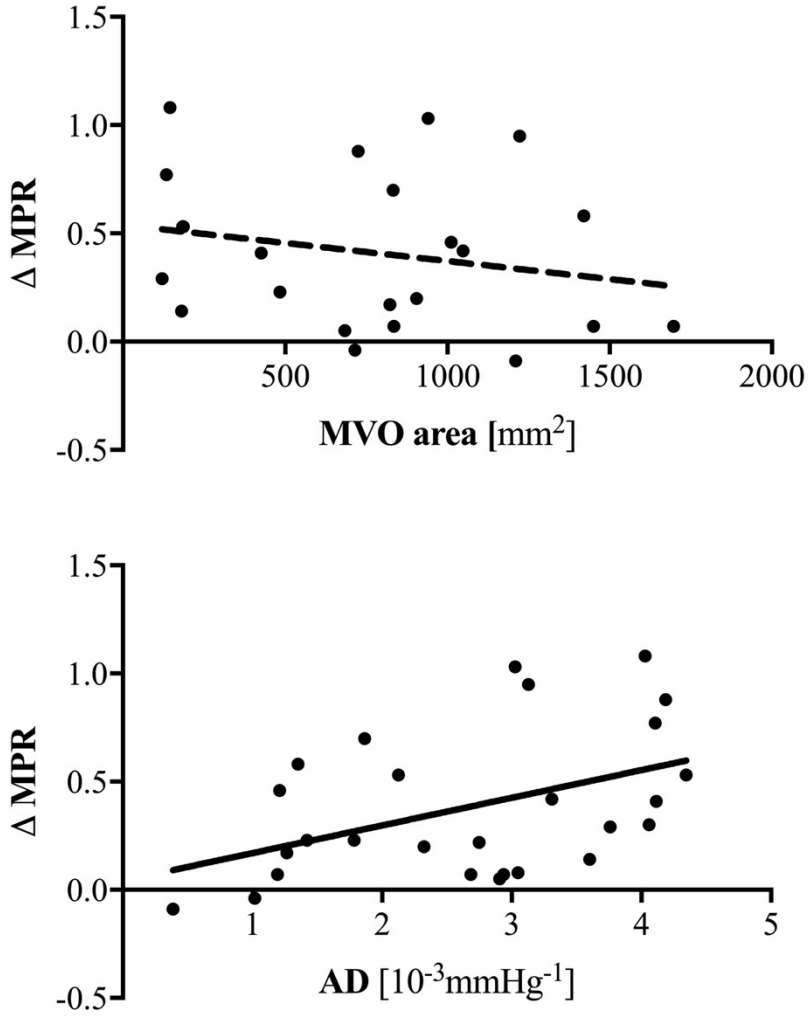

FIGURE 5.7 *Regression analysis between change in MPR and MVO (R^2 0.03, p = 0.314) and aortic distensibility (R^2 0.197, p = 0.020).*

5.6 DISCUSSION

This is the first study to evaluate the relationship between aortic stiffness and myocardial blood flow in infarct and non-infarct territories. Moreover, it is the first to evaluate how this relationship changes over time from day three to three months.

Arterial stiffness and **resting** myocardial blood flow

Resting MBF was not related to aortic distensibility in 'infarct' or 'non-infarct' myocardium at either *early* or *late* time points. Despite successful revascularisation, the microcirculation post infarct is often dysfunctional owing to distal embolisation (De Caterina et al. 2012), vasoconstriction (Cuculi et al. 2013) and oedema (Rezkalla & Kloner 2002). Determinants of *early* 'infarct' territory local blood flow are likely to be governed by mediators driving autoregulation (e.g. myocardial oxygen demand) and the resilience of microcirculatory function at a time where intracoronary resistance is known to be increased (Wijnbergen et al. 2016). Nonetheless, earlier studies (Leung et al. 2006), including our own (unpublished, *Chapter 2 and 3*) have shown a modest association between arterial stiffness and rMBF in non-ischemic and recently re-perfused myocardium. The disruption of a relationship post myocardial infarction suggests that any contribution to rMBF from large artery function is easily overwhelmed by local autoregulation or that at least some of the 'physiological' relationship may be determined by (or related to) an intact microcirculation. If a reduction in trans-coronary perfusion pressure was a significant determinant of rMBF, this effect ought to have been amplified at a time where microvascular resistance was at its greatest. This was not observed. At the *late* time point there had been no recovery of the relationship. Whether this reflects an ongoing, but potentially transient change in the relative determinants of rMBF, possibly through local remodelling or neurohormonal activity, remains to be seen.

The lack of association between rMBF and aortic stiffness in 'non-infarct' myocardium is unsurprising. Evolving evidence demonstrates the systemic sequelae of myocardial infarction such that the behaviour of 'non-infarct' myocardium appears different from truly 'normal' myocardium. Early invasive studies of post-infarct myocardium have been conflicting. Immediately following PCI, de Waard et al. (de Waard et al. 2016) found blood flow in remote myocardium to be increased compared to propensity-matched 'control' myocardium however Teunissen et al. (Teunissen et al. 2015) and Cuculi et al. (Cuculi et al. 2014), also within the first week, suggested no significant difference. In the post infarct setting, resting blood flow in these territories may be altered by increased filling pressures, compensatory hyperkinesis (Lew et al. 1985) and paracrine neurohormonal modulation of tone (Schafer et al. 2002). Regardless, these factors are likely to significantly overwhelm an already modest contribution to blood flow by large arterial function.

Arterial stiffness and **hyperaemic** blood flow

A stiff aorta was associated with reduced hyperaemic response to adenosine in both 'infarct' and 'non-infarct' myocardium. The relationship was similar at the *early* time point although strengthened in the 'non-infarct' compared to the 'infarct' myocardium by three months. The strengthening of the relationship in 'non-infarct' territories may represent recovery from an overwhelming post-infarct systemic inflammatory state characterised by circulating catecholamines and other neurohormonal vasoactive mediators such as vasopressin and angiotensin (Karlsberg et al. 1981; McAlpine et al. 1988). Larger infarcts are associated with a greater systemic inflammatory milieu impacting on haemodynamics and vasomotor tone (Frangogiannis 2014; Pietila et al. 1991), and thus theoretically may disrupt the aorto-coronary relationship to a greater extent. Adjustment for CMR derived infarct size at baseline and follow up, however, had no effect on the distensibility-hMBF correlation. An overall increase in non-infarct hyperaemic flow over time was observed which mirrored that of Teunissen et al.

(Teunissen et al. 2015). This supports the notion that a systemic, albeit transient, phenomenon could affect not only the absolute hyperaemic response, but also its relationship to large artery function.

In the 'infarct' myocardium, the aorto-coronary relationship remained statistically significant although unlike the 'non-infarct' myocardium, did not strengthen at three months and remained less significant when compared to our other studies in 'normal' myocardium. A number of changes are likely to occur at a local infarct artery, and territory, level which extend beyond the systemic milieu proposed to affect a remote territory. Hyperaemic blood flow relies on an intact microcirculatory response and patent capillaries, both of which can be significantly affected in the setting of a STEMI. At a microvascular level, vasoconstrictive mediators such as endothelin-1 (de Waard et al. 2016) are released from the endothelium and may mitigate a vasodilatory stimulation from adenosine. Peri-procedurally, microthrombi and atherosclerotic material released not only from the plaque event but also during instrumentation and stent deployment may plug capillaries and distal arterioles. Although well described in its fulminant form of 'no reflow' phenomenon (Wong et al. 2013), even PCI in stable coronary artery disease populations is associated with a transiently reduced CFVR (Uren, Crake, et al. 1994).

At an epicardial level, the presence of a stent in the infarct related artery may also contribute to reduced hyperaemic flow through the modification of its geometry thereby rendering the vessel less responsive to vasoactive stimuli for at least for the first three to six months (Hamilos et al. 2008; Togni et al. 2007). Although only felt to be a minor contributor to the overall hyperaemic response, the increase in flow generated by the microvasculature is believed to drive a secondary flow-mediated dilatation in the epicardial vessel. In a culprit coronary artery, endothelial dysfunction or the presence of a stent, or both, may reduce this component of hyperaemic flow.

This potential contributor is likely to improve over time and may be mitigated by current generation drug eluting stents (Hamilos et al. 2014).

Notwithstanding all of the above, a number of the phenomena are dynamic and improve by three months and yet the agreement between aorto-coronary haemodynamics remains more modest than in the non-infarct myocardium at this timepoint. This would suggest that these determinants may now be fixed and perhaps relate to irreversible myocardial damage and injury.

Myocardial damage and injury

Studies comparing CMR indices of myocardial injury with invasive measures of microvascular resistance (i.e. index of microcirculatory resistance) demonstrate a strong correlation between the presence of microvascular obstruction on CMR-DE imaging, larger infarct size and a persistent reduction in infarct territory hyperaemic blood flow (Cuculi et al. 2014). Supporting this is Teunissen et al. who demonstrated an increase in hyperaemic flow between one week and three months in the infarct territory however these values remained numerically less than both non-infarct and propensity matched control myocardium (Teunissen et al. 2015). At a pathophysiological level, if there has been significant myocyte necrosis and subsequently fewer functional microvessels, the myocardium may be less capable of reducing absolute microcirculatory resistance and thus, generate less absolute hyperaemic flow.

Our univariate analysis showed a trend for an association between MVO and hMBF although no interaction with infarct size. Partial adjustment did not significantly affect the strength of the aorto-coronary relationship, in fact it numerically weakened it. Furthermore, in univariate analysis, aortic distensibility was also associated with MVO area. It may be that individuals with increased arterial stiffness are more vulnerable to developing MVO. This could be through observed perturbations associated with

arterial stiffness such as baseline reduced relative arteriolar density associated with compensatory hypertrophy or via the unfavourable supply and demand equation observed with increased cardiac work associated with arterial stiffness. Seminal work from Kass supports the latter where an animal model of LAD occlusion was better tolerated by compliant compared to stiff vasculature with observed worsening of regional wall motion and extension of ischaemia (Kass et al. 1996). Of note this was largely driven by an increased sensitivity to lowering mean arterial pressure rather than a reduction in coronary blood flow directly.

A further explanation is that aortic stiffness may also be a generalised marker of vascular risk as it has been associated with both the presence of coronary artery disease and atherosclerotic burden generally (Danchin et al. 2004; Jankowski et al. 2004; McLeod et al. 2004).

Aortic stiffness and myocardial perfusion reserve

Myocardial perfusion reserve was strongly related to aortic stiffness in both 'infarct' and 'non-infarct' myocardium and over time this relationship strengthened. Not only did a stiff aorta predict less improvement in hMBF, but on multivariate analysis, aortic distensibility was an independent predictor of MPR improvement at three months.

Myocardial perfusion reserve is, by definition, affected by both resting and hyperaemic flow. Although it has been calculated by the quotient of absolute hyperaemic and resting blood flow, the established technique is the quotient of the hyperaemic and resting maximal upslopes of the signal-time intensity curve. The simplicity of this MPR analysis obviates the need for mathematical deconvolution and constraint, both of which are known to be sensitive to noise (Lee, DC & Johnson 2009) and, together with more robust reproducibility *(Chapter 3)*, may be a more reliable measure of physiological response than absolute hMBF alone. Moreover the use of a quotient or index, corrects for changes in signal between resting and stress sequences; a source of error that arises when evaluating either resting or hyperaemic indices in isolation.

An evolving body of data support the prognostic role of both coronary flow- and myocardial perfusion reserve as methods to evaluate microcirculatory dysfunction post infarct. An early study evaluating hyperaemic reserve post infarct was performed with digital subtraction angiography post successful PCI and was shown to correlate strongly with regional myocardial function at early follow up (Suryapranata et al. 1994). Some years later the use of stress echocardiography and LAD Doppler signals allowed the approximation of CFR which was shown to predict LV remodelling in a more robust manner than established angiographic indices (Rigo et al. 2004) and subsequently also predicted in-hospital outcomes (Meimoun et al. 2009). In the invasive setting, Doppler FloWire derived CFVR post infarct vessel PCI was shown to predict regional wall motion recovery in hospital (Mazur et al. 1998), global and regional recovery at six months (Bax et al. 2004) and more recently, hard cardiovascular endpoints at 12 months (Takahashi et al. 2007). Understanding the contributors to CFR or MPR improvement post infarct is likely to be clinically relevant.

Limitations

By dichotomising our segments into 'infarct' and 'non-infarct' on the basis of equiangular segmentation of subtended myocardium from the angiogram, border zone and non-infarct myocardium would have been included in some of the 'infarct' segments and vice-versa. In an attempt to simplify the analysis and adjudication of 'infarct' and 'non-infarct', this may have added noise to the signal and reduced the strength of associations with dependent variables. The assessment of all CMR indices (with the exception of LV volumes and ejection fraction) are made on three slices per study and thus represents an approximation and over-represents the apical myocardium in an 18-segment model.

Assessment of presumed microvascular dysfunction was inferred from its association with CMR-DE MVO. Although highly correlated, the two are not the same and it would have been preferable to have performed a more specific marker of microcirculatory function such as IMR. To extend our observations across a spectrum of STEMI, it would have been of interest to study a larger cohort of patients without MVO on CMR-DE imaging. In this cohort, only a paucity of our cohort was free of MVO and we did not have sufficient numbers to perform a subgroup analysis. There is little evidence to confirm the veracity of CMR-PI to evaluate myocardial blood flow in a known area of infarct (Panse et al. 2007; Wong et al. 2012). All of the validation data has been done in 'normal' or ischaemic myocardium. Although theoretically possible, the behaviour of gadolinium within oedematous and leaky myocardium is less predictable, and thus the Fermi modelling assumptions are likely to become inaccurate. The assessment of MPR, however, is less affected by these limitations and may provide some rationale for the observed consistency of those measurements in comparison to divergent MBF values. Further evidence of noise is the modest association between rMBF and MVO (R -0.3) which ought to be measuring similar physiological phenomena.

Vasoactive medications were not held prior to CMR studies as cessation for the purpose of research imaging would have been unethical. Although the absolute values may differ, there was minimal difference in medications between patients and time points and thus is unlikely to have appreciably altered the observed results.

Conclusion

This is the first study to interrogate the relationship between aortic stiffness and indices of myocardial perfusion following STEMI. A stiff aorta was associated with reduced *early* and *late* hyperaemic response to adenosine in both the infarct and non-infarct myocardium. Improvement in MPR was predicted by both MVO and aortic distensibility however on multivariable analysis, only aortic distensibility remained independently associated. A stiffer aorta may be a key predictor for worse outcomes post STEMI.

6. CHAPTER 6: Summary and future directions

6.1 AORTO-CORONARY HAEMODYNAMICS

6.1.1 Resting blood flow

In states of 'health', resting coronary blood flow (rCBF) is mainly governed by local autoregulatory mechanisms, the most dominant being cardiac work. Despite this, after indexing to RPP and correcting for age and gender, aortic distensibility was correlated with both rCBF and rMBF. In *Chapter 4*, associations between aortic stiffness and rMBF were overall less robust but remained statistically significant and stronger in the 'ischaemic' territories. The relative difference between 'ischaemic' and 'non-ischaemic' may represent changes in autoregulation, possibly from 'hibernating' myocardium or a difference in myocardial oxygen demand in critically ischaemic myocardium. This association was not observed in either 'infarct' or 'non-infarct' myocardium *early* or *late* post myocardial infarction. The ability to disrupt this relationship as late as three months post infarction suggests large artery function is only tenuously involved in governing rMBF. The persistence of microcirculatory dysfunction, some of which is likely to be irreversible, may be a key confounder in any aorto-coronary relationship. This is supported by the correlation between MVO area and distensibility and supports the notion that at least some of the aorto-coronary relationship is determined by, or related to, an intact microcirculation.

Acknowledging the differences between these cohorts (and thus, regression analysis not performed), plotting rMBF and distensibility for the three cohorts on the same axis reveals a relatively consistent slope. In a hypothesis generating manner, this would suggest a consistent, albeit modest, relationship between large artery function and rMBF across both health and disease. Observed differences in aorto-coronary relationships within cohorts, therefore, might represent changes in the relative composition and activity from more local mediators of rMBF during states of ischaemia and. infarction.

Regardless, the agreement between distensibility and resting blood flow overall was modest at best, and far less robust than with hyperaemic flow. This easily disrupted relationship suggests aortic stiffness is likely to be only a minor contributor to overall resting perfusion.

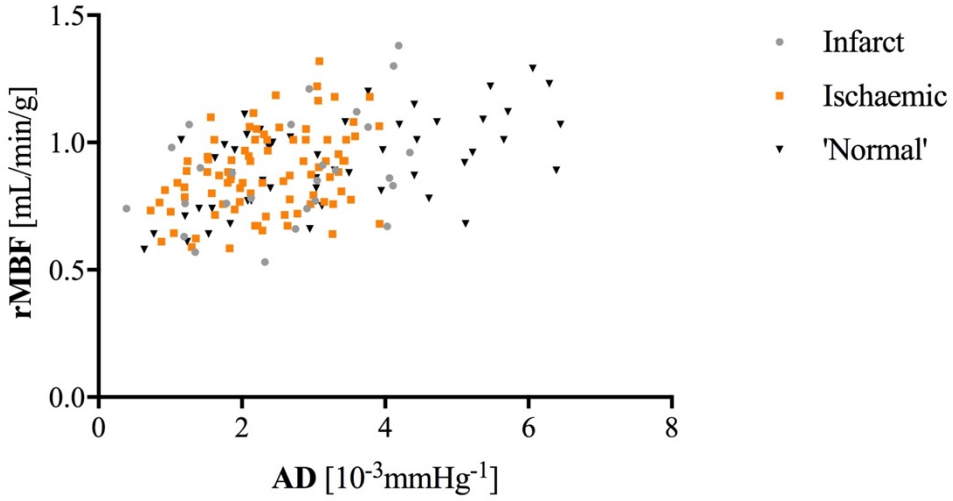

FIGURE 6.1 *Resting myocardial blood flow plotted against distensibility in all three cohorts: 'normal', 'ischaemic' and 'infarct'.*

6.1.2 Response to adenosine: hyperaemic flow and adenosine

Aortic distensibility was strongly associated with hyperaemic blood flow across all cohorts. This relationship was observed in individuals with near 'normal' coronaries, severe epicardial disease and following an infarct. In 'ischaemic' myocardium, the agreement was numerically stronger than 'non-ischaemic' myocardium however the divergence of the curves raises the potential for a 'threshold' effect from large artery function in a near maximally dilated microcirculation. In post-infarct myocardium, aortic stiffness continued to associate with hMBF, however this was less robust than with non-infarct myocardium at follow up. The failure to return to a 'strong' relationship at the follow up time point may be explained by a submaximal response to hyperaemia in remodelling myocardium or potentially an irreversible loss of microcirculatory function. As above, acknowledging these subjects come from different studies, and in the interest of hypothesis generation, plotting hMBF and

distensibility for the three CMR based studies demonstrates a similar slope of agreement. This is supporting evidence of a similar impact from distensibility on this perfusion metric across groups, but with variable contribution from other mediators of blood flow in both ischaemia and post-infarct.

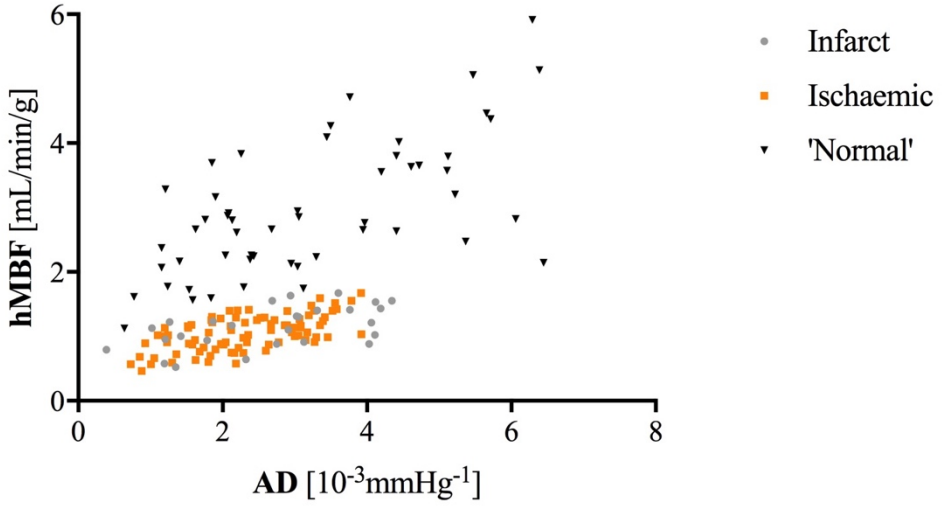

FIGURE 6.2 *Hyperaemic myocardial blood flow plotted against distensibility in all three cohorts: 'normal', 'ischaemic' and 'infarct'.*

6.1.3 Mechanistic basis for the aorto-coronary relationship

These studies have demonstrated variable strengths in the correlation of large artery function with indices of myocardial blood flow in relative 'health', ischaemia and following infarction. Our investigations were not designed, nor are they able, to elucidate mechanisms for these associations, but provide fertile thought for further enquiry. Increased aortic stiffness is associated with a number of perturbations associated with potential impact on coronary blood flow. Firstly, arterial stiffening increases left ventricular afterload, which promotes hypertrophy and a relative reduction in arteriolar density per unit of myocardium, thereby predisposing to ischaemia. Secondly, there is observed increase in intracoronary diastolic resistance, which may be contributed to by both increased intraventricular pressures and wall stress observed with increased arterial stiffness. Thirdly, increased pulse pressure observed with arterial stiffness is believed to cause microvascular rarefaction and possible arteriosclerotic changes, rendering them less responsive to vasomotor stimuli

not only in distant vascular beds, but also the myocardium. Fourthly, an increase in late systolic pressure through aortic impedance or reflected wave phenomena, can reduce central diastolic pressure and thus, potentially reduce coronary diastolic driving pressure. Although all of these are potential contributors, it is also possible that aortic stiffness and microvascular disease occur together, and that aortic stiffness is an integrative marker, or reflection, of microvascular function.

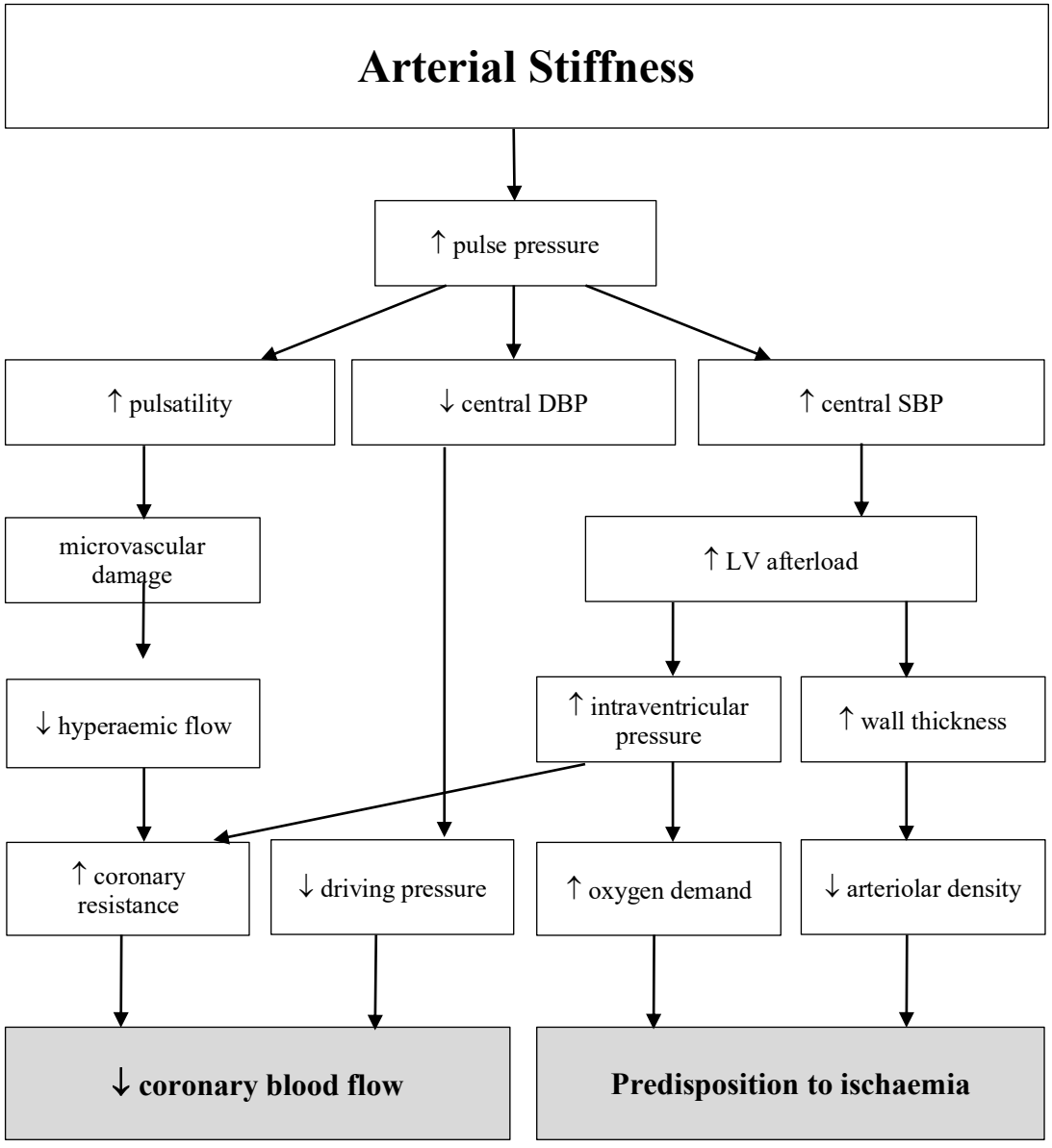

FIGURE 6.3 *Mechanistic diagram of potential contributors to the aorto-coronary relationship*

6.2 CMR PERFUSION IMAGING

This thesis has demonstrated CMR perfusion imaging to be a reproducible and robust modality for evaluating aorto-coronary haemodynamics. The ability to assess myocardial perfusion rather than coronary blood flow offers potential incremental insight as this represents the interface between tissue characteristics as well as blood supply. This clearly comes with limitations as the measures are less exact and prone to noise when compared to invasive, high fidelity imaging. Evidence of this are our regression coefficients in *Chapter 2* ($R^2 \sim 0.5$) compared to *Chapter 3* ($R^2 \sim 0.3$) in what were very similar cohorts. The assessment at a coronary or segment level is time consuming and, without any automation or standardisation, prone to inter-observer variability and inconsistency across centres. Perfusion imaging requires contrast, and thus comes with risks, particularly with repeated exposure and with underlying renal dysfunction. Newer sequences using arterial spin labelling may obviate this requirement. In the last 12 months, our group has also been exploring changes of hyperaemic flow within the coronary sinus (the vessel responsible for venous drainage of the myocardium) as a marker of perfusion reserve. Invasive studies and proof-of-concept phase contrast CMR studies have shown changes in coronary sinus flow in response to adenosine (coronary sinus flow reserve) to approximate that with coronary artery flow reserve (CFR). In a small cohort of 'normal' patients, we have demonstrated a similar relationship with aortic distensibility and an intraclass correlation of >90% when compared with MPR.

Nonetheless, if the aorto-coronary relationship is an endpoint worth evaluating, the assessment must evolve to be semi-automated and ultimately, gadolinium free. Together with ventricular structure and function, CMR provide a valuable, 'one stop shop' cardiovascular dynamic structure and function assessment.

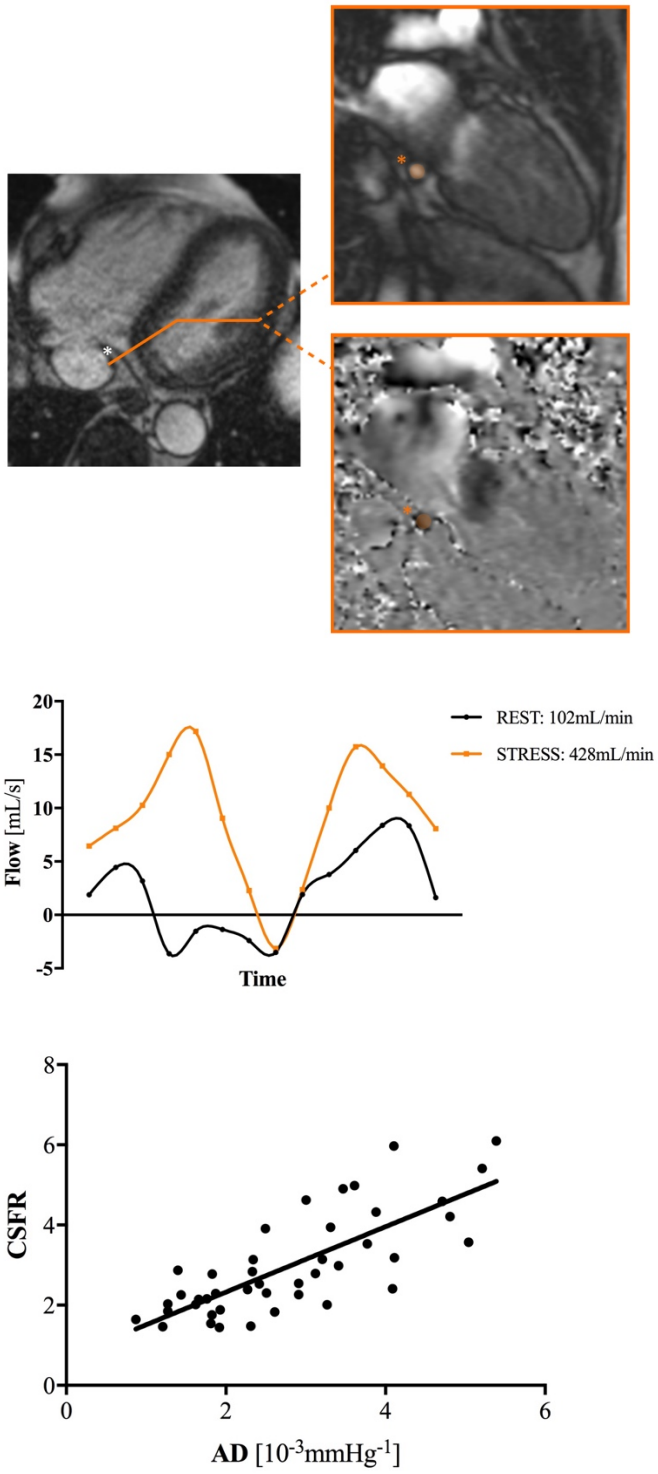

FIGURE 6.4 *Top: CMR image demonstrating transection of coronary sinus (asterisk) with phase contrast cross-sectional image highlighted in orange. Middle: phase contrast flow curves of coronary sinus flow at rest (black) and at hyperaemia (orange). Bottom: regression analysis of coronary sinus flow reserve against aortic distensibility.*

6.3 FUTURE DIRECTIONS

Ongoing work needs to take two directions. Firstly, a deeper understanding is required to decipher the underlying mechanistic basis for the aorto-coronary relationship and its interaction with microvascular function. Dissecting the relative contributions from aorto-coronary driving pressure from coronary microcirculatory resistance will require invasive measures of both pressure, flow and coronary resistance, together with indices of arterial stiffness. Understanding the dominant contributions from both components in different disease states and under different conditions will allow a more complete understanding of how this relationship changes and how therapies could be directed. Secondly, further studies will need to demonstrate that altering the aorto-coronary relationship has relevance, i.e. reducing arterial stiffness can improve anginal threshold, or in the context of an infarct, improve flow. Both would need to be demonstrated independent of blood pressure effects. Agents directed at altering arterial stiffness, particularly in patients with a deleterious aorto-coronary relationship, may derive additional benefit if there are clear changes at an end-organ level. Furthermore, the greatest potential for end-organ benefit is likely to be at the level of the coronary circulation. Ongoing understanding at this interface may provide a greater impetus to move our focus of care in coronary artery disease away from the epicardial vessel to a more integrated assessment of large artery and microcirculatory relationships.

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